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Elevated atmospheric CO_2 in open top chambers increases net nitrification and potential denitrification

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Abstract

The control of soil nitrogen (N) availability under elevated atmospheric CO_2 is central to predicting changes in ecosystem carbon (C) storage and primary productivity. The effects of elevated CO_2 on belowground processes have so far attracted limited research and they are assumed to be controlled by indirect effects through changes in plant physiology and chemistry. In this study, we investigated the effects of a 4-year exposure to elevated CO_2 (ambient + 400 μmol mol^{-1}) in open top chambers under Scots pine (*Pinus sylvestris* L) seedlings on soil microbial processes of nitrification and denitrification. Potential denitrification (DP) and potential N_2O emissions were significantly higher in soils from the elevated CO_2 treatment, probably regulated indirectly by the changes in soil conditions (increased pH, C availability and NO_3^- production). Net N mineralization was mainly accounted for by nitrate production. Nitrate production was significantly larger for soil from the elevated CO_2 treatment in the field when incubated in the laboratory under elevated CO_2 (increase of 100%), but there was no effect when incubated under ambient CO_2. Net nitrate production of the soil originating from the ambient CO_2 treatment in the field was not influenced by laboratory incubation conditions. These results indicate that a direct effect of elevated atmospheric CO_2 on soil microbial processes might take place. We hypothesize that physiological adaptation or selection of nitrifiers could occur under elevated CO_2 through higher soil CO_2 concentrations. Alternatively, lower microbial NH_4 assimilation under elevated CO_2 might explain the higher net nitrification. We conclude that elevated atmospheric CO_2 has a major direct effect on the soil microbial processes of nitrification and denitrification despite generally higher soil CO_2 concentrations compared to atmospheric concentrations.

Keywords: elevated CO_2, global change, nitrification, open top chambers, potential denitrification

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Introduction

There remains little doubt that the global earth climate has been changing over the 20th century (IPCC, 2001). Strong evidence suggests that these changes are linked to an increase, owing to human activities, in the concentration and the radiative forcing of the atmospheric greenhouse gases CO_2, CH_4 and N_2O. Concentrations of the most important greenhouse gas, CO_2, are likely to increase from the current concentrations of 360 μmol mol^{-1} up to 970 μmol mol^{-1} through the 21st century, and stabilization will only occur if urgent measures to reduce its emission are taken. Such elevated CO_2 concentrations are most likely to influence the global carbon (C) fluxes through the biosphere as well as the functioning of all biological systems.

Despite the importance of soil microbial activities in the control of the functioning of forest ecosystems, most studies so far have focused on net primary production and C allocation (Laitat *et al.*, 2000), tree physiology (Jarvis, 1998), litter quality (Cotrufo *et al.*, 1998a,b), decomposition (Cotrufo & Ineson, 1996) and rhizosphere processes (Janssens *et al.*, 1998; Zak *et al.*, 2000a). However, the microbial processes of nitrification and denitrification are of crucial importance. Nitrification influences

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primary productivity (Jorns & Hecht-Buchholz, 1985), may cause soil acidification (Reuss & Johnson, 1986), may cause cation and nitrate leaching (Carnol *et al*., 1997a,b; Carnol, 1999), is linked to N_2O emissions (Maag & Vinther, 1996) and may be linked to forest decline (Weissen *et al*., 1990). Soil nitrogen (N) availability is also an important regulator in ecosystem C storage in above and belowground biomass under elevated CO_2 (Pregitzer *et al*., 2000; Zak *et al*., 2000a).

Current experimental data on the effects of elevated CO_2 on the N cycle are inconclusive, showing large increases and decreases of N mineralization under elevated CO_2 (with few significant effects) with a high degree of variability within and between soil under graminoid, herbaceous and woody plant species (Zak *et al*., 2000b). Explanations for lower soil N availability under elevated CO_2 include decreased leaf N concentrations, leading to litter that is more recalcitrant to decomposition (Cotrufo *et al*., 1998b) and increased labile C exudated by a higher fine root biomass, causing increased microbial N assimilation (Janssens *et al*., 1998). However, some studies suggest that N mineralization could increase in response to enhanced labile C supply from roots (Clarholm, 1985; Zak *et al*., 1993). As N_2O emissions from soils are mainly owing to nitrification and denitrification (Firestone & Davidson, 1989), changes in substrate availability (litter quantity and chemistry, and root exudates) for nitrifiers and denitrifiers may also affect N_2O emissions. Modification of trace gas fluxes to the atmosphere under elevated CO_2 could be an important feedback mechanism influencing atmospheric chemistry. So far, few studies have investigated emissions of the important greenhouse gas N_2O under elevated CO_2 (Huntgate *et al*., 1997).

Soil CO_2 concentrations are on average 10 times higher than atmospheric concentrations (Sotomayor & Rice, 1999). Therefore, the possibility of increased atmospheric CO_2 directly affecting belowground microorganisms and processes has so far been largely ignored, despite reports on the dependence of microbial processes on soil CO_2 concentrations (Santruckova & Simek, 1994; Santruckova & Simek, 1997). Indirect effects through increased C allocation to roots, stimulating fine root production, root respiration and root exudation are thought to be predominant (O'Neill, 1994; Norby *et al*., 1999).

The objective of this study was to examine the direct and indirect effects of four years of elevated CO_2 treatment in the field on net N mineralization, potential denitrification (DP) and potential N_2O emissions under laboratory conditions. Our hypothesis was that the modification of soil conditions by changed litter and root exudation would influence the soil N biogeochemical cycle. To test the hypothesis of an adaptation of the microbiota or a direct effect of elevated CO_2, the laboratory mineralization experiment was performed under both ambient and elevated CO_2.

Materials and methods

Soil sampling and site description

Intact soil columns (8 cm dia. by 15 cm deep) were taken on January 18, 2000, in four open-top fumigation chambers (OTCs, Macathesm, Meslin-l'Eveque, Belgium) at the campus of the University of Antwerp (UIA). Each decagonal OTC (3 m dia., 4 m height) had been planted in March 1996 with 11 three-year-old, pot grown, dormant Scots pine (*Pinus sylvestris L)* seedlings in a circular pattern (Jach & Ceulemans, 1997; Jach & Ceulemans, 1999; Jach *et al*., 2000). Before planting, the original heavy loam soil was excavated to a depth of 0.5 m and replaced with a poor forest soil (about 0.12% N on a dry mass basis). After the harvest of half of the trees at the end of 1998, an additional poor forest soil layer was added to each OTC to fill the holes and equalize the soil during early 1999, without mixing the two layers. The upper horizon had therefore only been subjected to 1 year of CO_2 treatment. Mean annual temperature and rainfall at the site are 12 °C and 769 mm, respectively.

Two OTCs were subjected to current CO_2 concentrations (AMB, about 350 μmol mol^{-1}) and two to elevated atmospheric CO_2 concentrations (ELE, current concentration + 400 μmol mol^{-1}). The treatment was applied starting on April 1, 1996 on a 24-h basis continuously throughout the year. The remaining half of the trees were harvested during end November 1999 and the CO_2 treatment was then ended. For a more detailed description of experimental conditions, see Jach & Ceulemans (1999, 2000).

Five paired intact soil columns were sampled in each OTC at 20 cm from the remaining tree trunk. As the root system extended to approximately 35 cm around the tree, soil samples had been subjected to the influence of the tree roots under ambient or elevated CO_2. The columns were placed in polythene bags and transported to the University of Liège for laboratory experiments. Samples were stored at 4 °C until experiments commenced (within 1 week).

Soil chemical characteristics

One column of each pair was visually divided into the two layers resulting from the successive soil additions (mean heights upper horizon, UP: 4.7 cm, lower horizon, LOW: 9.6 cm). Layers were clearly distinct, indicating that no soil mixing had taken place. Samples were sieved (4 mm) and analysed for initial soil characteristics and DP (see below). Water content was determined as weight

loss after overnight drying at 105 °C. Loss on ignition (LOI) was determined as overnight weight LOI at 430 °C. Soil pH_{H2O} was measured potentiometrically after 1 h agitation and settling for 30 min in a 1:3 suspension (w/v) in distilled water (25 g fresh soil plus 62.5 mL distilled water). The exchangeable mineral N content was then extracted by adding 62.5 mL KCl 12% (final concentration 6%) and a further agitation of 1 h (Allen, 1989). The pH_{KCL} was measured after settling for 30 min in this 1:6 (w:v) suspension. NO_3-N, NO_2-N et NH_4-N were measured by continuous flow analysis (methods 824–87T and 795–86T; Bran and Luebbe Analysing Technologies, Elmsford, New York, USA). Mineral N was present only in the forms of NO_3-N and NH_4-N. Carbon and N were analysed from oven dried (105 °C), milled (1 mm) soil samples by a dynamic flush combustion method with an NC 2100 soil autoanalyser (Carlo Erba Strumentazione, Rodano, Italy). Exchangeable cations were extracted after 1 h agitation of 6 g dry weight (dw) in 60 mL 0.1M $BaCl_2$, after Hendershot & Duquette, 1986), and analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES) with a VISTA charge coupled device (CCD) simultaneous ICP (Varian Optical Spectroscopy Instruments, Mulgrave, Victoria 3170, Australia).

Nitrogen mineralization

One column of each pair was incubated in controlled phytotrons (Conviron, Wynnipeg, Canada) in the dark at 20 °C and a constant humidity for 10 weeks under ambient (400 μmol mol^{-1}) and elevated CO_2 (800 μmol mol^{-1}) for net N mineralization and nitrification measurements. After incubation, the columns were divided and analysed for extractable mineral N, as described above. Net N production was expressed as the difference between the contents before and after incubation.

DP and N_2O emissions

The DP was measured according to Simek *et al*. (2000), with some modifications. Portions of 10 g field wet soil were placed in 310-mL glass bottles in 5 mL water containing 3 mg N (KNO_3) and 3 mg C (glucose). Bottles were sealed with butyl rubber stoppers and air in the bottles was replaced with helium (99.99%) by flushing three times for 45 s. Acetylene (acetone-free, Hoek Loos, Schiedam, NL), 10% (10 kPa), inhibiting the reduction of N_2O to N_2 was added to samples for DP measurements. Potential N_2O–N production (N_2Opot) was determined under the same conditions, except that incubations were performed without acetylene. After 48 h, 20 mL of the atmosphere in the bottles was sampled into 10-mL vacutainers (Terumo Europe, Leuven, Belgium) and analysed for N_2O on a gas chromatograph (ThermoFinnigan Italia S.p.A., Rodano, Italy, 8000 Top). The overpressure allowed the transfer of the sample into the 2.5-mL preevacuated loop of the gas chromatograph. The chromatograph was equipped with an electron capture detector (ECD) fitted with a 3-m long, I-D 3.175 mm, column packed with 80–100 mesh Poropak Q. The column was operated at 70 °C, using He as carrier gas with a flow rate of 20 mL min^{-1} and Ar–CH_4 as makeup gas with a flow rate of 50 mL min^{-1}. We included a cleaning procedure for vacutainer stoppers by storing them for at least 24 h under vacuum (Heinemeyer & Kaiser, 1996). The amount of N_2O–N emitted was corrected for gas dissolved in the liquid phase (Tiedje, 1994). In this experiment, dissolved N_2O–N represented less than 1.1% of the total emission.

Denitrifying enzyme activity (DEA) was measured as N_2O emission from 25 g wet soil in a 25-mL suspension containing 1 mM glucose, 1 mM KNO_3 and 1 g L^{-1} chloramphenicol (Tiedje, 1994; Simek *et al*., 2000). The 240-mL bottles containing helium and 10% acetylene (see above) were placed on a rotary shaker. The N_2O production was calculated by the increase in concentration from 30 to 90-min incubation. Gas was sampled and analysed as described above, except that the column was operated at 40 °C using Ar–CH_4 (5%) as carrier gas with a flow rate of 25 mL min^{-1}.

Statistical analysis

As there were only two OTCs (replicates) for each treatment, we applied a *t*-test to determine significant differences between the two OTCs of the same treatment. This test was significant for N_2O production and total N content. For these measurements, the variable 'chamber' was included in the statistical analyses. In all other cases, each sample was assumed to be a real replicate. The effects and interactions of the CO_2 treatment in the field (TRMT: ambient-AMB, elevated-ELE), soil horizon (HOR: upper-UP, lower-LOW) and the laboratory incubation conditions for N mineralization measurements (INCUB: normal-NOR, with CO_2-CO_2) were determined by two-way (denitrification) or three-way (N mineralization) analysis of variance (ANOVA). When interactions between conditions were significant a *t*-test or two-way ANOVA for separate conditions (CO_2, HOR, INCUB) was applied (Cody & Smith, 1991). All analyses were performed using SAS (SAS Institute Inc., 1989). Differences discussed in the text are significant at $p < 0.05$.

As the experimental design, initial soil characteristics, air temperature and photon flux density at plant level were similar in all the four OTCs (Jach & Ceulemans, 1999, 2000), we consider that significant differences at the end of the experiment are owing to the CO_2 treatment in the field.

Table 1 Soil characteristics (means with standard errors in parentheses, $n = 10$; except N: $n = 5$) of two soil horizons (HOR: upper-UP and lower-LOW) sampled in OTCs under current CO_2 atmospheric concentration (350 μmol mol^{-1}, TRMT = treatment, AMB) and current CO_2 atmospheric concentration + 400 μmol mol^{-1} (ELE). The LOI: loss on ignition (%), exchangeable cations, CEC: sum of exchangeable cations and mineral nitrogen ($mmol_c\,kg^{-1}$), carbon (C) and nitrogen (N), (%). Asterisks indicate significant effects of elevated CO_2 within one layer (*t*-test, *, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.001$). Significant differences between soil horizons are indicated in the text

HOR	TRMT	pH_{H2O}	pH_{KCl}	Al	Ca	Fe	K	Mg	Mn	Na	Zn	CEC	NH_4^+–N	NO_3^-–N	N (%)	C (%)	C/N	L.O.I. (%)
UP	AMB	6.2	5.7	0.14	33.5	0.020	5.2	4.4	0.050	0.37	0.14	43.6	0.40	0.21	0.08	1.19	14.7	2.54
				(0.04)	(1.2)	(0.004)	(0.7)	(0.4)	(0.015)	(0.05)	(0.02)	(1.7)	(0.01)	(0.05)	(0.005)	(0.09)	(0.5)	(0.16)
	ELE	6.4*	5.9**	0.05**	36.0	0.022	3.7*	4.4	0.116**	0.35	0.09**	44.6	0.38	0.25	0.07	1.07	14.8	2.15**
				(0.02)	(1.2)	(0.007)	(0.3)	(0.2)	(0.001)	(0.10)	(0.01)	(1.4)	(0.01)	(0.03)	(0.003)	(0.05)	(0.5)	(0.08)
LOW	AMB	5.1	4.2	3.58	19.3	0.040	2.0	1.7	0.076	0.42	0.18	27.4	0.37	0.19	0.10	1.70	16.5	3.24
				(0.3)	(0.8)	(0.02)	(0.3)	(0.1)	(0.017)	(0.06)	(0.02)	(1.0)	(0.01)	(0.03)	(0.002)	(0.02)	(0.1)	(0.06)
	ELE	5.4***	4.8	1.87***	24.2***	0.010*	1.6	2.8***	0.021***	0.15***	0.16	30.8	0.38	0.16	0.10	1.8	17.2**	3.35
				(0.19)	(0.8)	(0.001)	(0.3)	(0.1)	(0.004)	(0.05)	(0.01)	(0.9)	(0.01)	(0.02)	(0.003)	(0.04)	(0.2)	(0.08)

Results

Soil characteristics

The soil was characterized by a low amount of total exchangeable cations, ranging from 27.4 to 44.6 $mmol_c$ kg^{-1} dw in the two horizons (Table 1). Under ambient conditions, the exchange complex was largely dominated by Ca (71–77%) in both horizons, followed by K (12%) and Mg (10%) in the UP horizon and by Al (13%) and K (7%) in the LOW horizon. The C and N contents were low, with a C/N ratio of 14.7–16.5.

Results from a two-way ANOVA showed a significant TRMT*HOR interaction for most variables. Significant effects were therefore tested by separate *t*-tests for each HOR (TRMT effect) and TRMT (HOR effect).

Comparing both the soil horizons under AMB conditions, the UP horizon had a lower LOI, C, N, C/N ratio and exchangeable Al than the LOW horizon. Exchangeable Ca, K, Mg, soil pH_{H2O}, and pH_{KCL} were higher in UP than in LOW.

In the UP horizon, the effect of elevated atmospheric CO_2 was reflected by a significant increase in pH_{KCL}, and decrease in soil organic matter and in exchangeable Al, Mn and Zn (Table 1). In the LOW horizon, elevated atmospheric CO_2 caused a significant increase in the pH_{H2O}, C/N ratio, exchangeable Ca and Mg, and a decrease in exchangeable Al, Na and Mn.

N_2O emissions

Results from a three-way ANOVA showed a significant TRMT*HOR interaction and significant effects were therefore tested by a separate two-way ANOVA for each HOR (TRMT and CHAMBER effect) and TRMT (HOR and CHAMBER effect).

Under AMB conditions, the UP horizon showed significantly higher potential N_2O production (N_2Opot) and DP than the LOW horizon (Table 2). Both N_2Opot and DP were below a mean of 6.5 μg N_2O–N g^{-1} over 48 h in LOW, but reached 10.5 and 24.6 μg N_2O–N g^{-1} over 48 h, respectively, in UP. The presence of acetylene in this horizon also caused a clear increase in N_2O–N emissions.

Elevated CO_2 caused a significant increase by 27% in DP in UP. In LOW, both potential N_2O production and DP were higher for soil originating from the OTCs under elevated CO_2, showing an increase of 140% and 160%, respectively. The DEA was undetectable under all conditions in both the horizons.

Nitrogen mineralization

Net ammonium production was low (0.2 ± 0.9 mg kg^{-1} over 10 weeks, for all incubation conditions) and not

significantly influenced by any treatment. Mineralized N was therefore rapidly transformed to nitrate, representing the majority of inorganic N in this soil. Nitrite concentrations were below detection limits.

Table 2 Potential N_2O–N production (N_2Opot) and potential denitrification (DP) (means with standard errors in parentheses, $n = 5$) of two soil horizons (HOR: upper-UP and lower-LOW) sampled in OTCs under current CO_2 atmospheric concentration (*ca.* 350 μmol mol^{-1}, AMB) and current CO_2 atmospheric concentration + 400 μmol mol^{-1} (ELE) after incubation of 48 h (μg N_2O–N g^{-1} 48 h^{-1}). Asterisks indicate significant effects of elevated CO_2 within one layer (Two-way ANOVA, *, $P < 0.05$; **, $P < 0.001$). Significant differences between soil horizons are indicated in the text

HOR	TRMT	N_2Opot	DP
UP	AMB	10.5	24.6
		(1.9)	(2.6)
	ELE	9.3	31.3*
		(1.3)	(1.4)
LOW	AMB	5.3	6.3
		(0.6)	(0.7)
	ELE	12.7**	16.4**
		(1.3)	(2.4)

Because the results from the three-way ANOVA showed significant interactions between CO_2 treatment in the field and CO_2 laboratory incubation conditions, significant effects were tested by a two-way ANOVA for each incubation (INCUB) condition (TRMT and HOR effect) and field CO_2 (TRMT) treatment (INCUB and HOR effect).

Net nitrate production was influenced by both CO_2 treatment in the field and CO_2 incubation conditions in the laboratory (Fig. 1, Table 3). There was no significant

Table 3 Summary of the results of significant effects of the CO_2 treatment in the field (ambient-AMB, elevated-ELE) and the laboratory incubation conditions (normal-NOR, with CO2-CO2) for N–NO_3 production (mg kg^{-1} 10 $weeks^{-1}$), as determined by two-way ANOVA (***: $P < 0.001$). Means across soil horizons with standard errors in parentheses, $n = 10$

	CO_2 treatment in the field		Effect
Laboratory incubation	AMB	ELE	
under elevated CO_2	8.9	17.9	***
	(1.8)	(1.4)	
	Laboratory incubation		
Soil from the elevated	NOR	CO_2	
CO_2 treatment in the field	10.8	17.9	***
	(1.4)	(1.4)	

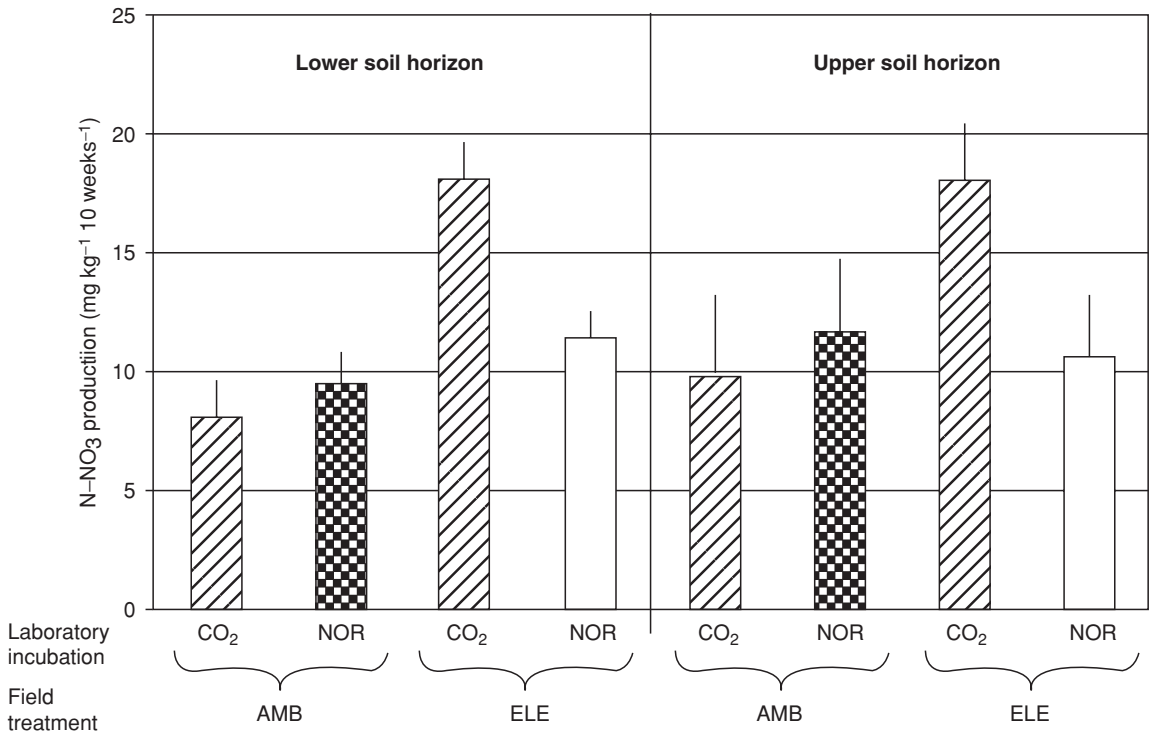

Fig. 1 Mean net nitrate production (mg kg^{-1} over 10 weeks) from the upper and lower soil horizon, having received 4 years of field ambient (AMB) or elevated CO_2 (ELE) and being incubated in the laboratory under ambient (NOR) or elevated CO_2 (CO_2). Vertical bars indicate standard errors of means.

difference between the soil horizons. Elevated CO_2 in the field caused significantly increased NO_3 production for both the horizons when laboratory incubations were performed under elevated CO_2 (comparison AMB-CO_2 to ELE-CO_2). Averaged across soil horizons this increase reached 100% (Table 3). Elevated CO_2 in the laboratory caused significant increased NO_3 production for both horizons having been treated with elevated CO_2 in the field (comparison ELE-CO_2 to ELE-NOR). Averaged across soil horizons this increase reached 66% (Table 3). The absence of significant differences between the soils from AMB in CO_2 and NOR laboratory incubation confirmed that the increased NO_3 production under CO_2 laboratory incubation was not owing to a difference between the two laboratory incubation chambers, other than the CO_2 concentration.

Discussion

The control of soil N availability under elevated atmospheric CO_2 is central to predicting changes in ecosystem C storage and primary productivity. The effects of elevated CO_2 on belowground processes have so far attracted limited research and they are assumed to be regulated by indirect effects through changes in plant physiology and chemistry. Our results demonstrate significant effects of elevated CO_2 on the soil microbial processes of nitrification and denitrification, and we provide evidence of a direct effect of elevated atmospheric CO_2 on net nitrate production.

Gas emission measurements performed in this study reflect different soil microbiological characteristics. The DEA is a measure of the active denitrifying enzyme concentration in the soil at the time of sampling. As these enzymes are inducible, their content reflects whether soil conditions were suitable to induce the process (Tiedje, 1994). In contrast, DP and potential N_2O emissions (N_2Opot) express the ability of denitrifiers to respond to substrate (NO_3^-) and electron donor (C source: glucose) abundance (Simek *et al*., 2000) with possible *de novo* synthesis of enzymes. During these measurements, inhibition of denitrification by O_2 and the occurrence of nitrification are excluded by the preparation of an anaerobic slurry.

No DEA was detected in this study, indicating that denitrification was not a significant microbial process in the OTCs at the time of sampling. The absence of DEA could be explained by low temperatures, aerobic soil conditions, low moisture contents or insufficient substrate availability preceding soil sampling.

Measured rates of DP from soils under ambient conditions at 12.3 and 3.15 μg N_2O–N g^{-1} over 24 h were well in the range reported in the literature (Simek *et al*., 2000). For example, Simek *et al*. (2000) reported mean values of 6.6–52 μg N g^{-1} over 24 h from 13 agricultural sites differing in physico–chemical and biological characteristics in the Czech Republic. Henrich & Hasselwandter (1991) reported DP of 5 μg N g^{-1} over 24 h from the top 10 cm of an acid Norway spruce site. The higher potential N_2O (N2Opot) production and DP in the upper horizon compared to the lower horizon observed in this study could be linked to the higher pH, as soil pH and DP are correlated (Simek *et al*., 2000).

Both DP and N_2Opot were increased in the LOW horizon, in response to the four-year treatment with elevated CO_2 in the field. In the UP horizon submitted to 1 year of elevated CO_2 treatment, only DP was increased. Elevated CO_2 has been shown to increase rhizosphere denitrifier activity (Smart *et al*., 1997) and N_2O fluxes from the soil (Arnone & Bohlen, 1998), corroborating increased DP observed in our study. In contrast, elevated CO_2 was reported by Huntgate *et al*. (1997) to have no effect on soil N_2O emissions. As DP is a measure of the ability of the denitrifier community to develop quickly under favourable conditions, it is much dependent on the chemical and biological state of the soil (Simek *et al*., 2000). In particular, C is thought to be an important regulator of the denitrifier populations in nature (Tiedje, 1988). We observed a significant effect of elevated atmospheric CO_2 on soil chemical conditions. Soil pH and some exchangeable base cations (Ca^{2+}, Mg^{2+}) increased, whereas acid cations (Al^{3+}, Mn^{2+}) decreased. Increased fine root biomass and root respiration under elevated CO_2 were observed during the first 2 years of this OTC study (Janssens *et al*., 1998). This might be responsible for higher exudation of organic substances from roots, increasing nutrient availability (Grayston *et al*., 1997) and in particular, exchangeable and soluble base cations in the rhizosphere (Gobran *et al*., 1998). A positive correlation between N_2O emissions and base saturation has been reported by Davidson & Swank (1986). Increased DP and N_2Opot in our study might therefore be regulated indirectly by the effects of elevated CO_2 on soil pH, C availability and NO_3^- production (see below) and consequently on microbial activities.

Net nitrification and net N mineralization result from a balance between gross N mineralization and N immobilization. In the present study, net ammonium production was low, so net nitrification and net N mineralization were equivalent. Net nitrate production was significantly increased for the soil originating from the elevated CO_2 treatment in the field when incubated in the laboratory under elevated CO_2. When this soil was incubated in the laboratory under ambient CO_2 concentrations, net nitrate production remained unchanged. Soil originating from the ambient field treatment showed no differences between incubation under both CO_2 levels. This result confirmed that the observed difference for the soil from the

elevated CO_2 treatment in the field was not owing to an artefact between incubation chambers, but conditioned by the continuous 4-year exposition to elevated CO_2 combined with the high exposition during laboratory incubation.

In a recent literature review (Zak *et al.*, 2000a), only 2 out of 19 reviewed studies were statistically significant and showed an increase in net nitrification. Increasing net nitrification under elevated CO_2 could be owing to enhanced labile C supply from roots causing increased or more active microbial populations (Zak *et al.*, 1993) or stimulating directly mixotrophically growing nitrifiers (Watson *et al.*, 1989). However, these explanations do not hold in our study, as the increase in net nitrification was conditioned by the presence of elevated CO_2 during laboratory incubation. In case of a 'labile C effect', soil from the elevated treatment in the field incubated under ambient CO_2 in the laboratory should also exhibit greater nitrification rates. However, we cannot conclude that labile C inputs are insignificant in N cycling under elevated CO_2 as in our study labile C might have been consumed in the interval between tree removal and soil sampling. These results indicate that there could have been a direct effect of elevated atmospheric CO_2 on the nitrifying bacteria.

CO_2 concentrations in the upper soil horizons can be below the optimum for maximal nitrification rates, which have been reported between 0.6 and 2.9% CO_2 (Clark, 1968). For example, in the upper soil horizons of forest soils, concentrations were near ambient during winter (0.035%) and lower than 0.2% during the whole season (Castelle & Galloway, 1990), or ranging from 0.10 to 0.35% (Fernandez & Kosian, 1987). However, in view of these data it is difficult to imagine that doubling of the atmospheric CO_2 concentration to 0.08% in the elevated treatments would have a major effect on nitrification rates. One possible explanation would be a selection of NH_4 oxidisers with higher affinity for CO_2 (Kinsbursky & Saltzman, 1990), causing higher nitrification rates when this soil is exposed to elevated atmospheric CO_2. Alternatively increased soil CO_2 may decrease soil heterotrophic activity and microbial biomass (Santruckova & Simek, 1997), reducing competition for ammonium. However, CO_2 concentrations to reduce microbial biomass were above 5% and this mechanism is more likely to occur in deeper soil horizons.

Results call for a more profound consideration of belowground processes and for integrated research including simultaneous measurements of plant level responses, N fluxes and microbiological parameters in field and laboratory experimentation. A further investigation of the controlling factors for the nitrification process is essential to provide an explanation of the divergent responses observed in nature (Zak *et al.*, 2000b). Investigation of the community composition of ammonia-oxidisers by molecular methods could also open new perspectives. Soil CO_2 measurements and measurement of gross N mineralization rates by ^{15}N tracer studies in experiments with elevated CO_2 would provide further insight into the hypotheses formulated in this contribution.

We conclude that elevated atmospheric CO_2 has a major effect on the soil microbial processes of nitrification and denitrification and that the increase in net nitrification is conditioned by the continued presence of elevated CO_2. Furthermore, we advance the hypothesis that this increase could be owing to a physiological adaptation or selection of nitrifiers, or a decrease in soil heterotrophic activity and microbial biomass, reducing competition for ammonium under elevated CO_2.

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