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ORIGINAL PAPER

Increased moisture and methanogenesis contribute to reduced methane oxidation in elevated CO_2 soils

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Abstract Awareness of global warming has stimulated research on environmental controls of soil methane (CH_4) consumption and the effects of increasing atmospheric carbon dioxide (CO_2) on the terrestrial CH_4 sink. In this study, factors impacting soil CH_4 consumption were investigated using laboratory incubations of soils collected at the Free Air Carbon Transfer and Storage I site in the Duke Forest, NC, where plots have been exposed to ambient (370 μL L^{-1}) or elevated (ambient+200 μL L^{-1}) CO_2 since August 1996. Over 1 year, nearly 90% of the 360 incubations showed net CH_4 consumption, confirming that CH_4-oxidizing (methanotrophic) bacteria were active. Soil moisture was significantly ($p<0.01$) higher in the 25–30 cm layer of elevated CO_2 soils over the length of the study, but soil moisture was equal between CO_2 treatments in shallower soils. The increased soil moisture corresponded to decreased net CH_4 oxidation, as elevated CO_2 soils also oxidized 70% less CH_4 at the 25–30 cm depth compared to ambient CO_2 soils, while CH_4 consumption was equal between treatments in shallower soils. Soil moisture content predicted ($p<0.05$) CH_4 consumption in upper layers of ambient CO_2 soils, but this relationship was not significant in elevated CO_2 soils at any depth, suggesting that environmental factors in addition to moisture were influencing net CH_4 oxidation under elevated CO_2. More than 6% of the activity assays showed net CH_4 production, and of these, 80% contained soils from elevated CO_2 plots. In addition, more than 50% of the CH_4-producing flasks from elevated CO_2 sites contained deeper (25–30 cm) soils. These results indicate that subsurface (25 cm+) CH_4 production contributes to decreased net CH_4 consumption under elevated CO_2 in otherwise aerobic soils.

Keywords Elevated CO_2 · CH_4 · Methanotrophy · Methanogenesis

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Introduction

Soil methane (CH_4) consumption by CH_4-oxidizing (methanotrophic) organisms is of crucial importance to the global CH_4 budget. It is the largest terrestrial CH_4 sink, oxidizing nearly half of all soil-produced CH_4, and it consumes 40–60 Tg per year of CH_4 directly from the atmosphere (Watson et al. 1990; Reeburgh et al. 1993). CH_4 oxidation is strongly influenced by soil moisture, organic matter content, texture, and nutrient status, but quantifying their effects on net CH_4 consumption in soils has been a challenge for microbial ecologists, in part because of the relatively recent finding that CH_4 production, a process dependent on an absence of O_2, can occur in macroscopically oxic soils (Yavitt et al. 1995; Horz et al. 2002). As a result, modeling efforts can be stymied by the existence of soil CH_4 sources and sinks in close proximity to one another, even within the same soil particle (Nedwell 1996; von Fischer and Hedin 2002).

Moisture is clearly one of the most important environmental factors influencing atmospheric CH_4 consumption in unsaturated soils (Born et al. 1990; Koschorreck and Conrad 1993). CH_4 diffusion is 10,000-fold slower in water than in air, and thus, increased soil moisture reduces diffusive transport of atmospheric CH_4 to the subsurface methanotrophs, limiting rates of soil CH_4 consumption (Koschorreck and Conrad 1993; Castro et al. 1994). Increased soil moisture can also

promote soil anoxia and subsequent methanogenesis that may contribute to diminished net CH_4 consumption (Sexstone and Mains 1990; Yavitt et al. 1995).

Recent research efforts into environmental controls of soil CH_4 consumption have focused on the effects of increasing atmospheric carbon dioxide (CO_2) on the terrestrial CH_4 sink, studies stimulated by an increased awareness of global warming and the roles that these two greenhouse gases will play in mediating future climate change. At some sites, soil CH_4 consumption has been shown to decrease by 23 to 67% under conditions of elevated atmospheric CO_2 (Ineson et al. 1998; Ambus and Robertson 1999; Phillips et al. 2001b), but the mechanisms controlling these reductions are largely unclear. Plant-mediated soil moisture increases have corresponded to decreased net methanotroph activity at some elevated CO_2 sites (Ambus and Robertson 1999; McLain et al. 2002). However, decreases in CH_4 oxidation under elevated CO_2 with no concomitant increases in soil moisture have also been reported (Phillips et al. 2001b), raising the possibility that other changes to the soil environment contribute to reduced CH_4 oxidation.

Over 2 years (March 1999 to February 2001), we monitored the effects of elevated atmospheric CO_2 on CH_4 cycling at the Free Air Carbon Transfer and Storage (FACTS)-I facility in the Duke University forest, a loblolly pine (*Pinus taeda* L.) ecosystem exposed to Free Air Carbon Dioxide Enrichment (FACE) technology. These soils embody a substantial CH_4 sink, consuming more than 200 mg CH_4 m^{-2} $year^{-1}$ (McLain et al. 2002), but from 1999 to 2001, atmospheric CO_2 enrichment decreased net CH_4 consumption by 26%, an inhibition that corresponded to 20% higher volumetric soil moisture in the elevated CO_2 plots (McLain et al. 2002). While soil moisture accounted for a significant proportion of the variability in the surface CH_4 fluxes, statistical analyses showed that the moisture differences mediated only half of the measured decrease in CH_4 consumption in elevated CO_2 plots (McLain et al. 2002). Laboratory incubations using sieved soils collected at 0–15-cm depths on three sampling dates (May to October 1999) confirmed the decreased CH_4 consumption but revealed no causative factors such as moisture increases or CH_4 production (Phillips et al. 2001a).

This work extends the findings of Phillips et al. (2001a) by reporting the results of laboratory activity assays conducted using soils collected at three sampling depths (0–25 cm) on ten sampling dates over 11 months (February to December 2000). By obtaining laboratory observations of CH_4 cycling activity from soil samples collected at individual depths over four seasons and by incubating these unsieved soils under atmospheric CH_4 concentrations closely resembling those found in situ, we hoped to more closely identify the changes to the soil ecology that were contributing to the observed year-round decreases in surface CH_4 flux in FACTS-I-elevated CO_2 soils. In addition, by incubating soils under a range of depths from 0 to 30 cm, we hoped to identify the soil layer in which methanotroph activities were most strongly inhibited. Results of this intensive study will aid in the understanding of potential changes to CH_4 cycling that may occur in future climates.

Materials and methods

Site description

The FACTS-I site consists of three control and three treatment plots, each 30 m in diameter, in a 25-year-old loblolly pine plantation in the Duke Forest, Orange County, NC (35°58′N, 70°05′W). Since fumigation began in August 1996, CO_2 has been delivered to maintain a concentration of ~200 μL L^{-1} above ambient or 570±30 μL L^{-1} in treatment plots (Hendrey et al. 1999), a level projected to occur within the next 50 years if current rates of atmospheric CO_2 increase are sustained (Schlesinger 1997). Control plots are fumigated with ambient air to replicate micrometeorological effects of the FACE technology.

The FACTS-I forest is dominated by a loblolly pine plantation. Soils at the site are of the Enon Series, Ultic Alfisols, with low nitrogen and phosphorus availability typical of many upland areas in the Southeast. The soils are derived from igneous rock, yielding an acidic (pH≈5.75), well-developed profile, consisting of a thick organic layer at the soil surface (0–5 cm) underlain by mixed clay mineralogy (25+ cm) (USDA-SCS 1977).

Soil sampling and physical analyses

Each FACTS-I plot is divided radially into four equal-sized sampling sectors. On each sampling date, soil cores (1.9×~35 cm) were removed from two sectors of each ring at random locations, but avoiding sites used for previous cores. Samples were obtained with an unslotted stainless steel soil recovery probe (AMS, American Falls, ID) fitted with a butyrate plastic liner (Forestry Suppliers, Jackson, MS) and a slide-hammer attachment (AMS). After the probe was withdrawn, the liner was removed, clipped to remove unfilled portions, sealed with polyethylene caps (Forestry Suppliers), and transported to the laboratory on ice.

In the laboratory, soil cores were placed in an anoxic glovebox (Coy Laboratory Products, Grass Lake, MI) under a N_2/H_2 ratio of 98:2 to prevent introduction of O_2. Subsamples at 0–5-, 15–20-, and 25–30-cm depths were analyzed for gravimetric water content (mL H_2O g^{-1} dry soil) by oven drying samples at 105°C for 48 h. Soil water-holding capacities (WHCs; mL H_2O g^{-1} dry soil) were

measured three times during the study period using a pressure plate (Soilmoisture Equipment, Santa Barbara, CA) and the method of Klute (1965). Briefly, a saturated soil sample was placed on a porous ceramic plate inside a pressure cylinder, whereupon N_2 was forced into the apparatus to attain a net pressure, forcing water from the soil. WHC was calculated as the amount of water held by a soil sample between pressures of 0.1 (field capacity) and 15 bars (permanent wilting point). The gravimetric water content of each soil subsample was converted to %WHC by dividing the water content by the WHC (mL H_2O g^{-1} dry soil) and multiplying by 100. Soil pH was measured using the method of Van Lierop (1990), in which 2.5 g of sieved, air-dried soil and 5 mL of ultrapure H_2O were combined and vortexed vigorously for ~30 s. Solids were allowed to settle, and the pH of the supernatant was determined with an Orion 420A meter and a Ross model 8005 electrode (Thermo Fisher Scientific, Waltham, MA). Soil C and N contents were determined by a dry combustion analyzer interfaced with a Europa Hydra 20/20 IRMS (Europa Scientific, Crewe, UK).

To minimize destructive sampling inside the FACTS-I rings, bulk densities (BD) at the 0–5-, 15–20-, and 25–30-cm soil depths were measured directly outside of the fumigated plots using the metal ring sampler technique (Foth et al. 1982). Briefly, a 30-cm-deep trench was dug, and a metal cylinder with an interior volume of 161.1 cm^3 was inserted into the side of the trench at the given depth to remove a known volume of soil. Soil samples were oven dried at 105°C for 48 h, weighed, and the BD (g cm^{-3} dry soil) was calculated.

Methane consumption activity assays

During construction of the FACTS-I site, soil gas wells were installed at 15- and 30-cm depths in each sector of each ring, allowing for the collection of porespace gases from individual depths corresponding to the soil core subsample depths. The gas wells consist of a 5 cm diameter×10 cm height pipe (internal volume ~200 cm^3) situated vertically in the soil such that its open bottom rests at the depth of interest, while its top is closed with a two-holed rubber stopper. Plastic tubes extend from the buried cylinder to the soil surface where they are sealed by Kynar® caps attached with stainless steel Swagelok connectors. On each sampling date, the plastic tubes were evacuated by pulling 45 cm^3 of gas from the buried cylinder, whereupon soil gas samples were withdrawn from the cylinder using a stainless steel vacuum manifold designed according to Andrews et al. (1999) and 75 cm^3 Whitey stainless steel cylinders that had been evacuated to 10^{-5} Pa before sampling. Gas samples were then transported to the laboratory to be used as headspace gas in the activity assays. Gas sampling from these cylinders was performed at a minimum of 2-week intervals to allow for equilibration between the cylinders and the porespace air. Additional information on the cylinder design and use can be found in Andrews et al. (1999).

Net CH_4-cycling activity was measured ten times in vitro from February through December 2000 using subsamples of the soil cores described above. A total of 360 incubations (ten sampling dates × six FACE rings × two soil samples per ring × three depths) were performed in which individual subsamples (3.0 g fresh weight) from three depths (0–5, 15–20, and 25–30 cm) were transferred from the soil cores into sterile 30-mL serum bottles and sealed with teflon-lined butyl rubber stoppers with crimp seals. Each serum bottle was purged with N_2 for 1 min, evacuated, and refilled with soil gases from the corresponding depth to reproduce atmospheric conditions in situ as closely as possible. Bottles were pressurized to 1.1 atm to prevent vacuum development during subsequent sampling. Replicate bottles without soil controlled for potential gas emission by rubber stoppers, and sterile controls were obtained by autoclaving core samples at 121°C for 1 h on each of 3 consecutive days.

Bottles were incubated at their respective field temperatures (4–20°C) in the dark, and 0.5-mL gas samples were removed from each bottle at 0, 4, 8, 12, 24, and 48 h and analyzed for CH_4 by gas chromatography/flame ionization detector (Varian 3400, GS-Q divinylbenzene homopolymer-fused silica capillary column [J&W Scientific, Folsom, CA], 30 m×0.53 mm inner diameter, at 150°C with 250°C detector and 30 mL min^{-1} He carrier gas). Certified CH_4 standards were used for calibration (Scott Specialty Gases, Plumsteadville, PA). Data revealed first-order decreases or increases in CH_4 with time, and net CH_4 cycling rates were calculated, by dividing the slope from the linear portion of the exponential decrease or increase (Koschorreck and Conrad 1993) of CH_4 within the incubation by the molar volume of the gas held in each incubation flask, to achieve a CH_4-cycling rate in moles. After completion of the assay, incubated soils were oven dried at 105°C for 48 h to determine gravimetric water content, and each CH_4 cycling rate was corrected for the moisture content of the soil sample.

Statistical analyses

Differences in CH_4 cycling between treatments were assessed using repeated-measures analysis of variance, with Tukey's post-hoc test to locate differences between the means. Fixed-effects linear regression models were used to investigate the effects of soil moisture on CH_4 cycling. All statistical estimation procedures and null hypothesis tests for regression coefficients within each model were per-

formed using the software package Splus2000 (Professional release 1; copyright 1998–1999 MathSoft). All models were fit using Splus functions *lm*, *lme*, and *aov*.

Results and discussion

Soil physical and chemical analyses

Analysis of cores collected within each ring showed that elevated CO_2 soils had significantly ($p=0.03$) greater surface (0–5 cm) organic C contents (Table 1), in agreement with other studies performed at the FACTS-I site (Schlesinger and Lichter 2001). No differences in C or N between the ambient and elevated CO_2 soils were detected in any other depth. Soil pH values were similar at all depths (Table 1), and no differences in pH were found between ambient and elevated CO_2 soils.

Many studies of microbial production and consumption of gases express activity in absolute terms (e.g., CO_2 production g^{-1} dry soil h^{-1}). However, it has been reported that the expression of methanotroph activity on a gravimetric basis in soils of differing BD can lead to biases in data analysis (Bradford et al. 2001). FACTS-I soils showed increased ($p<0.01$) BD with depth (Table 1), with no differences in BD found ($p=0.46$) between ambient and elevated CO_2 soils. Thus, data resulting from soil incubations was standardized using the BD of the corresponding soil depth to express CH_4-cycling activity on a volumetric soil basis.

Methane oxidation activity: depth dependence

Throughout the study, a total of 360 activity measurements, representing two replicates of each sample from each depth (0–5, 15–20, and 25–30 cm), were performed. Initial headspace concentrations of CH_4 within the incubation flasks decreased with depth, but these concentrations were equal between CO_2 treatments (Table 2). Activity assays confirmed that CH_4-cycling microbial communities were active throughout the 0–30-cm layer of FACTS-I soils, as 87.0% (313 flasks) showed net CH_4 consumption during the 48-h assay, while 6.1% (22 flasks) had no net CH_4 consumption or production, and 6.9% (25 flasks) exhibited net CH_4 production. As reported in a variety of temperate ecosystems (Koschorreck and Conrad 1993; Castro et al. 1994; Roslev et al. 1997), the strongest CH_4 sink was located below the surface (0–5 cm) layer. The 15–20-cm soils consumed the most ($p<0.01$) CH_4 by volume, averaging -3.7 ± 1.3 ng CH_4 cm^{-3} soil day^{-1} over the study period (Fig. 1), while consumption rates in surface soils (0–5 cm; -2.2 ± 0.8 ng CH_4 cm^{-3} soil day^{-1}) were equal ($p=0.52$) to those in the 25–30-cm layer (-2.0 ± 1.3 ng CH_4 cm^{-3} soil day^{-1}).

Results of the in vitro activity assays agreed with in situ surface flux measurements (Phillips et al. 2001b; McLain et al. 2002) in that net CH_4 consumption was inhibited in elevated CO_2 soils (Fig. 1). At 0–5 cm, CH_4 oxidation in ambient CO_2 rings (-2.5 ± 0.8 ng CH_4 cm^{-3} soil day^{-1}) was 26% higher than that of elevated CO_2 rings (-1.9 ± 0.8 ng CH_4 cm^{-3} soil day^{-1}), but this inhibition was only marginally significant ($p=0.07$). No significant differences in CH_4 consumption were found between treatments in the 15–20-cm depth, where ambient CO_2 soils consumed -4.1 ± 1.1 ng CH_4 cm^{-3} soil day^{-1}, and elevated CO_2 soils consumed -3.3 ± 1.5 ng CH_4 cm^{-3} soil day^{-1}, a difference of 21%. Significant ($p<0.01$) inhibition in CH_4 oxidation was measured in the elevated CO_2 treatment soils collected from the 25–30-cm depth, which consumed 70% less CH_4 (-0.9 ± 1.3 ng CH_4 cm^{-3} soil day^{-1}) than the same depth of ambient CO_2 soils (-3.0 ± 1.0 ng CH_4 cm^{-3} soil day^{-1}).

The CH_4 oxidation rates in the 0–15-cm soil layer were lower than those reported by Phillips et al. (2001a) in FACTS-I soil laboratory incubations (-3.0 to -6.2 ng CH_4 cm^{-3} day^{-1}). However, Phillips et al. (2001a) performed incubations using sieved (5 mm) soils, while measurements in the current study were performed using intact soil core subsamples. It is highly likely that sieving the FACTS-I soils increased soil diffusivity (Bradford et al. 2001), thereby increasing the rates of CH_4 oxidation found by Phillips et al. (2001a).

Soil moisture: depth dependence

WHCs of FACTS-I soil samples showed some variation with depth (Table 3). The organic 0–5-cm soils had higher

Table 1 Physical and mineral properties of FACTS-I soils. Numbers indicate average values of all assays (±1 standard deviation)

CO_2 treatment	Depth	Organic C (g kg^{-1})	Total N (g kg^{-1})	C/N	pH	Bulk density (g cm^{-3})
Ambient	0–5	23.58 (3.50)	0.94 (0.21)	25.08	5.66 (0.08)	0.84 (0.15)
	15–20	14.27 (2.10)	0.67 (0.11)	21.30	5.75 (0.10)	1.14 (0.08)
	25–30	5.34 (1.48)	0.42 (0.12)	12.71	5.60 (0.19)	1.47 (0.12)
Elevated	0–5	28.79 (2.65)	1.18 (0.33)	24.40	5.52 (0.29)	0.86 (0.11)
	15–20	12.98 (2.65)	0.72 (0.12)	18.03	5.63 (0.18)	1.16 (0.15)
	25–30	6.21 (2.43)	0.41 (0.08)	15.15	5.56 (0.24)	1.43 (0.10)

Table 2 Initial methane concentrations in headspace of incubation flasks, Feb–Dec 2000. Initial headspace concentrations of methane were equal at each depth in ambient and elevated CO_2 flasks

Depth (cm)	Ambient CO_2 ($\mu L\ L^{-1}$)	Elevated CO_2 ($\mu L\ L^{-1}$)	p H_o: Ambient CO_2=Elevated CO_2*
0–5	1.89±0.23	1.83±0.19	0.52
15–20	1.23±0.02	1.25±0.28	0.60
25–30	0.91±0.28	0.94±0.32	0.26

* Student's t test, n=3; H_o cannot be rejected at any depth.

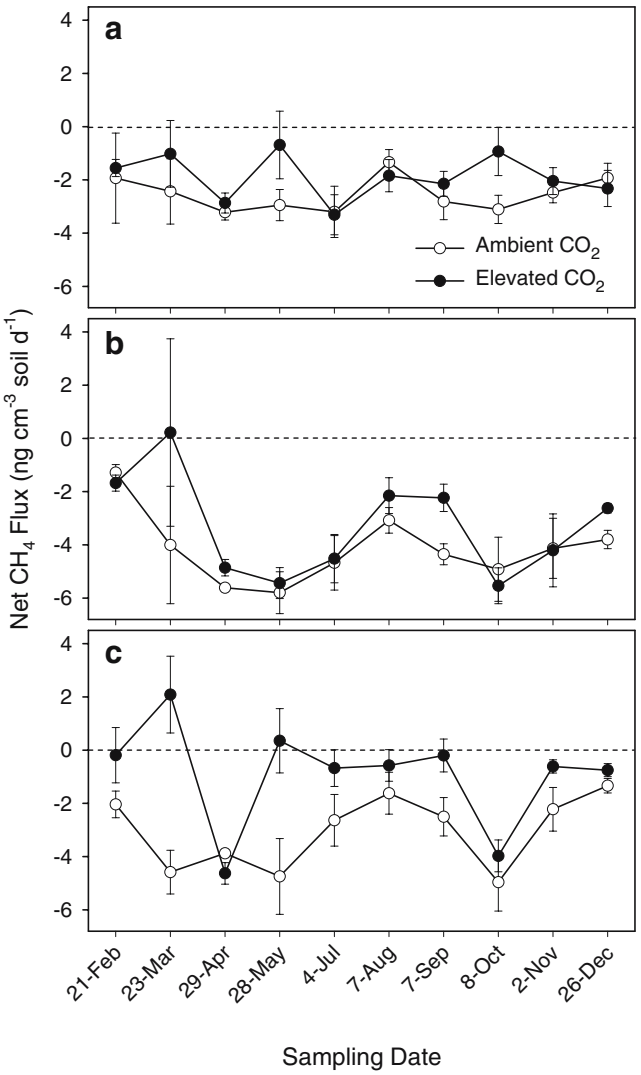

Fig. 1 Net CH_4 consumption in soil subsamples collected at 0–5- (**a**), 15–20- (**b**), and 25–30-cm (**c**) depths in FACTS-I sites over the 11-month sampling period. *Symbols* represent the average of two duplicate incubations performed at each depth under each CO_2 treatment (elevated and ambient), ±1 standard deviation. *Dotted line* represents division between CH_4 production (*above*) and consumption (*below*)

(p<0.01) WHC than the deeper mineral layers, while WHC in the 15–20- and 25–30-cm depths were equal (p=0.65). There were no significant differences in WHC between the six FACTS-I rings, and the average (0–30 cm) WHC of elevated CO_2 soils was equal to that of the ambient CO_2 soils (Table 3). These significant differences in WHC precluded the use of absolute expressions of water content (g H_2O g^{-1} dry soil) in this study for comparison of moisture effects on microbial activities (Gulledge and Schimel 1998). Instead, percent WHC, a parameter that parallels percent water-filled porespace (Linn and Doran 1984) and can thus provide an index of diffusivity across a range of physically different soils (Aulakh et al. 1991; Gulledge and Schimel 1998), was utilized to compare moisture effects on microbial activities between the three measured depths.

Moisture content measurements of the soil subsamples revealed seasonal patterns in the upper (0–5 and 15–20 cm) soil layers, where soils were wettest in mid-winter and drier in the summer and early fall (Fig. 2). One exception to this occurred on the August sampling date, after a rainfall event on August 1–5 during which more than 75 mm of precipitation fell at the site (State Climate Office of North Carolina 2004). Moisture contents measured in soil cores were statistically equal in the ambient and elevated CO_2 rings at the 0–5- (p=0.83) and 15–20-cm (p=0.30) depths. At the 25–30-cm soil depth, elevated CO_2 soil moisture contents were significantly (17.2%; p=0.01) higher than ambient. In situ measurements using time domain reflectometry probes confirmed that from February to December 2000, volumetric soil moisture averaged 17.0% higher in the 0–30-cm layer of elevated CO_2 soils compared to ambient (Duke FACE Facility 2007). Similar increases in soil moisture under CO_2 fumigation have also been reported in grasslands (Owensby et al. 1997; Niklaus et al. 1998) and in hardwood stands (Ambus and Robertson 1999).

The causes of the increased soil moisture in elevated CO_2 plots of FACTS-I soils have been the subject of debate. The moisture differences increased over time from a difference of 0.03±0.01 mL cm^{-3} during the first year of CO_2 fumigation (1997) to 0.08±0.05 mL cm^{-3} in 1999 and

Table 3 Water holding capacity (WHC) at three depths for FACTS-I soils

Depth (cm)	Ambient CO_2 (mL H_2O g^{-1} soil)	Elevated CO_2 (mL H_2O g^{-1} soil)	p H_o: Ambient CO_2=Elevated CO_2*
0–5	1.14±0.04	1.11±0.08	0.72
15–20	0.70±0.02	0.69±0.03	0.73
25–30	0.66±0.04	0.71±0.04	0.44
All (0–30)	0.84±0.04	0.83±0.01	0.98

*Student's t test, n=3; H_o cannot be rejected at any depth.

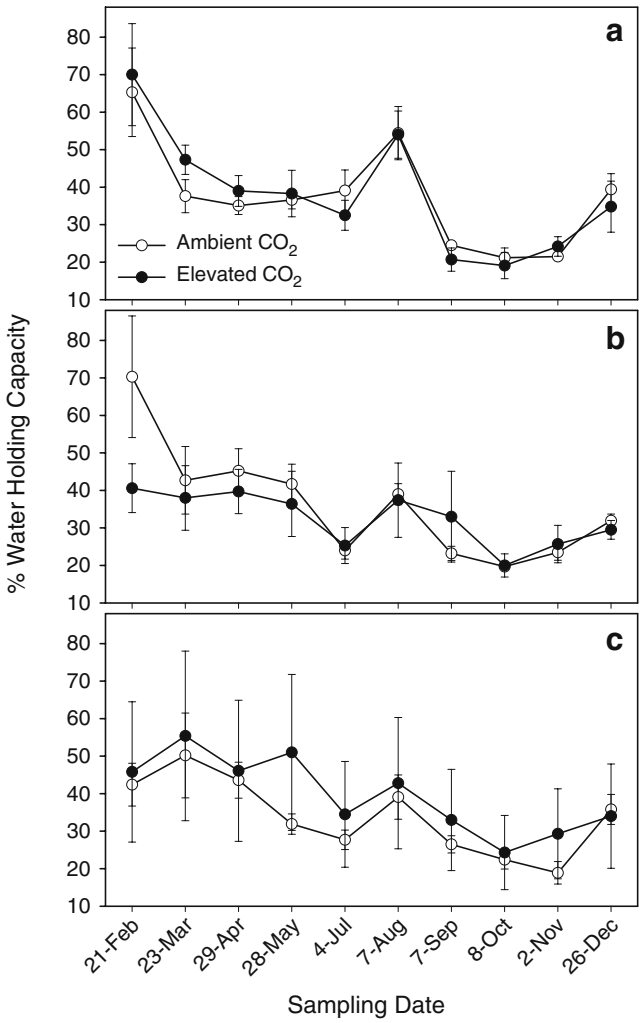

Fig. 2 Moisture contents (percent water-holding capacity) of soil subsamples collected at 0–5 (**a**), 15–20 (**b**), and 25–30-cm (**c**) depths in FACTS-I sites over the 11-month monitoring period. *Symbols* represent the average of two duplicate incubations performed at each depth under each CO_2 treatment (elevated and ambient), ±1 standard deviation

to 0.10±0.05 mL cm^{-3} in 2000 (Schäfer et al. 2002). The moisture increases in elevated CO_2 plots may reflect a reduction in water loss through evaporation from the soil surface. Increased plant biomass accumulation resulted in 29% more litter deposited in elevated CO_2 plots relative to ambient in 1999 (Schlesinger and Lichter 2001), and this thicker litter layer may inhibit surface evaporation, possibly increasing soil moisture retention under elevated CO_2 in FACTS-I soils (Schäfer et al. 2002).

Methane oxidation activity measurements: moisture dependence

Soil moisture is a key regulator of diffusive transport of atmospheric CH_4 into soils (Born et al. 1990; Dörr et al. 1993). Researchers have often reported negative correlations between soil moisture and CH_4 consumption in field soils (Koschorreck and Conrad 1993; Castro et al. 1995), and in situ surface CH_4 flux in FACTS-I soils was significantly ($p<0.01$) negatively correlated with volumetric moisture from March 1999 to February 2001 (McLain et al. 2002). Linear regression confirmed that the average %WHC from 0 to 30 cm accounted for significant variability in CH_4 consumption (also averaged 0–30 cm) in the soil incubations from both the ambient ($p=0.05$) and elevated ($p=0.02$) CO_2 rings (Fig. 3), with decreasing CH_4 oxidation correlated with increasing soil moisture. A significant relationship between net soil CH_4 oxidation and WHC has also been reported for European forest soils (Koschorreck and Conrad 1993).

Although a significant relationship was found between CH_4 consumption and %WHC when averaged over 0–30 cm (Fig. 3), %WHC did not account for significant variability in CH_4 oxidation in the 0–5 cm FACTS-I soils from the elevated CO_2 rings ($p=0.37$), while the relationship in the ambient CO_2 rings was near significant ($p=0.07$). At the 15–20-cm depth, %WHC was not correlated with CH_4-cycling activity in the elevated CO_2 rings ($p=0.61$) but was significantly so in the ambient CO_2 rings ($p=0.04$). Thus, while methanotroph activity results show strong moisture/CH_4 oxidation correlations in the upper soil layers under ambient CO_2, results from the elevated CO_2 rings suggest the presence of additional factors impacting net CH_4 oxidation. Soil moisture was not strongly correlated with CH_4 oxidation in the 25–30-cm layer ($p=0.18$ and 0.81 in elevated and ambient CO_2 rings, respectively). The higher

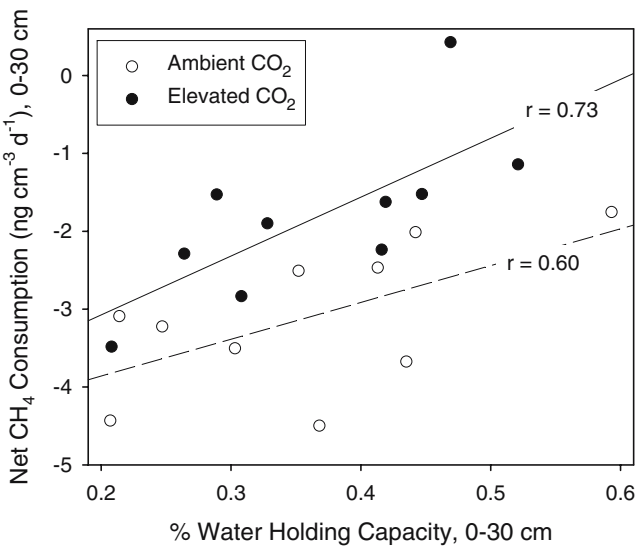

Fig. 3 Net CH_4 consumption in relation to 0–30-cm soil moisture (percent water holding capacity) in FACTS-I soil samples over the 11-month sampling period. *Regression lines* are shown for ambient (*dotted line*) and elevated (*solid line*) CO_2 treatments

clay content of the deeper soils most probably accounted for this observation, as soil gas diffusion is more strongly controlled by texture than by moisture content in finer-textured soils (Dörr et al. 1993).

Net CH_4 production

Of the 360 activity assays performed during this study, a total of 25 flasks showed net CH_4 production at ambient temperature and moisture (Fig. 4). Thus, although FACTS-I soils are macrosopically oxic from 0 to 30 cm (McLain et al. 2002), localized anaerobic conditions that stimulate the activity of methanogens may increase in response to environmental stimuli (e.g., root respiration or moisture increases). The consistent dominance of methanotroph activity, evidenced by net surface CH_4 consumption at all sites during a 2-year period (McLain et al. 2002) and the dominance of net CH_4 oxidation in activity flasks, indicates that only very low levels of CH_4 production may be occurring. Yet, methanogen activity has been documented in the upper layers of macroscopically oxic soils in other ecosystems (Yavitt et al. 1995; Castro et al. 2000; Horz et al. 2002), as O_2-deficient microniches exist in generally oxic soils (Sexstone et al. 1985), and several studies have reported enhancements in soil CH_4 production in response to environmental disturbance (Steudler et al. 1996; Castro et al. 2000).

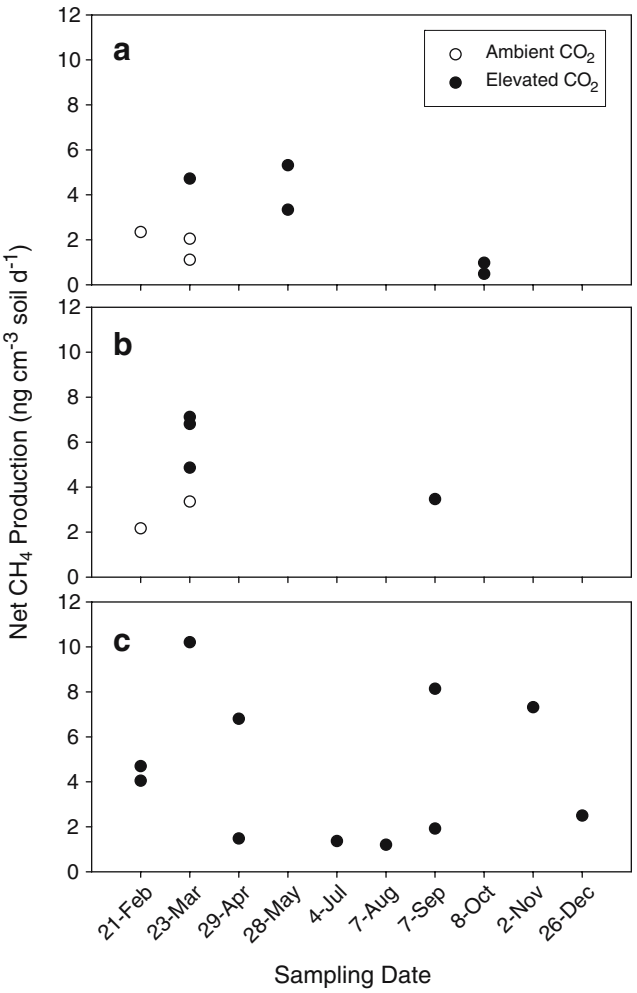

Fig. 4 Net CH4 production in soil subsamples collected at 0–5- (**a**), 15–20- (**b**), and 25–30 cm (**c**) depths in FACTS-I sites over the 11-month sampling period

The CH_4-producing flasks were equally distributed among soil depths, but 80% (20 of 25) of the CH_4-producing samples were collected from elevated CO_2 soils (Fig. 4). Moreover, more than half of the CH_4-producing flasks from elevated CO_2 soils (11 of 20) contained soils from 25 to 30 cm. Increased rates of respiration in elevated CO_2 soils (Andrews et al. 1999; McLain et al. 2002) may have depleted porespace O_2 in these deeper, finer-textured soils, producing microsites where CH_4 could be generated (Verchot et al. 2000). Although McLain et al. (2002) reported that porespace O_2 concentrations were indistinguishable between CO_2 treatments in the top 30 cm of FACTS-I soils, detection of small differences in O_2 may have been hampered by a relatively high detection limit (0.1%) of the instrument used. In deeper soils (70–200 cm), O_2 concentrations were lower at under elevated CO_2, with differences averaging ~6% across sampling dates (McLain et al. 2002).

These findings of in vitro CH_4 production differ from those of Phillips et al. (2001a), who reported no CH_4 production after applying a methanotroph inhibitor to laboratory incubations of sieved FACTS-I soils. However, this lack of agreement may be due to differing sampling and incubation protocols between the two studies. The sieving of soils by Phillips et al. (2001a) may have destroyed aggregates where microsites supporting the activity of methanogens would form in an otherwise aerobic soil (Smith 1980; Sexstone et al. 1985). In addition, while the headspace of incubation flasks used in the Phillips et al. (2001a) study contained ambient air, this study utilized porespace air, exposing the soils to slightly lower O_2 concentrations and possibly encouraging stronger methanogen activity.

The presence of net methanogenesis under macroscopically aerobic conditions suggests that net CH_4 fluxes in FACTS-I soils reflect a balance between soil production and consumption. Furthermore, increased methanogenesis in soils exposed to elevated CO_2 indicate that the fundamental changes occurring in these soils, including porespace moisture and CO_2 increases, are inducing CH_4 production. While the incubations utilized in the current study revealed interesting patterns in the net consumption and production of CH_4, it is hoped that these findings will stimulate further work utilizing isotope pool dilution experiments (Andersen et al. 1998; von Fischer and Hedin 2002) to separate and quantify gross CH_4 cycling in elevated CO_2 soils.

Conclusions

Efforts to understand increases in tropospheric CH_4 have focused primarily on increased emissions of CH_4 from biogenic sources to the atmosphere. However, recent research has indicated that a portion of the increase may also result from reduced strength of the CH_4 sink. Our work confirms that soil CH_4 consumption is reduced under elevated CO_2 in temperate forest soils and that increased moisture levels and anoxia in deep (25+ cm) soils are critical factors contributing to this inhibition. Our results further suggest that CH_4 production must also be considered as a contributor to decreased net CH_4 consumption under elevated CO_2 in some otherwise aerobic soils. By using a suite of approaches, including activity measurements, in situ net oxidation measurements, and statistical analyses, important insights into the CH_4 dynamics of a soil were obtained, allowing for more confident predictions of the response of CH_4 flux to future environmental change.

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