



## Fluxes of CH<sub>4</sub> and N<sub>2</sub>O in aspen stands grown under ambient and twice-ambient CO<sub>2</sub>

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### Abstract

Elevated atmospheric CO<sub>2</sub> has the potential to change below-ground nutrient cycling and thereby alter the soil-atmosphere exchange of biogenic trace gases. We measured fluxes of CH<sub>4</sub> and N<sub>2</sub>O in trembling aspen (*Populus tremuloides* Michx.) stands grown in open-top chambers under ambient and twice-ambient CO<sub>2</sub> concentrations crossed with 'high' and low soil-N conditions.

Flux measurements with small static chambers indicated net CH<sub>4</sub> oxidation in the open-top chambers. Across dates, CH<sub>4</sub> oxidation activity was significantly ( $P < 0.05$ ) greater with ambient CO<sub>2</sub> ( $8.7 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$ ) than with elevated CO<sub>2</sub> ( $6.5 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$ ) in the low N soil. Likewise, across dates and soil N treatments CH<sub>4</sub> was oxidized more rapidly ( $P < 0.05$ ) in chambers with ambient CO<sub>2</sub> ( $9.5 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$ ) than in chambers with elevated CO<sub>2</sub> ( $8.8 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$ ). Methane oxidation in soils incubated in serum bottles did not show any response to the CO<sub>2</sub> treatment. We suggest that the depressed CH<sub>4</sub> oxidation under elevated CO<sub>2</sub> in the field chambers is due to soil moisture which tended to be higher in the twice-ambient CO<sub>2</sub> treatment than in the ambient CO<sub>2</sub> treatment.

Phase I denitrification (denitrification enzyme activity) was 12–26% greater under elevated CO<sub>2</sub> than under ambient CO<sub>2</sub> in the 'high' N soil; one sampling, however, showed a 39% lower enzyme activity with elevated CO<sub>2</sub>. In both soil N treatments, denitrification potentials measured after 24 or 48 h were between 11% and 21% greater ( $P < 0.05$ ) with twice-ambient CO<sub>2</sub> than with ambient CO<sub>2</sub>. Fluxes of N<sub>2</sub>O in the open-top chambers and in separate 44 cm<sup>2</sup> cores  $\pm$  N fertilization were not affected by CO<sub>2</sub> treatment and soil N status.

Our data show that elevated atmospheric CO<sub>2</sub> may have a negative effect on terrestrial CH<sub>4</sub> oxidation. The data also indicated temporary greater denitrification with elevated CO<sub>2</sub> than with ambient CO<sub>2</sub>. In contrast, we found no evidence for altered fluxes of N<sub>2</sub>O in response to increases in atmospheric CO<sub>2</sub>

### Introduction

Elevated concentrations of atmospheric CO<sub>2</sub> have the potential to increase rates of below-ground as well as above-ground plant production in terrestrial ecosystems (Rogers et al., 1994). For example, Pregitzer et al. (1995) found that growth and turnover of fine roots of aspen trees increased under twice-ambient CO<sub>2</sub> and that inputs of carbon to soil roughly doubled. Similarly, Rouhier et al. (1996) observed increased root pro-

duction and rhizodeposition from chestnut seedlings grown under elevated CO<sub>2</sub>.

Increased C allocation below-ground in response to elevated CO<sub>2</sub> is likely to fuel below-ground heterotrophic processes and increase microbial biomass (Klironomos et al., 1996; Niklaus and Körner, 1996; Rice et al., 1994; Schenk et al., 1995; Zak et al., 1993) and soil respiration (Niklaus and Körner, 1996; Ross et al., 1995, 1996). Microbial responses to elevated CO<sub>2</sub> vary and may not be distinct in systems with low nutrient availability (Klironomos et al., 1996; Niklaus and Körner, 1996). Although some have sug-

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gested that increased below-ground productivity may reduce nutrient availability through increased sequestration in microbial biomass (Diaz et al., 1993), others (e.g. Rice et al., 1994) note that soil nutrient turnover may increase if the labile carbon pool increases. In accordance with this postulate, Zak et al. (1993) observed greater short-term net mineralization under *Populus grandidentata* grown with elevated CO<sub>2</sub>, but, in contrast Ross et al. (1995, 1996) found that soil N availability was apparently unaffected by growing grass clover pastures under elevated CO<sub>2</sub>.

In addition to producing fundamental changes in net primary productivity and soil nutrient cycling, CO<sub>2</sub> enrichment also has the potential to increase plant water-use efficiency. Working with tallgrass prairie systems, Owensby et al. (1993) and Rice et al. (1994) demonstrated that growth responses by both plants and microbes to elevated CO<sub>2</sub> were greater under dry conditions due to greater plant water-use efficiency than under ambient CO<sub>2</sub>.

Soil N availability and moisture are strong regulators of microbial CH<sub>4</sub> oxidation and N<sub>2</sub>O production in upland soils, so it is likely that ecosystem responses to elevated CO<sub>2</sub> will also include changes in fluxes of these trace gases (Robertson et al., 1989). Based on the literature, however, it is unclear whether CO<sub>2</sub> enrichment will feed back into the soil-atmosphere exchange of CH<sub>4</sub> and N<sub>2</sub>O. More specifically, reduced N availability may provide a basis for higher methanotrophic activity (Schnell and King, 1994), whilst N gas production will be diminished. With increased soil moisture, on the other hand, CH<sub>4</sub> oxidation is likely to be diminished due to diffusional constraints (Dörr et al., 1993), whereas the reduced O<sub>2</sub> availability will induce conditions favourable for N<sub>2</sub>O and CH<sub>4</sub> production. Hitherto very few studies (Hungate et al., 1997; Arnone and Bohlen, 1998; Ineson et al., 1998) have focused on this issue in upland soils.

The objective of this study was to determine the extent to which elevated CO<sub>2</sub> concentrations may change soil-atmosphere exchange of CH<sub>4</sub> and N<sub>2</sub>O in trembling aspen (*Populus tremuloides* Michx.) stands grown under ambient and twice-ambient CO<sub>2</sub> conditions for two growing seasons in soils of low and 'high' N availability. Soil surface fluxes of CH<sub>4</sub> and N<sub>2</sub>O, soil CH<sub>4</sub> oxidation and soil denitrification, respectively, was examined and compared with patterns of soil moisture and inorganic N across the different CO<sub>2</sub> and soil N treatments.

## Materials and methods

### Field studies

The experimental site is located at the University of Michigan Biological Station near Pellston, MI (45° 34' N, 84° 40' W). Twenty open-top chambers were arranged in a randomized complete 2 × 2 factorial design (CO<sub>2</sub> × N fertility), with each treatment replicated five times; the experimental design was similar to that described in Pregitzer et al. (1995). Briefly, aspen trees were grown in 3.6 m square by 0.2 m deep open-bottom root boxes. Two contrasting N treatments were achieved by filling the boxes with 100% locally excavated topsoil (Kalkaska series, Typic Haplorthod) or a mixture of 20% top soil and 80% C horizon sand (Entic Haplorthod). These two soil treatments will be termed 'high' N (quotation marks to separate from a truly high N soil) and low N, respectively. Open-top chambers (3 m diameter by 3.4 m high) were placed on all root boxes during the growing season to manipulate atmospheric CO<sub>2</sub>. The aspens were planted as cuttings two years prior to the experiment and had been exposed to ambient and twice-ambient atmospheric CO<sub>2</sub> for two growing seasons.

Sampling took place on three occasions in 1996: on May 1 prior to canopy development, on May 28 following canopy development, and on July 6 shortly before the experiment was terminated by destructive harvest.

Soil-atmosphere gas fluxes were measured using two-piece static gas chambers (Ambus et al., 1993). A 27 cm × 27 cm × 10 cm high aluminum frame was permanently installed 8 cm into the ground in each root box. When gas sampling took place, a 29 cm × 29 cm × 14 cm high white ABS plastic lid was fitted into a water filled groove on top of the aluminum base, providing a gas tight enclosure of 12 L. A rubber septum in the lid allowed gas sampling using a needle and syringe. The chamber remained sealed for 3 h and headspace samples were removed at 1 h intervals for analysis of CH<sub>4</sub> and N<sub>2</sub>O.

Sampling involved a two step procedure. First, a 15-mL sample was withdrawn and used to flush a 2-mL crimp-sealed vial. Second, a 5-mL sample was withdrawn and stored in the now pressurized vial. Gas samples were analyzed within two days after collection. Gas fluxes were calculated as the linear increase or decrease in gas concentration inside the flux chamber over the 3 h sampling period.

Methane and N<sub>2</sub>O were analyzed in 0.5 mL samples on a Hewlett Packard 5890 gas chromatograph equipped with flame-ionization (125 °C for CH<sub>4</sub>) and electron-capture detectors (350 °C for N<sub>2</sub>O).

#### Laboratory studies

At each field sampling soil samples (0-15 cm depth) was taken from each root box using a 20 mm dia. auger. In order to minimize disturbance of plant roots, only one auger sample was taken from each root box per sampling time, and soil from the five replicate boxes was mixed to obtain adequate material for subsequent analysis. The mixed samples were sieved (<4 mm), and sub-samples were analyzed for gravimetric (105 °C; 24 h) water content, which was expressed as % of water filled pore space (WFPS; Linn and Doran, 1984) and 1 M KCl (soil:KCl=1:10 w/v) extractable NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N (Alpkem Autoanalyzer). Soil total N and total C was determined by dry combustion (Carlo Erba CN Analyzer) and pH (air dry soil:water=1:2.5 w/v) were also measured; average values of these three parameters did not vary among CO<sub>2</sub> treatments and dates, and are expressed as means in Table 1.

Table 1. Total N, total C, and pH of soil from root boxes with 'high' and low N treatments, respectively. Data are means ( $n=6 \pm SE$ ) among CO<sub>2</sub> treatments and sampling dates

	Total (%)	Total C (%)	pH
Low N soil	0.04±0.002	0.29±0.03	6.3±0.04
'High' N soil	0.14±0.007	1.39±0.12	5.6±0.01

Methane oxidation and soil respiration was measured in 20 g field moist samples incubated with ambient laboratory air (2 ppm CH<sub>4</sub>) in 160 mL sealed serum bottles at 20 °C. In addition, one set of samples was incubated with laboratory air with 100 ppm CH<sub>4</sub> achieved by injecting 1 mL of a 1% CH<sub>4</sub> in N<sub>2</sub> mixture into the bottle atmosphere. Headspace samples of 0.5 mL were withdrawn several times during a 72 h incubation period and analyzed immediately for CH<sub>4</sub> and CO<sub>2</sub>. Carbon dioxide was analyzed on a Beckman Model 865 Infrared Gas Analyzer. Incubations for CH<sub>4</sub> oxidation and respiration were not performed on July 6.

Denitrification potential was determined in 10 g moist soil samples placed in 20 mL of nutrient solution (0.36 g KNO<sub>3</sub> L<sup>-1</sup> and 1.25 g glucose L<sup>-1</sup>) in 120

mL sealed serum bottles. The bottles were made anaerobic by evacuating and refilling them with N<sub>2</sub> three times. Acetylene was added to 10 % v/v (10 kPa). A 1-mL headspace sample was withdrawn at 1 h intervals during the 0-3 h of incubation and then again at 24 h and 48 h (May 28 and July 6), and stored in 3-mL N<sub>2</sub>-flushed Venoject vials until analysis for N<sub>2</sub>O.

An additional experiment was undertaken on July 6 to examine gas fluxes in response to fertilization. Two PVC cylinders, 16 cm long and 7.5 cm internal diameter, bevelled at one end, were pushed 10 cm into the ground in each root box and then removed by excavation. The cylinders were wrapped at the bottom with polyethylene film. Sixteen hours prior to gas measurements the two sets of cores were amended with either 45 mL of water core<sup>-1</sup> or 45 mL of an NH<sub>4</sub>NO<sub>3</sub> solution (1 g N L<sup>-1</sup>) core<sup>-1</sup>, respectively. For gas flux measurements the cylinders were placed on trays filled to a depth of 1 cm with water, and the headspace around the individual cylinders was then sealed by 3.2 L metal cans placed open end down to achieve gas-tight seals. The cans were fitted with rubber septa for gas sampling, which was carried out as described for the field boxes. Pure N<sub>2</sub>, equivalent to the amount removed for sampling, was injected into the cans to compensate for underpressure. Headspace samples were taken after 0, 2.5, 5, 7.5, 10, and 24 h of incubation at 20 °C.

Statistical analyses were performed on LOG-transformed data to meet the assumptions of equal variances. Multiple means were compared using Tukey's Studentized Range Test of Proc GLM at  $P=0.05$  (SAS Institute, 1990).

## Results and discussion

### Methane

Net CH<sub>4</sub> consumption was observed at all samplings at rates from 3.5 to 14.5 μg C m<sup>-2</sup> h<sup>-1</sup> (Figure 1). These rates are at the low end of CH<sub>4</sub> uptake measured in temperate forest stands (Ambus and Christensen, 1995; Castro et al., 1995) but similar to rates observed in cropping systems (Ineson et al, 1998). The disturbance of the soil in the root boxes two years prior to the study may have depressed the CH<sub>4</sub> oxidation activity, as documented in comparative studies on cultivated and uncultivated sites (Hütsch et al., 1994).

Methane oxidation in the twice-ambient CO<sub>2</sub> treatment was on average 22% (range 2–61%) lower than

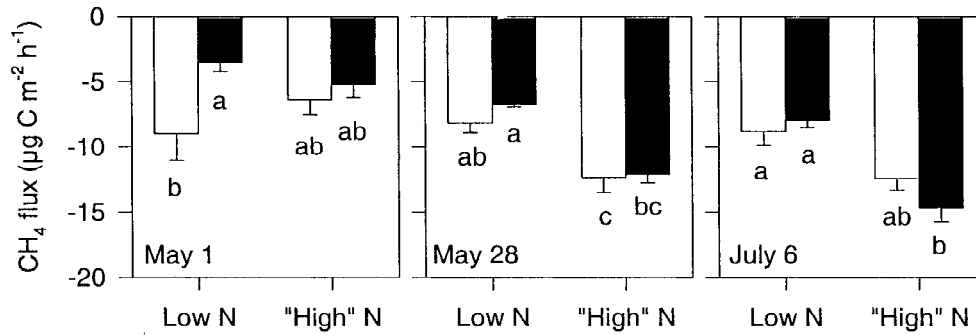


Figure 1. Fluxes of CH<sub>4</sub> from aspen (*Populus tremuloides*) stands grown in open top chambers under ambient (open bars) and twice-ambient (solid bars) CO<sub>2</sub> concentrations. Negative numbers indicate net CH<sub>4</sub> uptake. The CO<sub>2</sub> treatments were crossed with two soil N treatments. Numbers are means of five replicate chambers ( $\pm$ SE). Different letters indicate significant differences at each sampling time ( $P < 0.05$ ).

Table 2. Soil moisture and total inorganic N contents in soil from aspens (*Populus tremuloides*) grown under ambient CO<sub>2</sub> and twice-ambient CO<sub>2</sub> crossed with low N and 'high' N soil treatments. Numbers are mean of duplicate ( $\pm$ SE) aliquots of composite samples from five field replicates

GO <sub>2</sub> treatment	Date	% WFPS	Inorganic N (mg kg <sup>-1</sup> )
Low N soil			
Ambient	May 1	N.d. <sup>a</sup>	N.d.
	May 28	8.8 <sup>b</sup>	0.9 $\pm$ 0.03
	July 6	12.3 $\pm$ 1.2	1.0 $\pm$ 0.07
Twice-ambient	May 1	25.4 $\pm$ 0.4	0.8 $\pm$ 0.06
	May 28	10.7	1.1 $\pm$ 0.04
	July 6	13.5 $\pm$ 0.2	1.0 $\pm$ 0.03
'High' N soil			
Ambient	May 1	45.1 $\pm$ 0.03	3.6 $\pm$ 0.1
	May 28	27.5	3.4 $\pm$ 0.2
	July 6	14.5 $\pm$ 0.5	2.7 $\pm$ 0.02
Twice-ambient	May 1	46.1 $\pm$ 0.5	3.9 $\pm$ 0.3
	May 28	30.5	4.0 $\pm$ 0.3
	July 6	15.8	2.9 $\pm$ 0.1

<sup>a</sup>N.d.= no data; <sup>b</sup>one aliquot analyzed.

CH<sub>4</sub> oxidation in the ambient CO<sub>2</sub> treatment, excluding the 'high' N soil on July 6 (Figure 1). Averaging rates over sampling dates revealed a significantly ( $P < 0.05$ ) lower CH<sub>4</sub> oxidation in twice-ambient CO<sub>2</sub> plots ( $6.5 \pm 0.6 \mu\text{g C m}^{-2} \text{ h}^{-1}$ ) than in ambient CO<sub>2</sub> chambers ( $8.7 \pm 0.8 \mu\text{g C m}^{-2} \text{ h}^{-1}$ ) with low N soil. In the 'high' N soil CH<sub>4</sub> oxidation was not different between the two CO<sub>2</sub> treatments. Across dates and regardless of soil N treatment, twice-ambient CO<sub>2</sub> plots oxidized CH<sub>4</sub> at a rate of  $8.8 \mu\text{g C m}^{-2} \text{ h}^{-1}$  which is significantly lower than that for ambient CO<sub>2</sub> plots at  $9.5 \mu\text{g C m}^{-2} \text{ h}^{-1}$  ( $P < 0.05$ ; Mann Whitney non

parametric statistics). Differences in CH<sub>4</sub> oxidation rates are coincident with trends in other soil parameters. First, the elevated CO<sub>2</sub> treatment tended to have higher soil water contents than the ambient CO<sub>2</sub> plots, on average 11% (Table 2). This is probably due to a higher water use efficiency of the aspens grown under elevated CO<sub>2</sub> (Owensby et al., 1993). Increased soil moisture constrains CH<sub>4</sub> diffusion and will therefore inhibit CH<sub>4</sub> oxidation (Dörr et al., 1993). Ineson et al. (1998) hypothesized that depressed CH<sub>4</sub> uptake in a ryegrass field in response to elevated CO<sub>2</sub> was caused by an increase in CH<sub>4</sub> production. A similar mechanism may also have been operative in our study. The importance of soil moisture in the temporal control of CH<sub>4</sub> uptake fluctuations was indicated by the significant negative correlations between the temporal variations in soil moisture and CH<sub>4</sub> uptake, viz.  $r = -0.911$  ( $n = 6$ ;  $P < 0.05$ ) and  $r = -0.915$  ( $n = 5$ ;  $P < 0.05$ ) for the low N and 'high' N soil, respectively. Secondly, inorganic N pools tended to be higher in the twice-ambient CO<sub>2</sub> plots than in the ambient CO<sub>2</sub> plots (Table 2). The differences, however, were very small, and would be unlikely to have the potential to depress CH<sub>4</sub> oxidation (Schnell and King, 1994). Moreover, the CO<sub>2</sub> treatments did not affect net- or gross N mineralization in the root boxes (Zak, 1998 – pers. comm.). Overall, this suggests that neither inorganic N pool sizes nor N turnover was important for CH<sub>4</sub> turnover in this study.

Methane oxidation in laboratory-incubated soils occurred at constant rates during the 72 h incubation period (not shown). In Table 3 we report the oxidation rates calculated from linear regressions of CH<sub>4</sub> concentration vs. time. In incubations with 2 ppm CH<sub>4</sub>, the CH<sub>4</sub> oxidation activity showed no response

Table 3. Rates of CH<sub>4</sub> oxidation (ng C kg<sup>-1</sup> h<sup>-1</sup>) in soil from aspen (*Populus tremuloides*) grown under ambient CO<sub>2</sub> and twice-ambient CO<sub>2</sub> crossed with low N and 'high' N soil treatments. The soil was incubated under 2 ppm CH<sub>4</sub> and under 100 ppm CH<sub>4</sub> in serum bottles. Numbers are means (±SE) of triplicate aliquots of composite samples from five field replicates; for each N treatment different letters indicate significant differences ( $P < 0.05$ )

CO <sub>2</sub> treatment	2 ppm CH <sub>4</sub>	2 ppm CH <sub>4</sub>	100 ppm CH <sub>4</sub>
Low N soil	May 1	May 28	
<i>Ambient</i>	N.d. <sup>a</sup>	22±1b	1650±334b
<i>Twice-ambient</i>	11±2a	17±2b	1274±96b
'High' N soil			
<i>Ambient</i>	54±8a	60±13a	3378±78a
<i>Twice-ambient</i>	71 ±6a	80±4a	2820±835a

<sup>a</sup>N.d.= no data

to the CO<sub>2</sub> treatment (Table 3). In incubations with 100 ppm CH<sub>4</sub> the data suggest that twice-ambient CO<sub>2</sub> depressed CH<sub>4</sub> uptake by 17 to 23%, in accordance with the results from the field chambers, but the variability among replicates was high and differences among CO<sub>2</sub> treatments were not significant. Evidently the mechanism responsible for depressed CH<sub>4</sub> uptake in response to enhanced CO<sub>2</sub> in the field was not operative in the incubated soil. This supports the finding discussed above that the reduced CH<sub>4</sub> oxidation *in situ* in the elevated CO<sub>2</sub> experiment was caused by lowered diffusion rather than changes in N availability; in incubated soil, aeration is much facilitated as compared with conditions *in situ*. On the other hand, differences in soil moisture was very small, and it can be debated whether they would be sufficient to influence the CH<sub>4</sub> oxidation. Nevertheless, Whalen et al. (1990) observed a 20-fold change in CH<sub>4</sub> oxidation when soil moisture changed from 5 to 11%, probably as a combination of changes in physical gas exchange and microbial water stress at the low moisture content (Adamsen and King, 1993).

#### Denitrification and nitrous oxide

Denitrification potentials were significantly enhanced by elevated CO<sub>2</sub> in the May 1 and July samples in the 'high' N soil (Figure 2). Phase I denitrification (existing enzyme activity; Smith and Tiedje, 1979) was increased 26% ( $P < 0.05$ ) by twice-ambient CO<sub>2</sub> on July 6. In contrast, on May 28, elevated CO<sub>2</sub> depressed Phase I denitrification by 39%; this difference, however, was only transient and not apparent after 48 h of incubation (Figure 2). Following 24 h (May 1)

or 48 h (July 6) of incubation, the high N soil from twice-ambient CO<sub>2</sub> chambers had denitrified 19% and 11% more, respectively, than soil from ambient CO<sub>2</sub> chambers. In the low N soil, Phase I denitrification was similar for the two CO<sub>2</sub> levels. After 48 h of incubation on May 28, however, 21% more N was denitrified in soil from elevated CO<sub>2</sub> chambers than in soil from ambient CO<sub>2</sub> chambers.

From these data it can be concluded that the CO<sub>2</sub> treatments did not consistently affect concentrations of denitrifying enzymes whereas following prolonged exposure to denitrifying conditions denitrification was enhanced by the twice-ambient CO<sub>2</sub> treatment. As denitrifying organisms are dominated by heterotrophic bacteria (Tiedje, 1988), this increase may be due to increased substrate availability governed by increased below-ground C allocation by elevated-CO<sub>2</sub> aspens. Under denitrifying conditions, e.g. shortly after rain (Corre et al., 1995), soil N<sub>2</sub>O fluxes may thus be greater from elevated CO<sub>2</sub> aspens than from ambient CO<sub>2</sub> aspens. However, the potential for such an effect in these sandy soils is limited (Groffman and Tiedje, 1989).

Both Phase I denitrification and denitrification after 24 or 48 h were significantly greater in the 'high' N soil than in the low N soil (Figure 2). This difference was also apparent for CH<sub>4</sub> oxidation activity in incubated soil (Table 3) and in chamber measurements on May 28 and July 6 (Figure 1), and may be ascribed to an overall greater microbial biomass in the 'high' N soil than in the low N soil. It is noteworthy, however, that phase I denitrification was 92-fold greater in the 'high' N soil than in the low N soil, whereas denitrification after 24 or 48 h was only 4.5-fold greater in the 'high' N soil. This suggests a higher potential for induction of denitrification in the low N soil than in the 'high' N soil.

Soil respiration was also determined in incubated soil samples and it was found that soil from twice-ambient CO<sub>2</sub> chambers on average had a 17% greater ( $P < 0.05$ ) activity than soil from ambient CO<sub>2</sub> chambers (Table 4); this calculation includes the non-significant differences on May 28 in 2 ppm CH<sub>4</sub> bottles. The often greater heterotrophic activity associated with soils from twice-ambient CO<sub>2</sub> chambers is in agreement with their increased denitrification potentials, which were probably fueled by increased below-ground C allocation by the elevated-CO<sub>2</sub> grown aspens. Among all data sets ( $n=3$ ), respiration in the 'high' N soil was 5 times greater than in the low N soil,

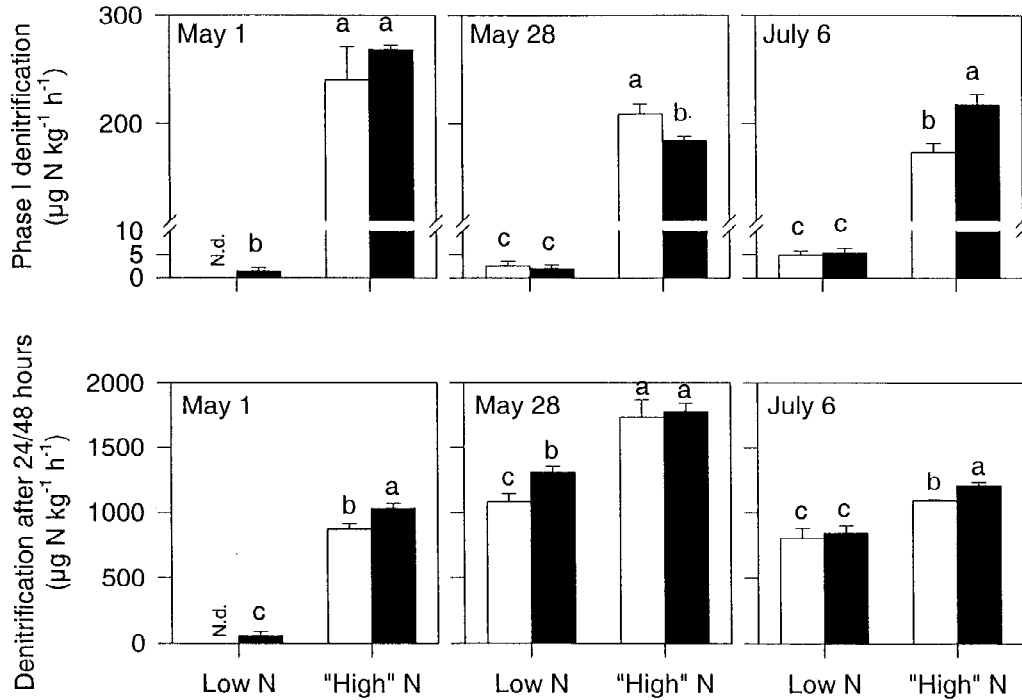


Figure 2. Denitrification potentials in soil samples taken from aspen (*Populus tremuloides*) stands grown in open top chambers under ambient (open bars) and twice-ambient (solid bars) CO<sub>2</sub> concentrations. The CO<sub>2</sub> treatments were crossed with two soil N treatments. Denitrification was measured regularly during 0-3 h of incubation, and again after 24 h (May 1) and 48 h (May 28 and July 6) of incubation. Numbers are means of triplicate aliquots ( $\pm$ SE) of a composite sample from five field replicates. Different letters indicate significant differences at each sampling time ( $P < 0.05$ ).

Table 4. Rates of CO<sub>2</sub> evolution ( $\mu\text{g C kg}^{-1} \text{h}^{-1}$ ) in soil from aspen (*Populus tremuloides*) grown under ambient CO<sub>2</sub> and twice-ambient CO<sub>2</sub> crossed with low N and 'high' N soil treatments. The soil was incubated under 2 ppm (ambient) CH<sub>4</sub> and under 100 ppm CH<sub>4</sub> in serum bottles. Numbers are means ( $\pm$ SE) of triplicate aliquots of composite samples from five field replicates; for each N treatment different letters indicate significant differences ( $P < 0.05$ )

CO <sub>2</sub> treatment	2 ppm CH <sub>4</sub>	2 ppm CH <sub>4</sub>	100 ppm CH <sub>4</sub>
Low N soil	May 1	May 28	
Ambient	N.d. <sup>a</sup>	83 $\pm$ 4b	75 $\pm$ 0.4d
Twice-ambient	118 $\pm$ 8c	83 $\pm$ 1b	81 $\pm$ 2c
'High' N soil			
Ambient	622 $\pm$ 7b	338 $\pm$ 8a	285 $\pm$ 9b
Twice-ambient	743 $\pm$ 6a	529 $\pm$ 168a	352 $\pm$ 0.5a

<sup>a</sup>N.d.= no data.

and is consistent with the differences in CH<sub>4</sub> oxidation and denitrification discussed above.

Fluxes of N<sub>2</sub>O from the root boxes ranged between 0.1 and 2.1  $\mu\text{g N m}^{-2} \text{h}^{-1}$  (Figure 3) and were similar to those obtained from poplar stands in South-

west Michigan (Robertson, 1997 – unpublished data) and from Massachusetts pine and hardwood stands (Bowden et al., 1990). Fluxes from replicate boxes, however, were variable and showed no consistent response to the CO<sub>2</sub> treatments. Likewise, N<sub>2</sub>O fluxes did not differ among the N treatments except on May 28 when N<sub>2</sub>O fluxes under twice-ambient CO<sub>2</sub> were 80 times greater from the 'high' N soil (2.5  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ) than from the low N soil (0.03  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ). In a recent study in a California grassland, Hungate et al. (1997) also observed unaltered N<sub>2</sub>O emissions in response to CO<sub>2</sub> treatments whereas Ineson et al. (1998) and Arnone and Bohlen (1998) observed increased outputs of N<sub>2</sub>O under elevated CO<sub>2</sub> in grass covered land.

Incubated cores without added inorganic N emitted N<sub>2</sub>O at rates between 1.6 and 3.4  $\mu\text{g N m}^{-2} \text{h}^{-1}$  (Figure 4). These rates are somewhat greater than those measured *in situ* in the open-top chambers in July when the cores were sampled (Figure 3), probably due to the wetting of the soil prior to incubation, but as

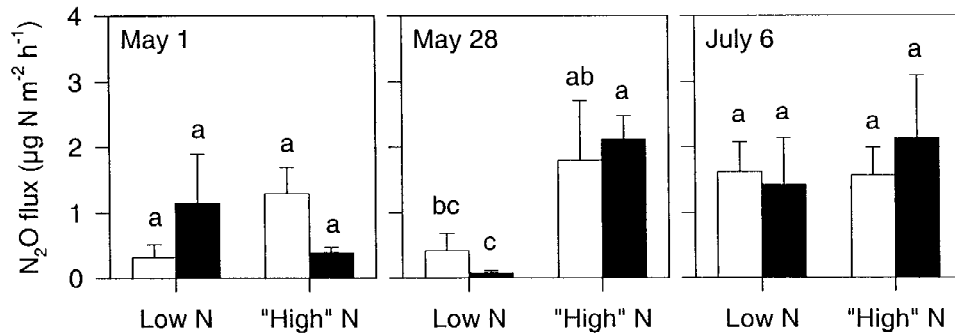


Figure 3. Fluxes of N<sub>2</sub>O from aspen (*Populus tremuloides*) stands grown in open top chambers under ambient (open bars) and twice-ambient (solid bars) CO<sub>2</sub> concentrations. See caption for Figure 1.

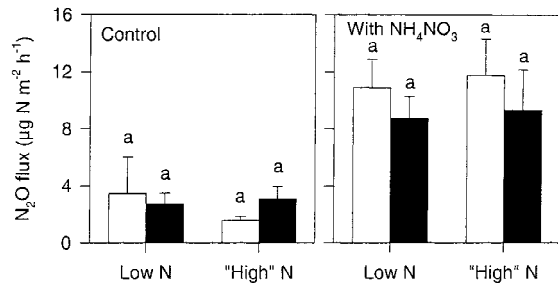


Figure 4. Fluxes of N<sub>2</sub>O from incubated cores taken from aspen (*Populus tremuloides*) stands grown in open top chambers under ambient (open bars) and twice-ambient (solid bars) CO<sub>2</sub> concentrations. The CO<sub>2</sub> treatments were crossed with two soil N treatments. One set of cores was incubated with ambient inorganic N (control) and another set received additional N (NH<sub>4</sub>NO<sub>3</sub>) prior to incubation. Numbers are means of five replicate cores ( $\pm$ SE). Different letters indicate significant differences for each treatment ( $P < 0.05$ ).

with the field measurements (Figure 3) did not differ among CO<sub>2</sub> treatments nor *in situ* soil N treatments.

The N<sub>2</sub>O fluxes in all treatments appeared to be limited by available N as fluxes in N-amended cores increased 3- to 4-fold (Figure 4). As for the un-amended cores, N<sub>2</sub>O fluxes from N-fertilized cores did not differ among CO<sub>2</sub> treatments or *in situ* soil N treatments. The similarity of the N<sub>2</sub>O fluxes from the two *in situ* soil N treatments was unexpected, since both denitrification potentials (Figure 2) and total organic C (Table 1) were higher in the 'high' N soil than in the low N soil. Perhaps processes uncoupled from denitrification and C availability, i.e. autotrophic nitrification, were important for the N<sub>2</sub>O formation under the prevailing field conditions. In the root boxes, WFPS did not exceed 46% (Table 2) and in the laboratory-incubated cores soil moisture was equivalent to 41% and 39% WFPS for low N and 'high' N soil, respectively. These soil moisture values are all less than 60%

WFPS, below which denitrification N<sub>2</sub>O production is likely to be negligible (Davidson, 1991; Linn and Doran, 1984).

## Conclusions

We found evidence for depressed CH<sub>4</sub> oxidation by soil in aspen stands grown under elevated CO<sub>2</sub> conditions compared with aspens under ambient CO<sub>2</sub> conditions. As such, the anticipated global increase in atmospheric CO<sub>2</sub> may have a negative feedback on the terrestrial CH<sub>4</sub> cycle, increasing CH<sub>4</sub> emissions from upland soil indirectly by inhibiting CH<sub>4</sub> oxidation. In contrast, N<sub>2</sub>O fluxes were identical under the different CO<sub>2</sub> conditions. However, we found evidence for temporal greater denitrification in the soil from twice-ambient CO<sub>2</sub> aspens than in soil from the ambient-CO<sub>2</sub> aspens. Denitrification conditions did not apply in our field study, but it can be speculated that induction of denitrification conditions e.g. by rain events may lead to greater N<sub>2</sub>O fluxes from elevated-CO<sub>2</sub> aspens than from ambient-CO<sub>2</sub> aspens. This needs further investigation.

Our results suggest that increased atmospheric CO<sub>2</sub> may induce global warming to a stronger degree than current estimates because changes in soil conditions may ultimately lead to depressed oxidation of atmospheric CH<sub>4</sub> in upland soils.

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