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Plant diversity enhances ecosystem responses to elevated CO$_2$ and nitrogen deposition

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Human actions are causing declines in plant biodiversity, increases in atmospheric CO$_2$ concentrations and increases in nitrogen deposition; however, the interactive effects of these factors on ecosystem processes are unknown[1,2]. Reduced biodiversity has raised numerous concerns, including the possibility that ecosystem functioning may be affected negatively[1–4], which might be particularly important in the face of other global changes[5,6]. Here we present results of a grassland field experiment in Minnesota, USA, that tests the hypothesis that plant diversity and composition influence the enhancement of biomass and carbon acquisition in ecosystems subjected to elevated atmospheric CO$_2$ concentrations and nitrogen deposition. The study experimentally controlled plant diversity (1, 4, 9 or 16 species), soil nitrogen (unamended versus deposition of 4 g of nitrogen per m^2 per yr) and atmospheric CO$_2$ concentrations using free-air CO$_2$ enrichment (ambient, 368 μmol mol^{-1}, versus elevated, 560 μmol mol^{-1}). We found that the enhanced biomass accumulation in response to elevated levels of CO$_2$ or nitrogen, or their combination, is less in species-poor than in species-rich assemblages.

In the twenty-first century humans will live in, manage and depend on ecosystems that are less diverse[1,2] and subjected to higher CO$_2$ levels and nitrogen (N) deposition rates than in recorded human history[1]. Although we are beginning to understand the individual impacts of each of these factors on terrestrial ecosystems, our understanding of their interactive effects is poor at best[1–10]. Net primary productivity and carbon (C) input to ecosystems are usually enhanced by elevated CO$_2$, but this seems to be related to the extent and type of other limitations[11–20]. Nitrogen-poor ecosystems have often shown less response to elevated CO$_2$ than more fertile systems[18–25], which is important given that, worldwide,

productivity in most terrestrial ecosystems is limited by N (ref. 26) and rates of N deposition are expected to increase in the future[1,2,6]. In addition, it has been proposed that species-poor communities may be less responsive to elevated CO_2 or N compared with diverse communities[7].

Our study focuses on the influence of plant diversity on the response of ecosystem functions, such as productivity, to elevated CO_2 and N supply rates. Theoretically, because the range in plant traits associated with acquisition of C and N is frequently correlated with the species or functional diversity of an ecosystem[7,10], ecosystem responses to elevated CO_2 and N deposition may be sensitive to variation in levels of biodiversity[4,7,10,11,13–19]. High-diversity plant communities frequently have a greater range of plant functional traits that affect C (for example, C_3 and C_4 photosynthetic pathways) and N (for example, legumes associated with N-fixing symbionts and non-leguminous species) cycling than have species-poor communities[7,10], and therefore have potentially greater responsiveness of ecosystem functions to elevated CO_2 and N deposition. This can occur because diverse assemblages have a greater likelihood of containing species with strong responses to resources and strong impacts on ecosystem processes compared with species-poor assemblages (that is, a sampling effect)[27,28]. Alternatively, this can occur because the greater range of traits extant in diverse assemblages positively affects competitive associations and interactions (such as niche complementarity and positive species interactions)[3,7,10,28].

Testing the influence of plant diversity on the impacts of elevated CO_2 and N to ecosystem functioning requires simultaneous manipulation of plant species composition, CO_2, and N. Although there have been glasshouse studies[8] of species responses to elevated CO_2 in monocultures versus mixtures, and field studies of monocultures and bi-species mixtures[9,15], this is the first field study, to our knowledge, to test the hypothesis[7] that plant species diversity influences ecosystem-scale biomass responses to elevated CO_2 and N levels. The BioCON experimental facility (see Methods) in Minnesota, USA, was designed expressly for the simultaneous manipulation of these three factors in experimental grassland plots under field conditions, using a well-replicated split-plot experiment comprising a full factorial combination of treatment levels in a completely randomized design.

Our study includes 296 individual plots (each 2×2 m) distributed among six 20-m diameter experimental areas (rings). In three elevated CO_2 rings, a free-air CO_2 enrichment system[29] was used during the 1998 and 1999 growing seasons to maintain the CO_2 concentration at an average of $560 \, \mu mol \, mol^{-1}$, a concentration likely to be reached this century[1,2,6]. Three ambient rings ($368 \, \mu mol \, mol^{-1}$ CO_2) were treated identically but without additional CO_2. All plots were planted with 1, 4, 9 or 16 perennial grassland plant species randomly chosen from 16 species in 4 functional groups (C_3 grasses, C_4 grasses, C_3 legumes, C_3 non-legume forbs). Hence high-diversity treatments incorporate greater species and functional group diversity. Native and secondary grasslands in Minnesota typically contain a mixture of these four functional types. Beginning in 1998, half the plots received additional N equivalent to $4 \, g \, N \, m^{-2} \, yr^{-1}$, which is comparable to high rates of N deposition observed in industrialized regions[1].

Diversity, CO_2 and N treatments had significant main effects on total biomass (Table 1, Fig. 1). Above-ground and below-ground biomass both increased markedly with increasing species diversity. Elevated CO_2 predominantly stimulated below-ground biomass, whereas enriched N largely increased above-ground biomass (Table 1). Elevated CO_2 decreased and enriched N increased plant N concentration, total plant N, soil net N mineralization and soil solution N pools (Table 1).

There was no significant interaction between CO_2 and N, and elevated levels of both resources increased total biomass by 27% on average compared with ambient plots (Fig. 1). In essence, the productivity response to elevated CO_2 was not constrained by N limitation in unfertilized plots, even those lacking N-fixing legumes (data not shown). This result differs from many earlier studies[11,18,20], perhaps because we added smaller amounts of N ($4 \, g \, N \, m^{-2} \, yr^{-1}$) to represent elevated N deposition, whereas other studies (such as ref. 18) added high amounts (up to $56 \, g \, N \, m^{-2} \, yr^{-1}$) to mimic agricultural N addition.

Although we did not detect a CO_2 and N interaction, there were significant biomass interactions between diversity and CO_2 as well as between diversity and N (Fig. 1; and Methods), largely owing to responses of below-ground biomass, which comprised three-quarters of the total biomass. As the response to elevated CO_2 and N varied among diversity treatments, we tested their effects within diversity levels, using post-hoc comparisons of the mean responses averaged over all four harvests. In the 16-species plots, all treatments
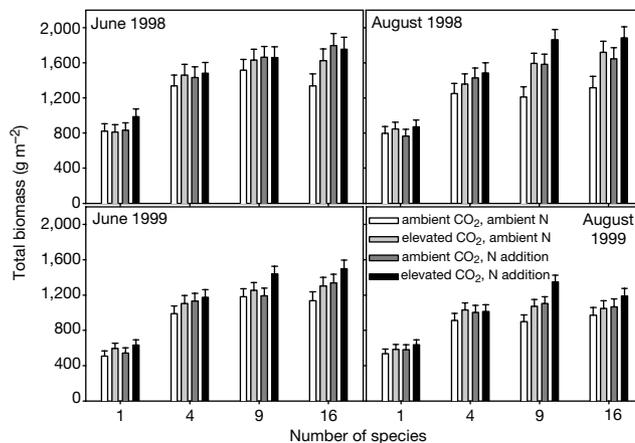

Figure 1 Total biomass (above-ground plus below-ground, 0–20 cm depth) ($\pm$ 1 s.e.) for plots planted with either 1, 4, 9 or 16 species, grown at four combinations of ambient ($368 \, \mu mol \, mol^{-1}$) and elevated ($560 \, \mu mol \, mol^{-1}$) concentrations of CO_2, and ambient N and N addition ($4 \, g \, N \, m^{-2} \, yr^{-1}$). Biomass data are shown for each of four harvests (June and August in both 1998 and 1999). The biomass (total and/or below-ground) response to elevated CO_2, enriched N, or both, differed significantly among diversity treatments at every harvest.

Table 1 Average two-year responses of experimental grassland communities to elevated atmospheric CO_2 concentrations, N deposition, and species diversity

Parameter	R^2	F ratio	CO_2 % change	N % change	Species diversity % change
Total biomass	0.54	16.9	+12**	+13***	+98***
Above-ground biomass	0.39	9.2	+8	+23***	+81***
Below-ground biomass	0.50	15.0	+14*	+9*	+105***
Whole plant %N	0.29	5.9	−13***	+14***	−26***
Total plant N content	0.47	12.9	−2	+29***	+64***
Soil net N mineralization	0.23	4.2	−15	+64*	−68***
Soil solution N (0–20 cm)	0.21	3.9	−14	+36	−76**

R^2 and F ratio shown for the whole model (see Methods), for which P values were always less than 0.001. Main effects (% difference, pooled across all other treatments) and significant level (‡, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) shown are based on measurements in all 296 plots. Species diversity % effects shown compare 16-species versus 1-species plots.

with elevated levels of either CO_2 or N had significantly greater biomass than the ambient CO_2/ambient N plots. In the 9-species plots, only the elevated CO_2/elevated N treatment had significantly greater biomass than the ambient/ambient treatment. In contrast, there was no significant effect of CO_2 or N treatment, alone or in combination, on total biomass for either the 1- or 4-species levels.

Thus, across the four harvests in 1998 and 1999, the enhancement of biomass owing to either elevated CO_2 or enriched N decreased with declining diversity. In the unamended N treatment, the average stimulation of total biomass in response to elevated CO_2 was 22% in 16-species plots, 18% in 9-species plots, 10% in 4-species plots and 7% in monoculture plots (Fig. 2). Differences in plant species diversity accounted for a fivefold difference in the impacts of CO_2 fertilization on biomass accumulation. For example, whereas elevated CO_2 increased biomass by $258 \, \text{g m}^{-2}$ on average in the most diverse (16-species) plots, it increased biomass by only $47 \, \text{g m}^{-2}$ on average in the monocultures (Fig. 2).

Similarly, under ambient CO_2, the average enhancement of total biomass in response to N addition ranged from 25% in 16-species plots to 18%, 11% and 2% in 9-species, 4-species and monoculture plots, respectively (Fig. 2). Thus, biomass enhancement by N enrichment was much less in low-diversity plots—N deposition increased biomass by almost $300 \, \text{g m}^{-2}$ in the diverse plots and by only $15 \, \text{g m}^{-2}$ in the monocultures. For plots subjected to both elevated CO_2 and enriched N, biomass increased by more than $400 \, \text{g m}^{-2}$ in both the 16- and 9-species plots (+35%), but only by

roughly $100-150 \, \text{g m}^{-2}$ in 4-species plots (+15%) and monocultures (+17%).

By sorting above-ground biomass to species, we found that the increase in biomass with increased diversity at each resource level was due largely to the four species that were most abundant in mixtures. The four species *Achillea*, *Bromus*, *Lupinus* and *Poa* together made up more than 80% of the above-ground biomass in 16-species plots (Table 2) and represent all three of the C_3 functional groups. Three of these species were among the four most productive species in monocultures. However, all four of the species abundant in mixtures had lower average monoculture biomass at a given resource level than the average 16-species mixtures at the same resource level (Table 2) and no one species comprised more than one-third of the above-ground biomass in the 16-species mixture at any level. Thus, consistent with a multiple-species 'sampling effect', there was collective dominance in 16-species mixtures by three of the more productive members of the species pool, but this dominance was moderate, and other species also contributed to the greater biomass of the high-diversity plots.

The effects of species diversity on biomass accumulation responses to elevated CO_2 and N deposition also appear to result from the combination of multiple-species sampling effects and niche complementarity or positive species interactions. The four dominant species (*Achillea*, *Bromus*, *Lupinus* and *Poa*) were responsible for much of the increase in biomass in 16-species plots at elevated resource levels (that is, elevated CO_2, enriched N, or both) and showed large responses to these treatments when grown in monoculture (Table 2; for example, *Achillea* and *Lupinus* for elevated CO_2; *Poa* and *Bromus* for elevated N).

Although the response of *Poa* monocultures to N fertilization was large enough to suggest that it may have the capability of explaining most of the increased biomass response of the 16-species mixture if heavily dominant, *Poa* was not more than one-third of the total above-ground biomass in those mixtures, indicating that other species are also important. For elevated CO_2 at ambient N, the stimulation of total biomass ($+258 \, \text{g m}^{-2}$) in 16-species mixtures was greater than the stimulation in monoculture of any species to elevated CO_2. Thus, the increase in biomass of 16-species plots with increasing resource supply was substantially due to the presence and response of species that do respond strongly in monocultures (that is, interpretable as mainly a sampling effect), but they collectively increased more when together than when in monoculture (a niche effect).

In addition, different species were responsible for the enhanced responses to different combinations of CO_2 and N, again suggesting niche differentiation. The increased total biomass response of 16-species plots to elevated CO_2 at ambient N (of +22%, Figs 1 and 2) was largely due to the response of *Achillea*, *Lupinus* and *Bromus*, each of which maintained a roughly similar fraction of a higher total above-ground biomass under elevated than ambient CO_2 (Table 2). At either ambient or elevated CO_2, the enhanced biomass in N-enriched 16-species plots as compared with ambient/ambient plots was due to increases in biomass by *Poa*, *Bromus* and *Achillea*, in that
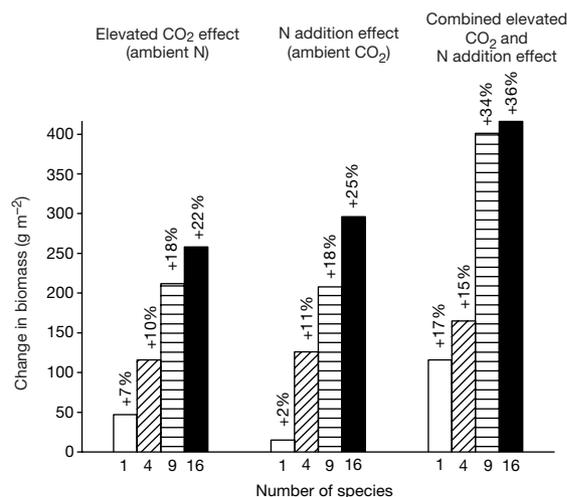

Figure 2 Change in total (above-ground plus 0–20 cm below-ground) biomass (compared with ambient levels of both CO_2 and N) in response to elevated CO_2 alone (at ambient soil N), to enriched N alone (at ambient CO_2), and to the combination of elevated CO_2 and enriched soil N, for plots containing 1, 4, 9 or 16 species. Data were averaged for 4 harvests over 2 yr. Per cent change is shown above each histogram for each diversity treatment.

Table 2 Total biomass of 16-species mixtures and of monocultures of the dominant species in the 16-species mixtures

	Ambient CO_2, ambient N		+CO_2, ambient N		Ambient CO_2, +N		+CO_2, +N	
Biomass (g m^{-2}) of 16-species mixtures	1,165		1,423		1,462		1,581	
Species	Monoculture biomass (g m^{-2})	% of 16-species mixture	Monoculture biomass (g m^{-2})	% of 16-species mixture	Monoculture biomass (g m^{-2})	% of 16-species mixture	Monoculture biomass (g m^{-2})	% of 16-species mixture
Achillea	1,124	34%	1,314	33%	848	31%	1,321	34%
Poa	979	22%	1,089	19%	1,322	33%	1,407	27%
Lupinus	509	15%	672	17%	449	6%	670	9%
Bromus	1,021	11%	989	12%	1,228	14%	1,082	14%
All other species	590	18%	615	19%	587	16%	664	16%

Totals are above-ground plus below-ground biomass, at all four combinations of CO_2 and N. Also shown is the % of total above-ground biomass for each of the dominant species in the 16-species mixtures at each CO_2 and N combination.

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order, despite a decline in *Lupinus* biomass.

These results suggest that multiple-species sampling effects, niche complementarity and positive species interactions[3,7,10,28] jointly help to explain the greater responses of diverse than species-poor plots to elevated levels of the two major global change factors: atmospheric CO_2 and N deposition. Moreover, the central finding of this study—that changes in plant diversity influence the magnitude of CO_2 and N impacts on ecosystem functioning—is important regardless of which set of mechanisms are operating.

Our study raises concerns about the consequences of widespread changes in plant composition and diversity in ecosystems world-wide for responses to other global changes[1,2], because the results show that, in response to elevated levels of CO_2 and N, ecosystems with decreased diversity may acquire less C and biomass than ecosystems with greater diversity. It is unclear whether the responses observed in 1998–1999 will change with time as the composition of the communities changes, the climate varies, and long-term soil microbial feedbacks occur[20–25], or whether natural or managed vegetation would respond similarly to combinations of diversity, CO_2 and N. Nonetheless, our results suggest that the reduction of diversity occurring globally may reduce the capacity of ecosystems to capture additional C under conditions of rising atmospheric CO_2 concentrations and N deposition levels. □

Methods

BioCON experiment

The BioCON (Biodiversity, CO_2 and N) experiment (http://swan.lter.umn.edu/biocon/) is located at the Cedar Creek Natural History area in Minnesota, USA. Plots were established on a secondary successional grassland on a sandy outwash soil after removing the previous vegetation. The experimental treatments were arranged in complete factorial combination of CO_2 (ambient or 560 μmol mol^{-1}), species number (1, 4, 9, and 16) and N level (control and fertilized). The species numbers were chosen as the squares of 1, 2, 3 and 4 to represent roughly equal effective differences in diversity, on the basis of earlier studies[4]. Each plot was planted in 1997 with 12 g m^{-2} of seed partitioned equally among all species planted in a plot.

The design consisted of a split-plot arrangement of treatments in a completely randomized design. CO_2 treatment is the whole-plot factor and is replicated three times among the six rings. The subplot factors of species number and N treatment were assigned randomly and replicated in individual plots among the six rings. For each of the four combinations of CO_2 and N levels, pooled across all rings, there were 32 randomly assigned replicates for the plots planted to 1 species, 15 for those planted to 4 species, 15 for 9 species, and 12 for 16 species. Beginning in 1998, the plots assigned to the N addition treatment were amended with 4 g N m^{-2} yr^{-1}, applied over three dates each year. CO_2 was added in elevated treatments during all daylight hours from 9 April to 16 October 1998, and from 20 April to 9 November 1999. Although there was modest variation in CO_2 concentrations spatially within and across rings, the average CO_2 concentrations were not more than 1–2 μmol mol^{-1} different among rings, or among diversity or N treatment levels averaged within and across rings (see Supplementary Information).

Species and biomass measurements

The 16 species used in this study were all native or naturalized to the Cedar Creek Natural History Area. They include four C$_4$ grasses (*Andropogon gerardii, Bouteloua gracilis, Schizachyrium scoparium, Sorghastrum nutans*), four C$_3$ grasses (*Agropyron repens, Bromus inermis, Koeleria cristata, Poa pratensis*), four N-fixing legumes (*Amorpha canescens, Lespedeza capitata, Lupinus perennis, Petalostemum villosum*) and four non-N-fixing herbaceous species (*Achillea millefolium, Anemone cylindrica, Asclepias tuberosa, Solidago rigida*), and all are referred to by genus elsewhere. Monocultures of all species were replicated twice at all CO_2 and N levels. The 4- and 9-species plots were random selections from all species. Plots were regularly weeded to remove unwanted species. In June and August of each year, we assessed above- and below-ground (0–20 cm) biomass, plant C and N, and soil N (see Supplementary Information). Soil net N mineralization rates were measured once each year. Above-ground biomass was sorted to species at each harvest. The species richness of clipped above-ground biomass samples was 1.0, 3.8, 8.0 and 13.6 species on average for plots planted with 1, 4, 9 and 16 species, respectively, on the basis of the number of species identified in each plot from the four above-ground harvests (each 0.1 m^2).

Statistical analysis

In analysis of variance all treatment effects were considered fixed. Using F-tests, the effect of CO_2 (1 degree of freedom, d.f.) was tested against the random effect of ring nested within CO_2 (4 d.f.). The main effects of species number (3 d.f.) and N (1 d.f.), and interactions between CO_2 and N were tested against the residual error. The main effect of species number and its first-order interaction terms were partitioned into single-degree-of-freedom contrasts for linear, quadratic and cubic terms to test for interactions between diversity and either CO_2 or N treatments, and additionally to test hypotheses about predetermined contrasts of elevated resource levels (elevated CO_2, enriched N, or both) versus the ambient/ambient conditions. There were interactions for below-ground biomass between species diversity and CO_2 treatments in the August 1998 ($P < 0.05$), June 1999 ($P < 0.10$) and August 1999 ($P < 0.10$) harvests, and between species diversity and N treatments in the June 1999 ($P < 0.05$) and August 1999 ($P < 0.05$) harvests. For the pre-planned contrast between the ambient/ambient and the elevated CO_2/enriched N treatments, there were significantly different responses (for below-ground and total biomass) for different diversity treatments at the June 1998 ($P < 0.05$), August 1998 ($P < 0.05$) and June 1999 ($P < 0.05$) harvests. Given interactions between species diversity and resource treatments, post-hoc Student's t-tests were also conducted to compare individual resource treatments within species diversity levels. All analyses were conducted for each harvest, for each year (pooling harvest data by plot), and across years averaged (pooling data for all harvests by plot), with similar results, although there was harvest-to-harvest variation (see Fig. 1).

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