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Short-term uptake of ^{15}N by a grass and soil micro-organisms after long-term exposure to elevated CO_2

Romain Barnard[1,2,3], Laure Barthes[2] & Paul W. Leadley[2]
[1]*Institute of Plant Sciences, ETH Zürich, Universitätstrasse 2, CH-8092 Zürich, Switzerland.* [2]*Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Sud, UMR CNRS 8079, F-91405 Orsay, France.* [3]*Corresponding author**

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Abstract

This study examines the effect of elevated CO_2 on short-term partitioning of inorganic N between a grass and soil micro-organisms. ^{15}N-labelled NH_4^+ was injected in the soil of mesocosms of *Holcus lanatus* (L.) that had been grown for more than 15 months at ambient or elevated CO_2 in reconstituted grassland soil. After 48 h, the percentage recovery of added ^{15}N was increased in soil microbial biomass N at elevated CO_2, was unchanged in total plant N and was decreased in soil extractable N. However, plant N content and microbial biomass N were not significantly affected by elevated CO_2. These results and literature data from plant–microbial ^{15}N partitioning experiments at elevated CO_2 suggest that the mechanisms controlling the effects of CO_2 on short- vs. long-term N uptake and turnover differ. In particular, short-term immobilisation of added N by soil micro-organisms at elevated CO_2 does not appear to lead to long-term increases in N in soil microbial biomass. In addition, the increased soil microbial C:N ratios that we observed at elevated CO_2 suggest that long-term exposure to CO_2 alters either the functioning or structure of these microbial communities.

Introduction

Rising atmospheric CO_2 concentrations and a consequent increase in C allocation to soils via plants are likely to alter the competition for N between plants and micro-organisms (Hu et al., 1999). Such changes in the competition for N by elevated CO_2 have been shown to affect plant growth (Diaz et al., 1993; Hungate, 1999; Zak et al., 1993) and ecosystem N cycling (Berntson and Bazzaz, 1998; Hungate et al., 1997).

Elevated atmospheric CO_2 generally increases the C:N ratios of organic inputs to soils via rhizodeposition (Cotrufo and Gorissen, 1997; Pendall et al., 2004; van Ginkel et al., 2000). This increased availability of C may lead to increased microbial demand for N at elevated CO_2, and result in increased microbial N immobilisation (Diaz et al., 1993; Hungate, 1999; Rice et al., 1994). On the other hand, elevated CO_2 often increases root biomass and root length (Pritchard et al., 1999; Rogers et al., 1994; Wand et al., 1999) and may sometimes lead to increased root physiological nitrogen uptake capacity (Bassirirad, 2000), all of which may increase plant nitrogen uptake. Although there are records of increased plant N uptake at elevated CO_2, findings are not consistent (see review by Bassirirad, 2000).

Barnard et al. (2004b) measured the dynamics of plant N content and microbial biomass N in

* FAX No: +41-44-632-11-53.
E-mail: romain.barnard@ipw.agrl.ethz.ch

mesocosms of *Holcus lanatus* exposed to elevated atmospheric CO_2 over a period of 15 months. Plant N-limitation was observed at elevated CO_2 in the early stage of the experiment, while soil microbial biomass N increased. By the end of the experiment, however, plants did not appear to be N-limited. The authors attributed this pattern of response to an increase in the competitive capacity of soil micro-organisms at elevated CO_2 in the short term that did not persist in the long-term. A more rapid turnover of microbial biomass compared to plant roots may explain these findings (Barnard et al., 2004b; Hodge et al., 2000). In the present study, a $^{15}NH_4^+$ pulse-labelling procedure was used at the end of this long-term (15 months) CO_2 fumigation experiment. The objective was to determine whether the uptake capacity of soil micro-organisms had remained higher at elevated CO_2, and to shed further light on the short-term (48 h) partitioning of N between plants and soil micro-organisms.

Materials and methods

Experimental design and treatment

The experimental design has been described in detail by Barnard et al. (2004b). Briefly, monocultures of *Holcus lanatus* L. (Yorkshire fog), were grown in mesocosms (14×19×50 cm PVC pots) in naturally lit, CO_2 controlled chambers, in reconstituted sandy-loam soil. *Holcus lanatus* is a tuft-forming, perennial C3 grass with a very large ecological amplitude (Grime et al., 1988). It can dominate mesic grassland communities, especially on moderately acid soils (pH 5–6), and has particularly fine roots, even in comparison with other pasture grasses (C. Picon-Cochard, personal communication).

Soil was dug out from two soil horizons (0–20 cm and 20–40 cm) of a semi-natural grassland (Foljuif, France, 48°17′ N, 2°41′ E), and sieved using a 1 cm mesh sieve. The pots were filled successively with 9 cm sand, 19 cm soil from the 20–40 cm horizon, and 19 cm soil from the 0–20 cm horizon. The soil of the top horizon had a cation exchange capacity of 4.1 cmol + kg^{-1}, 28.8 g kg^{-1} SOM , 1.27 g kg^{-1} total N, 16.77 g kg^{-1} total C and pH 5.3 (soil–water suspension). Soil texture was 71% sand, 8% clay and 21% silt. The soil of the second horizon had a cation exchange capacity of 3.4 cmol + kg^{-1}, 19.5 g kg^{-1} SOM, 0.74 g kg^{-1} total N, 11.32 g kg^{-1} total C and pH 5.6 (soil–water suspension). Soil texture was 73% sand, 7% clay and 20% silt.

Seeds of *Holcus lanatus* were set to germinate on 4 March, 2001, and seedlings transferred to mesocosms on 14 March, 2001. The experimental design consisted of 12 naturally lit chambers (wooden frame and clear plastic walls, 65×67×100 cm high) set up in a large greenhouse. Each chamber contained nine mesocosms. Six chambers were ventilated with ambient air taken from outside the greenhouse ($[CO_2]$ = ca. 365 μmol mol^{-1}), and the other six were ventilated with ambient air enriched with 300 μmol mol^{-1} CO_2. CO_2 enrichment started on 7 April, 2001. CO_2 concentrations and temperature were monitored in all chambers. The use of closed chambers, as opposed to open-top chambers, allowed more precise control of the CO_2 concentrations, with only infrequent deviation of more than 50 μmol mol^{-1} from their setting points and a more homogeneous mixing inside the chamber. To avoid positional effects inside the chambers, mesocosms were rotated randomly after each of the five harvests during the 15-month experiment. Mesocosms received equal amounts of water to meet plant requirements.

Tracer addition and sample analyses

Ammonium solution ($^{15}NH_4Cl$ at 99% ^{15}N, 0.01 *M*) was injected into the mesocosms on 30 July, 2002. Total quantity of N added was 5 mg pot^{-1}, corresponding to 188 mg N-NH_4^+ m^{-2}. A three-dimensional grid was used for the injections, consisting of 12 points with 3 injection depths (1.5, 5 and 7.5 cm) for each of these points. This resulted in 36 injection locations in the 0–10 cm soil layer. In each mesocosm, 1 mL of solution was injected in each of the 36 locations. Plants and soil were harvested 48 h after adding the tracer. Plant biomass was separated into five fractions: aboveground biomass at height 0–5 cm (leaf bases) and height > 5 cm (leaf blades), senescent leaf material (yellow leaves still attached to the plant or yellow tips of leaves), and belowground biomass in the 0–10 cm (roots and stem bases) and (roots

only) 10–30 cm soil layers. Samples were dried for 48 h at 60 °C, weighed and ground. Total N and ^{15}N concentrations for all plant fractions were analysed using an elemental analyser (NA-1500, Carlo Erba Instruments, Milan, Italy). Total plant biomass was calculated from the dry weights of belowground and aboveground biomass. Correction for the natural abundance of ^{15}N was made based on the atomic ratio of atmospheric nitrogen (see Hu et al., 2001). Natural abundance of ^{15}N in the plant, soil and microbial compartments may deviate slightly from that in the atmosphere, however, these differences are very small compared to the enrichment in ^{15}N that we measured in these compartments after labelling. The percentage of ^{15}N in plant N was calculated as $100\times{}^{15}N/[{}^{15}N+{}^{14}N]$. Microbial biomass N, ^{15}N and C were measured in the 0–10 cm soil layer. Five grams of soil was sieved (1 mm mesh) and then fumigated 24 h with chloroform vapour (Brookes et al., 1985). Control samples were not fumigated. Microbial N and C were extracted by vigorous shaking in 30 m*M* K_2SO_4 for 30 min and lyophilised. N, ^{15}N and C contents were analysed using continuous-flow gas isotope-ratio mass spectrometry (Thermo-Finnigan Delta Plus mass spectrometer interfaced with a Carlo Erba 2100 elemental analyser) at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, USA). Nitrogen in the soil microbial biomass was calculated as [(total N in fumigated soil) − (total N in non-fumigated soil)]/0.54 (Brookes et al., 1985). Correction for the natural abundance of ^{15}N was based on the atomic ratio of atmospheric nitrogen. Microbial biomass C was calculated in a similar way with an extraction efficiency coefficient of 0.45 (Wu et al., 1990). Soil extractable N and ^{15}N were measured in the unfumigated extracts. Gravimetric soil water content was determined in each pot using samples of approx. 5 g fresh soil.

Statistical analysis

Analysis of variance was carried out using R 1.9.0 (R Development Core Team, 2004), with $n=6$. When necessary, variables were transformed (inverse or square root transformation) to correct a skewed distribution or unequal variances. A Wilcoxon nonparametric test was used when simple transformations could not correct the distribution and variance of the data. The values per pot were converted to values per m^2 by multiplying them by 37.6 pot m^{-2}, to facilitate comparisons with field data and since pots were kept close together to form a closed canopy for most of the duration of the experiment. A percent response ratio for the CO_2 effect was calculated using the following formula: % effect $=100\times$[elevated CO_2 − ambient CO_2]/ambient CO_2.

Results

Plant and microbial pools

Elevated CO_2 increased plant biomass of the upper leaf blades and senescent leaf fractions, but had no significant effects on the other plant fractions and total plant biomass (Table 1). Above- and belowground plant N contents were not affected by elevated CO_2, while N concentration decreased in the aboveground plant parts and in the whole plant (Table 1).

Elevated CO_2 had no significant effect on microbial biomass N, but microbial biomass C was significantly increased at elevated CO_2 (Table 1), resulting in an increased microbial C:N ratio (+13% CO_2 effect, $p=0.009$). Soil extractable N in the 0–10 cm soil layer was not affected by elevated CO_2 (Table 1). Gravimetric soil water content was lower in the ambient CO_2 treatment ($4.1\pm0.4\%$) than in the elevated CO_2 treatment ($5.7\pm0.6\%$, CO_2 effect $=+40\%$, $P=0.049$)

^{15}N partitioning

The percentage recovery of ^{15}N increased at elevated CO_2 in microbial biomass N, while it decreased in soil extractable N, and was unchanged in total plant N (Figure 1). There was a mean increase of 0.073 μg ^{15}N g^{-1} dry soil in soil microbial biomass N, and a mean decrease of 0.095 μg ^{15}N g^{-1} dry soil in soil extractable N at elevated compared to ambient treatment CO_2. Total recovery of added ^{15}N was not significantly affected by elevated CO_2, and averaged 32% across treatments.

Table 1. Biomass, N content and N concentration in different fractions of *Holcus lanatus* (grown in mesocosms for over 15 months) and microbial biomass N and C and soil extractable N in the 0–10 cm soil layer at ambient and elevated CO_2

	Treatment		CO_2 effect (%)	*P*
	Ambient CO_2	Elevated CO_2		
Plant biomass (g m^{-2})				
Total plant biomass	555.8 ± 74.3	596.2 ± 17.9	7	0.39
Aboveground biomass	399.7 ± 53.2	444.7 ± 12.6	11	0.39
Leaves 0–5 cm	144.9 ± 52.5	90.5 ± 8.5	−38	0.81
Leaves > 5 cm	202.7 ± 9.9	269.0 ± 10.1	33	< 0.001
Senescent leaves	52.1 ± 6.0	85.2 ± 5.2	64	0.002
Belowground biomass	156.0 ± 24.2	151.6 ± 14.4	−3	1
0–10 cm	57.9 ± 20.7	36.1 ± 1.9	−38	0.49
10–30 cm	98.1 ± 13.4	115.4 ± 13.7	18	0.39
Plant N content (g N m^{-2})				
Total plant	6.4 ± 0.5	6.0 ± 0.2	−6	0.69
Aboveground	4.7 ± 0.4	4.3 ± 0.2	−8	0.41
Belowground	1.7 ± 0.2	1.7 ± 0.2	0	1
Plant N concentration (%)				
Total plant	1.18 ± 0.06	1.01 ± 0.03	−15	0.03
Aboveground	1.22 ± 0.07	0.97 ± 0.03	−20	0.01
Belowground	1.10 ± 0.04	1.10 ± 0.02	1	0.89
Soil N and C				
Microbial N (μg N g^{-1} dry soil)	17.6 ± 1.0	19.1 ± 0.9	9	0.26
Microbial C (μg C g^{-1} dry soil)	105.3 ± 4.3	132.7 ± 6.2	26	0.007
Soil extractable N (μg N g^{-1} dry soil)	5.8 ± 0.3	6.1 ± 0.6	5	0.93

Values indicate mean ± SE. CO_2 effect = 100×[elevated − ambient]/ambient.

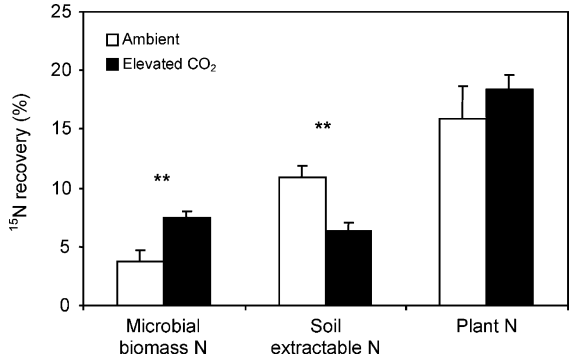

Figure 1. Recovery of added ^{15}N in microbial biomass N, soil extractable N and total plant N in mesocosms of *Holcus lanatus* exposed to ambient or elevated CO_2 concentrations for over 15 months. Open and closed bars indicate mean ± SE at ambient and elevated CO_2, respectively. Significance between treatments: $^{**}0.01 > P > 0.001$.

Of all plant fractions, ^{15}N recovery was only affected in the leaf blades (+48%, Figure 2a). N content was not affected by elevated CO_2 in any plant fraction (Figure 2b). However, the percentage of ^{15}N in plant N significantly increased in leaves > 5 cm (Figure 2c), resulting in a significant increase in aboveground and total percentage of ^{15}N in plant N (+39%, $P = 0.002$ and +25%, $P = 0.03$, respectively). The amount of ^{15}N per unit of plant biomass was not significantly affected in any plant fraction (data not shown). Short-term acquisition (measured as the

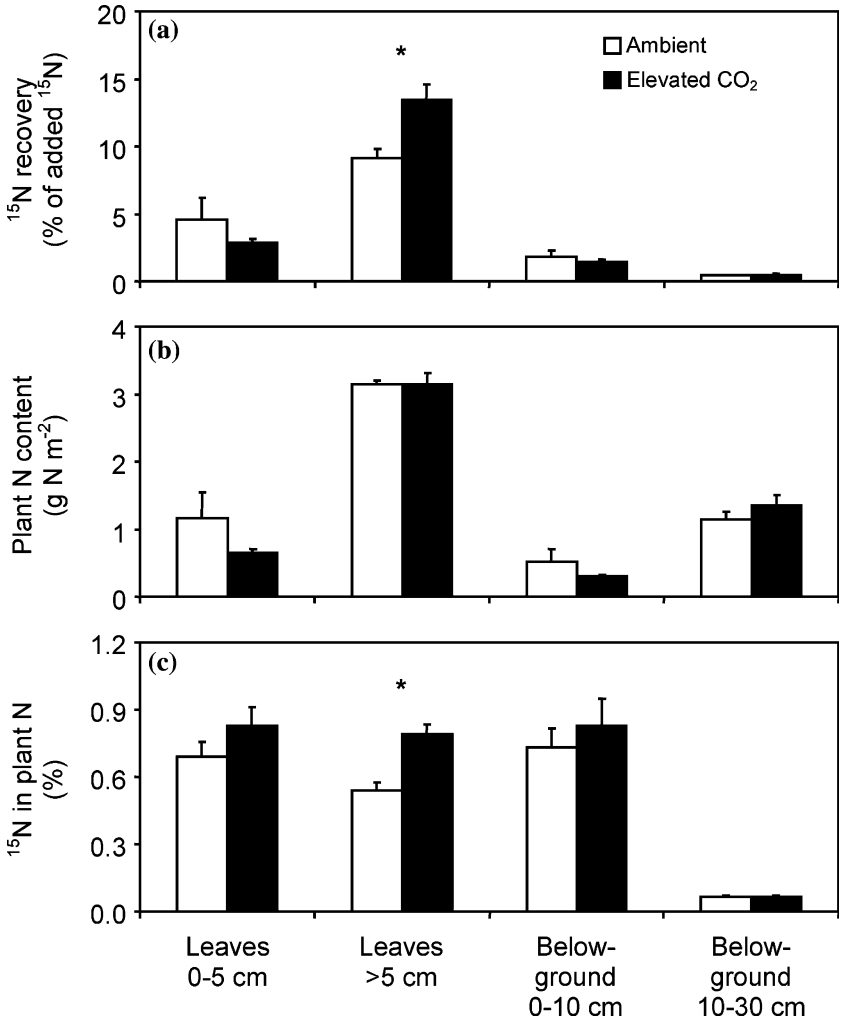

Figure 2. Recovery of added ^{15}N (a), plant N content (b), and percentage of ^{15}N in plant N (c) in different fractions of *Holcus lanatus* grown for over 15 months in mesocosms exposed to ambient or elevated CO_2. Open and closed bars indicate mean ± SE at ambient and elevated CO_2, respectively. Significance between treatments: $*0.05 > P > 0.01$.

percentage ^{15}N of total plant N) was not affected by the CO_2 treatment in the belowground fractions.

Discussion

Plant–microbial N partitioning

After more than 15 months of fumigation, elevated CO_2 had a significant effect on the partitioning of ^{15}N-labelled ammonium between plants and soil microbial biomass in our well-established grass mesocosms. Short-term partitioning of ^{15}N between plants and soil micro-organisms is thought to depend on their respective biomass, uptake kinetics per unit of biomass, and diffusion constraints in the soil. Soil micro-organisms are generally considered to be the better short-term competitors for N because of their high surface area to biomass ratio and their distribution throughout the soil (Hodge et al., 2000). Forty-eight hours after pulse-labelling, the percentage recovery of ^{15}N in microbial biomass was increased at elevated CO_2. Part of this increase may be explained by higher microbial biomass (i.e., higher microbial C, Table 1) although increased uptake kinetics per unit biomass may

also have played a role, since the CO_2 effect on ^{15}N uptake was much larger than that on microbial biomass. The reduction in ^{15}N remaining in the soil extractable N pool that we measured at elevated CO_2 may reflect this increased uptake by soil micro-organisms. The increase in soil microbial uptake of ^{15}N was not reflected in decreased plant ^{15}N uptake, despite the strong reduction in soil extractable ^{15}N. These results point towards increased microbial N demand at elevated CO_2, which is consistent with the hypothesis that elevated CO_2 leads to higher belowground input of labile C. Elevated CO_2 may also have altered ^{15}N partitioning via changes in soil water content. Soil water content commonly increases in herbaceous ecosystems at elevated CO_2 (e.g., Barnard et al., 2004b; Niklaus et al., 1998; Rice et al., 1994), and this could affect ^{15}N partitioning by modifying physiological or diffusion limitations on uptake of N by plants and micro-organisms. Findings from short-term ^{15}N partitioning (< 10 days incubation) in CO_2 experiments are highly variable, and no clear pattern emerges (Table 2). This may be partially explained by strong plant species-specific effects (see further discussion below).

As opposed to results from short-term ^{15}N labelling experiments, measurements of N accumulated in plant or microbial biomass typically integrate N uptake over sufficiently long periods for growth and turnover rates of micro-organisms and plants to play a dominant role in determining N partitioning (Berntson and Bazzaz, 1998; Hodge et al., 2000). Our results show that soil microbial N and total plant N were not affected by over 15 months of exposure to elevated CO_2, despite the observed short-term advantage of the soil microbial community for rapid uptake of readily available N (as measured by ^{15}N recovery after pulse-labelling). The long-term unresponsiveness of microbial biomass N at elevated CO_2 in our study is in line with findings from long-term field studies (Barnard et al., 2004a; Finzi and Schlesinger, 2003; Niklaus and Körner, 1996; Niklaus et al., 2003). Both of the grassland field studies of long-term ^{15}N partitioning that are available (Table 2, > 1 month incubation) are also coherent with this response. Six weeks after pulse-labelling the soil in a annual Mediterranean grassland that had been exposed to elevated CO_2 for 5 years, Hu et al. (2001) measured no significant effect of elevated CO_2 on the recovery of ^{15}N in microbial biomass, while recovery of ^{15}N in plant biomass increased, resulting in altered interaction between plants and microbes in favour of plant N utilisation. In tallgrass prairie that had been fumigated for 8 years, elevated CO_2 had no significant effect on the recovery of ^{15}N either in microbial biomass or in plant biomass after 5 months after pulse-labelling the soil (Williams et al., 2001).

Table 2. Effect of elevated CO_2 on the relative recovery of ^{15}N between plants and soil microbes in elevated CO_2 studies in grassland systems. CO_2 effect $= 100 \times$ [elevated − ambient]/ambient

System	CO_2 effect (%)			N added (mg N m^{-2})	Duration of incubation	Duration of CO_2 enrichment	Reference
	% ^{15}N recovery		Root biomass				
	Plants	Microbes					
Grassland microcosms (native sp., no fert.)	−25	+13	na	25	24 hours	5 months	Hungate et al. (1996)
Grassland microcosms (introduced sp., no fert.)	+38	+95	na	25	24 hours	5 months	Hungate et al. (1996)
Holcus mesocosms	+15	**+101**	−3	188	48 hours	15 months	Our study
Grassland microcosms (after wet-up)	−47	**+61**	**+29**	105	9 days	1 year	Hungate et al. (1997)
Grassland	**+21**	−16	+25	4	1.5 months	5 years	Hu et al. (2001)
Tallgrass prairie	−19	−10	Increase[a]	270	5 months	8 years	Williams et al. (2001)

Significant responses are indicated in bold.
na: Data not reported.
[a] Significance level not reported.

While short-term ^{15}N partitioning is often poorly correlated with long-term N partitioning between plants and soil micro-organisms (Hodge et al., 2000), short-term labelling experiments may provide valuable insight into the dynamics of nutrient uptake at critical periods when new soil nutrient patches are exploited or during N flushes following soil disturbance. In the early stages of our experiment, when microbial biomass N was rapidly increasing, N pool data showed that elevated CO_2 stimulated N sequestration in soil micro-organisms (Barnard et al., 2004b, Figure 3). This increased microbial N sequestration was coupled with a large reduction in plant N, which was similar to the CO_2 response observed by Diaz et al. (1993). This response is also consistent with the hypothesis that elevated CO_2 leads to higher belowground input of labile C, inducing higher microbial N demand.

Hungate et al. (1996) examined ^{15}N uptake in native and introduced plant species in California grassland soil and found that the effects of CO_2 on ^{15}N partitioning were highly species-dependent. Moreover, these authors found no evidence of a trade-off (i.e. negative relationship) between ^{15}N uptake in plants and soil micro-organisms, a pattern that also held true for accumulated N in plant and microbial biomass over 5 months. This latter finding is in agreement with the results of our experiment that the CO_2 effects on ^{15}N uptake in soil micro-organisms were poorly correlated with those on plant biomass N. While our ^{15}N partitioning data after 15 months of CO_2 exposure agree with the findings of Hungate et al. (1996), the experimental conditions were very different. In particular, in the unfertilised treatment of Hungate et al. (1996): (i) the plants were about one order of magnitude smaller than ours, (ii) there was no effect of elevated CO_2 treatment on soil extractable ^{15}N, and (iii) plant ^{15}N uptake was very low (about 2% of added ^{15}N compared to an average of 17% in our study). Nonetheless, taken together, these two N partitioning studies suggest that elevated CO_2 does not induce a trade-off between N accumulation in biomass between soil micro-organisms and plants during the early phases of vegetation establishment (Hungate et al., 2000) or later on when plant and soil microbial biomass levels have stabilised (present study, also see Barnard et al., 2004b). In contrast, CO_2-induced trade-offs between plant and microbial N uptake may be restricted to periods when competition for N is particularly intense, e.g. during phases when microbial populations and plant biomass are "expanding" (*sensu* Niklaus and Körner, 1996). Such a phase of fast microbial and plant growth may have induced the trade-off in N uptake at the beginning of the experiment, reported in Barnard et al. (2004b).

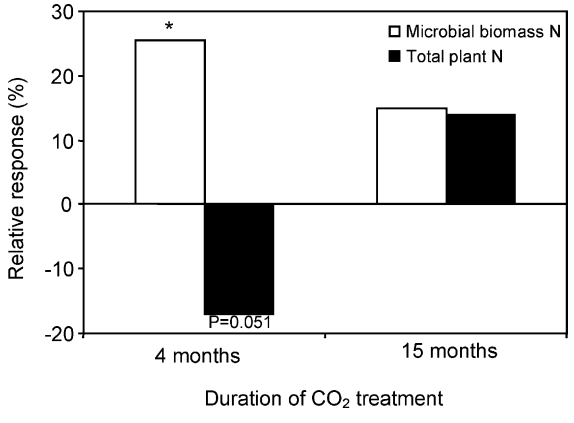

Figure 3. Relative response of microbial biomass N in the whole pot (open bars) and total plant N (full bars) to elevated CO_2 concentration in September, 2001 and July, 2002, after 4 months and 15 months of CO_2 fumigation, respectively. % effect = 100×[elevated CO_2 − ambient CO_2]/ambient CO_2. Significance level: $*0.05 > P > 0.01$.

Microbial C:N ratio

We measured a significant increase in microbial C:N ratio at elevated CO_2 which indicates that CO_2 may have altered the functioning or the structure of the soil microbial community. First, the soil micro-organisms may have become more strongly N-limited at elevated CO_2 due to a C:N imbalance, which is consistent with the observed increase in microbial recovery of ^{15}N in soil microbial biomass at elevated CO_2. Second, elevated CO_2 may have altered the structure of the soil microbial community in favour of micro-organisms with higher C:N ratios (Grayston et al., 1998). The microbial C:N ratio has been proposed as an indicator for the relative contribution of fungal (high C:N ratio) as opposed to bacterial (low C:N ratio) component of soil microbial biomass and the overall microbial demand for newly available N (Hodge et al., 2000; Klironomos et al., 1997). The microbial

community in the present mesocosm experiment may have adapted to changes in the quality of soil organic inputs at elevated CO_2, which could have important implications for long-term C and N cycling at elevated CO_2 (Hungate et al., 2003).

N allocation in the plant

The pattern of N and ^{15}N allocation among fractions of biomass was roughly similar in both CO_2 treatments in our *H. lanatus* plants, except for the very low amount of ^{15}N in the roots of the 10–30 cm deep layer in both treatments (below the tracer injection zone). Hungate et al. (1996) also found that ^{15}N and biomass N followed similar patterns in their unfertilised treatment. The small amount of ^{15}N in the deep root fraction might be explained by low ^{15}N uptake because the ^{15}N was added to the surface soil layer, or may have resulted from low translocation rates of ^{15}N to these roots from the surface roots. Only the upper part (>5 cm) of the grass leaves, also the most responsive plant fraction in terms of increased biomass, contained more ^{15}N at elevated CO_2 (Table 1, Figure 2c). Our results are in general agreement with those of Hungate et al. (1996), who found for most species in their study that exposure to elevated CO_2 did not alter ^{15}N allocation patterns under conditions of no fertilisation.

Conclusion

The differences in ^{15}N recovery in this mesocosm experiment between the grass *H. lanatus* and the soil microbial community after pulse-labelling shows that elevated CO_2 affects the plant–microbial relationship. This CO_2 effect on the functioning of our plant–soil system may also have implications at the ecosystem-level. Our findings suggest that short-term immobilisation of inorganic soil N or exploitation of nutrient pulses may be altered under conditions of elevated atmospheric CO_2 concentration. The absence of apparent relationship between biomass N and short-term ^{15}N partitioning stresses the need for mechanistic studies to better understand the factors that determine biomass turnover and changes in the structure of the soil microbial community.

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