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Response of soil methanotrophic activity to carbon dioxide enrichment in a North Carolina coniferous forest

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Abstract

Rates of methane oxidation in soils from a forest that was enriched with carbon dioxide at 200 $\mu l\ l^{-1}$ above ambient CO_2 for 3 years were compared with rates in control soils from a forest that was aerated with ambient air. Laboratory incubations were performed on homogenized soil samples collected from the 0 to 7.5 and 7.5 to 15 cm soil zones at 8-week intervals from May through October. Methane was oxidized consistently at both depths. A repeated measures analysis of variance indicated that net CH_4 consumption was significantly lower in soils collected under elevated CO_2, despite similar moisture, temperature, NO_3^-, and NH_4^+ contents. The effect of CO_2 enrichment on CH_4 consumption was greatest in May and lowest in October. Potential differences in the labile fraction of organic matter in soils from CO_2-enriched plots were not responsible for lower CH_4 oxidation, as rates measured did not change for both ambient and elevated CO_2 soils during a 2-week incubation. Net CH_4 oxidation rates were unaffected by additions of 0.5 $\mu mol\ NH_4^+\ g^{-1}$ soil, but were significantly reduced in all soils at 1.0 $\mu mol\ NH_4^+\ g^{-1}$. Nitrate addition did not influence net CH_4 oxidation at either concentration. Methane production (methanogenesis) was not evident after application of difluoromethane, an inhibitor of CH_4 oxidation, indicating that the observed changes in headspace CH_4 concentration during lab incubations resulted solely from methanotrophic activity. Methane consumption from May through October for elevated CO_2 soils was 47% less overall than for ambient CO_2 soils. The contemporary increase in atmospheric CO_2 may negatively affect the soil CH_4-oxidizing community of upland forest soils and reduce the sink strength of these ecosystems in the atmospheric CH_4 budget.

Keywords: Methane; Methanotrophs; Greenhouse gases; Elevated carbon dioxide

1. Introduction

Methane is photochemically and radiatively reactive in the Earth's atmosphere, and it is 20 to 25 times more effective per molecule than CO_2 as a greenhouse gas (Blake and Rowlands, 1988; Rodhe, 1990). The atmospheric CH_4 concentration has more than doubled over the last 250 years, and this sharp rise is primarily attributed to anthropogenic activities (Houghton et al., 1994). Most CH_4 in the atmosphere is destroyed through reactions with the hydroxyl (OH) radical, but approximately 10% is consumed biologically in well-drained upland soils by aerobic CH_4-oxidizing microbes (methanotrophs). Methanotrophs in upland soils also consume CH_4 produced by methanogens in anaerobic microzones that may diffuse upward from sources lower in the soil profile (Conrad, 1995). Worldwide, methanotrophs in upland soils consume about 40 Tg $year^{-1}$ of atmospheric CH_4, which is equivalent to the annual atmospheric increase (Reeburgh et al., 1993).

The primary control on rates of CH_4 oxidation in upland soils is the rate of diffusion of atmospheric CH_4 into soil (Born et al., 1990), which is highly dependent upon soil moisture content (Czepiel et al., 1995). Other physicochemical factors that directly or indirectly affect methanotrophic activity include land-use, soil pH, temperature, and nitrogen and organic carbon (Nesbit and Breitenbeck, 1992; Castro et al., 1994; Castro et al., 1995; King, 1996; Amaral et al., 1997). Rates of net CH_4 consumption can be influenced by simultaneous CH_4 production (methanogenesis), which has been reported for some forest soils (Yavitt et al., 1995).

Increasing concentrations of atmospheric CO_2 can alter the flux of carbon through ecosystems (Zak et al., 1993). Ecosystems maintained at high CO_2 often have greater plant productivity (DeLucia et al., 1999), higher root turnover (Pregitzer et al., 1995), and improved nutrient and

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water-use efficiency (Jackson et al., 1994; Hungate et al., 1997). These plant responses to elevated CO_2 may in turn influence soil microbial activity (Zak et al., 1993; Sadowsky and Schortmeyer, 1997), but the effects of extended, large-scale CO_2 enrichment on soil biotic processes such as methanotrophy are not well defined (Schimel and Gulledge, 1998). As forests occupy one-third of the Earth's terrestrial surface, a detailed understanding of the effects of elevated CO_2 on net CH_4 oxidation in forest soils is critical to predicting the future contributions of these ecosystems to the atmospheric CH_4 budget.

An ecosystem-scale experiment was implemented in 1996 to examine the response of a coniferous forest to continuous CO_2 enrichment at 200 $\mu l\ l^{-1}$ above ambient CO_2, or approximately 560 $\mu l\ CO_2\ l^{-1}$. Laboratory incubations, using soils from both the elevated CO_2 and ambient CO_2 plots, were conducted to determine if rates of CH_4 uptake might differ after 3 years of continuous CO_2 enrichment. The objectives were to: (a) evaluate the effect of elevated CO_2 on rates of CH_4 consumption at in situ levels of moisture (% H_2O by mass), temperature, NO_3^-, NH_4^+, and pH over a growing season; (b) determine if changes in the labile organic carbon pool resulting from CO_2 enrichment affected rates of net CH_4 oxidation; (c) assess the effect of NH_4^+ and NO_3^- additions on CH_4 consumption under ambient and elevated CO_2; and (d) test if CO_2 enrichment influenced the role of methanogenesis in determining net rates of CH_4 oxidation.

2. Methods

2.1. Field site

Soils were collected from a Free-Air CO_2 Enrichment (FACE) site that is located in Orange County, North Carolina, USA (35°58′N, 79°05′W). The site consists of a 90-ha parcel of even-aged loblolly pine (*Pinus taeda* L.) that was planted on a clay loam soil in 1983. Topography is flat, and soils are Ultic Alfisols of the Enon Series (D. Richter, pers. comm.). Average seasonal air temperature ranges from 1°C in January to 36°C in July. Precipitation averages 1150 mm and is evenly distributed throughout the year (State Climate Office, 1999).

Six circular, 30-m diameter plots (herein referred to as 'rings') were established within the forest. Each of the six rings was divided into four pie-shaped sections, for a total of 24 individual sectors. Triplicate rings served as controls to which ambient air was added, while the remaining three rings were fumigated daily to maintain atmospheric CO_2 concentrations at ~560 $\mu l\ l^{-1}$, or 200 $\mu l\ l^{-1}$ above the ambient concentration of ~360 $\mu l\ l^{-1}$. Fumigation of both enriched and control plots ensured that both groups were treated similarly, with the exception of CO_2 concentration. Air or CO_2 was allowed to gently circulate freely into and out of each plot to closely similate condition in nature. Treatment began on 27 August 1996 and has continued daily without interruption. Sensors within rings continuously monitor the CO_2 concentration. Actual mean enrichments ranged from 199 to 203 $\mu l\ l^{-1}$, with concentrations inside the CO_2-treated rings varying from 550 $\mu l\ l^{-1}$ to 570 $\mu l\ l^{-1}$ (Hendrey et al., 1998).

2.2. Soil sampling and physicochemical analyses

Soil cores (5.5 cm diameter × 15 cm length) were collected four times during the 1999 growing season (May–October). One core was collected at random within each sector from each ring for a total of 24 soil cores at each sampling date. Cores were separated into 0–7.5 cm and 7.5–15 cm depth intervals. For each depth increment, soil collected from each of the rings was combined, sieved (5 mm mesh), and mixed. Soil organic matter content was determined by loss on ignition (550°C for 4 h) from three oven-dry subsamples per ring at each depth. Soil moisture was determined gravimetrically by oven drying samples at 105°C for 24 h and is reported here as percentage of water holding capacity (%WHC). Soil particle density was measured pycnometrically, and bulk density was computed as the quotient, oven-dried mass divided by field volume. Soil pH was measured potentiometrically on 1:2 soil:deionized water slurries equilibrated for 24 h. Methodologies follow Carter (1993).

2.3. General procedure for rate determinations

Unless otherwise specified, the following procedures on field-moist soils were used for all CH_4 oxidation experiments. Four 35-g samples of soil from each of the six rings were sieved and weighed within 24 h of collection. The samples were placed into 133-ml jars fitted with Swagelok O-seal fittings and acclimated at 10°C overnight. Rates of CH_4 oxidation were determined the following day from an initial concentration of approximately 2 $\mu l\ CH_4\ l^{-1}$. Headspace CH_4 determinations were made at approximately 1.5-h intervals (minimum four observations) for a maximum of 24 h by removing 2-ml gas headspace samples using 10-ml glass syringes. Methane was measured within 4 h after sampling using a Shimadzu model GC-8A flame ionization detector gas chromatograph with a 1 m mol. sieve 5A (60/80 mesh) column and an ultrapure N_2 carrier gas (33 ml min^{-1}). Column and injector temperatures were 90 and 140°C, respectively. The precision of analysis expressed as a coefficient of variation for ten replicate injections of a 1.01 $\mu l\ CH_4\ l^{-1}$ standard was <3%. The minimum detectable concentration change was ±0.10 $\mu l\ CH_4\ l^{-1}$. Concentration changes within this range were considered to be no different from zero. Autoclaved soil samples were used as controls to confirm that changes in headspace CH_4 concentration were caused by biological activity. Net CH_4 oxidation rates were calculated from the time-linear change in headspace CH_4 concentration and are reported on a dry soil mass basis.

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2.4. CH_4 consumption experiment

Methane consumption was measured four times during the growing season using soils collected every 8 weeks to assess how FACE might alter CH_4 consumption relative to ambient CO_2 control soils.

2.5. CH_4 production experiment

Rates of CH_4 production were measured using soils collected in August 1999 by inhibiting methanotrophic activity with difluoromethane (Miller et al., 1998). Quadruplet samples for each depth and for each ring were injected with 50 Pa difluoromethane (DFM) and allowed to equilibrate overnight. The time-linear change in headspace CH_4 concentration was measured over an 6-h time period both prior to headspace amendment with DFM and after a 12-h incubation. Pre-DFM changes in headspace CH_4 concentrations represent CH_4 oxidation plus CH_4 production, while post-DFM data represent CH_4 production only. Post-DFM data were used to determine if CH_4 production was significant.

2.6. Labile carbon experiment

The residence time of labile carbon, defined as the carbon readily respired by soil microorganisms during a 50 day incubation period, had been determined to be about 12 days for soils from both ambient and elevated CO_2 plots (Andrews et al., 2000). Consequently, in June 1999 CH_4 consumption rates were measured approximately every 36 h for 14 days, using soils from both CO_2 treatment groups to determine if a decline in labile carbon affected net CH_4 oxidation. All samples were vented and re-equilibrated at 2 $\mu l\ l^{-1}$ prior to each rate determination as described above.

2.7. Nitrogen addition experiment

The effect of NH_4^+ and NO_3^- additions on CH_4 oxidation at 0.5 and 1.0 $\mu mol\ g^{-1}$ soil were determined using soils collected in October from the 7.5 to 15 cm depth zone. Forty-eight sieved 20 g samples (eight per ring) were placed in jars, and each soil sample was uniformly amended with a 2 ml solution of either KNO_3, KCl, NH_4Cl, or deionized water (one at each dose). All salt amendments were equinormal with respect to cations at both the 0.5 and 1.0 $\mu mol\ g^{-1}$ concentrations. Final water content after treatment with deionized water or salt solution was about 40% WHC, which is within the range reported for optimal CH_4 oxidation in a wide variety of soils (Gulledge and Schimel, 1998). The jars were acclimated 12 h prior to determination of net CH_4 consumption rates.

2.8. Statistical analyses

A one-way analysis of variance was used on each sampling date to test for differences in soil moisture among homogenized samples collected at a specific depth, and a two-way analysis of variance was used to test for differences in net CH_4 consumption by CO_2 treatment and between groups of salt solution for each dose. Rates of CH_4 consumption were analyzed by soil depth using a mixed hierarchical linear model with repeated measures (SAS, 1998). The model included the random effect of ring nested inside CO_2 treatment and the fixed effect of CO_2 enrichment. This model was also utilized to analyze the persistence of the treatment effect for the June incubations and to test if the effect of treatment varied during repeated measurements over 14 days. Methane production rates (following DFM amendment) were analyzed with a linear regression to test if changes in CH_4 headspace concentration over time were significantly different from 0. Differences in percent soil organic matter between elevated and ambient plots were determined with a mixed, repeated measures analysis of variance at each depth interval. A significance level of $\alpha = 0.05$ was used for all tests.

3. Results

3.1. Soil physical properties

Soil particle density and pH for both soil depth zones was 2.5 $g\ cm^{-3}$ and 6.0, respectively. Bulk density was 1.1 $g\ cm^{-3}$ in the 0–7.5 cm zone and 1.3 $g\ cm^{-3}$ in the 7.5–15 cm zone. Soil organic matter varied from 6.4 to 9.0% from 0 to 7.5 cm and 3.6–6.3% from 7.5 to 15 cm. Soil nitrate and ammonium averaged approximately 0.1 and 2 $\mu g\ N\ g^{-1}$ soil, respectively (A. Finzi, pers. comm.). None of the physicochemical properties measured were significantly affected by CO_2 treatment except for soil organic matter. Mean percent soil organic matter for plots under FACE (elevated CO_2) were consistently higher than in controls for each month, and treatment differences ranged from 4 to 25%, depending on month of collection and depth (Fig. 1). A significant difference between enriched and ambient CO_2 soils was found for the 7.5–15 cm zone. Mean differences between FACE and control soils for this zone were greatest in August, with 24% higher soil organic matter under FACE. Treatment differences were less pronounced in October, where mean soil organic matter for elevated CO_2 plots was only 18% higher than controls for the 7.5–15 cm zone.

Field moisture content ranged from 27 to 47% WHC, depending upon the collection date, with the lowest moisture in August and the highest in May. Within a specific month and depth, samples did not show significant differences in soil moisture.

3.2. Net methane oxidation

Monthly mean net CH_4 oxidation rates averaged between 70 and 400 $pg\ g^{-1}\ h^{-1}$, with the highest rates in May and the lowest in October (Fig. 2). Carbon dioxide enrichment significantly decreased net CH_4 oxidation for these soils at both depths throughout the sampling period. Net CH_4

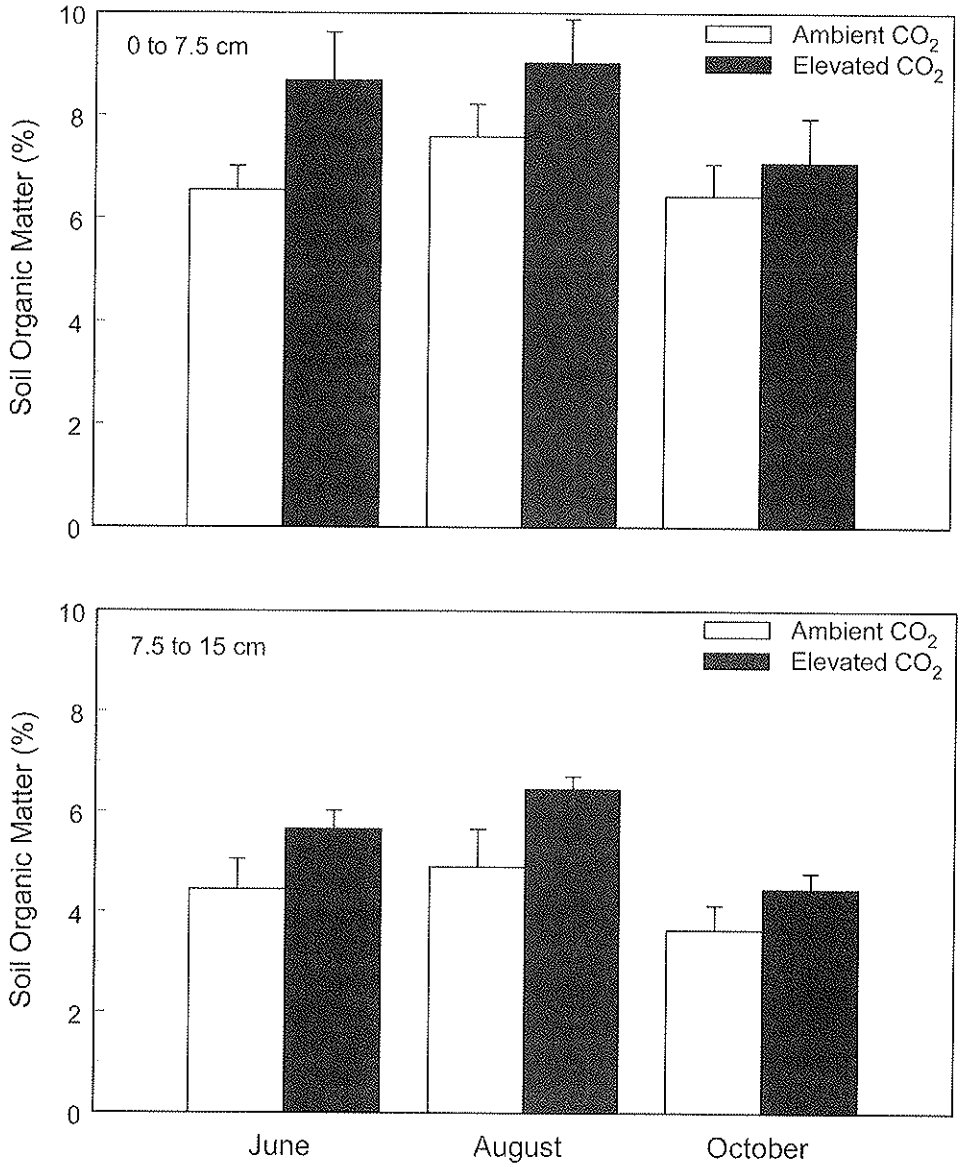
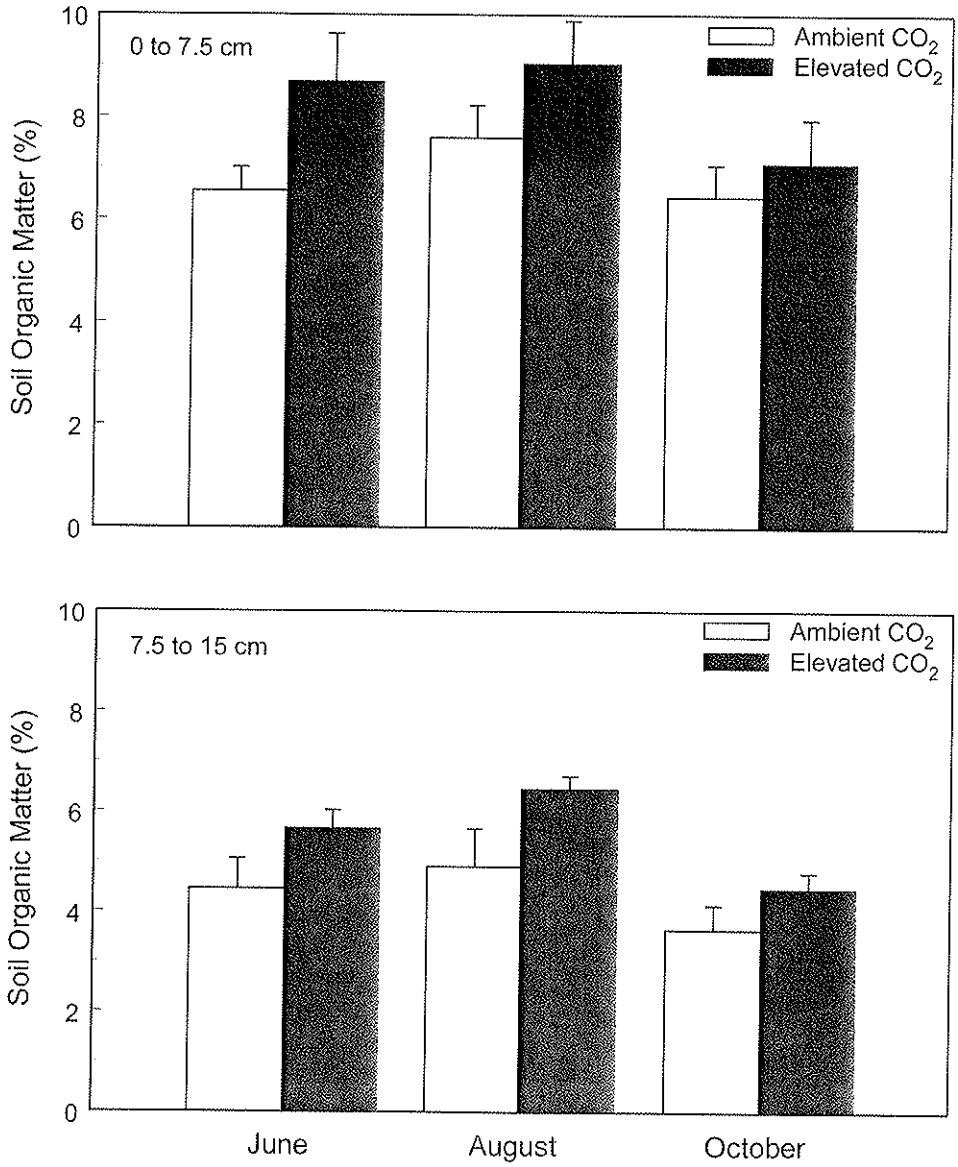


Fig. 1. Soil organic matter by treatment for two soil sampling zones that had been exposed for ~3 years to ambient or elevated CO_2. Error bars are ±one standard deviation.

oxidation rates for FACE soils in the 0–7.5 cm zone ranged from 104 to 198 pg g^{-1} h^{-1} and rates in the 7.5–15 cm zone ranged from 66 to 138 pg g^{-1} h^{-1}. In contrast, net CH_4 oxidation rates for controls ranged from 221 to 399 pg g^{-1} h^{-1} in the 0 to 7.5 cm zone and from 98 to 269 pg g^{-1} h^{-1} in the 7.5–15 cm zone. The smallest difference between treatments was found in August for the 7.5–15 cm soil depth, and the greatest difference was found in May for the 0–7.5 cm soil depth. The overall mean (±one standard deviation) rate of net CH_4 oxidation in FACE plots, averaged across all collection times at both depths, was over 47% lower (125 ± 71 pg g^{-1} h^{-1}; $n = 72$) than in control plots (234 ± 115 pg g^{-1} h^{-1}; $n = 72$). Soil moisture content for each depth and collection date was similar for both treatment groups.

Methane production was assessed by measuring changes in headspace CH_4 concentration over time before and after inhibition of methanotrophy for soils collected in August 1999. Prior to DFM application, overall mean CH_4 oxidation rate in the 0–7.5 cm soil depth zone (267 ± 119 pg g^{-1} h^{-1}; $n = 24$) was higher than mean CH_4 oxidation rate in the 7.5–15 cm soil depth zone (142 ± 45 pg g^{-1} h^{-1}; $n = 24$). Headspace CH_4 concentrations remained unchanged following DFM application (slopes of time courses were not significantly different from zero), indicating that methanogenesis was absent. Therefore, observed rates of CH_4 oxidation determined prior to DFM application resulted from methanotrophic activity alone. Hence, all measured rates represent not only net, but also gross rates of CH_4 oxidation.



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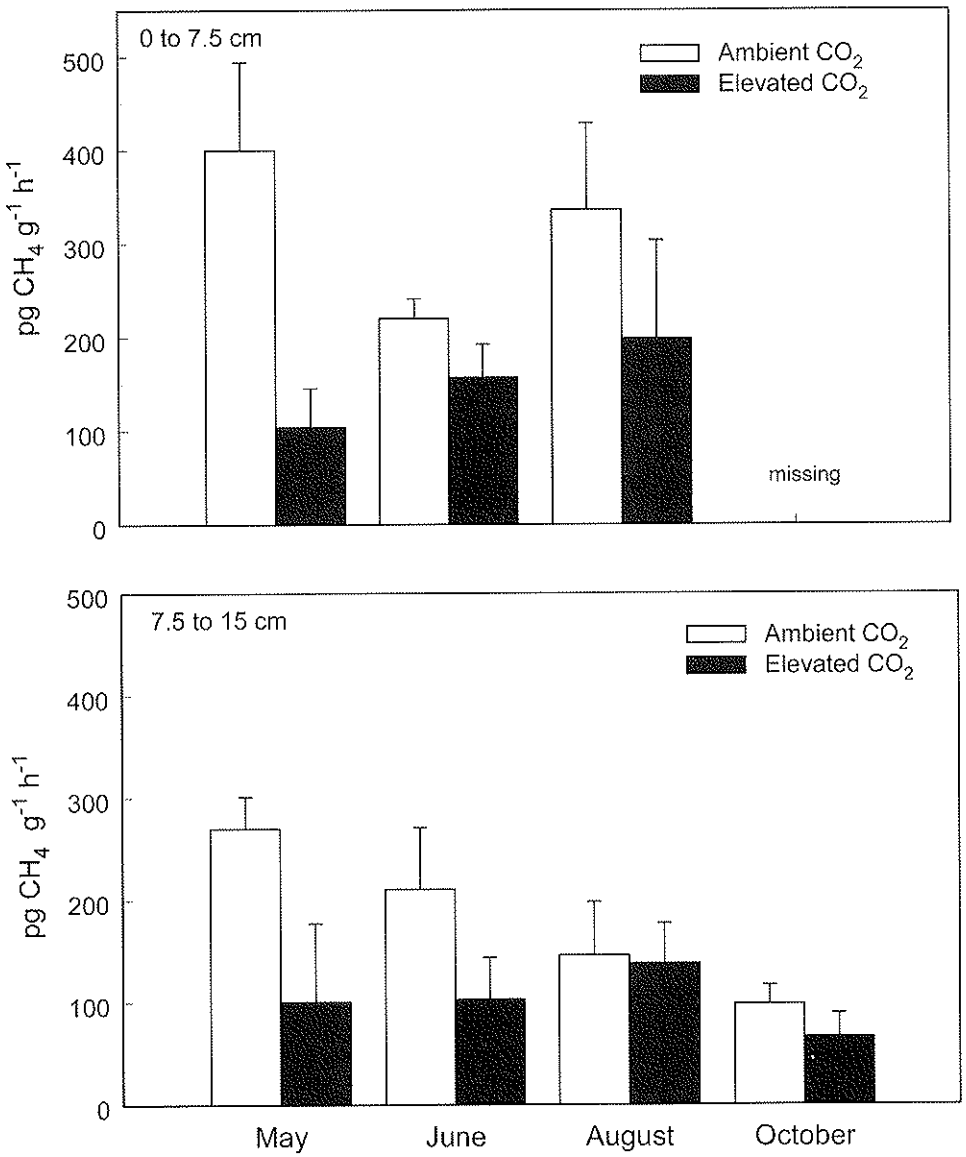


Fig. 2. Methane oxidation rates by treatment for two soil sampling zones that had been exposed for ~3 years to ambient or elevated CO_2. Rates were determined during laboratory incubations with soils collected every 8 weeks. Error bars are ±one standard deviation.

In the experiment designed to test the influence of labile carbon on CH_4 consumption, FACE soils consistently consumed less CH_4 than controls during the 14 days following soil collection (Fig. 3), and the effect of CO_2 treatment did not vary throughout the experiment. Differences in methanotrophic activity between elevated and ambient CO_2 persisted for 2 weeks, with a 30% lower mean net CH_4 oxidation rate under elevated CO_2 (159 ± 52 pg g^{-1} h^{-1}; $n = 63$) than in controls (227 ± 47 pg g^{-1} h^{-1}; $n = 63$) for the 0–7.5 cm soil depth. The mean net CH_4 oxidation rate was 47% lower under FACE (114 ± 40 pg g^{-1} h^{-1}; $n = 62$) than in controls (215 ± 64 pg g^{-1} h^{-1}; $n = 58$) for the 7.5–15 cm depth.

The addition of nutrient solutions did not affect CH_4 consumption at a concentration of 0.5 μmol g^{-1} soil, when compared with controls amended with deionized water. However, NH_4Cl at 1 μmol g^{-1} soil did inhibit CH_4 consumption, and this response did not vary with CO_2 treatment. The KCl amendment did not inhibit net CH_4 oxidation at either concentration, indicating that inhibition of net CH_4 oxidation by 1.0 μmol g^{-1} NH_4Cl was due to NH_4^+ rather than to a salt effect. Mean CH_4 consumption for the KCl, KNO_3, and H_2O treatments was 160–190 pg g^{-1} h^{-1} at both doses. However, the high dose of NH_4Cl reduced CH_4 consumption to 99 pg g^{-1} h^{-1}.

4. Discussion

Methane oxidation rates for soils from control plots (100–400 pg g^{-1} h^{-1}) found in this study are similar to those reported in laboratory incubations of other forest soils. Comparable studies have found atmospheric CH_4 consumption rates of about 400 pg CH_4 g^{-1} h^{-1} for Louisiana soils

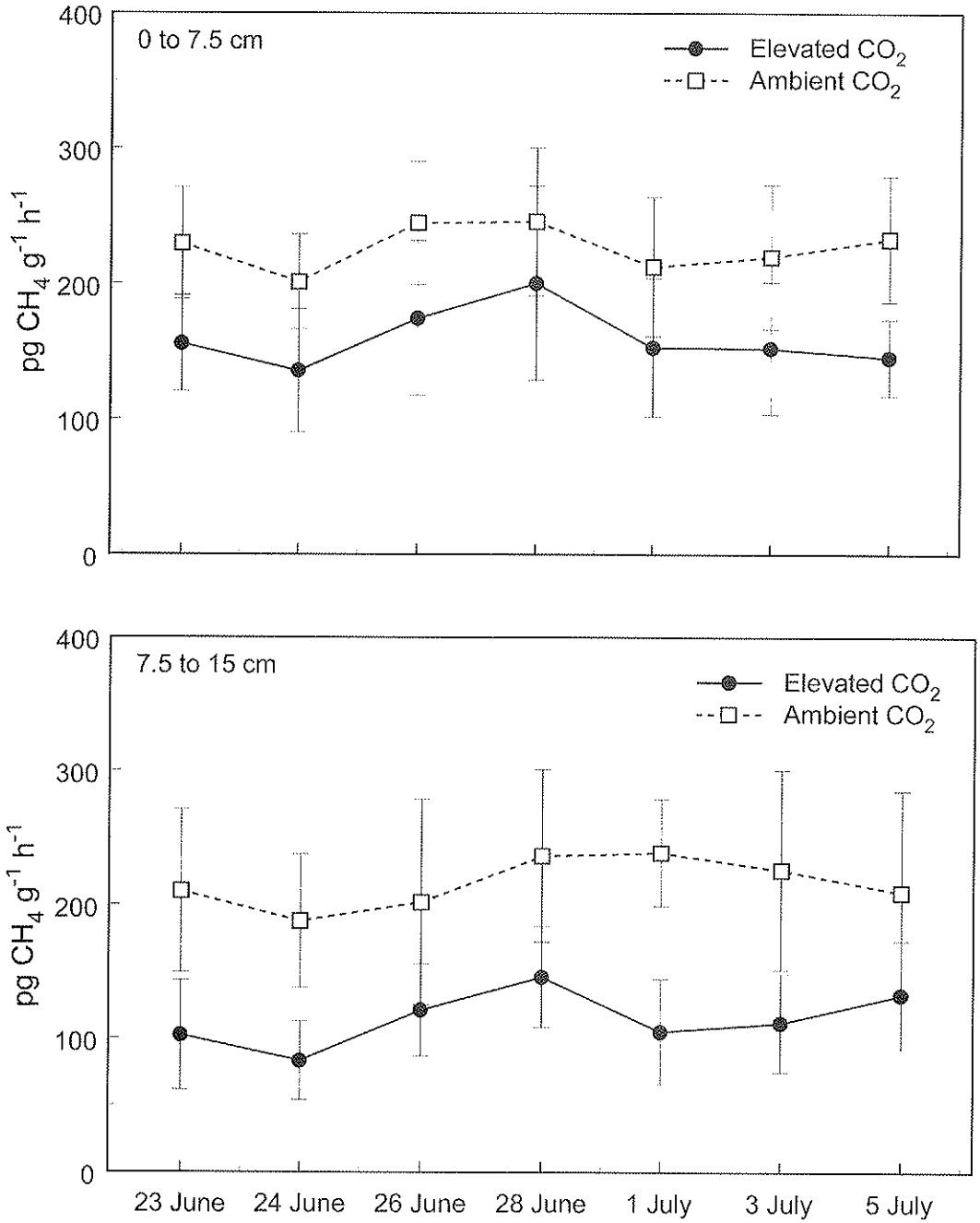
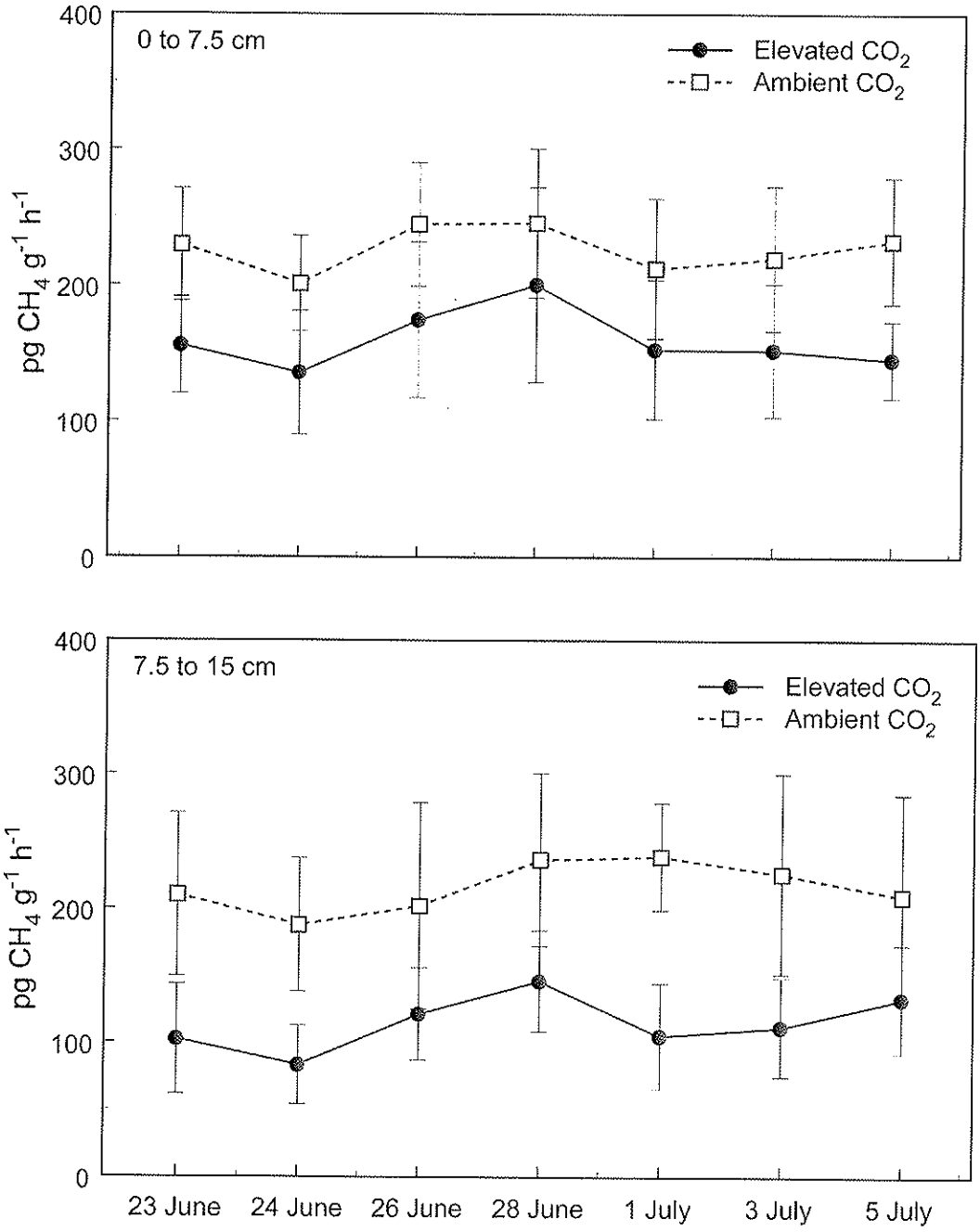


Fig. 3. Rates of methane oxidation over 14 days for two soil sampling zones, as influenced by ~3 years of CO_2 fumigation. Rates were determined during laboratory incubations with soils collected in June. Error bars are ±one standard deviation.

(Nesbit and Breitenbeck, 1992) and approximately 10 pg CH_4 g^{-1} h^{-1} for Maine soils at comparable soil water contents (Adamsen and King, 1993). Rates of CH_4 consumption for soils incubated at 4°C from a northern US hardwood forest were approximately 40 pg CH_4 g^{-1} h^{-1} for the mineral soil and 200 pg CH_4 g^{-1} h^{-1} for the lower organic horizon (Yavitt et al., 1995).

Methane flux measurements in other ecosystems, utilizing the static chamber technique, also indicate that an enriched CO_2 atmosphere may reduce net CH_4 flux. Ineson et al. (1998) found reduced CH_4 flux in a CO_2–enriched grassland relative to controls, but environmental factors (moisture, temperature, and nutrients) that may have contributed to variability in CH_4 flux were not measured. Ambus and Robertson (1999) reported a 22% reduction in net CH_4 flux when aspen cuttings grown in low nitrogen soils were exposed to 700 μl CO_2 l^{-1}. This decrease was attributed to an 11% increase in soil moisture in the elevated CO_2 plots (as CH_4 flux was highly correlated with soil moisture) which limited CH_4 diffusion. However, the authors observed no differences in net CH_4 oxidation between elevated and ambient CO_2 during soil laboratory



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Methane flux measurements in other ecosystems, utilizing the static chamber technique, also indicate that an enriched CO_2 atmosphere may reduce net CH_4 flux. Ineson et al. (1998) found reduced CH_4 flux in a CO_2–enriched grassland relative to controls, but environmental factors (moisture, temperature, and nutrients) that may have contributed to variability in CH_4 flux were not measured. Ambus and Robertson (1999) reported a 22% reduction in net CH_4 flux when aspen cuttings grown in low nitrogen soils were exposed to 700 μl CO_2 l^{-1}. This decrease was attributed to an 11% increase in soil moisture in the elevated CO_2 plots (as CH_4 flux was highly correlated with soil moisture) which limited CH_4 diffusion. However, the authors observed no differences in net CH_4 oxidation between elevated and ambient CO_2 during soil laboratory

incubations. Results of our study are in agreement with previous studies that showed a reduction in the CH_4 flux under elevated CO_2 (Ineson et al., 1998; Ambus and Robertson, 1999), although differences in methanotrophic and methanogenic activity specifically were not determined.

The environmental variables that typically control activity of methanotrophs cannot be invoked to explain the significantly reduced rates of net CH_4 oxidation for FACE plots relative to controls in our monthly rate measurements. There were no treatment-wise differences in %WHC, diffusivity, pH, land-use, inorganic-N concentrations, or incubation temperature. Further, N turnover times were similar for both elevated and ambient CO_2 soils (Allen et al., 2000), and the nutrient addition experiment demonstrated that the amount of NH_4^+ required to inhibit CH_4 oxidation in these soils greatly exceeds in situ concentrations. Increased methanogenesis under FACE could have reduced the rate of net CH_4 oxidation relative to controls. Other studies have reported low rates of methanogenesis in upland forest soils at ambient atmospheres (Adamsen and King, 1993; Yavitt et al., 1995) as well as enhanced methanogenesis in CO_2–amended plots (Dacey et al., 1994; Megonigal and Schlesinger, 1997). However, results of the DFM experiment clearly indicate that treatment-wise differences in net CH_4 oxidation were due solely to differences in methanotrophic activity, as methanogenesis was not observed in soils for either treatment.

Microbial biomass and community structure may be affected by changes in the quality and quantity of the carbon flow belowground (Sadowsky and Schortmeyer, 1997). Increased organic matter content in CO_2-enriched plots (Fig. 1) at this FACE site has been attributed to enhanced fine root production (Matamala and Schlesinger, 2000). Although we lack information (due to limited opportunity for destructive sampling) regarding the size and chemical composition of the labile carbon pool, it is clear that any differences between treatments did not affect the activity of the methanotrophs. Data are limited regarding the influence of organic compounds on CH_4 oxidation, and the results are equivocal. In agreement with our data, Schnell and King (1995) found that CH_4 oxidation was not affected by the addition of methanol, acetate, formate or glucose. Naturally occurring organic substrates, however, have been reported to inhibit CH_4 consumption in forest soils (Amaral et al. 1997). Carbon dioxide enrichment reduced methanotrophy under FACE, but we did not find evidence linking CH_4 oxidation to the labile fraction of soil organic carbon. Lower rates of net CH_4 oxidation under elevated CO_2 may not be directly related to soil organic matter content, yet additional soil carbon may induce changes in microbial community ecology and modify population dynamics at an ecosystem scale (Ringelberg et al., 1997). Altered methanotrophic activity in CO_2-enriched soils may result from increased predation (Groffman, 1999) or by a community shift among populations of organisms that utilize simple carbon compounds, but this was not examined in this study.

Reduced methanotrophic activity with CO_2 enrichment may represent acclimation rather than equilibration. Down-regulation, or a reduced metabolic response to treatment with time, has been reported for some biological processes in other CO_2–enriched environments (Tissue et al., 1997). The duration of the CO_2 effect in this pine forest is unknown and long-term monitoring will be necessary to determine if the decrease in net CH_4 oxidation is an equilibrium response. Moreover, similar, long-term measurements are necessary in other FACE sites to determine the spatio-temporal effect of CO_2 enrichment on atmospheric CH_4 consumption. This information should improve predictive capabilities for consumption and emission of greenhouse gases by soils in projected future climates.

5. Conclusions

We have observed differences in methanotrophic activity between soils from an upland forest ecosystem grown for 3 years under enriched and ambient CO_2. Soils evaluated from May through October indicate that this coniferous forest ecosystem has responded to increased CO_2 with a 47% reduction in methanotrophic activity. A significant CO_2 enrichment effect persisted for 2 weeks after soils were sampled, suggesting that reduced CH_4 oxidation under FACE continued beyond the residence time of labile soil carbon. Also, differences between treatments resulted from altered methanotrophic activity as opposed to altered rates of methanogenesis. Consistently lower rates of CH_4 oxidation for FACE soils as compared with controls is evidence of altered ecosystem function as a result of exposure to an elevated CO_2 atmosphere. Possible long-term consequences of a rising CO_2 atmosphere may be a reduction in the strength of the CH_4 soil sink and a positive feedback to the greenhouse effect. Further studies are needed to determine the cause of differences in CH_4 oxidation under FACE, whether the response is persistent, and whether the response is common among forests or across other ecosystems. A decrease in soil CH_4 consumption similar to the 47% observed here will significantly affect the atmospheric CH_4 budget and, consequently, future climates.

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