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Plant and microbial N acquisition under elevated atmospheric CO_2 in two mesocosm experiments with annual grasses

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Abstract

The impact of elevated CO_2 on terrestrial ecosystem C balance, both in sign or magnitude, is not clear because the resulting alterations in C input, plant nutrient demand and water use efficiency often have contrasting impacts on microbial decomposition processes. One major source of uncertainty stems from the impact of elevated CO_2 on N availability to plants and microbes. We examined the effects of atmospheric CO_2 enrichment (ambient + 370 μmol mol^{-1}) on plant and microbial N acquisition in two different mesocosm experiments, using model plant species of annual grasses of *Avena barbata* and *A. fatua*, respectively. The *A. barbata* experiment was conducted in a N-poor sandy loam and the *A. fatua* experiment was on a N-rich clayey loam. Plant–microbial N partitioning was examined through determining the distribution of a ^{15}N tracer. In the *A. barbata* experiment, ^{15}N tracer was introduced to a field labeling experiment in the previous year so that ^{15}N predominantly existed in nonextractable soil pools. In the *A. fatua* experiment, ^{15}N was introduced in a mineral solution [$(^{15}NH_4)_2SO_4$ solution] during the growing season of *A. fatua*. Results of both N budget and ^{15}N tracer analyses indicated that elevated CO_2 increased plant N acquisition from the soil. In the *A. barbata* experiment, elevated CO_2 increased plant biomass N by ca. 10% but there was no corresponding decrease in soil extractable N, suggesting that plants might have obtained N from the nonextractable organic N pool because of enhanced microbial activity. In the *A. fatua* experiment, however, the CO_2-led increase in plant biomass N was statistically equal to the reduction in soil extractable N. Although atmospheric CO_2 enrichment enhanced microbial biomass C under *A. barbata* or microbial activity (respiration) under *A. fatua*, it had no significant effect on microbial biomass N in either experiment. Elevated CO_2 increased the colonization of *A. fatua* roots by arbuscular mycorrhizal fungi, which coincided with the enhancement of plant competitiveness for soluble soil N. Together, these results suggest that elevated CO_2 may tighten N cycling through facilitating plant N acquisition. However, it is unknown to what degree results from these short-term microcosm experiments can be extrapolated to field conditions. Long-term studies in less-disturbed soils are needed to determine whether CO_2-enhancement of plant N acquisition can significantly relieve N limitation over plant growth in an elevated CO_2 environment.

Keywords: Avena barbata, *Avena fatua*, elevated CO_2, microbial biomass C and N, ^{15}N availability, N tracer, plant–microbial N partitioning

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Introduction

The concentration of atmospheric CO_2 has increased by nearly 100 ppm since preindustrial times (Keeling *et al.*, 1989) and is predicted to double within the next century

largely because of the increasing combustion of fossil fuel and land use changes (IPCC 2001). CO_2 is an important greenhouse gas contributing 55% of all the greenhouse gas forcing in the 1980s, and is estimated to have an even greater relative importance in the 1990s through to the 21st century (Lashof & Ahuja, 1990). Terrestrial ecosystems contain nearly three times as much C (ca. 2060 Gt C) as that currently present in the atmosphere (ca. 735 Gt C) (Houghton & Woodwell, 1989) and have the potential to be either a significant C sink or C source under future CO_2 scenarios (Raich & Potter, 1995; Hungate *et al*., 2003). Hence there is increasing interest in the possibility of modulating atmospheric CO_2 concentration via enhanced below ground carbon storage in managed terrestrial systems. The feasibility of this approach will depend to a large degree on our ability to successfully manage the 'plant C-fixation–microbial decomposition loop'. There has now been a substantial amount of work on elevated CO_2 and plant response. Over the short-term, elevated CO_2 often stimulates photosynthesis, enhances plant nutrient demand and nutrient use efficiency, and improves plant water use efficiency (Coleman & Bazzaz, 1992; Jackson *et al*., 1994). This can affect ecosystem C balance by increasing net primary production (NPP) (Melillo *et al*., 1993) and modifying decomposition processes (Ball, 1997). However, the long-term impacts on ecosystem C balance are not clear because those CO_2-induced alterations may have interactive, often contrasting, effects on soil microbial processes, particularly on soil N immobilization and mineralization (Richter *et al*., 2003).

The primary productivity of most temperate terrestrial ecosystems is N-limited (Vitousek & Howarth, 1991); hence a major source of uncertainty stems from the effect of CO_2 elevation on plant and microbial N acquisition and subsequent N cycling, which may significantly impact further ecosystem responses to CO_2 enrichment (Daepp *et al*., 2000; Schlesinger & Lichter, 2001; Gill *et al*., 2002; Luo *et al*., 2004). On the one hand, elevated CO_2 often increases NPP and litter C/N or lignin/N ratios, favoring C or N accumulation in plant biomass and soil organic matter (Rouhier *et al*., 1994; Oren *et al*., 2001), and slowing nutrient cycling (Cotrufo & Ineson, 1996; Hu *et al*., 2001). On the other hand, elevated CO_2 may enhance root exudation and C availability for microbes, stimulating microbial activity and N mineralization, and increasing the proportion of C and N undergoing rapid cycling (Zak *et al*., 1993; Hungate *et al*., 1997; Paterson *et al*., 1997; Pendall *et al*., 2004). Also, CO_2-led increases in plant C : N ratios are likely species-dependent and less significant in naturally senesced litters of tree species (Norby *et al*., 2001). Whether CO_2-enhancement of plant photosynthesis and subsequent C accumulation can be sustained will largely depend on the potential for microbes to obtain and/or release limited nutrients to balance the needs for continuous plant growth.

Studies on the effects of elevated CO_2 on microbial processes have generally emphasized the importance of enhanced C-input to microbial biomass and activity (Diaz *et al*., 1993; Zak *et al*., 1993; Paterson *et al*., 1997; Hu *et al*., 1999), assuming that microbes in soil are C-limited (Smith & Paul, 1990). However, both plants and microbes are commonly N-limited in terrestrial ecosystems (Kaye & Hart, 1997) and litter decomposition can be inhibited by plant competition for N (Wang & Bakken, 1997). CO_2 enrichment in the atmosphere alters resource availability (particularly the relative availability of C and N) for microbes, potentially altering the microbial community composition and functions (Montealegre *et al*., 2002). The vast majority of N in soil exists in the form of organic N with low C : N ratios ranging from 10–12 : 1 in agricultural soils to 20–25 : 1 in forest soils (Fog, 1988). Therefore, one important question is whether or not the generally enhanced C availability for microbes under elevated CO_2 stimulates microbial utilization of this old organic N (Zak *et al*., 1993; Cardon *et al*., 2001; Hu *et al*., 2001; Richter *et al*., 2003).

Little attention has been directed toward the effects of elevated CO_2 on plant–microbial competition for N and the subsequent effects on microbial N acquisition from old organic matter. Decomposition experiments, largely conducted in soils without the presence of actively growing plants, indicate that compared with ambient controls, the decomposition rate of litter produced under elevated CO_2 is slower over the short-term (Cotrufo & Ineson, 1996; Ball, 1997). On one hand, elevated CO_2 might promote microbial N utilization and subsequent immobilization because of increased C supplies to microbes (Diaz *et al*., 1993). On the other hand, it may promote plant N acquisition through enhancing the growth of fine roots and mycorrhizae (Rillig *et al*., 1999; Hu *et al*., 2001). In addition, results from other experiments suggest that enhanced microbial activities induced by elevated CO_2 may increase N mineralization (Zak *et al*., 1993; Hungate *et al*., 1997). However, clear information addressing the effects of elevated CO_2 on microbial N acquisition in the presence of active plants is very limited. This knowledge gap severely limits our ability to predict ecosystem C balances under the future conditions of elevated atmospheric CO_2 (Niklaus *et al*., 2001).

In this paper, we present data obtained from two microcosm experiments using ^{15}N as a tracer to examine the effects of elevated CO_2 on plant–microbial N partitioning and plant N acquisition. Our objectives were (1) to examine whether elevated CO_2 increases

plant and microbial N acquisition, and (2) to determine where the increased N in biomass originates if plant and microbes enhance their N uptake.

Materials and methods

Two growth chamber experiments were conducted to determine the effects of elevated CO_2 on plant–microbial N partitioning. In the first experiment, mineral ^{15}N was introduced in the previous season (1997) in a field labeling experiment, and ^{15}N was distributed among various soil pools (Hu *et al*., 2001). In the second experiment, ^{15}N was introduced during the growing season as soluble mineral N. In addition, soil texture and fertility were different between the two soils used, with experiment 1 on a nutrient-poor sandy loam and experiment 2 on a relatively N-rich clay loam. The two-model plant species used, annual graminoid *Avena barbata* and *A. fatua*, are common in California grasslands.

Experiment 1

Experimental setup. The first experiment was conducted in regular growth chambers at the University of California at Berkeley, CA. The soil was collected from an isotope (^{15}N)-labeled experiment at the Jasper Ridge Biological Preserve, Palo Alto, CA, USA (Hu *et al*., 2001). The field site was an annual grassland dominated by *A. barbata*. The ^{15}N tracer was introduced into the soil on 1 March, 1997 by injecting $(^{15}NH_4)_2SO_4$ solution (4.0 mg ^{15}N kg^{-1} soil) into a depth of about 10 cm. Soils (15 cm depth) were collected when plants senesced on 23 April, 1997 (Hu *et al*., 2001). The sandstone grassland soil contained 1.68% total organic C and 0.17% organic N as quantified after plant materials (both shoots and roots) were removed after the ^{15}N labeling experiment. The soil was sieved (2 mm mesh) and residues on the sieve were removed, leading to very low C availability for soil microbes. Because of the ^{15}N-labeling treatment in the previous season, the soil had a higher ^{15}N content equivalent to an addition of 2.66 μg ^{15}N g^{-1} soil. The ^{15}N largely existed in nonextractable pools (73%) and microbial biomass (21.7%), with a very small fraction in extractable mineral N (5.3%) (Hu *et al*., 2001). The soil had been kept at 4 °C for about 16 months until being used for this experiment.

Annual graminoid *A. barbata* seeds were collected from the Jasper Ridge Biological Preserve, Palo Alto, CA. Seeds of *A. barbata*, a nonnative annual grass, were pregerminated at room temperature for 48 h and two healthy germinating seeds were planted into each pot microcosm (10 cm × 10 cm × 10 cm). Each microcosm contained exactly 670 g soil (dry-soil equivalent). These microcosms were immediately transferred into growth chambers with a 12 h photoperiod, 25 °C day, 15 °C night, and 70% relative humidity. Two treatments, ambient and elevated CO_2 (ambient + 370 μmol mol^{-1}), were applied 1 week after seeding, with 10 replicates for each treatment. The microcosms were rotated biweekly between the two chambers to eliminate any potential chamber effects (Diaz *et al*., 1993) and microcosms were rotated within each chamber. During the whole growing season, the same amount of water was added to each pot using a syringe as needed to ensure adequate water was available (as shown by no plant withering) and watering was carefully conducted to ensure no water leakage. A saucer was also placed under each microcosm pot to further ensure no leaking of water occurred.

Sample collection and soil, plant and microbial analyses. Microcosm pots were destructively harvested when seeds of *A. barbata* matured on 16 March, 1999. Plant shoots and roots were manually separated and the soil samples were sieved (2 mm mesh). Roots on the sieve were manually sorted, washed thoroughly and oven-dried (65 °C, 72 h).

Microbial respiration in root-free soils was measured as total CO_2 evolution from incubation at 22 °C for 7 days at constant moisture (15% w/w, ca. 75% field water holding capacity). Microbial biomass C and N were determined by fumigation–extraction (Vance *et al*., 1987), using 0.5 M K_2SO_4 extraction after being shaken for 30 min. Soil extractable organic C in the K_2SO_4 extracts before and after the fumigation was quantified using a total C analyzer (Shimadzu TOC-5050A, Shimadzu Co., Kyoto, Japan). Soil extractable inorganic N (NH_4-N and NO_3-N) in the nonfumigated and fumigated soils was measured on a flow injection analyzer (Lachat Quickchem Systems, Milwaukee, WI, USA) after alkaline persulfate digestion (Cabrera & Beare, 1993). The total of extractable NH_4^+ and NO_3^- in K_2SO_4 extracts of nonfumigated samples was defined as extractable N.

The C and N concentrations of soil, shoots and roots and their isotope ratios were determined by combustion on a gas chromatograph-mass spectrometer (GC–MS) (Europa Scientific LTD., Crewe, UK), using finely ground subsamples. The ^{15}N ratio in the extractable pool of N was determined following diffusion and the microbial biomass ^{15}N ratio was determined following Kjeldahl digestion and diffusion (at University of California at Berkeley), and the diffusion process was carried out by using filter paper disks in PTFE (Teflon) traps in plastic specimen cups (Stark & Hart, 1996). Sample $\delta^{15}N$ (‰) values were converted to excess

nitrogen isotope (mg) and conversion of $\delta^{15}N$ (‰) to the absolute isotope ratio ($^{15}N/^{14}N$) of the sample was based on the atom ratio of atmospheric nitrogen. Sample ^{15}N content was then calculated from fractional abundance ($^{15}N/(^{15}N + ^{14}N)$) and total N content of the sample. Nonextractable ^{15}N content (mostly organic ^{15}N) was calculated by subtracting microbial biomass ^{15}N and K_2SO_4-extractable ^{15}N from total soil ^{15}N.

Experiment 2

The second experiment was conducted at the USDA Air-Quality CO_2 Facility, North Carolina State University, Raleigh, NC, USA. The facility consists of a 9 m × 12 m bay and contains 20 continuous stirred tank reactor (CSTR) chambers for exposure of plants to CO_2 (Booker *et al*., 2000). The chambers were built in a regular greenhouse and no extra light was applied. Each CSTR (1.2 m diameter × 1.4 m tall) can be used to expose plants to CO_2. Air sampling is accomplished by extracting a small quantity of gas exiting the CSTR and drawing it back through Teflon lines into the laboratory. A computer activates and deactivates solenoid valves (one per CSTR) to sample the air in each chamber for approximately 2 min and averages and collects CO_2 data for analysis.

Experimental setup. The soil was a fine-loam over clayey, mixed, mesic Ultic Haploxeralf (Sutherlin series) collected from an annual grassland at the University of California Hopland Research Station in Mendocino County, CA, USA (39°00′N latitude, 123°4′ W longitude). About 80 kg soil (sampled to 10 cm) was obtained, sieved (4 mm mesh), and well mixed before being shipped to North Carolina State University, Raleigh, NC, USA. The soil had been kept under 4 °C for 18 months until being used for this experiment. Two CO_2 concentrations, ambient and elevated (ambient + 370 μmol mol^{-1}), were applied, with three replicates (i.e. three CSTR chambers) for each CO_2 treatment. In each CSTR chamber, six microcosm pots (12 cm × 12 cm × 15 cm) were randomly placed and their positions were frequently rotated. Each pot contained exactly 1294.36 g of soil (dry soil equivalent). Twenty-five pregerminated *A. fatua* seeds (48 h) were planted into each pot and then thinned 1 week later so that each pot had 20 healthy plants and the CO_2 treatments immediately started thereafter. During the whole growing season, watering was carefully conducted as described in Experiment 1.

Introduction of ^{15}N tracer. Exactly 4.0 mg ^{15}N ($^{15}NH_4)_2SO_4$, 99.7% atom ^{15}N) per kg of soil were injected into soils on 10 March, 2001 in 60.0 mL of H_2O solution, introducing a total of 5.177 mg ^{15}N into each microcosm pot at six points to a 10 cm depth into the soil. Watering was carefully conducted to ensure no water leakage and associated ^{15}N leaching.

Collection and analyses of plant and soil samples. Microcosm pots were destructively harvested on 11 March, 24 April and 23 May (i.e. 1, 45 and 75 days after the ^{15}N introduction). These sampling dates corresponded approximately to the late tillering, flowering and maturing stages of *A. fatua*. The last sampling date physiologically corresponded to the harvest date for *A. barbata* in experiment 1. Microbial respiration in root-free soils was measured as total CO_2 evolution from incubation at 22 °C for 7 days at constant moisture (18% w/w, equivalent to 70–75% water holding capacity) (Hu & van Bruggen, 1997). Microbial biomass C and N were determined by fumigation–extraction as previously described. Plant shoot, root and soil subsamples were oven-dried (65 °C, 72 h) and finely ground using a ball mill. The C and N concentrations of soil, shoots, and roots were quantified on a Perkin-Elmer 2400 CHNS/O elemental analyzer (Norwalk, CT, USA) and their isotope ratios were determined by combustion on a ThermoFinnigan DELTAPlus continuous flow isotope ratio mass spectrometer (CF–IRMS) (ThermoFinnigan, Bremen, Germany), using finely ground subsamples (measured at the Soil Analytical Lab., North Carolina State University, Raleigh, NC, USA). Microbial biomass ^{15}N ratio was determined following persulfate digestion and diffusion (Stark & Hart, 1996) and ^{15}N in the nondigested samples were taken as the ^{15}N of the extractable inorganic N. Sample $\delta^{15}N$ (‰) and sample ^{15}N content were calculated as described above.

Mycorrhizal infection of plant roots. The percentage of root length colonized by arbuscular mycorrhizal (AM) fungi was measured on roots stained in trypan blue (Phillips & Hayman, 1970) using the gridline-intersect method (Giovannetti & Mosse, 1980). Briefly, roots (about 1 cm long) were cleared in KOH, acidified in HCl, and then stained with trypan blue solution. The stained roots were spread on a Petri dish with gridlines, and examined on a dissecting microscope at ×40 magnification. The intersections between roots and gridlines were counted. The percentage of the colonized roots was then calculated.

Data analyses. Plant biomass (root and shoot) and biomass N was converted to a base unit of per m^2 of soil. One-way variance analysis (ANOVA) was used to detect the effects of elevated CO_2 on plant shoot and root biomass, biomass N and ^{15}N, microbial biomass C, microbial biomass N, microbial respiration, and soil

extractable inorganic N ($P \leq 0.05$). For all statistical analyses, the SPSS V.10.0 (SPSS Inc., Chicago, IL, USA) software package was used.

Results

Shoot and root biomass and biomass N

Elevated CO_2 in the atmosphere significantly increased plant biomass in both *A. barbata* and *A. fatua* experiments (Fig. 1). In the first experiment, elevated CO_2 significantly increased *A. barbata* shoot biomass by 36.5% from 235 to 320 g m^{-2} (Fig. 1a) and shoot biomass N by 25.1% from 1.3 to 1.6 g N m^{-2} (Fig. 1b). However, root biomass (299 and 338 g m^{-2}, respectively) and biomass N (1.4 and 1.3 g m^{-2}, respectively) were not significantly different between the control and elevated CO_2. Still, CO_2 enrichment significantly increased total plant biomass N by 9.8%. In addition, elevated CO_2 significantly increased biomass C : N ratios from 73 to 80 in shoots and 85 to 100 in roots. By the final harvest, root biomass constituted ca. 50% of the total plant biomass in *A. barbata*.

In the second experiment, CO_2 enrichment significantly increased *A. fatua* shoot biomass by 11%, 53% and 42% at the March, April and May sampling dates, respectively (Fig. 1c). However it significantly increased total N in shoots only in the April and May samples (10.5% and 12.9% for shoots, respectively) (Fig. 1d). The final shoot biomass N was 7.1 and 8.1 g N m^{-2} soil for the control and elevated CO_2 treatments, respectively. Also, CO_2 enrichment significantly increased root biomass (77% and 60%, respectively) and biomass N (23% and 36%, respectively) in the April and May samples (data not shown). The root biomass N at the final harvest was 1.7 and 2.4 g N m^{-2} soil for the ambient control and elevated CO_2 treatment, respectively. Biomass C : N ratio significantly increased from 52 to 59 in roots and 55 to 63 in shoots from the control to elevated CO_2. By the final harvest, root biomass constituted less than 25% of the total plant biomass in *A. fatua*. In addition, total *A. fatua* biomass did not increase between the April and May sampling, but root biomass was slightly reduced. By the final harvest, total plant biomass N of *A. fatua* was 13.6 g m^{-2} soil under elevated CO_2, 17.8% higher than the ambient control (11.3 g m^{-2} soil).

K_2SO_4 extractable N in soil. Elevated CO_2 did not significantly reduce soil extractable N in the *A. barbata* experiment. Although the mean value of the extractable N was ca. 16% lower in the elevated CO_2 treatment (0.71 mg N kg^{-1} soil) than in the ambient control (0.85 mg N kg^{-1} soil), this CO_2-induced reduction was not statistically significant (Fig. 2a) and only accounted for ca. 5% of the increased biomass N. At the early stage of the *A. fatua* experiment when plants were small,

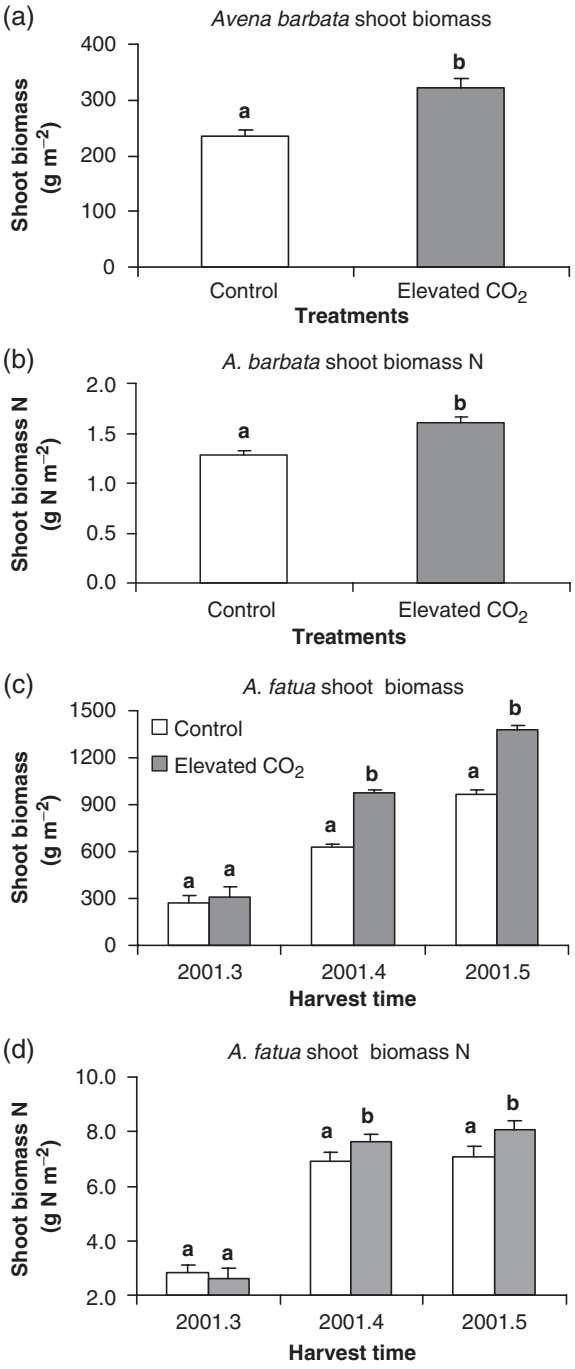

Fig. 1 The effects of CO_2 enrichment on plant biomass and biomass N of *Avena barbata* (a, b) and *A. fatua* (c, d). The unit was g m^{-2} for plant biomass and g N m^{-2} for plant biomass N. Bars depict treatment means and standard errors (SEM). Bars with the same letter at the sampling date are not significantly different at $P \leq 0.05$.

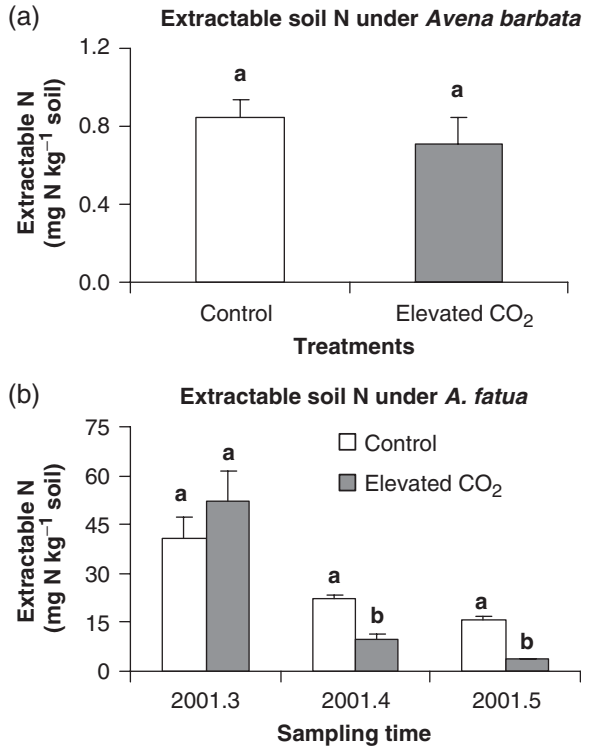

Fig. 2 Elevated CO_2 effects on soil extractable N under *Avena barbata* (a) and *A. fatua* (b). Bars depict treatment means and standard errors (SEM). Bars with the same letter at the sampling date are not significantly different at $P \leq 0.05$.

elevated CO_2 did not affect soil extractable N, as shown by samples collected on 11 March. But as the growing season advanced, CO_2-induced decreases in extractable N became significant. By the final harvest time, total extractable N was averaged at 3.84 mg N kg^{-1} soil in the elevated CO_2 pots, in comparison with 15.99 mg N kg^{-1} soil in the ambient control (Fig. 2b). The reduction in extractable N in soil (averaged at 12.15 mg N kg^{-1} soil) under elevated CO_2 was approximately equal to the increase in total plant biomass N (averaged at 12.24 mg N kg^{-1} soil).

Soil microbial biomass carbon and nitrogen. Microbial biomass C (fumigation flush) was significantly higher under elevated CO_2 in the *A. barbata* experiment (Fig. 3a), but microbial biomass N (fumigation flush) showed no difference between the ambient control (18.3 mg N kg^{-1} soil) and the elevated CO_2 (19.3 mg N kg^{-1} soil). In the *A. fatua* experiment, CO_2 enrichment did not increase microbial biomass C (Fig. 3b) but enhanced microbial respiration in the late growing stage (Fig. 3c). Microbial biomass N showed no significant difference between the treatments and among the sampling dates, averaging at 30.3 mg N kg^{-1} soil in the ambient control and 28.1 mg N kg^{-1} soil under elevated CO_2.

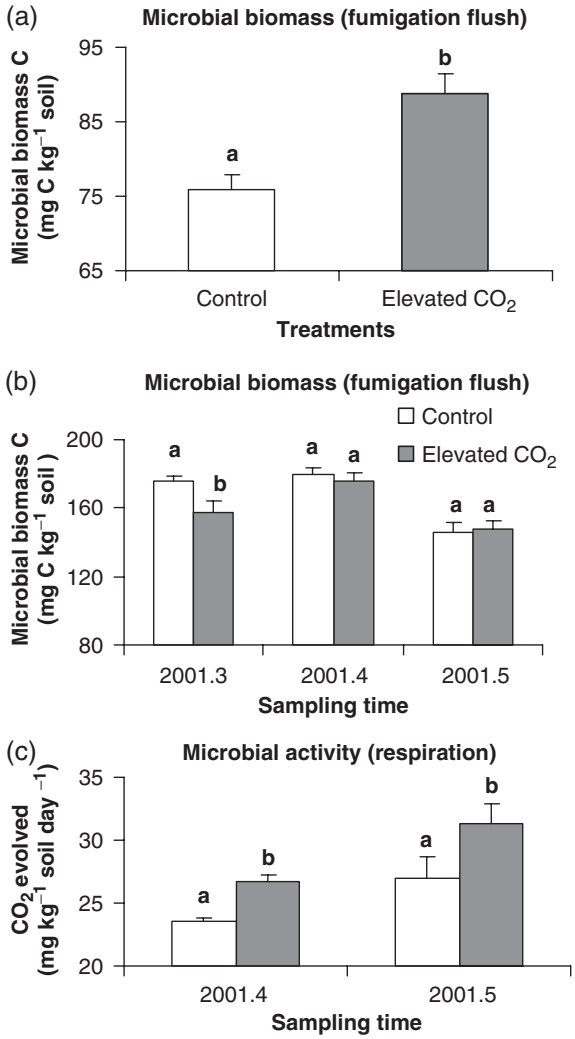

Fig. 3 Elevated CO_2 effects on soil microbial biomass C and microbial activity under *Avena barbata* (a) and *A. fatua* (b, c). Bars depict treatment means and standard errors (SEM). Bars with the same letter at the sampling date are not significantly different at $P \leq 0.05$.

^{15}N distribution among plant, soil and soil microbes. Elevated CO_2 altered ^{15}N distribution among plant shoots and roots, soil and soil microbes (Figs 4 and 5). Total ^{15}N in *A. barbata* shoots and roots was not significantly impacted by CO_2 elevation (data not shown). However, by the final harvest, percentage recovery of introduced ^{15}N in *A. fatua* materials increased from 2.6% to 4.6% in roots, and from 18.2% to 26.0% in shoots under elevated CO_2 (Figs 4a and b). Elevated CO_2 in the atmosphere reduced the ^{15}N concentration of *A. barbata* shoots and roots because of dilution effects of increased biomass, but increased both shoot and root ^{15}N concentrations in *A. fatua* (Table 1). ^{15}N in the extractable N pool was not different between the ambient control and elevated CO_2 in the *A. barbata*

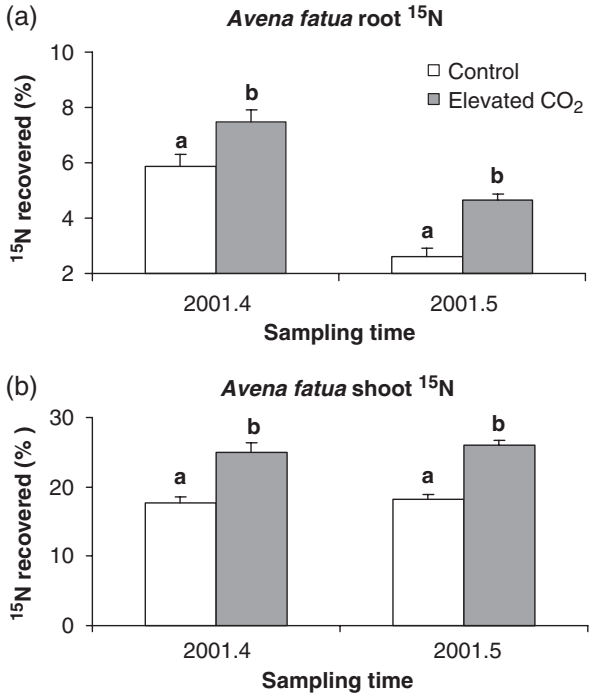

Fig. 4 Elevated CO_2 effects on plant ^{15}N acquisition of *Avena fatua*: shoot ^{15}N (a) and root ^{15}N (b). Bars depict treatment means and standard errors (SEM). Bars with different letters at the sampling date are significantly different at $P \leq 0.05$.

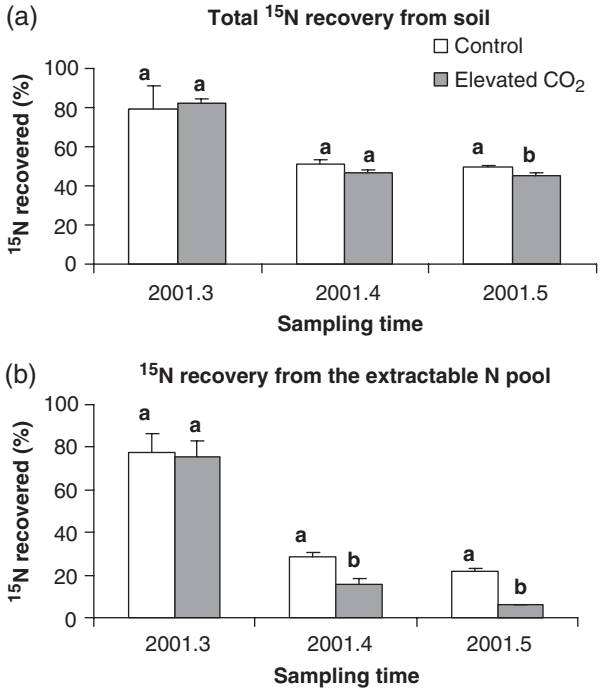

Fig. 5 Effects of CO_2 enrichment on soil ^{15}N distribution under *Avena fatua*: (a) total ^{15}N in soil, and (b) ^{15}N recovered from the extractable soil N pool. Bars depict treatment means and standard errors (SEM). Bars with the same letter at the sampling date are not significantly different at $P \leq 0.05$.

experiment (data not shown). However, in the *A. fatua* experiment, total ^{15}N in soil (% recovery) was significantly reduced with elevated CO_2 by the late growing season to 45.5%, compared with 49.4% in the ambient control (Fig. 5a). The reduction was highly significant in the extractable N pool (Fig. 5b). By the final harvest time, about 22% of introduced ^{15}N remained in the extractable soil N pool in the control, but this number reduced to 6.0% under elevated CO_2. Total recovery of ^{15}N (i.e. the sum of ^{15}N in soil and plant shoots and roots) was significantly higher in the elevated CO_2 (76% of total ^{15}N introduced) than in the control (70.2% of total ^{15}N introduced). Total ^{15}N in the microbial biomass N (ca. 1% of the total ^{15}N introduced) and microbial biomass $^{15}N:{}^{14}N$ ratios were not impacted by elevated CO_2 in either experiment (data not shown).

Mycorrhizal colonization of A. fatua *roots.* Mycorrhizal colonization of *A. fatua* roots was significantly higher under elevated CO_2 than in the ambient control (Fig. 6). CO_2-led increases in root mycorrhizal colonization were about 71% in the April samples and 48% in the May samples. For the samples collected at the final harvest, the magnitude of increases in mycorrhizal infection (48%) was close to that in aboveground plant biomass (42%, Fig. 1). However, there was no correlation between these two parameters ($R^2 = 0.03$; $P > 0.05$). Over time, root mycorrhizal colonization significantly increased from April (10.8% in the ambient control and 18.5% in the elevated CO_2) to May (21.0% and 31.1%, respectively).

Discussion

Results obtained from our experiments indicated that elevated CO_2 led to increased plant N uptake in both highly N-limiting and relatively N-rich soils (Fig. 1). However, microbial biomass N did not increase under elevated CO_2, in spite of increases in microbial biomass C under *A. barbata* (Fig. 3a) or microbial activity under *A. fatua* (Fig. 3c), suggesting differential effects of CO_2 enrichment on plant and microbial N acquisition.

The data from the ^{15}N tracer provided some direct evidence showing that elevated CO_2 increased N transfer from the soil to plants (Figs 1, 2, 4 and 5). These results were consistent with our previous observations in a grassland dominated by *A. barbata* (Hu *et al.*, 2001), suggesting that elevated CO_2 may favor plants over microbes for N acquisition. Similar results have recently been reported in several long-term

Table 1 Elevated CO_2 effects on ^{15}N concentrations ($^{15}N/(^{15}N + ^{14}N) \times 100$) of plant and soil samples†

	Sampling date					
	1		2		3	
CO_2 treatment	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Experiment 1 (*Avena barbata*)						
Shoots	ND	ND	ND	ND	1.24	1.08*
Roots					1.12	0.97*
Soil					0.49	0.49
Experiment 2 (*A. fatua*)						
Shoots	0.49	0.54	1.78	2.12*	1.77	2.09*
Roots	0.62	0.65	1.29	1.29	1.20	1.32*
Soil	0.54	0.54	0.48	0.47	0.48	0.46*

*Significant difference ($P \leq 0.05$) between the control and elevated CO_2 at its corresponding sampling date.
†Sampling dates 1, 2 and 3 approximately correspond to tillering, flowering and maturing stages of *A. barbata* (March 16, 1999 only) and *A. fatua* (March 11, April 23 and May 24, 2001, respectively).
ND means no data because plants and soils were not sampled.

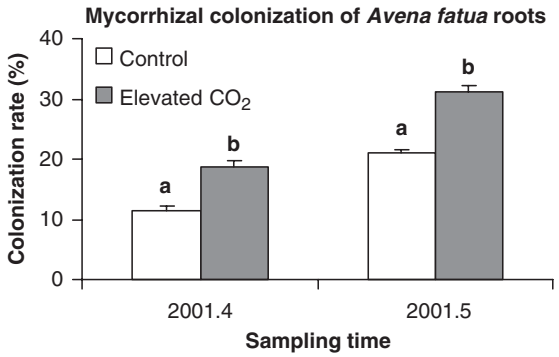

Fig. 6 Elevated CO_2 effects on mycorrhizal colonization of roots in *Avena fatua*. Bars depict treatment means and standard errors (SEM). Bars with different letters at the sampling date are significantly different at $P \leq 0.05$.

field experiments. For example, Richter *et al*. (2003) reported that in *Lolium perenne* and *Trifolium repens* swards, microbial N immobilization was not strongly affected by 7-years of exposure to elevated CO_2. Niklaus *et al*. (2003) also observed no changes in microbial biomass N in a nutrient-poor grassland after being exposed to elevated CO_2 for six growing seasons. Together, these results suggest that enhanced microbial immobilization may not be a major mechanism constraining plant response to elevated CO_2, as initially proposed (Diaz *et al*., 1993). The implications for this alteration of N partitioning between plants and microbes are not exactly known. However, an understanding of the mechanisms that govern CO_2 enhancement of plant N acquisition may help us predict the long-term consequences. Experimental evidence demonstrating CO_2-led increases in root biomass and C inputs is plentiful (Treseder & Allen, 2000; BassiriRad *et al*., 2001; Niklaus *et al*., 2003; Treseder *et al*., 2003; Pendall *et al*., 2004), similar to the CO_2-led increases in root biomass and mycorrhizal infection in our current experiments. This evidence indicates that plants under elevated CO_2 may be able to adjust their C allocation to exploit N (and possibly other nutrient) resources in the soil (i.e. compensatory adjustments to increase acquisition capacity for minerals, BassiriRad *et al*., 2001; Pendall *et al*., 2004). However, what is unknown is the potential of CO_2-enhancement of plant N uptake and where the enhanced N, if any, originates (Richter *et al*., 2003).

In many temperate ecosystems, the soil contains a total organic N that is at least two magnitudes larger than the annual needs of plants. Still, plant growth in these ecosystems is most likely N-limited (Vitousek & Howarth, 1991) because soil microbes do not release the N contained in plant residues and other older organic matter to permit plants uptake. Since elevated CO_2 often stimulates microbial activity through increasing C inputs to soil, it has been proposed that CO_2-enhancement of microbial activities would increase N mineralization, thereby sustaining N supply for plants (Zak *et al*., 1993; Hungate *et al*., 1997). A gradual conversion of this organic N into plant biomass with high C : N would sustain plant N requirements under elevated CO_2 over the long term, without the need for new N inputs. Results from our *A. barbata* experiment seemed to support this hypothesis as the CO_2-led increase in plant biomass N cannot be balanced unless microbes released some N from other organic N. CO_2 enhancement of plant biomass N but not total ^{15}N in *A. barbata* suggest that microbes might have released some N

from older organic pools. However, the data obtained from our *A. fatua* experiment was inconsistent with this hypothesis. There, enhanced N uptake under elevated CO_2 predominantly originated from the extractable N pool as increases in plant biomass N were accompanied by comparable decreases in extractable N. Atmospheric CO_2 enrichment only facilitated the transfer of extractable N to plants as evidenced by increased ^{15}N concentrations in *A. fatua* tissues. These results are consistent with those obtained from open-top chambers in the field by Cardon *et al*. (2001), who showed that CO_2-enhanced microbial activity as measured by respiration may largely stem from preferential consumption of newly fixed organic C, rather than N-enriched old organic materials. Similarly, Richter *et al*. (2003) examined gross N fluxes in a grassland soil exposed to elevated atmospheric CO_2 for 7 years and found that organic N turnover and microbial N mineralization were not affected. The difference in the results between our two experiments may be related to the initial nutrient status, particularly the N availability. Elevated CO_2 may prompt plants to acquire N in the extractable pool first before microbes access old organic N (if any). The magnitude of microbial conversion of older organic N under elevated CO_2 may be critically important in understanding plant and microbial response to progressive N limitation under elevated CO_2 (Hungate *et al*., 2003; Luo *et al*., 2004).

One interesting finding in our experiment is that CO_2-enhancement of mycorrhizal colonization of roots (Fig. 6) coincided with a marked decrease in soil extractable N in the *A. fatua* experiment (Fig. 2). Stimulation of mycorrhizae by elevated CO_2 has been documented in various experiments (Rillig *et al*., 1999; Treseder & Allen, 2000; Treseder *et al*., 2003). However, increased plant N uptake has not been attributed directly to the enhanced mycorrhizae. Instead, enhancement of plant N uptake has previously been attributed to higher soil moisture and extended plant growing periods under elevated CO_2 (Jackson *et al*., 1994), which is highly possible in some water-limiting systems (Hu *et al*., 2001; Billings *et al*., 2002). However, frequent watering was applied to avoid water limitation for the plants in our experiments. Also, plant roots essentially occupied the whole volume of the soil in our confined systems and mycorrhizal contribution for N uptake was expected to be minimal if their role is mainly extension of root surface. It is unknown why plant roots did not effectively uptake extractable N in the ambient control, although by the middle of the growing season plant leaves became yellowish in both treatments (similar to a N-limiting symptom). Rapid depletion of extractable N under elevated CO_2 suggests that mycorrhizal hyphae may still be able to reach N pools that plant roots are unable to. Alternatively, CO_2-enhancement of mycorrhizae may have stimulated plant uptake of other limiting nutrients such as P (Grunzweig & Korner, 2003), leading to a corresponding increase of plant N utilization. CO_2-stimulation of mycorrhizae and its resulting effects on N and P availability may have some long-term implications in mediating plant and ecosystem response to rising CO_2 in the atmosphere. In grasslands where AM fungi dominate, enhancement of mycorrhizae is unlikely to directly stimulate organic matter decomposition as AM fungi generally lack saprotrophic capacity (Allen, 1991; Read & Perez-Moreno, 2003). However, increased C inputs associated with enhanced mycorrhizal hyphae may indirectly stimulate residue decomposition by increasing microbial activity (Hodge *et al*., 2001; Pendall *et al*., 2004; Tu *et al*., unpublished data). Whether the resulting N release from organic matter can partially relieve N constraints on long-term plant and ecosystem responses to rising atmospheric CO_2 levels deserves more attention.

In summary, results obtained from our microcosm experiments showed that elevated CO_2 increased plant N acquisition without significantly impacting microbial biomass N. In the first experiment with a N-poor soil, the CO_2-enhanced N obtained by plants largely originated from the nonextractable pools. In the second experiment with a N-rich soil, it predominantly stemmed from the extractable N pool. However, the mechanisms that underlie the enhancement of plant N uptake are unclear and it is unknown whether results obtained in these short-term microcosm experiments can be extrapolated to the field. Potential effects of CO_2-enhancement of mycorrhizae on plant N and P acquisition warrant further investigation.

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