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REGULAR ARTICLE

Increased soil moisture content increases plant N uptake and the abundance of ^{15}N in plant biomass

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Abstract The natural abundance of ^{15}N in plant biomass has been used to infer how N dynamics change with elevated atmospheric CO_2 and changing water availability. However, it remains unclear if atmospheric CO_2 effects on plant biomass ^{15}N are driven by CO_2-induced changes in soil moisture. We tested whether ^{15}N abundance (expressed as $\delta^{15}N$) in plant biomass would increase with increasing soil moisture content at two atmospheric CO_2 levels. In a greenhouse experiment we grew sunflower (*Helianthus annuus*) at ambient and elevated CO_2 (760 ppm) with three soil moisture levels maintained at 45, 65, and 85% of field capacity, thereby eliminating potential CO_2-induced soil moisture effects. The $\delta^{15}N$ value of total plant biomass increased significantly with increased soil moisture content at both CO_2 levels, possibly due to increased uptake of ^{15}N-rich organic N. Although not adequately replicated, plant biomass $\delta^{15}N$ was lower under elevated than under ambient CO_2 after adjusting for plant N uptake effects. Thus, increases in soil moisture can increase plant biomass $\delta^{15}N$, while elevated CO_2 can decrease plant biomass $\delta^{15}N$ other than by modifying soil moisture.

Keywords Elevated CO_2 · Microbial biomass · ^{15}N natural abundance · Nitrogen cycling · Plant N uptake · Soil moisture

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Introduction

The variation in natural abundance of ^{15}N in various N pools can provide useful information about transformations, inputs, and outputs of N in ecosystems (Amundson et al. 2003; Högberg 1997; Robinson 2001). Recently, the natural abundance of ^{15}N in plant biomass has been used to better understand the effects of elevated atmospheric CO_2 on N cycling (BassiriRad et al. 2003; Billings et al. 2002; Johnson et al. 2000; Robinson and Conroy 1999). However, it is still unclear how N cycling is altered that causes variation in plant biomass $\delta^{15}N$ under elevated CO_2.

Different mechanisms have been suggested to explain the effects of elevated atmospheric CO_2 on plant biomass $\delta^{15}N$. Increased $\delta^{15}N$ in ponderosa pine (*Pinus ponderosa* Dougl.) needles in response to elevated CO_2 in an open top chamber study has been attributed to increased uptake of N from surface soil layers or recalcitrant soil N pools that are enriched in

^{15}N compared to other soil N pools (Johnson et al. 2000). Billings et al. (2002) found an increase in foliage $\delta^{15}N$ of Mojave desert shrubs under elevated CO_2 (from free-air CO_2 enrichment, FACE) and with time, and suggested that this was caused by an increase in microbial activity, which enriched pools of plant-available N and decreased N availability. In contrast, BassiriRad et al. (2003) observed mostly decreased foliage $\delta^{15}N$ with elevated CO_2 of 27 species measured at one time point from six different FACE experiments in the US. They suggested that an increase in the degree of mycorrhizal infection or internal fractionating processes of the N cycle within the plant may have caused the significant reduction in foliage $\delta^{15}N$ under high CO_2.

The change in foliage $\delta^{15}N$ with elevated CO_2 could be due to an elevated CO_2-induced change in soil moisture, although this has not been studied well. Soil moisture is one of the key factors influencing microbial activity, N mineralization and nitrification (Brady and Weil 2002). Nitrogen isotopic fractionation occurs during these microbial transformations (Högberg 1997; Robinson 2001), and thus by changing the rate of these transformations, soil moisture could modulate the $\delta^{15}N$ value of plant available N in the soil. Increased soil moisture could enhance net N mineralization, which has been shown to increase foliar $\delta^{15}N$ (Garten and Van Miegroet 1994; Koba et al. 2003). Increased soil moisture could also slow down nitrification (Brady and Weil 2002; Breuer et al. 2002) and increase uptake of relatively ^{15}N-enriched NH_4^+, resulting in increased foliage $\delta^{15}N$ (Garten and Van Miegroet 1994; Miller and Bowman 2002). It is further possible that increased soil moisture could stimulate mining of relatively ^{15}N-enriched organic pools (i.e., increase gross N mineralization) that could increase foliage $\delta^{15}N$ (Johnson et al. 2000). Finally, changes in the retention of N and ^{15}N in soil microorganisms, and mycorrhizae in particular (Hobbie and Colpaert 2003; Hobbie et al. 2000), with increased soil moisture content could alter plant biomass $\delta^{15}N$.

The aim of our study was to examine soil moisture and atmospheric CO_2 effects on plant biomass $\delta^{15}N$ of sunflower (*Helianthus annuus*). We hypothesized that an increase in soil moisture content increases plant biomass $\delta^{15}N$ of sunflower both at ambient and elevated atmospheric CO_2 conditions. We tested our hypothesis by growing sunflower at ambient (~380 ppm) and elevated (760 ppm) atmospheric CO_2 concentration, in soils with moisture contents kept at 45, 65 or 85% of field capacity, in a greenhouse experiment. Because we kept soil moisture content constant throughout the experiment in each treatment, potential CO_2-induced differences in soil moisture content were eliminated. Because the CO_2 treatment was not replicated (all plants were in one ambient CO_2 and one elevated CO_2 greenhouse), we were unable to statistically test for a CO_2 effect. Despite this shortcoming in our experimental design, our results are instructional for a better understanding of how atmospheric CO_2 affects plant biomass $\delta^{15}N$ other than by modifying soil moisture.

Materials and methods

We did our experiment in two greenhouses at the University of California, Santa Cruz. In one greenhouse (elevated CO_2 greenhouse) we raised the atmospheric CO_2 concentration to a constant level of 760 ppm by adding pure CO_2, controlled by an infrared gas analyzer (IRGA, LI-COR 820, LI-COR, Lincoln, NE). When the CO_2 concentration fell below 760 ppm, the IRGA opened a solenoid valve to let ^{13}C-depleted CO_2 from a gas tank into the greenhouse. The valve closed at 765 ppm. We reduced air leakage from the greenhouse by sealing off the joints of the greenhouse. In the other greenhouse (ambient CO_2 greenhouse), next to the elevated CO_2 greenhouse, we kept the atmospheric CO_2 concentration at ambient levels (~380 ppm). Two air-conditioners (Mitsubishu Electronic, Lawrenceville, GA) controlled the air temperature inside the elevated CO_2 greenhouse and an evaporative air cooler (AdobeAir Inc., Phoenix, AZ) in the ambient CO_2 greenhouse. The air temperature in both greenhouses was recorded every 15 min (Argus Control Systems Ltd., White Rock, BC). The average daily temperatures never deviated more than 1.5°C between the two greenhouses and was on average 21.3±4.0 and 21.4±4.7 (mean±SD) in the elevated and ambient CO_2 greenhouse, respectively. Both greenhouses were equipped with 1,100 W lights (P.L. Light Systems, Beamsville, ON) that went on whenever the natural light intensity fell below 200 Wm^2 between 7 A.M. and 6 P.M. (Argus Control Systems Ltd., White Rock, BC). In this way, light levels in both greenhouses were nearly identical.

We used a soil from a Kansas tall grass prairie (Mollisol, clay loam), with total C and N contents of 17 and 1.8 g kg^{-1}, and a pH of 7.05. The $\delta^{15}N$ of the soil N pool was 6.4‰. We air-dried and sieved (4 mm) soils, and removed most of the roots. In each of 24 PVC pots (diam. 15 cm, height 40 cm), capped at the bottom with an air inlet (Fig. 1), we placed a nylon bag filled with 2,500 g playground sand, before adding 6,000 g of air-dry soil. We buried a plastic watering tube (inner diam. 0.32 cm, total length 70 cm, buried length 50 cm), punctured with holes every 5 cm, while filling the pot with soil. The tube was buried in a spiral inside the pot, with a 100 ml reservoir at the top end of the tube that protruded from the soil (Fig. 1). Water added to the reservoir dripped slowly out of the holes in the tube, thereby maintaining uniform soil moisture content throughout the pot. In each pot we planted five seeds of sunflower (*H. annuus*, cultivar Sunbright). After germination we thinned each pot to one plant. We kept soil moisture content at 45, 65, and 85% of field capacity (corresponding to 0.19, 0.28, and 0.36 g water g^{-1} soil, eight pots for each soil moisture treatment) by weighing and watering the pots with DI water every day during the experiment. During the entire experiment, soil moisture never deviated more than 3% from the target value. Half of the pots (four pots of each soil moisture treatment) were placed in the elevated CO_2 greenhouse, and the other half in the ambient CO_2 greenhouse. Because of our frequent watering, we eliminated a potential atmospheric CO_2 effect on soil moisture. We connected the air inlet at the bottom of each pot to an aquarium pump and aerated the pots every 6 h for 15 min during the period of the experiment. We rotated pots inside the greenhouse each time plants were watered. Because the pots did not drain, and were regularly aerated, we kept losses of N through leaching and denitrification to a minimum.

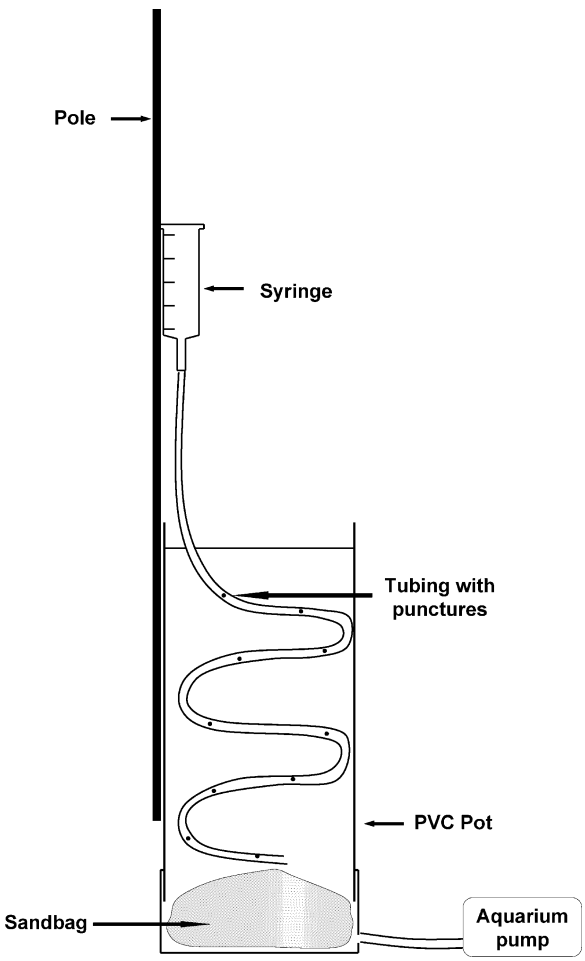

Fig. 1 Setup of the pots. The perforated tubing allows for homogeneous watering throughout the soil profile

Seventy days after planting, we harvested plants. We separated plant biomass into shoot and root biomass. We washed root samples with DI water. Plant materials were dried at 65°C to constant mass, weighed, and ground. We analyzed shoot and root biomass samples for total N and $\delta^{15}N$ ($\delta^{15}N$ to a precision of 0.3‰) on a Hydra 20–20 continuous flow isotope mass spectrometer (PDZ Europa, Chesire, UK). We calculated the $\delta^{15}N$ value of the total plant as the N-weighted average of the shoot and root $\delta^{15}N$ values.

After harvesting, we took one homogenized soil sample from each pot for inorganic and microbial N and $\delta^{15}N$ analyses. Because soil moisture affects the efficiency of K_2SO_4 extractable N (Haubensak et al. 2002), we moistened all soil samples (including two initial soil samples) to equal soil moisture content (85% field capacity) and incubated them in specimen cups for 24 h at room temperature. We use a modified method developed by Bruulsema and Duxbury (1996) to analyze microbial N and $\delta^{15}N$. We extracted one 15 g sub-sample with 30 ml of 0.05 M K_2SO_4, and fumigated another 15 g sub-sample with chloroform in a vacuum dessicator. After 48 h of fumigation, we extracted also these samples with 30 ml of 0.05 M K_2SO_4. We analyzed aliquots of the non-fumigated

extracts for NH_4^+ and NO_3^- (Lachat QuikChem 8000 flow injection analyzer, Milwaukee, WI, USA). After freeze-drying 10 ml of the non-fumigated and fumigated extracts, we analyzed the samples for total N and $\delta^{15}N$ on a Hydra 20–20 continuous flow isotope mass spectrometer (PDZ Europa, Chesire, UK). We calculated microbial N as the difference between total N in the fumigated and non-fumigated samples divided by 0.54 (Brookes et al. 1985). We calculated microbial $\delta^{15}N$ ($\delta^{15}N_{mic}$) as follows:

$$\delta^{15}N_{mic} = \left(\delta^{15}N_f{*}N_f - \delta^{15}N_e{*}N_e\right)/\left(N_f - N_e\right) \quad (1)$$

where $\delta^{15}N_f$ and N_f are the $\delta^{15}N$ value and amount of N in the fumigated extracts and $\delta^{15}N_e$ and N_e are the $\delta^{15}N$ value and amount of N in the non-fumigated extracts.

We used analyses of variance (ANOVA) and analyses of covariance (ANCOVA) with plant biomass N as a covariate to test for soil moisture effects on plant biomass $\delta^{15}N$ in the ambient and elevated CO_2 greenhouse separately. We used linear regressions to relate plant biomass N to plant biomass $\delta^{15}N$. We used ANOVA to test for soil moisture effects on soil extractable N pools, the $\delta^{15}N$ value of the total extractable N pool of the non-fumigated samples, and microbial N and $\delta^{15}N$. When the ANOVA was significant ($P<0.05$), we used post-hoc tests (Tukey's test) to test for differences among soil moisture contents. Because all pots treated with ambient and elevated atmospheric CO_2 were in separate greenhouses (i.e., one replicate for each CO_2 treatment), we were unable to statistically compare the ambient with the elevated CO_2 treatment. We used JMP 4.0.4 for all statistical analyses.

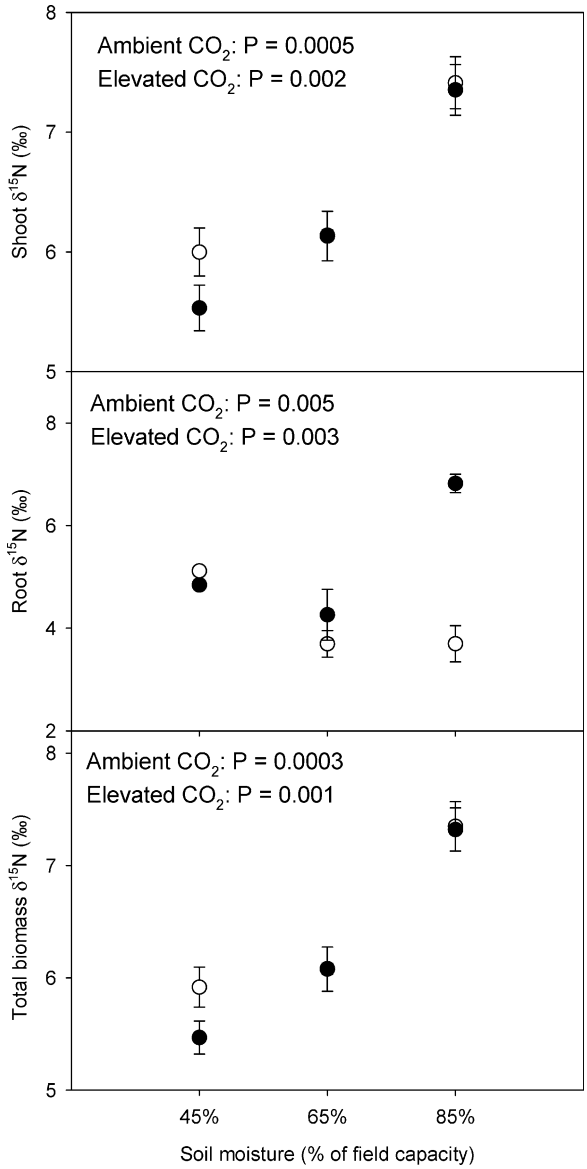

Fig. 2 Average $\delta^{15}N$ values in shoot, root, and total plant biomass (ambient CO_2: *open symbols*, elevated CO_2: *closed symbols*, some symbols are superimposed). Error bars are standard errors

Results

As we hypothesized, increased soil moisture content significantly increased plant biomass $\delta^{15}N$ in the elevated CO_2 greenhouse (both shoot and root, Fig. 2). In the ambient CO_2 greenhouse shoot biomass $\delta^{15}N$ also significantly increased, but root biomass $\delta^{15}N$ significantly decreased with increased soil moisture content. Root biomass was more ^{15}N-enriched at elevated CO_2 than at ambient CO_2 at the highest soil moisture content (by 3.1‰).

Plant biomass N pools showed similar patterns as their $\delta^{15}N$ values. Shoot and total plant biomass N pools significantly increased with increased soil moisture content in both greenhouses (Table 1). Root biomass N significantly increased with increased soil moisture content in the elevated CO_2 greenhouse, but did not significantly differ in the ambient CO_2 greenhouse. Tissue N concentrations were not affected by the soil moisture treatment in both greenhouses.

Table 1 Average N concentration and plant biomass N±SE at ambient and elevated atmospheric CO_2 and for three soil moisture treatments

	Soil moisture (% of field capacity)	Plant biomass N				
		Shoot		Root		Total
		%	mg pot^{-1}	%	mg pot^{-1}	mg pot^{-1}
Ambient CO_2	45	3.7±0.1	62±3	2.4±0.3	6.5±1.0	69±3
	65	3.6±0.1	198±18	2.0±0.1	6.1±1.2	204±18
	85	3.7±0.3	255±20	2.0±0.2	9.0±0.8	264±19
Soil moisture effects: ANOVA *P*-values		0.96	<0.0001	0.38	0.15	<0.0001
Elevated CO_2	45	2.4±0.1	170±17	1.9±0.5	15.4±7.9	185±44
	65	2.9±0.2	199±20	2.0±0.1	7.8±3.1	207±22
	85	2.9±0.1	366±27	2.2±0.1	24.6±2.0	390±28
Soil moisture effects: ANOVA P-values		0.17	0.002	0.72	0.02	0.002

We observed significant positive correlations between plant biomass N pools and their $\delta^{15}N$ values, except for root biomass in the ambient CO_2 greenhouse (Fig. 3). When we included plant biomass N pools as a covariate in the statistical models (ANCOVA), soil moisture effects became non-significant ($P>0.1$). Shoot and total plant biomass in the ambient CO_2 greenhouse showed greater $\delta^{15}N$ values than in the elevated CO_2 greenhouse when $\delta^{15}N$ values were compared at the same level of total biomass N. We observed no significant correlations between plant biomass $\delta^{15}N$ and plant biomass N concentration (data not shown).

Increased soil moisture content significantly reduced extractable NO_3^- in the soil (non-fumigated samples) in both greenhouses ($P=0.04$ and $P=0.005$ for ambient and elevated CO_2, respectively, Fig. 4), likely because of greater plant N uptake. Extractable NH_4^+ increased with increased soil moisture content (only significant in the elevated CO_2 greenhouse, $P=0.04$), but overall extractable NH_4^+ was of an order of a magnitude lower than NO_3^-. There was less extractable NH_4^+ and NO_3^- at the end of the experiment than at the start. Microbial N pools were not affected by soil moisture content in both greenhouses, and did not change compared to the microbial N pool in the soil at the start of the experiment.

As an indication of how much net N mineralization occurred during the experiment we calculated the difference between the final and initial available N pool in the soil (NH_4^+ and NO_3^-) plus the total plant biomass N pool (Table 2, assuming there was no denitrification, see discussion). Using this mass balance approach, net N mineralization only occurred in the treatment with the lowest soil moisture content under elevated CO_2, while all other treatments had net N immobilization. Net N mineralization and immobilization rates were small compared to the initial available N pool in the soil (680 mg pot^{-1}). Soil moisture had no significant effect on net N mineralization/immobilization, but N immobilization was always smaller in the elevated CO_2 than in the ambient CO_2 greenhouse.

The $\delta^{15}N$ value of the extractable N (NH_4^+, NO_3^-, and extractable organic N in non-fumigated samples) tended to increase with increased soil moisture content, although not significantly (Fig. 5). The $\delta^{15}N$ value of microbial N showed large variability and was not significantly affected by the soil moisture treatment. The large variability in $\delta^{15}N$ of microbial N was caused by the presence of large amounts of extractable N (mostly NO_3^-) in the non-fumigated samples, while the extractable N in the fumigated samples was only slightly larger. Consequently, subtraction of two large pools (see Eq. 1) reduced the precision of the microbial $\delta^{15}N$ value calculation.

Discussion

Our results indicate that the increase in shoot and total plant biomass $\delta^{15}N$ with increased soil moisture content was most likely a result of increased plant uptake of relatively ^{15}N-enriched N. While positive relationships between plant tissue N concentration and $\delta^{15}N$ have been observed elsewhere (Hobbie et al. 2000; Kitayama and Iwamoto 2001; Vitousek 1989), we found no such relationship.

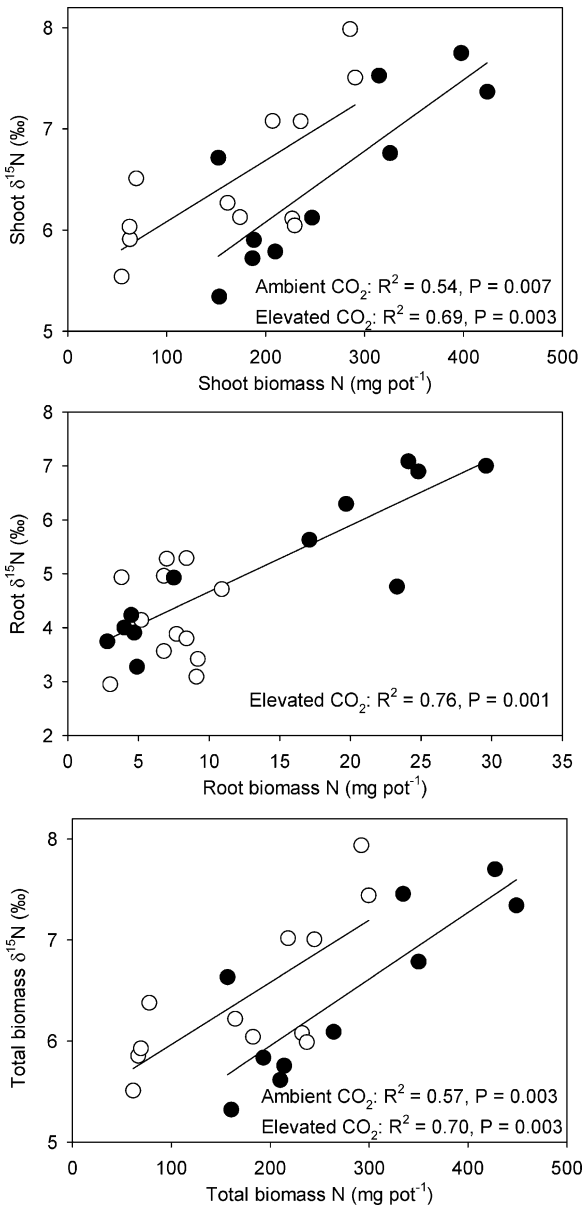

Fig. 3 Relationship between plant biomass N and plant biomass $\delta^{15}N$ for plants in the ambient (*open symbols*) and elevated CO_2 greenhouse (*closed symbols*)

The decrease in root biomass $\delta^{15}N$ with increased soil moisture content in the ambient CO_2 greenhouse may have been a result of internal fractionation processes within the plant (BassiriRad et al. 2003; Robinson et al. 1998). Overall, the root $\delta^{15}N$ values were lower than the shoot $\delta^{15}N$ values, particularly in the ambient CO_2 greenhouse. The significant increase in root biomass N with increased soil moisture content under elevated CO_2 was associated with an increase in root $\delta^{15}N$ (Table 1, Fig. 3), which may have concealed a more subtle effect of internal fractionation processes in the elevated CO_2 greenhouse.

Soil moisture can affect plant biomass $\delta^{15}N$ by changing microbial N retention, particularly in mycorrhizae (Hobbie and Colpaert 2003; Hobbie et al. 2000). While we did not measure mycorrhizal biomass or ^{15}N retention in mycorrhizae, we observed

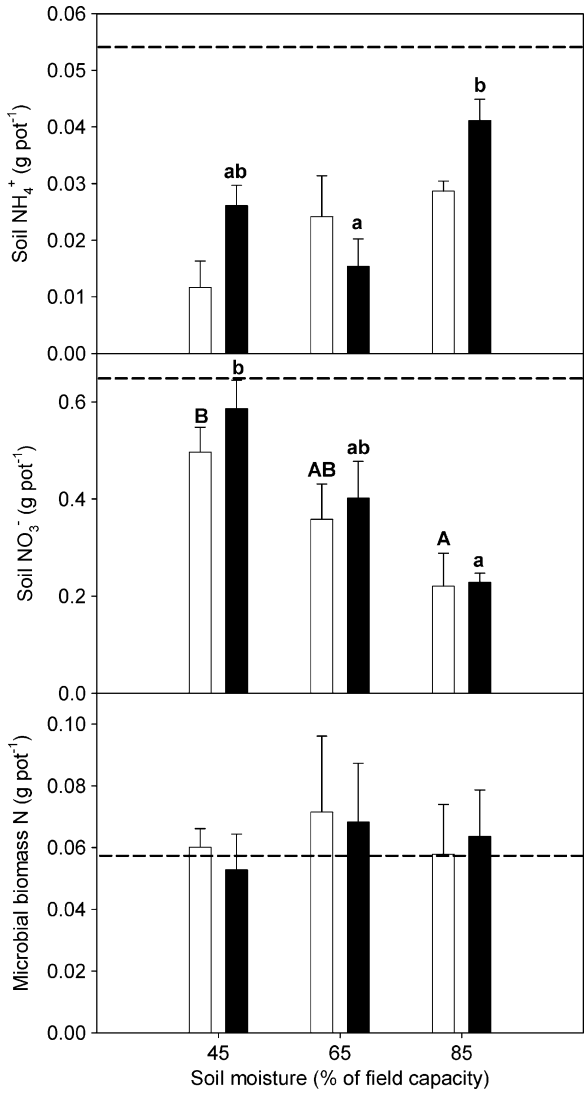

Fig. 4 Average extractable inorganic N (NH_4^+ and NO_3^- in non-fumigated K_2SO_4 extraction) and microbial N in the soil at the end of the experiment (ambient CO_2: *white bars*, elevated CO_2: *black bars*). *Dashed line* represents the inorganic and microbial N at the start of the experiment. Error bars are standard errors. Different letters (capitalized for ambient CO_2) denote significant differences among soil moisture contents ($P<0.05$, post-hoc Tukey's test)

Table 2 Average net N mineralization±SE (negative values indicate N immobilization) for ambient and elevated atmospheric CO_2 and three soil moisture treatments

Soil moisture (% of field capacity)	Net N mineralization (mg pot^{-1})	
	Ambient CO_2	Elevated CO_2
45	−123±49	47±119
65	−113±70	− 8±83
85	−187±86	− 4±45
ANOVA *P*-values	0.73	0.61

no soil moisture effects on microbial N and $\delta^{15}N$, suggesting that soil moisture effects on plant biomass $\delta^{15}N$ were not caused by changes in microbial biomass N or ^{15}N. However, we note that our microbial measurements had low precision.

Soil moisture can increase plant biomass $\delta^{15}N$ by increasing the rate of microbial processes that can increase plant available $\delta^{15}N$ such as gross N mineralization (Johnson et al. 2000), and cause isotopic fractionation such as net N mineralization (Garten and Van Miegroet 1994; Koba et al. 2003) and denitrification (Robinson and Conroy 1999). Although we did not directly measure gross N mineralization, we observed greater soil organic matter decomposition with increased soil moisture content and plant productivity in this system (Dijkstra and Cheng 2007). The $\delta^{15}N$ of the extractable N pool in the soil increased with increased soil moisture and plant N uptake, and approached the $\delta^{15}N$ of the total soil N pool (Fig. 4). This also suggests that gross N mineralization increased with increased plant N uptake, which may have increased plant biomass $\delta^{15}N$ in our study. On the other hand, the net amount of N mineralized during our experiment was not affected by soil moisture (Table 2), suggesting that soil moisture did not affect plant biomass $\delta^{15}N$ through changes in net N mineralization. Despite frequent aeration of all pots throughout the experiment, it is possible that denitrification occurred in anaerobic microsites (Parkin 1987) or by nitrifiers in the presence of oxygen (Firestone and Davidson 1989). However, soil moisture effects on plant biomass $\delta^{15}N$ became non-significant in the ANCOVA using plant biomass N as the covariate. This suggests that soil moisture effects on total plant biomass $\delta^{15}N$ were associated with changes in total plant N uptake (and N mineralization), rather than with changes in denitrification.

The $\delta^{15}N$ in plant biomass could increase due to isotopic fractionation when mycorrhizae transfer N to the plant (Hobbie et al. 2000), but also when plants increasingly assimilate more NH_4^+ than NO_3^- during

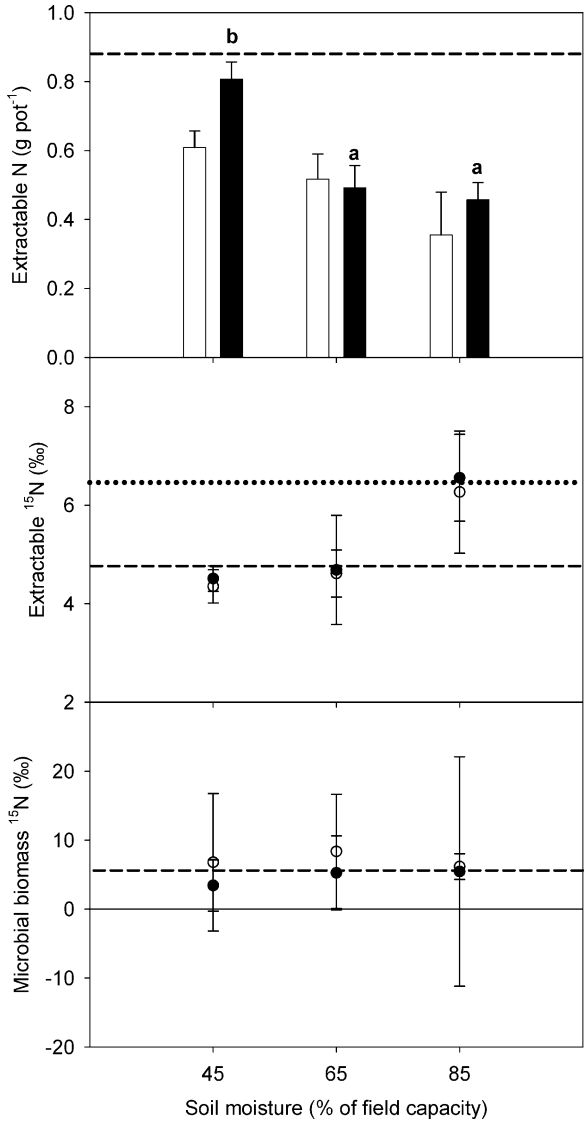

Fig. 5 Average amount of extractable N (non-fumigated K_2SO_4 extraction) and $\delta^{15}N$ values of extractable and microbial N (ambient CO_2: *open bars/symbols*, elevated CO_2: *closed bars/symbols*). *Dashed line* represents the amount of extractable N and the $\delta^{15}N$ value of extractable and microbial N at the start of the experiment. *Dotted line* represents the $\delta^{15}N$ value of total soil N. Error bars are standard errors. Different letters denote significant differences among soil moisture contents ($P<0.05$, post-hoc Tukey's test)

growth (Garten and Van Miegroet 1994; Miller and Bowman 2002). Unfortunately, we did not measure how N was taken up by the plant.

Plants grown under different CO_2 concentrations showed similar plant biomass $\delta^{15}N$ values, despite greater plant N uptake under elevated CO_2. When the effect of total plant N on plant biomass $\delta^{15}N$ value was adjusted by ANCOVA analysis, $\delta^{15}N$ values in shoot and total biomass were actually higher for plants grown in the ambient CO_2 greenhouse than in the elevated CO_2 greenhouse. It is doubtful that different climate conditions in the two greenhouses may have caused these differences, given that we kept the two greenhouses under similar temperature and light conditions. It is possible that lower plant biomass $\delta^{15}N$ values with elevated CO_2 was caused by increased root symbiotic association with mycorrhizal fungi (Alberton et al. 2005; Treseder 2004), and increased uptake of relatively ^{15}N-depleted NO_3^- as compared to NH_4^+ under elevated CO_2 (BassiriRad et al. 1996, 1997). Unfortunately, we did not measure mycorrhizal infection, or $\delta^{15}N$ in NH_4^+ and NO_3^- to substantiate these potential mechanisms. The CO_2 effects on plant biomass $\delta^{15}N$ are in disagreement with a study by Billings et al. (2002) who observed enrichment in ^{15}N of vegetation of the Mojave Desert under elevated CO_2. They suggested that this was caused by an increase in labile C substrates to the soil stimulating microbial activity thereby enriching plant-available N.

Although we cannot identify the specific mechanisms responsible for the soil moisture and elevated CO_2 effects on plant biomass $\delta^{15}N$, our results show that soil moisture content and elevated CO_2 can cause different effects on the natural abundance of ^{15}N in plants. If elevated CO_2 influences soil moisture, it is plausible that the resulting soil moisture changes could affect plant $\delta^{15}N$ apart from any additional elevated CO_2 effects. Although our controlled greenhouse experiment does not resemble field conditions where environmental factors such as temperature and soil moisture continuously fluctuate, results from our study provide important information on how the natural abundance of ^{15}N in plant biomass can be used to better understand the effects of soil moisture content on N cycling.

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