

BELOWGROUND RESPONSES TO CLIMATE CHANGE

Long-term enhancement of N availability and plant growth under elevated CO₂ in a semi-arid grassland

F. A. Dijkstra*¹, E. Pendall², A. R. Mosier³, J. Y. King^{4,5}, D. G. Milchunas⁶ and J. A. Morgan¹

¹USDA-ARS, Rangeland and Resources Research Unit, Fort Collins, CO 80526, USA; ²Department of Botany and Program in Ecology, University of Wyoming, Laramie, WY 82071, USA; ³USDA-ARS Soil–Plant–Nutrient Research Unit, Fort Collins, CO 80526, USA; ⁴Department of Soil, Water and Climate; and ⁵Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55108, USA; and ⁶Forest, Range and Watershed Stewardship Department, and Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, USA

Summary

1. While rising atmospheric CO₂ 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 CO₂ affects long-term soil N dynamics.
2. We used open-top chambers to examine the effect of 5 years of elevated atmospheric CO₂ 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% ¹⁵N, was added to each plot. We examined the effect of elevated CO₂ 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 CO₂. 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 CO₂. The amount and fraction of labelled N in the soil did not change with time and was unaffected by elevated CO₂. 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 CO₂. More of the mineralized N ended up in the above-ground biomass of *Stipa comata* and forbs (C₃) than in *Bouteloua gracilis* (C₄) under elevated CO₂.
4. Increased soil moisture under elevated CO₂ 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 CO₂ may be more persistent than in mesic systems where N mineralization is less constrained by soil moisture.

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

Introduction

The global atmospheric CO₂ 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 CO₂ concentration (hereafter also referred to as elevated CO₂) 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 CO₂ (Thompson *et al.* 2004). How-

ever, it remains uncertain if ecosystem responses to elevated CO₂ 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 CO₂ 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 CO₂ 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 CO₂. Therefore, PNL could eventually counteract the initial stimulation of plant growth

*Correspondence author. E-mail: feike.dijkstra@ars.usda.gov

and C sequestration in response to elevated CO₂ (Luo *et al.* 2004; Reich, Hungate & Luo 2006b).

Extensive research has been done on how elevated CO₂ affects soil N availability, but results have been inconsistent. Elevated CO₂ 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 CO₂ has been manipulated have been relatively short in duration (3 years or less), thereby reducing their utility for understanding long-term effects of elevated CO₂ on soil N availability.

Understanding how CO₂ 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 ¹⁵N 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 CO₂ (Zak *et al.* 2000; de Graaff *et al.* 2006; Reich *et al.* 2006b).

Net primary productivity and species composition responses to elevated CO₂ 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 CO₂ may be also important. When elevated CO₂ increases soil moisture and N availability, it could potentially delay the development of PNL. This effect of elevated CO₂ 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 CO₂ in this semi-arid grassland causing sustained greater plant N uptake and plant growth during 5 years of elevated CO₂. We used a novel ¹⁵N tracer technique to obtain an integrated long-term measurement of how elevated CO₂ 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 C₄ grass *Bouteloua gracilis* and the cool-season C₃ 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 CO₂ (360 ± 20 μmol/mol) and three with elevated CO₂ (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% ¹⁵N (both ammonium-N and nitrate-N were 99.9 atom% ¹⁵N, 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 m²) 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 C₃' and 'other C₄' 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 ¹⁵N 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:

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

where ¹⁵N_{plant,i}, ¹⁵N_{plant,96}, and ¹⁵N_{label} are the atom% ¹⁵N 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 ¹⁵N retention in the top 60-cm of the soil (¹⁵N_{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:

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

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

We used repeated-measures ANCOVA to test for CO₂ 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 CO₂ × time interaction effects ($P > 0.1$). We then tested for CO₂ 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 CO₂ treatment effects on N content in the July above-ground biomass among species and species groups over time, using repeated-measures ANCOVA (CO₂ 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 CO₂ and species effects on labelled N fractions in above-ground biomass (log-transformed) and soil, and ¹⁵N retention in plant and soil over time. We also used ANOVA to test for CO₂ effects on labelled N fractions in above-ground biomass in each year.

Results

Above-ground plant biomass under elevated CO₂ was significantly greater than under ambient CO₂ ($P < 0.05$, 5-year average) and had a significantly greater total N content by the time of senescence in October (up to 68%, $P < 0.05$, Fig. 1). The increase of the above-ground N pool in October under elevated CO₂ was sustained during the 5-year period (no significant time × CO₂ interaction using repeated measures ANCOVA). The N concentration in the July above-ground biomass was significantly lower under elevated CO₂ ($P < 0.001$) than under ambient CO₂, which resulted in similar amounts of above-ground plant N in July under elevated and ambient CO₂. 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 CO₂ 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 C₃ and other C₄ grasses)

Source of variation	d.f.	<i>F</i>	<i>P</i>
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 $P < 0.10$, and in bold when $P < 0.05$.

Species and species groups responded differently to elevated CO₂ in terms of their above-ground N content collected in July (CO₂ × species/species group interaction $P = 0.06$), and these species/species group-specific responses significantly changed over time (CO₂ × species/species group × year interaction $P = 0.04$, Table 1). Elevated CO₂ reduced above-ground N content in the C₄ grass *B. gracilis*, but increased above-ground N content in the C₃ 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 CO₂. 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 CO₂.

The ¹⁵N 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 ¹⁵N (i.e. directly after ¹⁵N application) followed by a progressive dilution of labelled N with unlabelled N. This progressive ¹⁵N

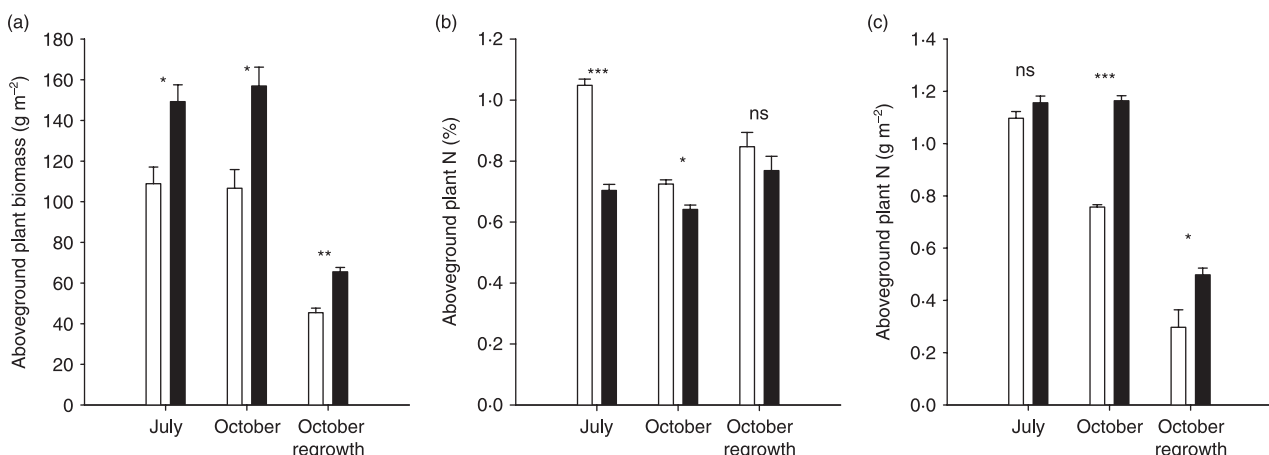


Fig. 1. (a) Average above-ground plant biomass, (b) N concentration, and (c) total N pool in above-ground biomass (5-year average) at ambient CO₂ (open bars) and elevated CO₂ (filled bars). All data were adjusted for pre-treatment (1996) values (least square means + SE from ANCOVA are shown). For October, 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, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

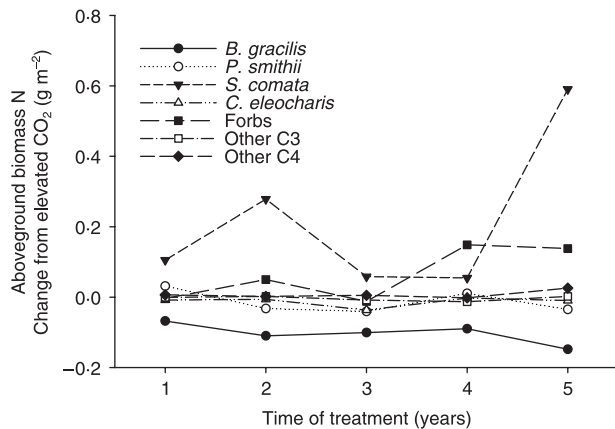


Fig. 2. The average change in above-ground biomass N content caused by elevated CO₂ (above-ground biomass N content at elevated CO₂ – above-ground biomass N content at ambient CO₂ in g m⁻²) over time for the different species and groups of species.

dilution was larger under elevated CO₂ than under ambient CO₂ (significant CO₂ × year interaction, $P < 0.05$). The relative difference in the labelled N fraction between the ambient and elevated CO₂ treatment steadily increased over time (Fig. 3d). The fraction of labelled N in above-ground biomass signifi-

cantly differed among species/species groups ($P < 0.0001$), and that difference changed with time (significant species/species group × year interaction, $P < 0.0001$). However, species and species groups did not respond differently to elevated CO₂ (no significant CO₂ × species/species group or CO₂ × species/species group × year interactions, Table 2). The increased ¹⁵N dilution with elevated CO₂ 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 CO₂ in terms of above-ground N content, Fig. 3e–h). The variability in the amount and fraction of ¹⁵N in below-ground biomass was large, and there was no CO₂ effect (data not shown).

The fraction of labelled N in the soil was not affected by CO₂ 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 CO₂ and with time was not caused by changes in the fraction of labelled N in the soil. Further, loss of ¹⁵N appeared not to have been affected by elevated CO₂, since ¹⁵N retention in plant and soil during the 5 years of measurement was similar under ambient and elevated CO₂ (Fig. 5, Table 2). However, ¹⁵N retention in plant and soil significantly declined over time. The loss of ¹⁵N during the first year (only c. 400 mg of ¹⁵N was retained of the 500 mg applied) may have been caused by a spike in ammonia volatilization directly after the application, while removal of ¹⁵N through plant harvesting

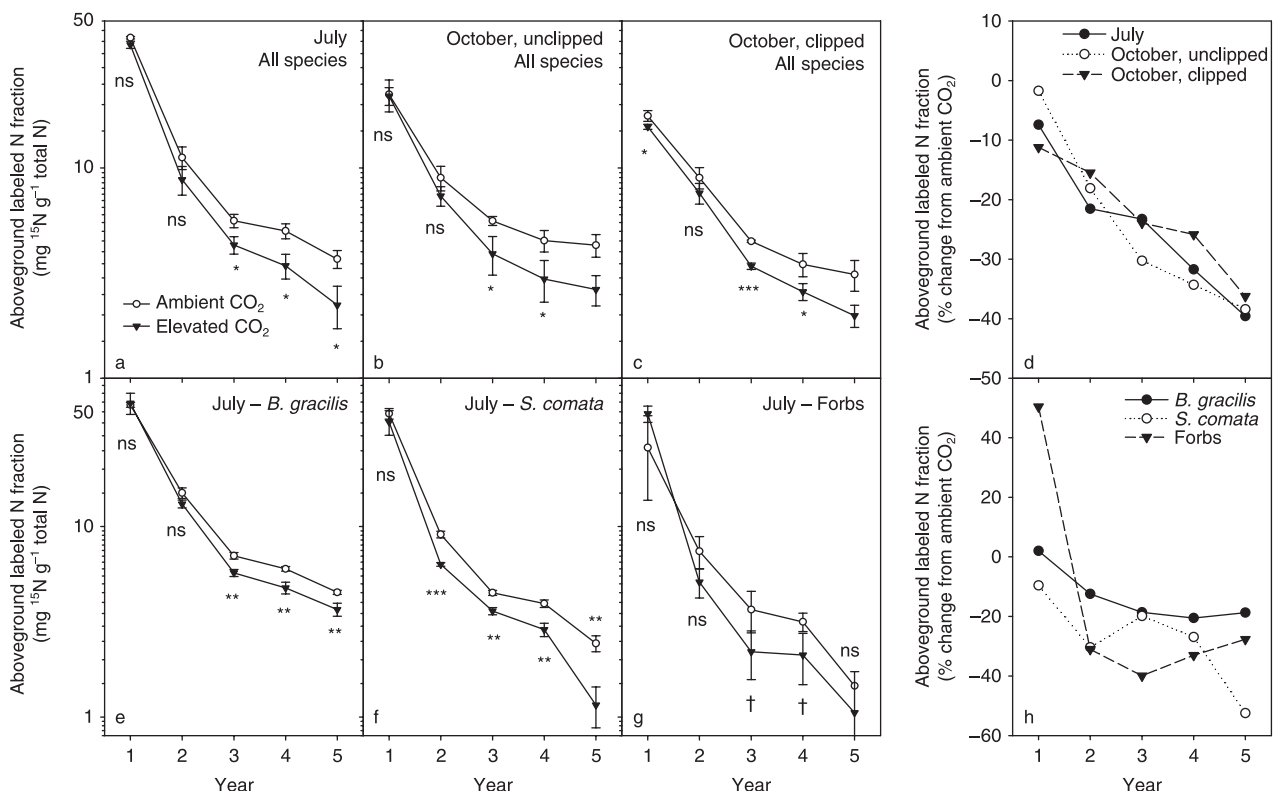


Fig. 3. Labelled N fractions (expressed per total amount of N) in above-ground biomass over time, (a) in July, (b) in October from grids that were not harvested that same year in July, (c) in October from grids that were harvested that same year in July, and (e–g) in July for *B. gracilis*, *S. comata* and forbs. For each year CO₂ treatment effects were tested with ANOVA (ns: not significant, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (d and h) The labelled N fraction in above-ground biomass from the elevated CO₂ treatment expressed as a relative percentage of the labelled N fraction in above-ground biomass from the ambient CO₂ treatment.

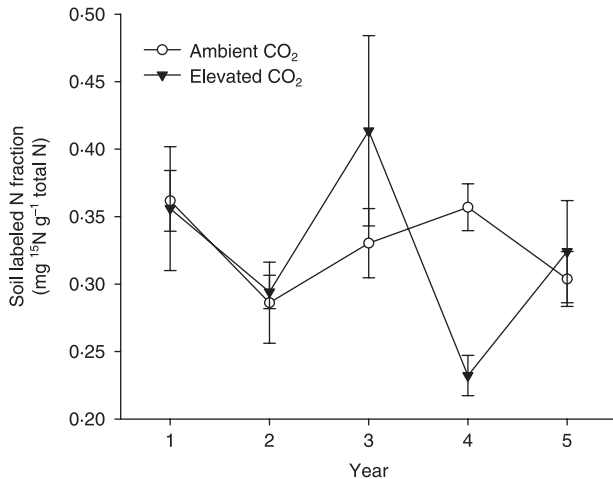


Fig. 4. Labelled N fractions (expressed per total amount of N) in soil to 60-cm soil depth over time.

and gaseous N loss may have caused a further decline in ¹⁵N retention with time. The total amount of ¹⁵N removed through harvesting after 5 years was 67 and 66 mg m⁻² under ambient and elevated CO₂, respectively.

Discussion

Our results indicate that enhanced plant production in response to elevated CO₂ did not lead to a progressive decline in soil N availability over 5 years. On the contrary, the persistently higher N uptake and greater ¹⁵N dilution in plants suggest that soil N availability remained higher after 5 years of elevated than ambient CO₂. Greater amounts of N in senescing above-ground biomass under elevated CO₂ by the end of the growing season (i.e. after N translocation to roots) indicate that plants under elevated CO₂ lost more N through litter-fall. However, elevated CO₂ 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 CO₂.

The ¹⁵N fraction in plants increased directly after the ¹⁵N application (year 1), which was followed by a dilution of ¹⁵N

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

Source of variation	d.f.	<i>F</i>	<i>P</i>
A. Labelled N fractions in above-ground biomass and soil (mg ¹⁵N g⁻¹ N)			
Above-ground biomass, July			
CO ₂	1	4.57	<i>0.09</i>
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	<i>0.94</i>
CO ₂ × year	4	2.23	<i>0.07</i>
Sp/sp gr × year	24	6.98	< 0.0001
CO ₂ × sp/sp gr × year	24	0.38	<i>0.99</i>
Above-ground biomass, October			
CO ₂	1	2.97	<i>0.16</i>
Year	4	161.57	< 0.0001
CO ₂ × year	4	2.94	0.05
Above-ground biomass, October, regrowth			
CO ₂	1	5.19	<i>0.09</i>
Year	4	310.77	< 0.0001
CO ₂ × year	4	2.34	<i>0.09</i>
Soil			
CO ₂	1	0.002	<i>0.97</i>
Year	4	1.52	<i>0.26</i>
CO ₂ *year	4	2.06	<i>0.15</i>
B. ¹⁵N retention (mg ¹⁵N m⁻²)			
Soil			
CO ₂	1	0.08	<i>0.79</i>
Year	4	1.25	<i>0.17</i>
CO ₂ × year	4	1.20	<i>0.16</i>
Plant + soil			
CO ₂	1	0.19	<i>0.69</i>
Year	4	7.75	0.001
CO ₂ × year	4	2.20	<i>0.12</i>

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

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 ¹⁵N 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

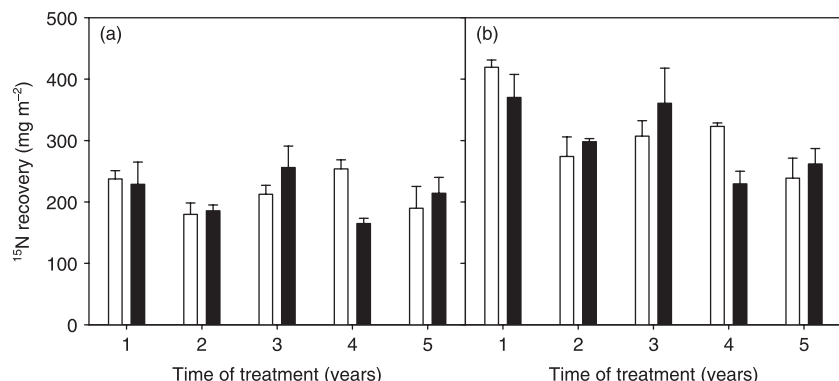


Fig. 5. Average ¹⁵N retention + SE (a) in soil to 60-cm soil depth and (b) in plant (above- and below-ground biomass) and soil under ambient (open bars) and elevated CO₂ (filled bars) over time. Data are from 20-cm diameter cores harvested each year in October.

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 $\text{mg }^{15}\text{N g}^{-1}$ 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 $\text{CO}_2 \times \text{species}/\text{species group}$ or $\text{CO}_2 \times \text{species}/\text{species group} \times \text{year}$ 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 CO₂. Indeed, at our site elevated CO₂ significantly increased SOM decomposition and enzyme activities (Pendall *et al.* 2003; Kandeler *et al.* 2006). Our ¹⁵N tracer method included potential rhizosphere priming effects on soil N availability. Moreover, by tracing the added ¹⁵N in plants over a 5-year period, we were able to integrate long-term effects of elevated CO₂ on soil N availability, which made our method very robust.

Our results suggest that elevated CO₂ 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 CO₂ 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 CO₂-induced increase in soil moisture can reduce constraints of available N on plant productivity. Plant growth responses to elevated CO₂ in semi-arid climates, where a CO₂-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 CO₂-induced change in soil moisture. Indeed, semi-arid ecosystems have been predicted to be some of the most responsive ecosystems to elevated CO₂ (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 CO₂ 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 CO₂ (Smith *et al.* 2000; Morgan *et al.* 2007). Shifts in species composition in response to elevated CO₂ 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 CO₂-rich environment requires an understanding of ecosystem responses to CO₂-induced changes in water and N availability.

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References

Ainsworth, E.A. & Long, S.P. (2005) What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist*, **165**, 351–372.

Burke, I.C., Lauenroth, W.K. & Parton, W.J. (1997) Regional and temporal variation in net primary production and nitrogen mineralization in grasslands. *Ecology*, **78**, 1330–1340.

Cheng, W. (1999) Rhizosphere feedbacks in elevated CO₂. *Tree Physiology*, **19**, 313–320.

de Graaff, M.A., van Groenigen, K.J., Six, J., Hungate, B. & van Kessel, C. (2006) Interactions between plant growth and soil nutrient cycling under elevated CO₂: a meta-analysis. *Global Change Biology*, **12**, 2077–2091.

Dufresne, J.L., Friedlingstein, P., Berthelot, M., Bopp, L., Ciais, P., Fairhead, L., Le Treut, H. & Monfray, P. (2002) On the magnitude of positive feedback between future climate change and the carbon cycle. *Geophysical Research Letters*, **29**, doi: 10.1029/2001GL013777.

Ebersberger, D., Niklaus, P.A. & Kandeler, E. (2003) Long term CO₂ enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. *Soil Biology and Biochemistry*, **35**, 965–972.

Finzi, A.C. & Schlesinger, W.H. (2003) Soil-nitrogen cycling in a pine forest exposed to 5 years of elevated carbon dioxide. *Ecosystems*, **6**, 444–456.

Gill, R.A., Polley, H.W., Johnson, H.B., Anderson, L.J., Maherali, H. & Jackson, R.B. (2002) Nonlinear grassland responses to past and future atmospheric CO₂. *Nature*, **417**, 279–282.

Hoosbeek, M.R., Lukac, M., van Dam, D., Godbold, D.L., Velthorst, E.J., Biondi, F.A., Peressotti, A., Cotrufo, M.F., de Angelis, P. & Scarascia-Mugnozza, G. (2004) More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): cause of increased priming effect? *Global Biogeochemical Cycles*, **18**, doi: 10.1029/2003GB002127.

Hungate, B.A., Chapin, F.S. III, Zhong, H., Holland, E.A. & Field, C.B. (1997) Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia*, **109**, 149–153.

Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y.Q. & Field, C.B. (2003) Nitrogen and climate change. *Science*, **302**, 1512–1513.

Intergovernmental Panel on Climate Change (2001) *Climate Change 2001: the Scientific Basis*. Cambridge University Press, New York.

Kandeler, E., Mosier, A.R., Morgan, J.A., Milchunas, D.G., King, J.Y., Rudolph, S. & Tschirko, D. (2006) Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. *Soil Biology and Biochemistry*, **38**, 2448–2460.

King, J.Y., Mosier, A.R., Morgan, J.A., LeCain, D.R., Milchunas, D.G. & Parton, W.J. (2004) Plant nitrogen dynamics in shortgrass steppe under elevated atmospheric carbon dioxide. *Ecosystems*, **7**, 147–160.

Kuzyakov, Y., Friedel, J.K. & Stahr, K. (2000) Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry*, **32**, 1485–1498.

LeCain, D.R., Morgan, J.A., Milchunas, D.G., Mosier, A.R., Nelson, J.A. & Smith, D.P. (2006) Root biomass of individual species, and root size characteristics after five years of CO₂ enrichment on native shortgrass steppe. *Plant and Soil*, **279**, 219–228.

Luo, Y., Su, B., Currie, W.S., Dukes, J.S., Finzi, A., Hartwig, U., Hungate, B., McMurtrie, R.E., Oren, R., Parton, W.J., Pataki, D.E., Shaw, M.R., Zak, D.R. & Field, C.B. (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience*, **54**, 731–739.

Melillo, J.M., McGuire, A.D., Kicklighter, D.W., Moore III, B., Vorosmarty, C.J. & Schloss, A.L. (1993) Global climate change and terrestrial net primary production. *Nature*, **363**, 234–240.

Milchunas, D.G., Mosier, A.R., Morgan, J.A., LeCain, D.R., King, J.Y. & Nelson, J.Y. (2005) Elevated CO₂ and defoliation effects on shortgrass steppe: forage quality versus quantity for ruminants. *Agriculture Ecosystems and Environment*, **111**, 166–184.

Morgan, J.A., Milchunas, D.G., LeCain, D.R., West, M. & Mosier, A.R. (2007) Carbon dioxide enrichment alters plant community structure and accelerates shrub growth in the shortgrass steppe. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 14724–14729.

Morgan, J.A., Mosier, A.R., Milchunas, D.G., LeCain, D.R., Nelson, J.A. & Parton, W.J. (2004a) CO₂ enhances productivity, alters species composition, and reduces digestibility of shortgrass steppe vegetation. *Ecological Applications*, **14**, 208–219.

Morgan, J.A., Pataki, D.E., Körner, C., Clark, H., DelGrosso, S.J., Grunzewig, J.M., Knapp, A.K., Mosier, A.R., Newton, P.C.D., Niklaus, P.A., Nippert, J.B., Nowak, R.S., Parton, W.J., Polley, H.W. & Shaw, M.R. (2004b) Water relations in grassland and desert ecosystems to elevated atmospheric CO₂. *Oecologia*, **140**, 11–25.

Mosier, A.R., Morgan, J.A., King, J.Y., LeCain, D. & Milchunas, D.G. (2002) Soil-atmosphere exchange of CH₄, CO₂, NO_x, and N₂O in the Colorado shortgrass steppe under elevated CO₂. *Plant and Soil*, **240**, 201–211.

Nelson, J.A., Morgan, J.A., LeCain, D.R., Mosier, A., Milchunas, D.G. & Parton, B.A. (2004) Elevated CO₂ increases soil moisture and enhances plant water relations in a long-term field study in semi-arid shortgrass steppe of Colorado. *Plant and Soil*, **259**, 169–179.

- Pendall, E., Del Grosso, S., King, J.Y., LeCain, D.R., Milchunas, D.G., Morgan, J.A., Mosier, A.R., Ojima, D.S., Parton, W.A., Tans, P.P. & White, J.W.C. (2003) Elevated atmospheric CO₂ effects and soil water feedbacks on soil respiration components in a Colorado grassland. *Global Biogeochemical Cycles*, **17**, doi: 10.1029/2001GB001821.
- Reich, P.B., Hobbie, S.E., Lee, T., Ellsworth, D.S., West, J.B., Tilman, D., Knops, J.M.H., Naeem, S. & Trost, J. (2006a) Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature*, **440**, 922–925.
- Reich, P.B., Hungate, B.A. & Luo, Y. (2006b) Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 611–636.
- Schimel, J.P. & Bennett, J. (2004) Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, **85**, 591–602.
- Smith, S.D., Huxman, T.E., Zitzer, S.F., Charlet, T.N., Housman, D.C., Coleman, J.S., Fenstermaker, L.K., Seemann, J.R. & Nowak, R.S. (2000) Elevated CO₂ increases productivity and invasive species success in an arid ecosystem. *Nature*, **408**, 79–82.
- Strain, B.R. & Bazzaz, F.A. (1983) Terrestrial plant communities. *CO₂ and Plants: The Response of Plants to Rising Levels of Carbon Dioxide* (ed. E. Lemon), pp. 177–222. AAAS, Washington D.C.
- Thompson, S.L., Govindasamy, B., Mirin, A., Caldeira, K., Delire, C., Milovich, J., Wickett, M. & Erickson, D. (2004) Quantifying the effect of CO₂-fertilized vegetation on future global climate and carbon dynamics. *Geophysical Research Letters*, **31**, 1–4.
- Van Groenigen, K.J., Six, J., Hungate, B.A., De Graaff, M.A., Van Breemen, N. & Van Kessel, C. (2006) Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 6571–6574.
- Williams, M.A., Rice, C.W. & Owensby, C.E. (2001) Nitrogen competition in a tallgrass prairie ecosystem exposed to elevated carbon dioxide. *Soil Science Society of America Journal*, **65**, 340–346.
- Zak, D.R., Holmes, W.E., Finzi, A.C., Norby, R.J. & Schlesinger, W.H. (2003) Soil nitrogen cycling under elevated CO₂: a synthesis of forest face experiments. *Ecological Applications*, **13**, 1508–1514.
- Zak, D.R., Pregitzer, K.S., King, J.S. & Holmes, W.E. (2000) Elevated atmospheric CO₂, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, **147**, 201–222.

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