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BELOWGROUND RESPONSES TO CLIMATE CHANGE

Long-term enhancement of N availability and plant growth under elevated CO_2 in a semi-arid grassland

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Summary

1. While rising atmospheric CO_2 has the potential to enhance plant growth and biomass accumulation, rates of these processes may be constrained by soil nitrogen (N) availability. Despite much effort, it is still uncertain how elevated CO_2 affects long-term soil N dynamics.

2. We used open-top chambers to examine the effect of 5 years of elevated atmospheric CO_2 concentration (720 vs. 368 p.p.m.) on N dynamics in a semi-arid grassland ecosystem in north-eastern Colorado, USA. In the first year 0·5 g m^{-2} of ammonium nitrate-N, 99·9 atom% ^{15}N, was added to each plot. We examined the effect of elevated CO_2 on N mineralization and plant N uptake by tracking the labelled and total N in plant and soil over the following 5 years.

3. Plant growth and plant N uptake remained significantly higher under elevated than under ambient CO_2. The fraction of labelled N (expressed per unit of total N) in above-ground biomass declined over time, and this decline was greater under elevated CO_2. The amount and fraction of labelled N in the soil did not change with time and was unaffected by elevated CO_2. These results suggest that with time, N released from mineralization in the soil diluted the labelled N in above-ground biomass and that this dilution effect caused by N mineralization was greater under elevated CO_2. More of the mineralized N ended up in the above-ground biomass of *Stipa comata* and forbs (C_3) than in *Bouteloua gracilis* (C_4) under elevated CO_2.

4. Increased soil moisture under elevated CO_2 likely supported higher rates of N mineralization, thereby reducing N constraints on plant growth. Therefore, in semi-arid systems, plant growth and species composition responses to elevated CO_2 may be more persistent than in mesic systems where N mineralization is less constrained by soil moisture.

Key-words: climate, ^{15}N tracer, nitrogen mineralization, nitrogen uptake, soil moisture

Introduction

The global atmospheric CO_2 concentration has increased from a pre-industrial value of about 280–368 p.p.m. in 2001, and could more than double by the end of this century (Intergovernmental Panel on Climate Change 2001). Increases in atmospheric CO_2 concentration (hereafter also referred to as elevated CO_2) stimulate plant growth (Ainsworth & Long 2005), alter species composition (Smith *et al.* 2000), and may increase soil C storage, thereby slowing the rate of further increases in atmospheric CO_2 (Thompson *et al.* 2004). However, it remains uncertain if ecosystem responses to elevated CO_2 can be sustained (Dufresne *et al.* 2002; Reich *et al.* 2006a).

In particular, soil nitrogen (N) availability for plant growth may constrain the extent to which rising atmospheric CO_2 enhances plant and soil C sequestration (Hungate *et al.* 2003; Luo *et al.* 2004; de Graaff *et al.* 2006; Reich *et al.* 2006a; Van Groenigen *et al.* 2006). It has been postulated that elevated CO_2 may reduce soil N availability, also referred to as the concept of progressive N limitation (PNL, Luo *et al.* 2004). Overall, PNL develops when proportionally more of the available soil N is fixed into long-lived plant biomass and soil organic matter (SOM) under elevated CO_2. Therefore, PNL could eventually counteract the initial stimulation of plant growth

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and C sequestration in response to elevated CO_2 (Luo *et al.* 2004; Reich, Hungate & Luo 2006b).

Extensive research has been done on how elevated CO_2 affects soil N availability, but results have been inconsistent. Elevated CO_2 can increase (Hungate *et al.* 1997; Ebersberger, Niklaus & Kandeler 2003), have no effect on (Williams, Rice & Owensby 2001; Finzi & Schlesinger 2003; Zak *et al.* 2003), or decrease soil N availability under field conditions (Gill *et al.* 2002; Reich *et al.* 2006a). Moreover, most field experiments in which CO_2 has been manipulated have been relatively short in duration (3 years or less), thereby reducing their utility for understanding long-term effects of elevated CO_2 on soil N availability.

Understanding how CO_2 affects soil N availability has been further hampered by difficulties measuring soil N availability under field conditions. Soil N availability has been estimated by measuring net N mineralization in soil incubations (in the field or in the laboratory, Gill *et al.* 2002; Ebersberger *et al.* 2003; Finzi & Schlesinger 2003; Reich *et al.* 2006a) or by measuring short-term gross mineralization and immobilization reactions (up to 48 h) through additions of ^{15}N to the soil (Hungate *et al.* 1997; Williams *et al.* 2001; Zak *et al.* 2003). However, soil incubations have severe limitations, in part because they are performed in the absence of plants (Schimel & Bennett 2004), and short-term measurements of gross N mineralization and immobilization are usually not sensitive enough to detect responses in soil N availability to elevated CO_2 (Zak *et al.* 2000; de Graaff *et al.* 2006; Reich *et al.* 2006b).

Net primary productivity and species composition responses to elevated CO_2 are predicted and have been shown to be among the largest in semi-arid and arid ecosystems (Strain & Bazzaz 1983; Melillo *et al.* 1993; Smith *et al.* 2000; Morgan *et al.* 2004b). While these responses may directly be related to improved water conditions, an increase in soil N availability under elevated CO_2 may be also important. When elevated CO_2 increases soil moisture and N availability, it could potentially delay the development of PNL. This effect of elevated CO_2 on soil moisture and N availability could particularly be important in semi-arid and arid ecosystems where soil moisture plays a key role in decomposition and mineralization. We hypothesize that soil N availability remains persistently higher under elevated than under ambient CO_2 in this semi-arid grassland causing sustained greater plant N uptake and plant growth during 5 years of elevated CO_2. We used a novel ^{15}N tracer technique to obtain an integrated long-term measurement of how elevated CO_2 influenced soil N availability. This new method further takes into account the direct effects of plants on N mineralization, thereby circumventing the pitfalls and shortcomings associated with soil incubation methods.

Methods

This experiment was established in 1996 on a native rangeland pasture at the USDA-ARS Central Plains Experimental Range, Colorado, USA. Mean annual precipitation is 321 mm, with the majority occurring in May, June and July, and mean air temperatures are 15·6 °C in July and 0·6 °C in January. Dominant species are the warm-season C_4 grass *Bouteloua gracilis* and the cool-season C_3 grasses *Pascopyrum smithii* and *Stipa comata* (together comprising *c.* 88% of the above-ground biomass in 1996). Other species include the sedge *Carex eleocharis* and the sub-shrub *Artemisia frigida* (each 4% of above-ground biomass in 1996). In March 1997 six open-top chambers (4·5 m diameter) were installed on the pasture, three chambers with ambient CO_2 (360 ± 20 μmol/mol) and three with elevated CO_2 (720 ± 20 μmol/mol, Morgan *et al.* 2004a). Chambers were removed in late October each year after plant senescence and reinstalled in March before plant growth began. Baseline plant data were collected in 1996 (same protocol used during 1997–2001, see below), prior to the installation of the chambers. In early April, 1997, we uniformly sprayed 0·5 g N m^{-2} of ammonium nitrate-N, 99·9 atom% ^{15}N (both ammonium-N and nitrate-N were 99·9 atom% ^{15}N, hereafter 'labelled N') as a solution (10 mm of water was applied during the N application) to the whole area of each of the plots.

Above-ground plant biomass was collected each year from 1997 to 2001 at the time of peak standing biomass (late July). A metal grid containing fifty-six 40·5 × 15·3 cm quadrats (total of 3·46 m^2) was placed inside the plots, and vegetation in every other quadrats (28 quadrats) was clipped to the crown. The 28 unclipped quadrats were clipped at peak standing biomass the next year. By October above-ground biomass had senesced and all 56 quadrats were harvested each year, including the 28 quadrats that were not clipped that previous summer and the 28 quadrats that were clipped (regrowth biomass). In July each year, the above-ground biomass was separated by the species *B. gracilis*, *P. smithii*, *S. comata* and *C. eleocharis*, while the remaining biomass was grouped into species groups 'forbs' (mostly the sub-shrub *A. frigida*), 'other C_3' and 'other C_4' grasses. Each year in October two 20-cm diameter cores to 60-cm depth were removed from each plot and separated into above- and below-ground plant biomass and soil. All above- and below-ground plant biomass and soil samples were dried at 60 °C and weighed. Plant and soil samples were ground and analysed for total N and ^{15}N by combustion/isotope ratio mass spectrometry.

We calculated the labelled N expressed as a fraction of total *N* in plant biomass in year '*i*' ($N_{label,i}$, in mg g^{-1}) using the following equation:

$$N_{label,i} = \frac{(^{15}N_{plant,i} - {}^{15}N_{plant,96})}{(^{15}N_{label} - {}^{15}N_{plant,96})} \times 1000$$

where $^{15}N_{plant,i}$, $^{15}N_{plant,96}$, and $^{15}N_{label}$ are the atom% ^{15}N in plant biomass measured in year *i*, in plant biomass measured in 1996 and of the labelled N added, respectively. We calculated the fraction of labelled N in the soil similarly. We assumed that a decrease in $N_{label,i}$ over time was due to uptake of unlabelled N from the soil, and that the rate of this dilution of labelled N with unlabelled N would be positively related to N mineralization in the soil (see discussion below). We calculated ^{15}N retention in the top 60-cm of the soil ($^{15}N_{retention,soil}$, the amount of labelled N recovered in the soil in mg m^{-2}) from the core samples harvested each year in October using the following equation:

$$^{15}N_{retention,soil} = N_{soil,i} \times \frac{(^{15}N_{soil,i} - {}^{15}N_{soil,96})}{(^{15}N_{label} - {}^{15}N_{soil,96})}$$

where $N_{soil,i}$ is the total amount of N in the soil measured in year *i* (mg m^{-2}), and $^{15}N_{soil,i}$ and $^{15}N_{soil,96}$ are the atom% ^{15}N in the soil measured in year *i* and in 1996, respectively. We also calculated ^{15}N retention in the whole ecosystem by summing the ^{15}N retention in the soil and in above- and below-ground biomass.

We used repeated-measures ANCOVA to test for CO_2 treatment effects on above-ground biomass, N concentration, and total N in

above-ground biomass (between-subject) over time (within-subject). Because of differences among plots in above-ground biomass, N concentration, and total N in above-ground biomass collected in 1996, we used these variables as covariates. There were no significant CO_2 × time interaction effects ($P > 0{\cdot}1$). We then tested for CO_2 treatment effects on average total N in above-ground biomass (average from 1997 to 2001) using ANCOVA with above-ground biomass, N concentration, and the total N in above-ground biomass collected in 1996 as a covariate. Because above-ground biomass collected in July was separated by species and species groups, we were able to test for CO_2 treatment effects on N content in the July above-ground biomass among species and species groups over time, using repeated-measures ANCOVA (CO_2 and species/species groups as main factors and above-ground N content in 1996 as a covariate). We used repeated-measures ANOVA to test for CO_2 and species effects on labelled N fractions in above-ground biomass (log-transformed) and soil, and ^{15}N retention in plant and soil over time. We also used ANOVA to test for CO_2 effects on labelled N fractions in above-ground biomass in each year.

Results

Above-ground plant biomass under elevated CO_2 was significantly greater than under ambient CO_2 ($P < 0{\cdot}05$, 5-year average) and had a significantly greater total N content by the time of senescence in October (up to 68%, $P < 0{\cdot}05$, Fig. 1). The increase of the above-ground N pool in October under elevated CO_2 was sustained during the 5-year period (no significant time × CO_2 interaction using repeated measures ANCOVA). The N concentration in the July above-ground biomass was significantly lower under elevated CO_2 ($P < 0{\cdot}001$) than under ambient CO_2, which resulted in similar amounts of above-ground plant N in July under elevated and ambient CO_2. Above-ground plant biomass was reported earlier by Morgan *et al.* (2004a) and plant N concentration and content for the first 3 years by King *et al.* (2004). Elevated CO_2 had no effect on below-ground plant biomass or N content measured each year in October (data not shown).

Table 1. Repeated-measures ANCOVA results for N content (g m^{-2}) in above-ground biomass collected in July by species (*B. gracilis*, *P. smithii*, *S. comata* and *C. eleocharis*) or species groups (forbs, other C_3 and other C_4 grasses)

Source of variation	d.f.	*F*	*P*
CO_2	1	0·11	0·74
Sp/sp gr	6	10·03	**< 0·0001**
Year	4	1·72	0·15
CO_2 × sp/sp gr	6	2·77	*0·06*
CO_2 × year	4	2·15	*0·08*
Sp/sp gr × year	24	5·08	**< 0·0001**
CO_2 × sp/sp gr × year	24	1·65	**0·04**
1996 above-ground biomass	1	61·36	**< 0·0001**

P-values in italics when $P < 0{\cdot}10$, and in bold when $P < 0{\cdot}05$.

Species and species groups responded differently to elevated CO_2 in terms of their above-ground N content collected in July (CO_2 × species/species group interaction $P = 0{\cdot}06$), and these species/species group-specific responses significantly changed over time (CO_2 × species/species group × year interaction $P = 0{\cdot}04$, Table 1). Elevated CO_2 reduced above-ground N content in the C_4 grass *B. gracilis*, but increased above-ground N content in the C_3 grass *S. comata* and forbs, particularly during the last 2 years of the experiment (Fig. 2). The other species and species groups did not respond to elevated CO_2. More than 80% of the above-ground N was tied up in the species *B. gracilis*, *P. smithii* and *S. comata* (data not shown). In the final year, above-ground N content in the forbs increased sevenfold in response to elevated CO_2.

The ^{15}N in plant biomass, expressed as a fraction of total plant N (labelled N fraction) clipped in July and in October, spiked in year 1, but then significantly declined over time (Fig. 3, Table 2), indicating an initial uptake of N enriched in ^{15}N (i.e. directly after ^{15}N application) followed by a progressive dilution of labelled N with unlabelled N. This progressive ^{15}N

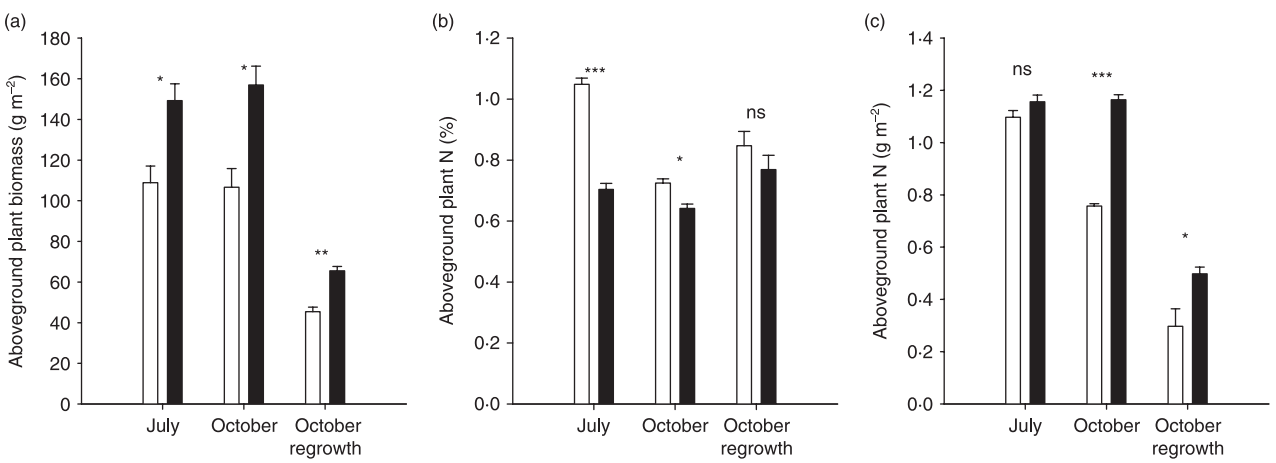

Fig. 1. (a) Average above-ground plant biomass, (b) N concentration, and (c) total N pool in above-ground biomass (5-year average) at ambient CO_2 (open bars) and elevated CO_2 (filled bars). All data were adjusted for pre-treatment (1996) values (least square means + SE from ANCOVA are shown). For October, results are shown for grids that were not harvested ('October') and for grids that were harvested ('October-regrowth') that same year in July. Significance levels shown: ns: not significant, $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

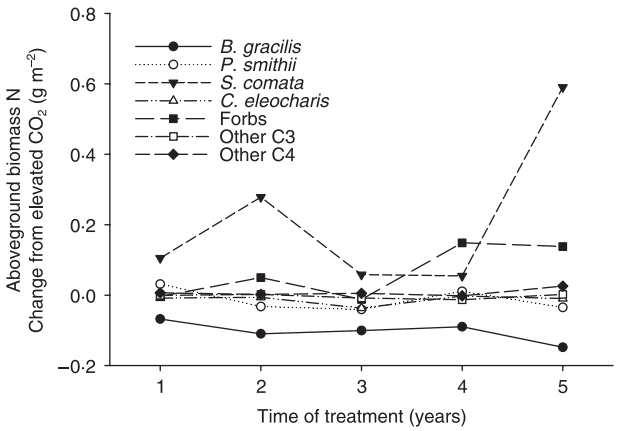

Fig. 2. The average change in above-ground biomass N content caused by elevated CO_2 (above-ground biomass N content at elevated CO_2 – above-ground biomass N content at ambient CO_2 in g m^{-2}) over time for the different species and groups of species.

dilution was larger under elevated CO_2 than under ambient CO_2 (significant CO_2 × year interaction, $P < 0{\cdot}05$). The relative difference in the labelled N fraction between the ambient and elevated CO_2 treatment steadily increased over time (Fig. 3d). The fraction of labelled N in above-ground biomass significantly differed among species/species groups ($P < 0{\cdot}0001$), and that difference changed with time (significant species/species group × year interaction, $P < 0{\cdot}0001$). However, species and species groups did not respond differently to elevated CO_2 (no significant CO_2 × species/species group or CO_2 × species/species group × year interactions, Table 2). The increased ^{15}N dilution with elevated CO_2 occurred in all species and species groups (only shown for *B. gracilis*, *S. comata* and forbs, the species and species group that responded most to elevated CO_2 in terms of above-ground N content, Fig. 3e–h). The variability in the amount and fraction of ^{15}N in below-ground biomass was large, and there was no CO_2 effect (data not shown).

The fraction of labelled N in the soil was not affected by CO_2 and did not change with time (Fig. 4, Table 2). This suggests that the dilution of labelled N in above-ground plant biomass under elevated CO_2 and with time was not caused by changes in the fraction of labelled N in the soil. Further, loss of ^{15}N appeared not to have been affected by elevated CO_2, since ^{15}N retention in plant and soil during the 5 years of measurement was similar under ambient and elevated CO_2 (Fig. 5, Table 2). However, ^{15}N retention in plant and soil significantly declined over time. The loss of ^{15}N during the first year (only *c.* 400 mg of ^{15}N was retained of the 500 mg applied) may have been caused by a spike in ammonia volatilization directly after the application, while removal of ^{15}N through plant harvesting

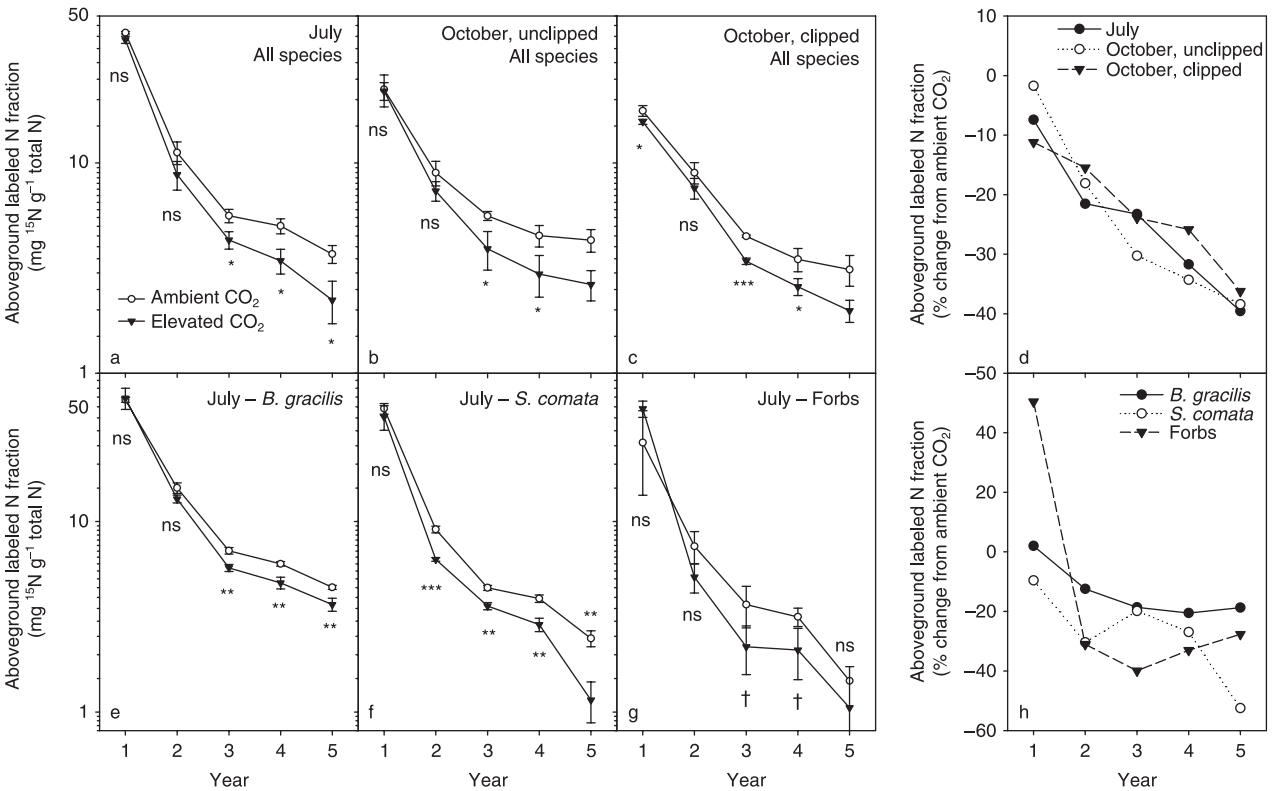

Fig. 3. Labelled N fractions (expressed per total amount of N) in above-ground biomass over time, (a) in July, (b) in October from grids that were not harvested that same year in July, (c) in October from grids that were harvested that same year in July, and (e–g) in July for *B. gracilis*, *S. comata* and forbs. For each year CO_2 treatment effects were tested with ANOVA (ns: not significant, †$P < 0{\cdot}1$, *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$). (d and h) The labelled N fraction in above-ground biomass from the elevated CO_2 treatment expressed as a relative percentage of the labelled N fraction in above-ground biomass from the ambient CO_2 treatment.

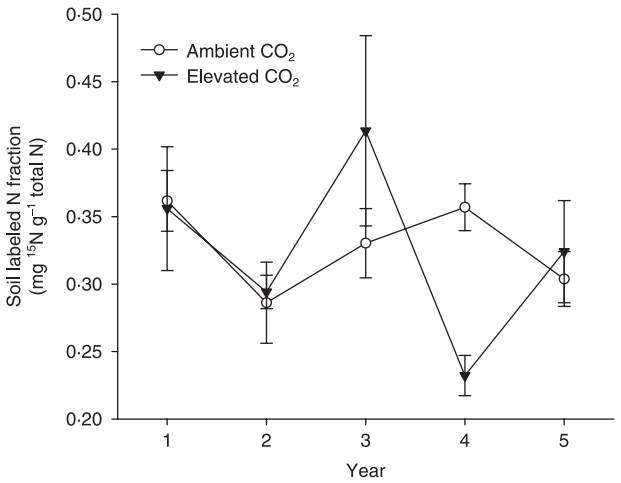

Fig. 4. Labelled N fractions (expressed per total amount of N) in soil to 60-cm soil depth over time.

and gaseous N loss may have caused a further decline in ^{15}N retention with time. The total amount of ^{15}N removed through harvesting after 5 years was 67 and 66 mg m^{-2} under ambient and elevated CO_2, respectively.

Discussion

Our results indicate that enhanced plant production in response to elevated CO_2 did not lead to a progressive decline in soil N availability over 5 years. On the contrary, the persistently higher N uptake and greater ^{15}N dilution in plants suggest that soil N availability remained higher after 5 years of elevated than ambient CO_2. Greater amounts of N in senescing above-ground biomass under elevated CO_2 by the end of the growing season (i.e. after N translocation to roots) indicate that plants under elevated CO_2 lost more N through litter-fall. However, elevated CO_2 resulted in a sustained larger N pool in above-ground biomass during the 5 years of study, suggesting that more N was taken up each year from the soil under elevated CO_2.

The ^{15}N fraction in plants increased directly after the ^{15}N application (year 1), which was followed by a dilution of ^{15}N in above-ground biomass over time. Most likely, progressive plant uptake of mineralized soil N that had a much lower fraction of labelled N diluted the ^{15}N in biomass over time. The fraction of labelled N in the first 60 cm of the soil profile was much lower than the labelled N fractions observed in above-ground plant biomass (compare Fig. 4 with Fig. 3). While the fraction of labelled N of mineralized soil N may have been slightly higher than the fraction of labelled N in the total

Table 2. Repeated-measures ANOVA results (A) for labelled N fractions in above-ground biomass and soil and (B) for ^{15}N retention in soil and plant + soil. Sp, species; sp gr, species group

Source of variation	d.f.	*F*	*P*
A. Labelled N fractions in above-ground biomass and soil (mg ^{15}N g^{-1} N)			
Above-ground biomass, July			
CO_2	1	4·57	*0·09*
Year	4	558·51	**< 0·0001**
CO_2 × year	4	3·81	**0·02**
Above-ground biomass, July, by species/species groups			
CO_2	1	5·29	**0.03**
Sp/sp gr	6	14·74	**< 0.0001**
Year	4	731·21	**< 0.0001**
CO_2 × sp/sp gr	6	0·27	0·94
CO_2 × year	4	2·23	*0.07*
Sp/sp gr × year	24	6·98	**< 0·0001**
CO_2 × sp/sp gr × year	24	0·38	0·99
Above-ground biomass, October			
CO_2	1	2·97	0·16
Year	4	161·57	**< 0·0001**
CO_2 × year	4	2·94	**0·05**
Above-ground biomass, October, regrowth			
CO_2	1	5.19	*0·09*
Year	4	310.77	**< 0·0001**
CO_2 × year	4	2.34	*0·09*
Soil			
CO_2	1	0.002	0.97
Year	4	1.52	0.26
CO_2*year	4	2.06	0.15
B. ^{15}N retention (mg ^{15}N m^{-2})			
Soil			
CO_2	1	0.08	0.79
Year	4	1.25	0.17
CO_2 × year	4	1.20	0.16
Plant + soil			
CO_2	1	0.19	0.69
Year	4	7.75	**0.001**
CO_2 × year	4	2.20	0.12

P-values in italics when $P < 0.10$, and in bold when $P < 0.05$.

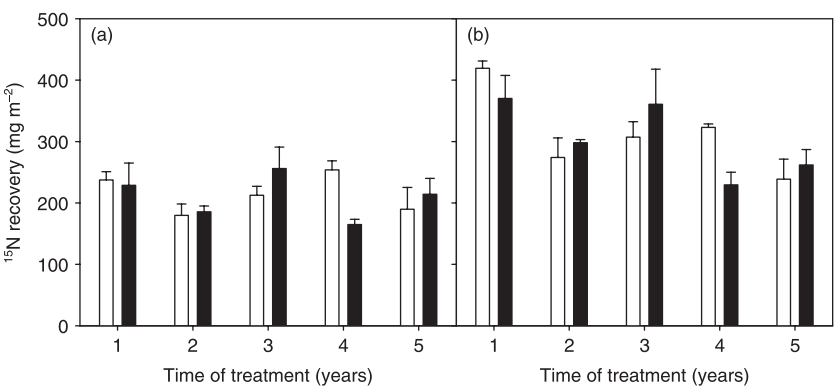

Fig. 5. Average ^{15}N retention + SE (a) in soil to 60-cm soil depth and (b) in plant (above- and below-ground biomass) and soil under ambient (open bars) and elevated CO_2 (filled bars) over time. Data are from 20-cm diameter cores harvested each year in October.

soil N pool (some of the non-labelled N in the soil may not have been accessible for microbes to mineralize), these data strongly suggest that the dilution of ^{15}N in above-ground plant biomass with time was largely caused by uptake of mineralized N with a generally low labelled N fraction. This ^{15}N dilution was observed to a greater degree under elevated than ambient CO_2, suggesting a greater mineralization rate under elevated CO_2. In a study with resin bags conducted during the first 3 years of the experiment we found no significant CO_2 effects on the amount of ammonium and nitrate absorbed (D.G. Milchunas, unpublished data). However, we believe that the amount of ammonium and nitrate absorbed on the bags may not only have been affected by N mineralization in the soil, but also by plant N uptake.

Atmospheric N deposition and atmospheric N fixation could potentially have diluted the labelled N fraction in the soil over time. However, the labelled N fraction in the soil did not significantly change with time (Table 2). It is unlikely that elevated CO_2 affected atmospheric N deposition, and atmospheric N fixation was most likely negligible (there were very few N-fixing plants).

It is possible that ^{15}N dilution was greater under elevated CO_2 because of greater exploration of non-labelled N from deep soil layers. However, there was no evidence for root growth to a greater depth to increase access of deep soil N under elevated CO_2 (LeCain *et al.* 2006) despite changes in species composition.

The greater dilution of ^{15}N in above-ground plant biomass under elevated CO_2 probably was not caused by treatment effects on ^{15}N loss from the ecosystem. Frequent harvesting of above-ground plant biomass and removal of ^{15}N did not result in greater dilution of plant ^{15}N under elevated CO_2. The total amount of ^{15}N removed after 5 years of harvesting was the same under ambient and elevated CO_2 (67 and 66 mg m^{-2}, respectively). The harvested biomass under elevated CO_2 had a slightly lower fraction of labelled N than under ambient CO_2 (5-year average of 12·2 and 10·2 mg ^{15}N g^{-1} total N for ambient and elevated CO_2, respectively), while both fractions were much greater than the soil labelled N fraction. Thus, under elevated CO_2, harvesting depleted the soil of ^{15}N slightly less than under ambient CO_2. Therefore, harvesting actually counteracted our observation of enhanced ^{15}N dilution in above-ground plant biomass under elevated CO_2. There was no evidence for CO_2 effects on N loss through nitrification or denitrification (Mosier *et al.* 2002). We did not directly measure N loss through leaching, but N loss through leaching (as well as through nitrification or denitrification) was most likely small because of a tight N cycle (Mosier *et al.* 2002). Further, elevated CO_2 did not affect ^{15}N retention in plant and soil during the 5 years of measurement, suggesting that there were no significant CO_2 effects on ^{15}N loss.

It is unlikely that the greater ^{15}N dilution was caused by reduced decomposition and mineralization of ^{15}N enriched litter produced during exposure to elevated CO_2 because above-ground litter and root decomposition experiments revealed no significant CO_2 treatment effects (J.Y. King and E. Pendall, unpublished data). Therefore, the increased N uptake in response to elevated CO_2 and the greater dilution of plant ^{15}N was likely caused by enhanced mineralization of relatively low-labelled soil N.

The C_3 grass *S. comata* and the forbs appeared to benefit more from the increase in N mineralization under elevated CO_2 than the C_4 grass *B. gracilis*. Nitrogen content in above-ground biomass of *S. comata* and the forbs increased in response to elevated CO_2, while it decreased in above-ground biomass of *B. gracilis*. Soil moisture was increased by elevated CO_2 (Nelson *et al.* 2004), which possibly enhanced N mineralization at depth and contributed to the increased response in productivity and abundance of *S. comata* and the sub-shrub *A. frigida*, both of which are more deeply rooted compared to *B. gracilis* (Morgan *et al.* 2004a; Morgan et al. 2007). Differences in the fraction of labelled N in above-ground biomass among plant species and species groups also suggest N uptake from different soil depths by the different plant species. The ^{15}N was added to the surface of the soil causing greater ^{15}N enrichment at the surface (data not shown). Therefore, with a shallow root structure, *B. gracilis* may have taken up a greater fraction of labelled N in the above-ground biomass compared to the other species. There were no significant $CO_2 \times$ species/species group or $CO_2 \times$ species/species group $\times$ year interaction effects on the fraction of labelled N in plant biomass, supporting the observation that elevated CO_2 did not alter rooting depth of individual species (LeCain *et al.* 2006).

Our results contrast with results from grassland field experiments in Texas and Minnesota in which elevated CO_2 reduced soil N availability within 2–6 years (Gill *et al.* 2002; Reich *et al.* 2006a). Elevated CO_2 increased soil moisture due to decreased plant transpiration at our site (Nelson *et al.* 2004), which could have stimulated microbial activity and N mineralization. This CO_2-induced soil moisture effect on microbial activity is likely to be more important in semi-arid grasslands than in mesic grasslands where microbial activity in the soil is less constrained by soil moisture. Although stimulation of plant growth may be directly related to increased water availability, a simultaneous increase in N mineralization may be an important 'hidden' variable controlling plant productivity in semi-arid grasslands (Burke, Lauenroth & Parton 1997). Thus, elevated CO_2 may have increased soil N availability through plant control of soil moisture thereby preventing progressive plant N limitation within the first 5 years of elevated CO_2.

Different patterns of soil N availability in response to elevated CO_2 in different studies may also stem in part from the different methods used to measure soil N availability. In the Texas and Minnesota studies, soil N availability was assessed by measuring net N mineralization in soil incubations. Because plants can interfere with these measurements through N uptake, measurements are typically done in the absence of plants. However, plant–soil interactions can significantly affect net N mineralization by enhancing SOM decomposition, known as the rhizosphere priming effect (Kuzyakov, Friedel & Stahr 2000). The rhizosphere priming effect could increase with elevated CO_2 (Cheng 1999; Hoosbeek *et al.* 2004). Thus, soil incubation assays that exclude plants could underesti-

mate N mineralization rates, particularly under elevated CO_2. Indeed, at our site elevated CO_2 significantly increased SOM decomposition and enzyme activities (Pendall *et al.* 2003; Kandeler *et al.* 2006). Our ^{15}N tracer method included potential rhizosphere priming effects on soil N availability. Moreover, by tracing the added ^{15}N in plants over a 5-year period, we were able to integrate long-term effects of elevated CO_2 on soil N availability, which made our method very robust.

Our results suggest that elevated CO_2 effects on ecosystem functioning may be larger and more persistent in semi-arid climates than in wetter climates. While increased plant productivity and biomass accumulation with elevated CO_2 is expected to be constrained by soil N availability (Hungate *et al.* 2003; de Graaff *et al.* 2006; Reich *et al.* 2006a; Van Groenigen *et al.* 2006), and even could result in PNL (Luo *et al.* 2004), our results suggest that in semi-arid climates, a CO_2-induced increase in soil moisture can reduce constraints of available N on plant productivity. Plant growth responses to elevated CO_2 in semi-arid climates, where a CO_2-induced increase in soil moisture has been shown to occur (Morgan *et al.* 2004b), may therefore be more pronounced and last longer than in climates with more effective precipitation and in which N mineralization is less influenced by a CO_2-induced change in soil moisture. Indeed, semi-arid ecosystems have been predicted to be some of the most responsive ecosystems to elevated CO_2 (Strain & Bazzaz 1983; Melillo *et al.* 1993; Morgan *et al.* 2004b). Other ecosystem properties that are influenced by N availability, such as plant species composition and C sequestration, may also show larger and more persistent responses to elevated CO_2 in semi-arid climates. For instance, growth of exotic annual grasses in the Mojave desert (NV, USA) and a weedy sub-shrub at our site showed dramatic increases in response to elevated CO_2 (Smith *et al.* 2000; Morgan *et al.* 2007). Shifts in species composition in response to elevated CO_2 could strongly impact overall forage digestibility (Morgan *et al.* 2004a; Milchunas *et al.* 2005) where grazing by domestic livestock is the primary land-use. Estimating long-term global changes in ecosystem functioning in a CO_2-rich environment requires an understanding of ecosystem responses to CO_2-induced changes in water and N availability.

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