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## Plant diversity enhances ecosystem responses to elevated CO<sub>2</sub> and nitrogen deposition

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Human actions are causing declines in plant biodiversity, increases in atmospheric CO<sub>2</sub> concentrations and increases in nitrogen deposition; however, the interactive effects of these factors on ecosystem processes are unknown<sup>1,2</sup>. Reduced biodiversity has raised numerous concerns, including the possibility that ecosystem functioning may be affected negatively<sup>1-4</sup>, which might be particularly important in the face of other global changes<sup>5,6</sup>. 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<sub>2</sub> 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<sup>2</sup> per yr) and atmospheric CO<sub>2</sub> concentrations using free-air CO<sub>2</sub> enrichment (ambient, 368 μmol mol<sup>-1</sup>, versus elevated, 560 μmol mol<sup>-1</sup>). We found that the enhanced biomass accumulation in response to elevated levels of CO<sub>2</sub> 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<sup>1,2</sup> and subjected to higher CO<sub>2</sub> levels and nitrogen (N) deposition rates than in recorded human history<sup>1</sup>. 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<sup>1-10</sup>. Net primary productivity and carbon (C) input to ecosystems are usually enhanced by elevated CO<sub>2</sub>, but this seems to be related to the extent and type of other limitations<sup>11-20</sup>. Nitrogen-poor ecosystems have often shown less response to elevated CO<sub>2</sub> than more fertile systems<sup>18-25</sup>, 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<sup>1,2,6</sup>. In addition, it has been proposed that species-poor communities may be less responsive to elevated CO<sub>2</sub> or N compared with diverse communities<sup>7</sup>.

Our study focuses on the influence of plant diversity on the response of ecosystem functions, such as productivity, to elevated CO<sub>2</sub> 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<sup>7,10</sup>, ecosystem responses to elevated CO<sub>2</sub> and N deposition may be sensitive to variation in levels of biodiversity<sup>4,7,10,11,13–19</sup>. High-diversity plant communities frequently have a greater range of plant functional traits that affect C (for example, C<sub>3</sub> and C<sub>4</sub> photosynthetic pathways) and N (for example, legumes associated with N-fixing symbionts and non-leguminous species) cycling than have species-poor communities<sup>7,10</sup>, and therefore have potentially greater responsiveness of ecosystem functions to elevated CO<sub>2</sub> 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)<sup>27,28</sup>. 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)<sup>3,7,10,28</sup>.

Testing the influence of plant diversity on the impacts of elevated CO<sub>2</sub> and N to ecosystem functioning requires simultaneous manipulation of plant species composition, CO<sub>2</sub>, and N. Although there have been glasshouse studies<sup>8</sup> of species responses to elevated CO<sub>2</sub> in monocultures versus mixtures, and field studies of monocultures and bi-species mixtures<sup>9,15</sup>, this is the first field study, to our knowledge, to test the hypothesis<sup>7</sup> that plant species diversity influences ecosystem-scale biomass responses to elevated CO<sub>2</sub> 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.

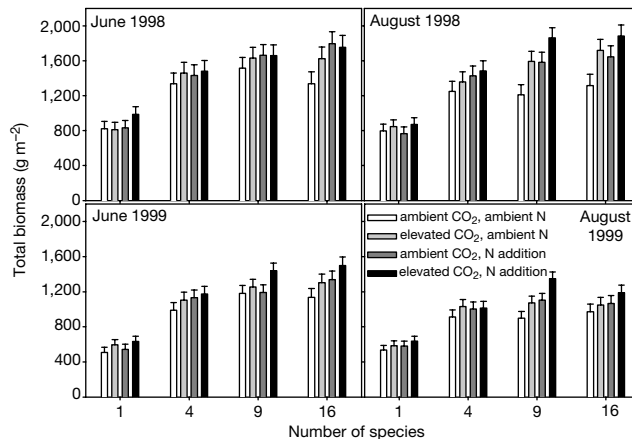
Our study includes 296 individual plots (each 2 × 2 m) distributed among six 20-m diameter experimental areas (rings). In three elevated CO<sub>2</sub> rings, a free-air CO<sub>2</sub> enrichment system<sup>29</sup> was used during the 1998 and 1999 growing seasons to maintain the CO<sub>2</sub> concentration at an average of 560 μmol mol<sup>-1</sup>, a concentration likely to be reached this century<sup>1,2,6</sup>. Three ambient rings (368 μmol mol<sup>-1</sup> CO<sub>2</sub>) were treated identically but without additional CO<sub>2</sub>. All plots were planted with 1, 4, 9 or 16 perennial grassland plant species randomly chosen from 16 species in 4 functional groups (C<sub>3</sub> grasses, C<sub>4</sub> grasses, C<sub>3</sub> legumes, C<sub>3</sub> 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 addi-

tional N equivalent to 4 g N m<sup>-2</sup> yr<sup>-1</sup>, which is comparable to high rates of N deposition observed in industrialized regions<sup>1</sup>.

Diversity, CO<sub>2</sub> 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<sub>2</sub> predominantly stimulated below-ground biomass, whereas enriched N largely increased above-ground biomass (Table 1). Elevated CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sup>11,18,20</sup>, perhaps because we added smaller amounts of N (4 g N m<sup>-2</sup> yr<sup>-1</sup>) to represent elevated N deposition, whereas other studies (such as ref. 18) added high amounts (up to 56 g N m<sup>-2</sup> yr<sup>-1</sup>) to mimic agricultural N addition.

Although we did not detect a CO<sub>2</sub> and N interaction, there were significant biomass interactions between diversity and CO<sub>2</sub> 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<sub>2</sub> 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  $\mu\text{mol mol}^{-1}$ ) and elevated (560  $\mu\text{mol mol}^{-1}$ ) concentrations of CO<sub>2</sub>, and ambient N and N addition (4 g N m<sup>-2</sup> yr<sup>-1</sup>). 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<sub>2</sub>, 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<sub>2</sub> concentrations, N deposition, and species diversity

Parameter	R <sup>2</sup>	F ratio	CO <sub>2</sub> % 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<sup>2</sup> 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 ( $\dagger$ ,  $P < 0.1$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ) 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<sub>2</sub> or N had significantly greater biomass than the ambient CO<sub>2</sub>/ambient N plots. In the 9-species plots, only the elevated CO<sub>2</sub>/elevated N treatment had significantly greater biomass than the ambient/ambient treatment. In contrast, there was no significant effect of CO<sub>2</sub> 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<sub>2</sub> or enriched N decreased with declining diversity. In the unamended N treatment, the average stimulation of total biomass in response to elevated CO<sub>2</sub> 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<sub>2</sub> fertilization on biomass accumulation. For example, whereas elevated CO<sub>2</sub> increased biomass by 258 g m<sup>-2</sup> on average in the most diverse (16-species) plots, it increased biomass by only 47 g m<sup>-2</sup> on average in the monocultures (Fig. 2).

Similarly, under ambient CO<sub>2</sub>, 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 g m<sup>-2</sup> in the diverse plots and by only 15 g m<sup>-2</sup> in the monocultures. For plots subjected to both elevated CO<sub>2</sub> and enriched N, biomass increased by more than 400 g m<sup>-2</sup> in both the 16- and 9-species plots (+35%), but only by

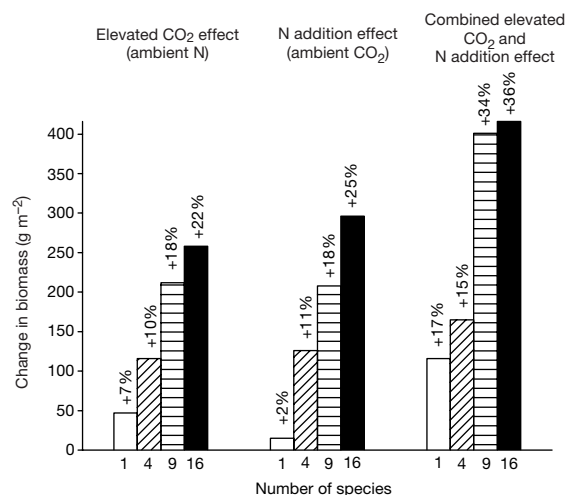
roughly 100–150 g m<sup>-2</sup> in 4-species plots (+15%) and monocultures (+17%).

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<sub>3</sub> 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<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub>; *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<sub>2</sub> at ambient N, the stimulation of total biomass (+258 g m<sup>-2</sup>) in 16-species mixtures was greater than the stimulation in monoculture of any species to elevated CO<sub>2</sub>. 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<sub>2</sub> and N, again suggesting niche differentiation. The increased total biomass response of 16-species plots to elevated CO<sub>2</sub> 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<sub>2</sub> (Table 2). At either ambient or elevated CO<sub>2</sub>, 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<sub>2</sub> and N) in response to elevated CO<sub>2</sub> alone (at ambient soil N), to enriched N alone (at ambient CO<sub>2</sub>), and to the combination of elevated CO<sub>2</sub> 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

Species	Ambient CO <sub>2</sub> , ambient N		+CO <sub>2</sub> , ambient N		Ambient CO <sub>2</sub> , +N		+CO <sub>2</sub> , +N	
	Monoculture biomass (g m <sup>-2</sup> )	% of 16-species mixture	Monoculture biomass (g m <sup>-2</sup> )	% of 16-species mixture	Monoculture biomass (g m <sup>-2</sup> )	% of 16-species mixture	Monoculture biomass (g m <sup>-2</sup> )	% of 16-species mixture
<i>Achillea</i>	1,124	34%	1,314	33%	848	31%	1,321	34%
<i>Poa</i>	979	22%	1,089	19%	1,322	33%	1,407	27%
<i>Lupinus</i>	509	15%	672	17%	449	6%	670	9%
<i>Bromus</i>	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<sub>2</sub> 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<sub>2</sub> and N combination.

order, despite a decline in *Lupinus* biomass.

These results suggest that multiple-species sampling effects, niche complementarity and positive species interactions<sup>3,7,10,28</sup> 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<sub>2</sub> and N deposition. Moreover, the central finding of this study—that changes in plant diversity influence the magnitude of CO<sub>2</sub> 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 worldwide for responses to other global changes<sup>1,2</sup>, because the results show that, in response to elevated levels of CO<sub>2</sub> 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<sup>20–25</sup>, or whether natural or managed vegetation would respond similarly to combinations of diversity, CO<sub>2</sub> 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<sub>2</sub> concentrations and N deposition levels. □

## Methods

### BioCON experiment

The BioCON (Biodiversity, CO<sub>2</sub> 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<sub>2</sub> (ambient or 560 μmol mol<sup>-1</sup>), 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<sup>4</sup>. Each plot was planted in 1997 with 12 g m<sup>-2</sup> 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<sub>2</sub> 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<sub>2</sub> 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<sup>-2</sup> yr<sup>-1</sup>, applied over three dates each year. CO<sub>2</sub> 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<sub>2</sub> concentrations spatially within and across rings, the average CO<sub>2</sub> concentrations were not more than 1–2 μmol mol<sup>-1</sup> 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<sub>4</sub> grasses (*Andropogon gerardii*, *Bouteloua gracilis*, *Schizachyrium scoparium*, *Sorghastrum nutans*), four C<sub>3</sub> 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<sub>2</sub> 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<sup>2</sup>).

### Statistical analysis

In analysis of variance all treatment effects were considered fixed. Using *F*-tests, the effect of CO<sub>2</sub> (1 degree of freedom, d.f.) was tested against the random effect of ring nested within CO<sub>2</sub> (4 d.f.). The main effects of species number (3 d.f.) and N (1 d.f.), and interactions between CO<sub>2</sub> 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<sub>2</sub> or N treatments, and additionally to test hypotheses about

predetermined contrasts of elevated resource levels (elevated CO<sub>2</sub>, enriched N, or both) versus the ambient/ambient conditions. There were interactions for below-ground biomass between species diversity and CO<sub>2</sub> 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<sub>2</sub>/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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Supplementary information is available on Nature's World-Wide Web site (<http://www.nature.com>) or as paper copy from the London editorial office of Nature.

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