

## Belowground factors mediating changes in methane consumption in a forest soil under elevated CO<sub>2</sub>

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[1] The sustained increase in atmospheric CO<sub>2</sub> concentration observed over the past century, and projected to continue into the next, is of great significance for atmospheric CH<sub>4</sub>. Effects of elevated CO<sub>2</sub> on microbial methane cycling are potentially mediated by its effects on plant physiology, which include enhancement of carbon assimilation, belowground carbon allocation, and water use efficiency. To determine the importance of such changes for methane cycling, belowground factors impacting soil CH<sub>4</sub> consumption were investigated at the Free Air Carbon Transfer and Storage (FACTS)-I site in the Duke Forest, North Carolina, in which plots have been exposed to ambient (370 ppm) or elevated (ambient + 200 ppm) CO<sub>2</sub> since August 1996. CH<sub>4</sub> fluxes at the soil surface, porespace concentrations of CH<sub>4</sub>, O<sub>2</sub>, and CO<sub>2</sub>, soil moisture, soil temperature, and soil pH were simultaneously measured over 24 months. Porespace CH<sub>4</sub> concentrations were highest (1.98 ± 0.25 ppm) at the soil surface and decreased to 0.65 ± 0.22 ppm at 30 cm, indicating that methanotrophic activity was depleting CH<sub>4</sub> in the upper soil layers and creating a gradient to draw atmospheric CH<sub>4</sub> into the soil. This was confirmed by surface CH<sub>4</sub> flux measurements, which averaged -1.54 ± 0.65 μmol/m<sup>2</sup>/h. Under elevated CO<sub>2</sub>, porespace CH<sub>4</sub> was 25–30% higher in the upper 70 cm of soils; CH<sub>4</sub> fluxes from the atmosphere into soil were diminished by ~25%; soil CO<sub>2</sub> increased by 10–70%; and volumetric soil moisture was greater by up to 40% during some seasons. Statistical modeling revealed that soil moisture strongly predicted variability in surface CH<sub>4</sub> fluxes and that soil CO<sub>2</sub> and soil moisture both predicted variability in soil CH<sub>4</sub>. Results also indicated that a portion of the net CH<sub>4</sub> sink inhibition in elevated CO<sub>2</sub> soils could be attributable to alterations in soil biological processes, suggesting that changes in the CH<sub>4</sub>-cycling microbial ecology had taken place. *INDEX TERMS*: 0315 Atmospheric Composition and Structure: Biosphere/atmosphere interactions; 0330 Atmospheric Composition and Structure: Geochemical cycles; 1610 Global Change: Atmosphere (0315, 0325); 1615 Global Change: Biogeochemical processes (4805); *KEYWORDS*: methane oxidation, elevated carbon dioxide, forest soil, methane flux, loblolly pine

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### 1. Introduction

[2] The biogeochemical cycles of methane and carbon dioxide are closely interconnected through microbial,

plant-mediated, and abiotic processes [Hanson and Hanson, 1996; Schlesinger, 1997]. Currently, however, the mechanisms by which atmospheric CO<sub>2</sub> affects methane cycling are incompletely understood. Atmospheric concentrations of both CO<sub>2</sub> and CH<sub>4</sub> have increased dramatically in recent decades [Whorf and Keeling, 1998], causing these mechanisms to become a matter of increasing concern in efforts both to understand and to predict future changes in atmospheric composition. Because microbial activity produces the majority of atmospheric methane, and also provides the single largest terrestrial methane sink [Born et al., 1990; Lelieveld et al., 1993], understanding

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microbial responses to elevated atmospheric CO<sub>2</sub> is of primary importance.

[3] Elevated atmospheric CO<sub>2</sub> consistently stimulates photosynthetic carbon assimilation and biomass accumulation [O'Neill et al., 1987; Ceulemans and Mousseau, 1994; Curtis, 1996; Drake et al., 1997; Ellsworth, 1999], as well as litterfall [DeLucia et al., 1999] and fine root production and turnover [Van Veen et al., 1991; Pregitzer et al., 1995; Rouhier et al., 1996; Matamala and Schlesinger, 2000] in a variety of plants and ecosystems. In addition, elevated CO<sub>2</sub> may increase exudation of organic compounds from roots [Zak et al., 1993; Hutchin et al., 1995; Magonigal and Schlesinger, 1997]. Together, these responses greatly enhance belowground carbon allocation in some systems, a change expected to have significant implications for soil microbial ecology [Schenk et al., 1995; Klironomos et al., 1996; Niklaus and Körner, 1996; Ambus and Robertson, 1999].

[4] Elevated atmospheric CO<sub>2</sub> has also been linked to increases in soil moisture. Numerous plant systems have exhibited diminished stomatal conductance under elevated CO<sub>2</sub> in growth chambers [Farquhar and Wong, 1984; Morison, 1985, 1993; Tyree and Alexander, 1993; Niklaus et al., 1998], and resultant increases in soil moisture within these systems have been attributed to reduced rates of evapotranspiration [Jackson et al., 1994; Field et al., 1995; Magonigal and Schlesinger, 1997; Owensby et al., 1997; Niklaus et al., 1998; Ambus and Robertson, 1999]. The applicability of these results to intact forest ecosystems is unclear, however: Many forest trees do not demonstrate this stomatal response to a significant degree [Ellsworth et al., 1995; Teskey, 1995; Curtis, 1996; Picon et al., 1996; Saxe et al., 1998], and results from mesocosm-scale experiments may not be valid for much larger, open systems [Hendrey and Kimball, 1994; Amthor, 1999].

[5] By stimulating heterotrophic microbial activity and inhibiting oxygen diffusion into soil, CO<sub>2</sub>-mediated increases in soil moisture and soil carbon deposition are likely to enhance methanogenesis [Yavitt et al., 1995; Castro et al., 2000; Verchot et al., 2000], inhibit methanotrophy [Ineson et al., 1998; Ambus and Robertson, 1999; Phillips et al., 2001a], or have both effects. Methane cycling may be further affected by moisture-related limitations on its diffusion into soils, as suggested by Ambus and Robertson [1999]; by direct inhibition of methanotroph activity by CO<sub>2</sub>, as proposed by Ineson et al. [1998]; and by changes in the methane-oxidizing microbial community, as hypothesized by Phillips et al. [2001a].

[6] While the mechanisms by which CO<sub>2</sub>-mediated changes in soil carbon, moisture, and other environmental factors may affect soil microorganisms remain mysterious, it is clear that a variety of microbial activities and communities are altered significantly in soils exposed to elevated CO<sub>2</sub> [Dacey et al., 1994; Klironomos et al., 1996; Niklaus and Körner, 1996; Zak et al., 1996; Ringelberg et al., 1997; Ineson et al., 1998; Ambus and Robertson, 1999]. Accordingly, it is through altering methanogenic and methanotrophic activities, either directly or indirectly, that elevated

CO<sub>2</sub> is expected to have its greatest effects on methane cycling.

[7] To quantify and understand these effects, multiple environmental parameters were investigated 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 [Hendrey, 1993] that is typical of temperate forests in the ability of its soils to consume large amounts of atmospheric methane [Phillips et al., 2001a]. Inhibition in net soil CH<sub>4</sub> oxidation under elevated atmospheric CO<sub>2</sub> was demonstrated in previous work at this site and potential mechanisms affecting CH<sub>4</sub> cycling were hypothesized [Phillips et al., 2001a, 2001b]. These mechanisms had also been hypothesized, but not tested, in similar systems [Ineson et al., 1998; Ambus and Robertson, 1999]. The work described here represents the first attempt to test the various hypotheses whereby elevated CO<sub>2</sub> affects CH<sub>4</sub> cycling in soils of temperate forests. Results showed that methane consumption was significantly inhibited by the CO<sub>2</sub> treatment. The strongest predictors of treatment effect were soil moisture, as hypothesized, as well as porespace CO<sub>2</sub>, date, and soil depth, while porespace O<sub>2</sub>, [H<sup>+</sup>], and temperature were not significant influences. Further analysis of spatial and temporal correlations between these parameters may reveal further influences on soil CH<sub>4</sub> oxidation and aid in a complete understanding of the mechanisms responsible for reduced CH<sub>4</sub> consumption under elevated atmospheric CO<sub>2</sub>.

## 2. Site Description

[8] The FACTS-I site consists of three control and three treatment plots, each 30 m in diameter, in an 18-year old loblolly pine plantation in the Duke Forest, Orange County, North Carolina (35°58'N, 70°05'W). In each plot, gases are delivered to a circular array of 32 vertical vent pipes that extend from the forest floor to the top of the canopy. Computer-adjustable ports regulate gas flow to maintain a concentration of ~200 ppm above ambient, or 570 ± 30 ppm in treatment plots [Andrews, 1999; Ellsworth, 1999], a level projected to occur within the next 50 years if current rates of atmospheric CO<sub>2</sub> increase are sustained [Hendrey, 1993]. Control plots are fumigated with ambient air to replicate micrometeorological effects of the Free Air Carbon Enrichment (FACE) technology. Fumigation began on 27 August 1996 and has been interrupted only during repairs, periods of low temperature (<5°C), and sustained periods of high wind (>5m/s).

[9] The FACTS-I forest is dominated by loblolly pine (1733 stems/ha), with significant sweetgum (*Liquidambar styraciflua*, 620 stems/ha) and winged elm (*Ulmus alata*, 226 stems/ha) as secondary associates [Matamala and Schlesinger, 2000]. Loblolly pine growth in the plantation is uniform, with a median height of 15 m, a mean diameter of ~15 cm, and a leaf area index of ~3.5 [Katul et al., 1997]. 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 with mixed clay mineralogy [U.S. Department of Agriculture/SCS, 1977].

### 3. Methods

#### 3.1. Soil Gas Sampling

[10] Each FACTS-I plot is divided radially into four equal-sized sampling sectors. During construction of the facility, soil gas wells were installed at 15, 30, and 70 cm depths in each sector, and wells at 100 and 200 cm were installed in two sectors per ring. Each well consists of a 5-cm-diameter polyvinylchloride (PVC) pipe 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. Two 0.6-cm OD Kynar<sup>®</sup> plastic tubes extend from the stopper to the soil surface where they are sealed by Kynar<sup>®</sup> caps attached with stainless steel Swagelok connectors.

[11] Soil gas samples were withdrawn using a stainless steel vacuum manifold designed according to Andrews [1999]. In the field, the manifold was evacuated with a hand pump, purged with two volumes of soil gas, and used to direct the gas into 75-cc Whitey stainless steel cylinders that had been evacuated to  $10^{-5}$  Pa prior to gas sampling. Additional 50-mL samples were then withdrawn from each well using a gas-tight syringe (Hamilton Co.) and immediately injected into an EGM-1 Environmental Gas Monitor with OP-1 oxygen probe attachment (PP Systems) for direct reading of O<sub>2</sub> concentrations. Certified O<sub>2</sub> standards were used for calibration (Scott Specialty Gases).

[12] In the laboratory, duplicate subsamples were removed from each cylinder with gas-tight syringes and analyzed for methane by GC-FID (Varian 3400, GS-Q divinylbenzene homopolymer fused silica capillary column (J&W Scientific), 30 m × 0.53 mm ID, at 150°C with 250°C detector and 30 mL/min He carrier gas). Certified CH<sub>4</sub> standards were used for calibration (Scott Specialty Gases). Additional duplicate gas subsamples were analyzed for CO<sub>2</sub> on a total organic carbon analyzer (Shimadzu TOC 5000), bypassing the combustion system and injecting samples directly into the nondispersive infrared detector. Certified CO<sub>2</sub> standards were used for calibration (Scott Specialty Gases).

[13] Gas samples were collected at intervals of at least 3 weeks to allow complete equilibration between soil gases and the gas wells. Sampling was delayed further, however, when wells were flooded or covered with snow. All other field sampling, described below, was performed on the same dates as porspace gas measurements.

#### 3.2. Net Methane Flux Measurements

[14] PVC flux collars, 22 cm in diameter, were permanently installed in each sector during site construction. Net methane fluxes at the soil surface were determined using the static chamber method [Hutchinson and Mosier, 1981; Ambus et al., 1992; Koschorreck and Conrad, 1993]. Portable static chambers each consisted of a polyethylene cylinder (4.9 L, 23 cm in diameter) fitted with a length of 0.6-cm Tygon tubing attached to a Mininert syringe valve to form a sampling port. Chambers were firmly affixed to the collars using silicone sealant, whereupon a 30-mL air

sample was removed through the sampling port using a gas-tight syringe and transferred to a preevacuated 30-mL serum bottle fitted with a Teflon-lined butyl rubber stopper (Wheaton) and aluminum crimp seal. Air samples were collected at 15-min intervals for 1 hour, transferred to individual serum bottles, and analyzed for methane by GC-FID. Net CH<sub>4</sub> fluxes were calculated from the exponential regression of the time series of CH<sub>4</sub> concentrations [Koschorreck and Conrad, 1993].

#### 3.3. Soil Sampling

[15] Soil cores (1.9 cm × ~35 cm) were removed from two sectors of each ring at locations chosen randomly. Cores were obtained with an unslotted stainless steel soil recovery probe (AMS, Inc.) fitted with a butyrate plastic liner (Forestry Suppliers) and a slide-hammer attachment (AMS, Inc.). After the probe was withdrawn, the liner was removed, clipped to remove unfilled portions and sealed with polyethylene caps (Forestry Suppliers). Because these samples were also used for molecular analyses not described in this paper, the soils were transported to the laboratory on ice.

[16] Pretreatment pH values of FACTS-I soils were determined from samples collected during the summer of 1996 when the site was under construction. Soil samples were collected at five different depths: 0–7.5, 7.5–15, 30–60, 60–90, and 100–200 cm, air-dried, sieved, and stored in glass jars at room temperature until analysis.

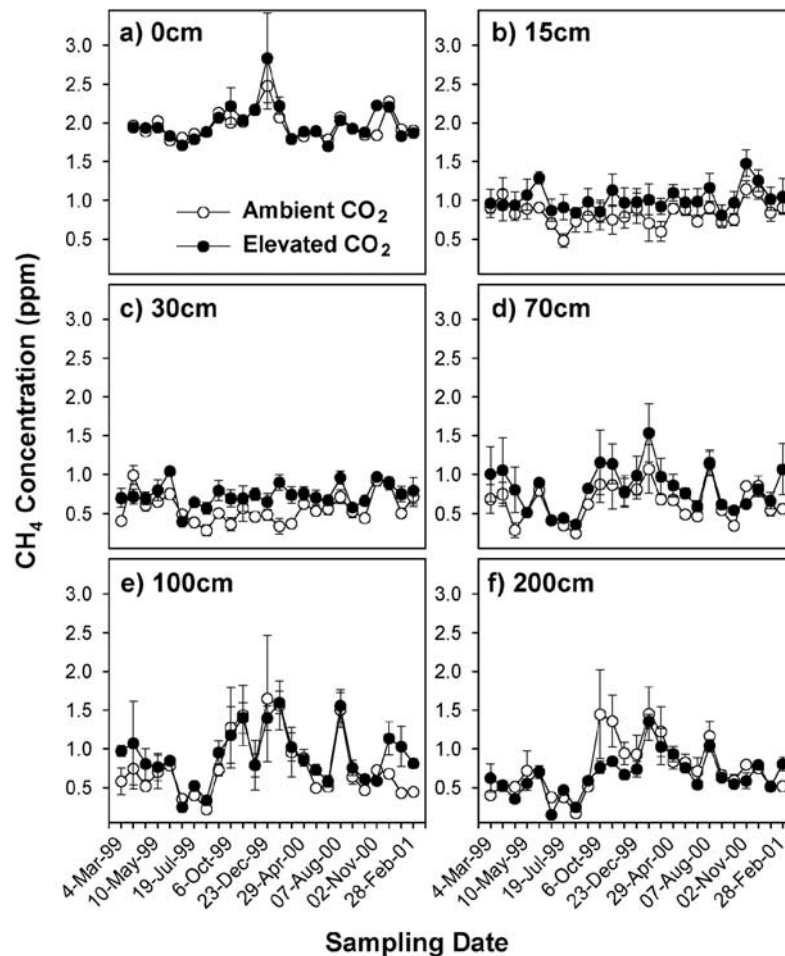
#### 3.4. Soil Analyses

[17] In the laboratory, soil cores were placed in an anoxic glovebox (Coy, Inc.) under N<sub>2</sub>:H<sub>2</sub>::98:2 to prevent introduction of O<sub>2</sub>; this precaution was necessary for microbiological experiments not described in this paper. Subcores at 0–5, 15–20, and 25–30 cm depths were homogenized, split into subsamples, and analyzed for pH and water content.

[18] 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<sub>2</sub>O 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.

[19] Gravimetric soil moisture content (g H<sub>2</sub>O/g dry soil) was determined by oven-drying samples at 105°C for 48 hours. In addition, an average volumetric soil moisture content (mL H<sub>2</sub>O/cm<sup>3</sup> soil) of the upper 30-cm soil layer was measured using time domain reflectometry (TDR) probes (CS615, Campbell Scientific) installed in each sector of the rings as described by Oren et al. [1998]. For each sector, a bulk density (g dry soil/cm<sup>3</sup> dry soil) was calculated from the depth-averaged gravimetric soil moisture and the measured TDR readings. These estimates of bulk density were consistent with bulk densities measured on pretreatment soil samples (N.-H. Oh and D.D. Richter, unpublished data, 1999). The average bulk density and gravimetric soil moisture contents within each sector were then used to calculate volumetric soil moisture at the three subcore depths.

[20] Soil temperature was measured using permanently installed thermocouple probes located adjacent to each



**Figure 1.** Methane concentrations at the soil surface (0 cm) and in the soil porespace (15, 30, 70, 100, and 200 cm depths) versus sampling date for March 1999 through February 2001 in forest plots exposed to ambient and elevated CO<sub>2</sub>. Data are presented as the mean  $\pm$  standard error ( $n = 3$ ). Fixed effect model results showing porespace CO<sub>2</sub>, porespace O<sub>2</sub>, soil moisture, soil temperature, and soil [H<sup>+</sup>] effects are presented in Table 1.

belowground gas well. Probes were assembled using PVC/PVC insulation wire connected to aboveground thermocouple connectors (Cole-Parmer), and temperatures were measured and recorded using a Digi-Sense Type K Thermometer (Cole-Parmer).

### 3.5. Statistical Analyses

[21] For each covariate measured, a mean for each experimental ring was calculated from the data of its four sectors. Each ring was then treated as an independent replicate, such that  $n = 3$ . A Ryan-Joiner test for data normality (Minitab 10.1, Minitab Inc.) revealed one significantly anomalous location (30 cm depth, Sector 4, Ring 1), in which measured CH<sub>4</sub> concentrations were  $\sim 5$  standard deviations above the mean. The cause of this anomaly is unclear, but could be related to the collection of water around the gas well. For this reason, data from this sector were omitted from all statistical analyses.

[22] The significance of treatment effects on the methane-cycling covariates (porespace CH<sub>4</sub> and CH<sub>4</sub> fluxes at the

soil surface) were determined using stratified error model ANOVAs, a class of models that encompasses both repeated measures and other mixed effect models. To examine the dependence of the treatment effect on depth, the corresponding linear mixed effects models were used. Both of these models include two sources of random variability: that of measurements within rings and that among rings. Among-ring variability results from systematic but unpredictable differences in the set of measured covariates as well as in unmeasured parameters such as soil texture and mineral composition, microbial and invertebrate populations, and nutrient content. The methane cycling variables showed great variation with date and depth, so these covariates were included as categorical fixed-effects variables in all models. The FACTS-I experimental design calls for this mixed effects treatment because it contains both fixed and random effects. Fixed effects are those that are quantitatively reproducible in replicate experiments, while random effects are those that cannot be quantitatively reproduced from one experiment to another but nevertheless

contribute in important ways to the variability in the measured responses.

[23] The linear mixed effects model used for analysis of porespace CH<sub>4</sub> was of the form

$$Y_{ijkl} = \beta_0 + \beta_1 D_j + \beta_2 T_k + \beta_3 \tau_i D_j + \rho_{il} + \varepsilon_{ijkl},$$

where  $Y_{ijkl}$  represents the porespace CH<sub>4</sub>, measured under the  $i$ th treatment (ambient or elevated CO<sub>2</sub>,  $\tau_i$ ) at the  $j$ th depth ( $D_j$ ) at the  $k$ th time ( $T_k$ ), or date, and within ring  $il$ .  $\beta$  is the regression coefficient for the fixed effects, and  $\rho_{il}$  represents the random effects within ring  $il$ , assumed to be normally distributed with mean 0 and variance independent of  $i$  and  $l$ . The  $\varepsilon$  is the residual variability, assumed normal with mean 0 and constant variance.

[24] Fixed effects linear regression models were used to investigate the effects of the other covariates on the CH<sub>4</sub>-cycling variables. The covariates considered were soil moisture, soil temperature, soil pH, and porespace O<sub>2</sub> and CO<sub>2</sub> concentrations. The base model again always included date and depth as categorical variables, and was of the form

$$Y_{ijkl} = \beta_0 + \beta_1 D_j + \beta_2 T_k + \beta_3 X_{ijkl}^1 + \beta_4 X_{ijkl}^2 + \dots + \varepsilon_{ijkl},$$

where  $X_{ijkl}^1, X_{ijkl}^2, \dots$  are the covariates under consideration at measurement  $ijkl$ .

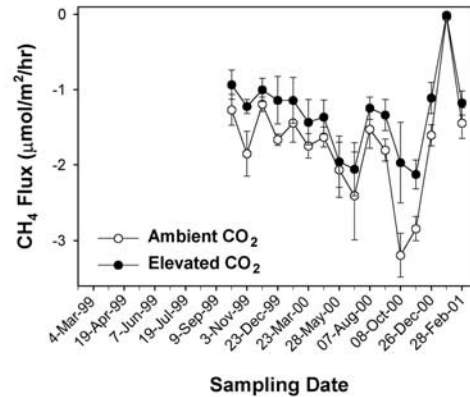
[25] All statistical estimation procedures and null hypothesis tests for regression coefficients within each model were performed using the software package Splus2000 (Professional release 1; copyright 1998–1999, MathSoft, Inc.). All models were fit using Splus functions *lm*, *lme*, and *aov*.

## 4. Results and Discussion

### 4.1. Soil Methane Concentrations

[26] Methane concentrations were measured at the soil surface and at 15, 30, 70, 100, and 200 cm depths (Figure 1). CH<sub>4</sub> concentrations at the soil surface averaged 2.0 ppm and showed no apparent differences between ambient and elevated CO<sub>2</sub> plots (Figure 1a). Atmospheric CH<sub>4</sub> levels were consistently higher than the global average of ~1.7 ppm [Lelieveld *et al.*, 1993; Schlesinger, 1997], apparently resulting from methane emissions of the nearby Orange County landfill: Aboveground CH<sub>4</sub> concentrations increased markedly between the FACTS-I site and the landfill, with a maximum measurement of 3.2 ppm near the landfill border (data not shown).

[27] At 15 cm below the soil surface, CH<sub>4</sub> concentrations dropped markedly, averaging 1.0 ppm in all forest plots, indicating that methanotrophic activity was depleting CH<sub>4</sub> in the upper soil layers and creating a concentration gradient that drew atmospheric CH<sub>4</sub> into the soil. Treatment effects were evident at this depth, with CH<sub>4</sub> concentrations consistently higher by ~25% in elevated CO<sub>2</sub> plots (Figure 1b), indicating that CO<sub>2</sub> fumigation could be stimulating CH<sub>4</sub> production, inhibiting CH<sub>4</sub> consumption, or both. Methane concentrations at the 30 cm depth were even lower, averaging 0.6 ppm, and showed a similar pattern in that they were consistently higher, by ~30%, under elevated CO<sub>2</sub> (Figure 1c). Similar results were found at 70 cm, where porespace concentrations averaged 0.7 ppm, and



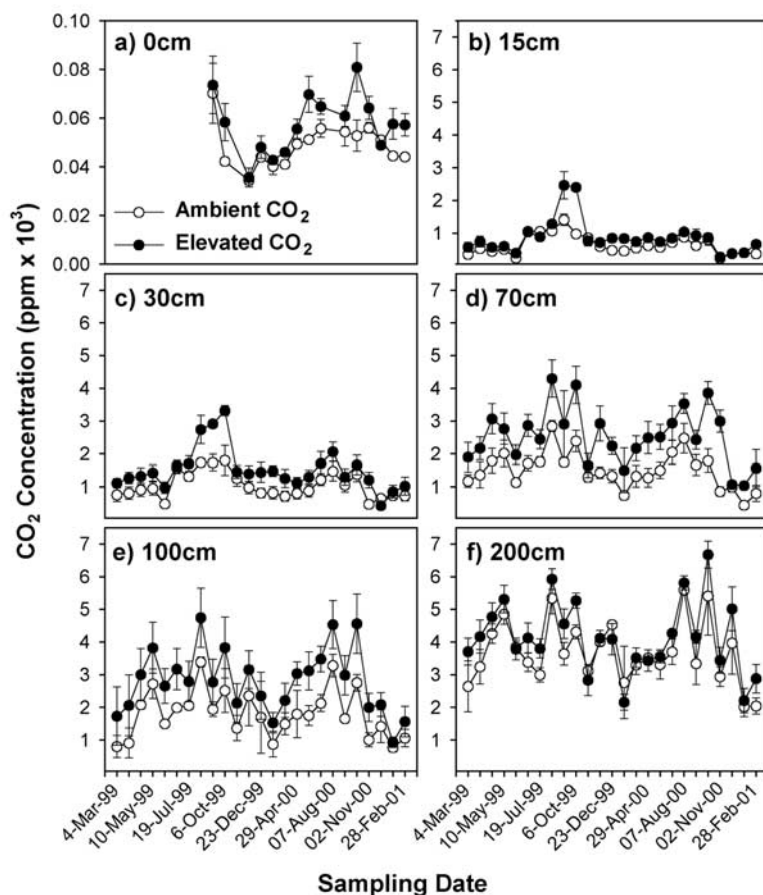
**Figure 2.** Methane fluxes at the soil surface versus sampling date for October 1999 through February 2001 in forest plots exposed to ambient and elevated CO<sub>2</sub>. Positive flux is directed upward. Data are presented as the mean  $\pm$  standard error ( $n = 3$ ). Fixed effect statistical model results showing porespace CO<sub>2</sub>, soil moisture, soil temperature, and soil [H<sup>+</sup>] effects are presented in Table 1.

were higher by ~25% in elevated CO<sub>2</sub> plots (Figure 1d). At 100 and 200 cm, porespace CH<sub>4</sub> concentrations averaged 0.8 and 0.7 ppm, respectively, with no apparent CO<sub>2</sub> treatment effects.

[28] Statistical analyses of these data, by comparison of linear mixed effect models using “ring” as a random variable, revealed that CO<sub>2</sub> treatment did not have a significant effect on atmospheric CH<sub>4</sub> concentrations ( $p = 0.6707$ ), but did have a significant effect on soil porespace CH<sub>4</sub> concentration overall ( $p < 0.0001$ ). In addition, this effect was highly dependent on soil depth: CH<sub>4</sub> concentrations were significantly higher under elevated CO<sub>2</sub> at 15 cm ( $p = 0.0062$ ), 30 cm ( $p = 0.0114$ ), and 70 cm ( $p = 0.0115$ ), but not at the 100 cm ( $p = 0.0857$ ) and 200 cm ( $p = 0.1838$ ) depths. These results suggest that the environmental factors that mediated the effects of elevated CO<sub>2</sub> on soil methane cycling were not as disparate in deeper layers, possibly due to the relative insulation of these layers from variations in moisture, temperature, and plant metabolism [Oren *et al.*, 1998; Ellsworth, 1999].

### 4.2. Methane Fluxes

[29] Methane fluxes at the soil surface were consistently negative throughout the sampling period (Figure 2), confirming that methanotrophic bacteria were active in FACTS-I soils. These fluxes, ranging from  $-0.01$  to  $-3.19$   $\mu\text{mol}/\text{m}^2/\text{h}$  (mean =  $-1.54 \pm 0.65$ ), are in close agreement with and extend those of Phillips *et al.* [2001a], who measured CH<sub>4</sub> fluxes of  $-1.39 \pm 0.10$   $\mu\text{mol CH}_4/\text{m}^2/\text{h}$  in FACTS-I soils from January through December 1999. These flux results are typical for a variety of unsaturated temperate soils, where fluxes are reported to average  $-1.39 \pm 1.18$   $\mu\text{mol}/\text{m}^2/\text{h}$  [Koschorreck and Conrad, 1993]. Comparison of stratified error (mixed effect) models confirmed that net methane consumption was significantly ( $p < 0.0040$ ) diminished in soils exposed to elevated CO<sub>2</sub> by an average of 26% (Figure 2). This diminished consumption was consis-



**Figure 3.** CO<sub>2</sub> concentrations at the soil surface (0 cm) and in the soil porespace (15, 30, 70, 100, and 200 cm depths) versus sampling date for March 1999 through February 2001 in forest plots exposed to ambient and elevated CO<sub>2</sub>. Data are presented as the mean  $\pm$  standard error ( $n = 3$ ). Fixed effect model results showing porespace O<sub>2</sub>, soil moisture, soil temperature, and soil [H<sup>+</sup>] effects are presented in Table 1.

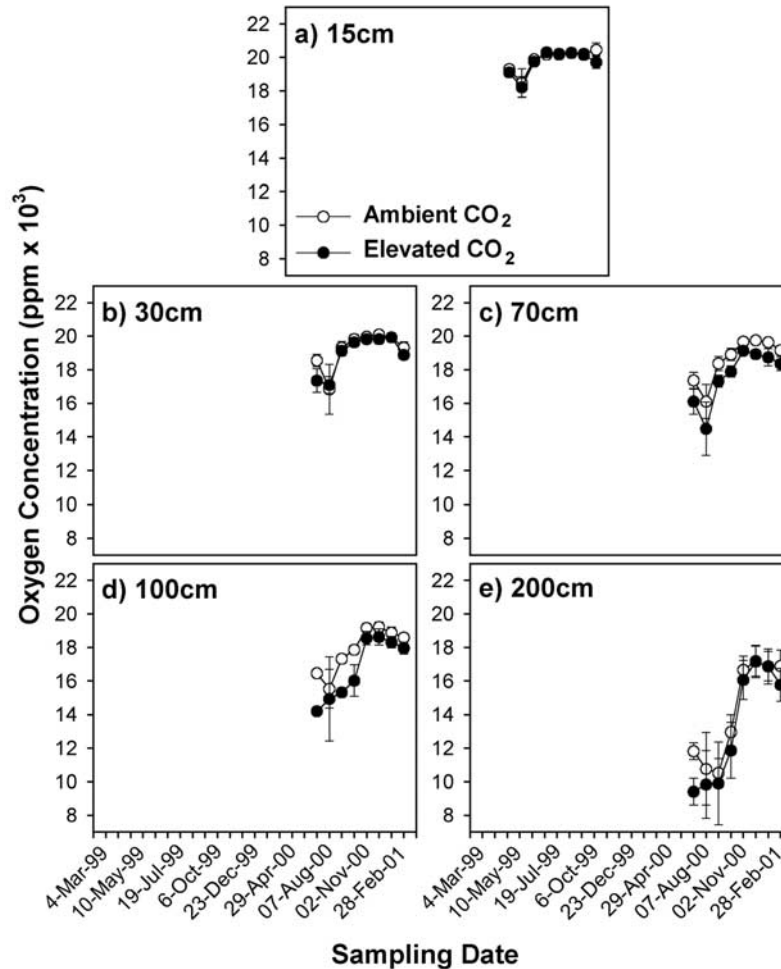
tent with observations of higher porespace CH<sub>4</sub> in elevated CO<sub>2</sub> soils (Figure 1), indicating that CH<sub>4</sub> uptake by soils was inhibited under elevated atmospheric CO<sub>2</sub>. These findings are also in agreement with those of many previous studies: Diminished net CH<sub>4</sub> consumption under elevated CO<sub>2</sub> has been observed in soils of forests [Ambus and Robertson, 1999; Rigler and Zechmeister-Boltenstern, 1999; Phillips *et al.*, 2001a], grasslands [Ineson *et al.*, 1998], and wetlands [Meronigal and Schlesinger, 1997; Dacey *et al.*, 1994].

#### 4.3. Soil CO<sub>2</sub>

[30] Soil CO<sub>2</sub> was monitored simultaneously with soil porespace CH<sub>4</sub> and CH<sub>4</sub> surface fluxes (Figure 3). At the soil surface, CO<sub>2</sub> was considerably higher than the atmospheric average of 370 ppm, presumably due to respiratory emissions by microorganisms and plant roots from soil [Cheng, 1999] (Figure 3a). Seasonal variations in these CO<sub>2</sub> emissions were evident, with low CO<sub>2</sub> concentrations at the surface in winter (November–February) and higher concentrations in the summer and fall (June–October). Within the soil profile, CO<sub>2</sub> concentrations increased consistently with depth, from a mean of

~5300 ppm at 15 cm to a mean of ~37,000 ppm at 200 cm (Figures 3b–3f).

[31] Preliminary correlation analyses revealed a strong log linear ( $r = -0.74$ ), rather than linear ( $r = -0.43$ ), relationship between porespace CO<sub>2</sub> and CH<sub>4</sub>; therefore, CO<sub>2</sub> concentration data were log-transformed prior to all statistical analyses. Impacts of elevated atmospheric CO<sub>2</sub> on porespace CO<sub>2</sub> were then analyzed using mixed effect linear models, which revealed a significant effect of treatment on soil CO<sub>2</sub> concentration overall ( $p < 0.0001$ ). CO<sub>2</sub> concentrations were significantly higher under elevated CO<sub>2</sub> at 15 cm ( $p = 0.0180$ ), 30 cm ( $p = 0.0013$ ), 70 cm ( $p < 0.0001$ ), and 100 cm ( $p < 0.0001$ ), but results were not significant at 200 cm ( $p = 0.2536$ ). These results are in close agreement with data collected from 1996 through 1999 showing that porespace CO<sub>2</sub> was significantly higher at all depths except 70 cm in CO<sub>2</sub>-fumigated plots [Andrews and Schlesinger, 2001]. The increase in porespace CO<sub>2</sub> under elevated CO<sub>2</sub> is attributable both to increased root respiration and to increased input of organic carbon followed by microbial decomposition [Allen *et al.*, 2000]. The evidence of plant-mediated CO<sub>2</sub> influence below the primary root zone of 0–30 cm was likely to



**Figure 4.** O<sub>2</sub> concentrations in the soil porespace (15, 30, 70, 100, and 200 cm depths) versus sampling date for July 2000 through February 2001 in forest plots exposed to ambient and elevated CO<sub>2</sub>. Data are presented as mean  $\pm$  standard error ( $n = 3$ ).

have been the result of growth of some roots below that zone (D. D. Richter, personal communication, 2001).

[32] At both 15 and 30 cm depths, soil CO<sub>2</sub> was particularly elevated in fumigated sites during the unusually warm, wet period in September 1999 during which North Carolina experienced Hurricanes Dennis and Floyd. This extremely wet period (rainfall 483 mm above normal) followed an extremely dry August (rainfall 25 mm below normal) (State Climate Office of North Carolina, Raleigh-Durham NC Climate Summary, North Carolina State University (available at <http://www.nc-climate.ncsu.edu/>) hereinafter referred to as State Climate Office data), with the apparent result that drought-limited respiration was suddenly relieved of this stress [Atlas and Bartha, 1998; Larcher, 1995]. The conspicuous increase in respiration in the fumigated sites was likely related to increases in soil organic matter content and fine root biomass under elevated CO<sub>2</sub> [Allen et al., 2000; Matamala and Schlesinger, 2000]. When water stress was relieved and root and microbial respiration increased to predrought levels, the response was correspondingly larger in elevated

CO<sub>2</sub> soils. Below the root zone, hurricane effects were less noticeable.

#### 4.4. Soil Oxygen

[33] Porespace O<sub>2</sub> was monitored simultaneously with all other factors beginning in July 2000 (Figure 4). Soil O<sub>2</sub> was significantly lower than atmospheric levels, presumably due to O<sub>2</sub> consumption by root and microbial respiration [Larcher, 1995; van Elsas et al., 1997]. Oxygen concentrations were indistinguishable between CO<sub>2</sub> treatments in the top 30 cm of the soil (Figures 4a and 4b), demonstrating that the established increase of root respiration under elevated CO<sub>2</sub> [Allen et al., 2000] did not significantly decrease soil O<sub>2</sub> concentrations in these zones. Detection of small differences may have been hampered, however, by a relatively high detection limit of 0.1% (1000 ppm). From 70 to 200 cm, porespace O<sub>2</sub> consistently decreased with depth (Figures 4c–4e). O<sub>2</sub> concentrations were lower at these depths under elevated CO<sub>2</sub>, with differences averaging ~6% across sampling dates. Seasonal patterns in O<sub>2</sub> concentrations emerged as well, with porespace O<sub>2</sub> levels

increasing at all depths in November and December as root respiration rates diminished [Andrews, 1999].

[34] Statistical analyses of these data by comparison of mixed effect linear models revealed that the CO<sub>2</sub> treatment did not have a significant effect on soil porespace O<sub>2</sub> concentration at any measured depth, although effects were nearly significant at 70 and 100 cm ( $p = 0.1241$  and  $p = 0.0807$ , respectively). It is important to note, however, that O<sub>2</sub> measurements were only collected over an 8-month period, and continued measurements may be necessary to reveal significant treatment effects.

#### 4.5. Soil Moisture

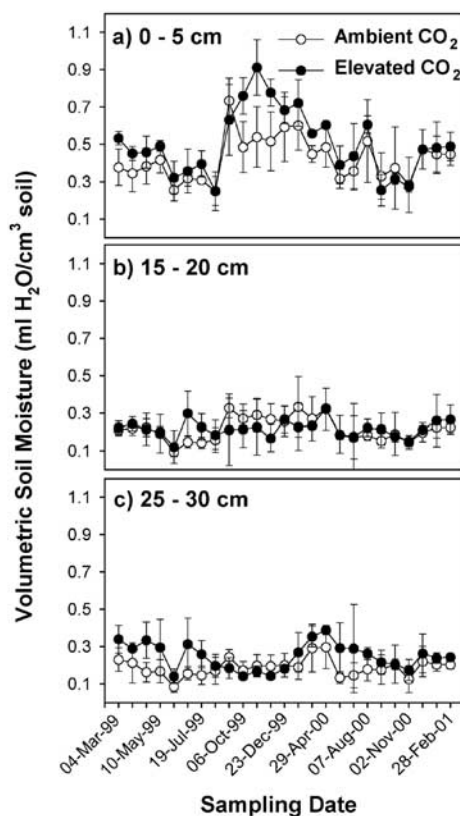
[35] Volumetric soil moisture was measured continuously at the FACTS-I site using permanently installed time domain reflectometry (TDR) probes, each of which produced a single moisture content reading for the top 30 cm of a soil locus. To examine depth-dependent interactions between soil moisture and CH<sub>4</sub> cycling, TDR values were resolved by depth by using gravimetric soil moisture and bulk density to calculate volumetric soil moisture in the 0–5, 15–20, and 25–30 cm layers.

[36] Soil moisture content of the uppermost layer was approximately twice as great as that of the deeper layers, which were similar to each other (Figure 5). In addition, great seasonal and spatial variability was observed at all depths throughout the sampling period, as expected due to small variations in topography, seasonal precipitation patterns (State Climate Office data), and diversity in soil structure and composition (D. D. Richter, unpublished data, 1999).

[37] Statistical analyses of these data by comparison of mixed effect linear models revealed that, considering all seasons, CO<sub>2</sub> treatment had a significant effect ( $p = 0.0489$ ) on volumetric soil moisture only at the 25–30 cm depth. During the spring months (March–June), however, volumetric soil moisture was also significantly higher ( $p = 0.0036$ ) under elevated CO<sub>2</sub> in the 0–5 cm layer. TDR values from the 0–30 cm soil layer confirmed that elevated CO<sub>2</sub> plots were significantly wetter than ambient CO<sub>2</sub> plots and showed that the volumetric soil moisture differences between treatments have increased over time, from a difference of  $0.03 \pm 0.01$  mL/cm<sup>3</sup> during the first year of CO<sub>2</sub> fumigation (1997) to  $0.08 \pm 0.05$  mL/cm<sup>3</sup> in 1999 and  $0.10 \pm 0.05$  mL/cm<sup>3</sup> in 2000. These data reveal that fumigation with elevated CO<sub>2</sub> has significantly ( $p < 0.0001$ , repeated measures ANOVA on daily means,  $n = 3$ ) increased soil moisture in treated plots [Schäfer et al., 2002].

[38] Higher soil moisture under CO<sub>2</sub> fumigation at the FACTS-I site may have resulted from increased soil organic carbon found in elevated CO<sub>2</sub> plots [Schlesinger and Lichter, 2001], as such a change typically augments a soil's water-holding capacity (WHC) [Vereecken et al., 1989; Hudson, 1994]. However, the overall WHC in the 0–30 cm soil layer was not significantly greater in elevated CO<sub>2</sub> plots (J. E. T. McLain and D. M. Ahmann, unpublished data, 2002), diminishing the likelihood that WHC was a dominant contributor to increased soil moisture.

[39] An alternative, and more plausible, mechanism leading to increased soil moisture under CO<sub>2</sub> fumigation may



**Figure 5.** Volumetric soil moisture (0–5, 15–20, and 25–30 cm depths) versus sampling date for March 1999 through February 2001 from forest plots exposed to ambient and elevated CO<sub>2</sub>. Data are presented as the mean  $\pm$  standard error ( $n = 3$ ).

have been the insulation of soils from evaporative moisture loss by the forest floor biomass, which was 30% greater per unit ground area in elevated CO<sub>2</sub> rings [Schlesinger and Lichter, 2001]. Microtopographic differences at the FACTS-I site have also been hypothesized to have contributed to pretreatment differences in soil moisture [Schäfer et al., 2002], but these would not have accounted for the increasing differences in soil moisture between elevated and ambient CO<sub>2</sub> plots over time.

[40] Regardless of the mechanism responsible for soil moisture increases, soil moisture was highly likely to have impacted methane cycling because of the great sensitivity of both methanotrophic and methanogenic bacteria to this factor. Reflecting this, direct links between moisture and methane-cycling activity have been well documented. In very moist soils, consumption of atmospheric CH<sub>4</sub> is strongly diminished by moisture-limited diffusion of both CH<sub>4</sub> and O<sub>2</sub> into the soils [Striegl, 1993; Castro et al., 1994; Mancinelli, 1995]. Although significant treatment-related O<sub>2</sub> differences were not found in FACTS-I soils, O<sub>2</sub> diffusion in surface soils has been shown to be significantly slowed at volumetric water contents above 0.25–0.30 mL/cm<sup>3</sup> [Kursar et al., 1995]. During our 8-month O<sub>2</sub> sampling period, volumetric water content in the 0–30 cm soil layer averaged 0.25 mL/cm<sup>3</sup> in ambient CO<sub>2</sub> soils and 0.28 mL/cm<sup>3</sup> in

**Table 1.** Environmental Effects on Porespace Methane, Surface Methane Flux, and Porespace Carbon Dioxide<sup>a</sup>

	Porespace Methane		Surface Methane Flux		Porespace Carbon Dioxide	
	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$
Log (Soil CO <sub>2</sub> )	<b>0.0466</b>	-0.1130	0.0605	-	-	-
Soil O <sub>2</sub>	0.0868	-	-	-	<b>0.0008</b>	<-0.0001
Soil Moisture	<b>&lt;0.0001</b>	0.4927	<b>0.0003</b>	1.9063	<b>0.0119</b>	0.2469
Soil Temperature	0.5299	-	0.8314	-	<b>0.0003</b>	0.0090
Soil [H <sup>+</sup> ]	0.0777	-	0.3194	-	<b>0.0168</b>	6 × 10 <sup>3</sup>

<sup>a</sup>Boldface entries are significant at  $\alpha = 0.05$ .

elevated CO<sub>2</sub> soils, suggesting that small differences in porespace O<sub>2</sub> concentrations between elevated and ambient CO<sub>2</sub> soils were present, but were not detected as a result of the limited duration of O<sub>2</sub> measurements and the high detection limit of the instrument used. High soil moisture also increases microsite anoxia, enhancing conditions conducive to methanogenesis. Moisture increases as small as 10% have significantly reduced net CH<sub>4</sub> uptake rates by soils [Sitaula *et al.*, 1995], and during the 2-year sampling period, volumetric soil moisture in the 0–30 cm layer averaged 20% higher under elevated CO<sub>2</sub>.

[41] Despite the probable great importance of soil moisture, two lines of evidence suggest that moisture was not the only parameter controlling CH<sub>4</sub> uptake by FACTS-I soils. First, laboratory incubations of control and treated soils, when brought to equivalent moisture contents, still did not show similar CH<sub>4</sub>-oxidizing activity [Phillips *et al.*, 2001b; J. E. T. McLain and D. M. Ahmann, unpublished data, 2002]. Second, porespace CH<sub>4</sub> concentrations would have been lower in elevated CO<sub>2</sub> soils compared to those of ambient CO<sub>2</sub> soils, not higher as observed, if gas diffusion were the only mechanism limiting CH<sub>4</sub> oxidation. The reason for this is that decreased CH<sub>4</sub> availability to methanotroph populations in elevated CO<sub>2</sub> soils would have allowed methanotrophs to consume CH<sub>4</sub> to the threshold uptake level of approximately 0.03 ppm [Conrad, 1994]. The higher CH<sub>4</sub> concentrations measured in elevated CO<sub>2</sub> soils indicate that prolonged exposure to elevated CO<sub>2</sub> may have brought about important changes in methane-cycling microbial ecology. Such changes in microbial ecology are quite likely to have occurred, based on evidence of methanotroph community changes under other environmental stressors [Roslev and King, 1994; Amaral and Knowles, 1995; Schnell and King, 1996; Amaral *et al.*, 1998].

#### 4.6. Soil pH

[42] Prefumigation soil pH values measured in archived soil samples were consistently near 4.9 in the top 30 cm. No significant differences were found among sites destined for treatment versus control status using Student's *t*-test (data not shown).

[43] Soil pH was monitored at 0–5, 15–20, and 25–30 cm depths simultaneously with porespace gas concentrations and surface CH<sub>4</sub> fluxes beginning in August 1999. Surface pH varied in all plots from approximately 4.5 to 6.0 (data not shown), reflecting normal seasonal fluctuations [Fisher *et al.*, 2000]. Mean pH values were frequently lower, by up to one-half unit, in surface soils of elevated CO<sub>2</sub> plots, but mixed effect linear models showed

that the CO<sub>2</sub> treatment effect was not significant at any depth ( $p = 0.1106$  at 0–5 cm,  $p = 0.6192$  at 15–20 cm, and  $p = 0.4625$  at 25–30 cm).

[44] While reductions in soil pH have been found in laboratory incubations of elevated CO<sub>2</sub> soils [Rigler and Zechmeister-Boltenstern, 1999], lowered pH would be unlikely to diminish net soil methane consumption because methanotrophic bacteria are tolerant of a wide range of pH values (~5–8) [Dunfield *et al.*, 1993; Bender and Conrad, 1995; Syamsul Arif *et al.*, 1996], and methanogenic activity typically diminishes as pH is lowered below neutral [Dunfield *et al.*, 1993; Sitaula *et al.*, 1995]. Nevertheless, diminished soil pH may have had indirect effects on soil microbiota, as lower pH values promote solubility of many metals, including aluminum, copper, and iron, known to inhibit microbial activities [Brady and Weil, 1996; Nester *et al.*, 1998; Nanba and King, 2000]. Therefore, pH variations in FACTS-I soils may have influenced methanotrophy and/or methanogenesis in ways that were not statistically apparent during this study, but that may have important long-term consequences.

#### 4.7. Soil Temperature

[45] Soil temperatures showed pronounced seasonal fluctuations, but measurements were statistically equivalent between ambient and elevated CO<sub>2</sub> sites (data not shown). This fact does not diminish the potential contributions of temperature to interactions between elevated CO<sub>2</sub> and other soil parameters, however. Soil temperature was therefore monitored simultaneously with porespace gas concentrations and surface CH<sub>4</sub> fluxes and included in statistical models described below.

#### 4.8. Statistical Models

[46] To explore further the mechanisms mediating elevated CO<sub>2</sub> influences on soil CH<sub>4</sub> cycling, a series of fixed effect statistical models were developed. Note that pH data were transformed to hydrogen ion concentrations ([H<sup>+</sup>]) for modeling purposes.

##### 4.8.1. Porespace CH<sub>4</sub>

[47] Fixed effect modeling of influences on porespace CH<sub>4</sub> revealed that log soil CO<sub>2</sub> and soil moisture were significant predictors of porespace CH<sub>4</sub>, while soil O<sub>2</sub>, temperature, and [H<sup>+</sup>] were nearly significant influences (Table 1). Log soil CO<sub>2</sub> was negatively correlated with soil CH<sub>4</sub> (Table 1, row 1), an inverse relationship that is typical of temperate forest soils in which aerobic methane oxidation is the dominant microbial methane-cycling process and diffusion-limited gas exchange occurs between

**Table 2.** Proportion of Variability Explained By Environmental Covariates

Response Variable	Best Model	% of Variance Explained by Base Model	% of Excess Variance Explained
Soil CH <sub>4</sub>	CH <sub>4</sub> = β <sub>1</sub> (date) + β <sub>2</sub> (depth) + β <sub>3</sub> (soil moisture)	85.3	10.5
Surface CH <sub>4</sub> flux	CH <sub>4</sub> flux = β <sub>1</sub> (date) + β <sub>2</sub> (soil moisture)	64.7	10.3
Soil CH <sub>4</sub>	CH <sub>4</sub> = β <sub>1</sub> (date) + β <sub>2</sub> (depth) + β <sub>3</sub> (treatment)	75.7	3.5
Surface CH <sub>4</sub> flux	CH <sub>4</sub> flux = β <sub>1</sub> (date) + β <sub>2</sub> (treatment)	64.7	21.6
Log (soil CO <sub>2</sub> )	log (soil CO <sub>2</sub> ) = β <sub>1</sub> (date) + β <sub>2</sub> (depth) + β <sub>3</sub> (temperature)	89.6	1.6
[H <sup>+</sup> ]	[H <sup>+</sup> ] = β <sub>1</sub> (date) + β <sub>2</sub> (depth) + β <sub>3</sub> (oxygen) + β <sub>4</sub> (log CO <sub>2</sub> )	17.4	9.1

soils and the atmosphere [Crill, 1991; Hansen *et al.*, 1993; Sitaula *et al.*, 1995]. In these soils, CH<sub>4</sub> decreases with depth as a result of methanotrophic activity [Whalen *et al.*, 1992] while porespace CO<sub>2</sub> increases due to microbial and root respiration [Crill, 1991; Johnson *et al.*, 1994]. While higher porespace CO<sub>2</sub> could theoretically have promoted CH<sub>4</sub> production by serving as a substrate for methanogenesis [Rigler and Zechmeister-Boltenstern, 1999], methanogenesis is not typically CO<sub>2</sub>-limited [Whiticar *et al.*, 1986], and direct effects of elevated porespace CO<sub>2</sub> on methane production have not been reported to date. Considering these alternatives, it appears unlikely that increased porespace CO<sub>2</sub> affected CH<sub>4</sub> cycling directly; instead, increased soil CO<sub>2</sub> and reduced net CH<sub>4</sub> consumption were most likely to have been linked by the atmospheric CO<sub>2</sub>-mediated influences of other soil properties, as discussed below.

[48] O<sub>2</sub> was a nearly significant predictor of porespace CH<sub>4</sub> (Table 1, row 2), suggesting that methanogenesis may have been stimulated by reductions in O<sub>2</sub> even under macroscopically oxic conditions. This result is consistent with previous observations that methanogenic bacteria can thrive in anoxic microzones in the interior of soil particles, even in unsaturated soils [Topp and Pattey, 1997; Verchot *et al.*, 2000].

[49] A positive correlation was found between soil moisture and soil CH<sub>4</sub> (Table 1, row 3), almost certainly reflecting the ability of moisture to promote anoxia in soil microsites, inhibiting methanotrophy and stimulating methanogenesis [Striegl, 1993; Mancinelli, 1995; Castro *et al.*, 2000]. This positive correlation highlights the importance of diminished methanotroph activity to net CH<sub>4</sub> cycling in these soils: if moisture-limited CH<sub>4</sub> diffusion had been the only limitation on CH<sub>4</sub> oxidation, the correlation between CH<sub>4</sub> and CO<sub>2</sub> would have been negative, as discussed previously.

[50] The absence of a correlation between temperature and porespace CH<sub>4</sub> (Table 1, row 4) is not surprising: although methanotrophic activity is markedly diminished at temperatures below ~5°C [Dunfield *et al.*, 1993; van den Pol-van Dasselaar *et al.*, 1998], such low temperatures were observed only once during this study, and other factors such as soil moisture control methanotrophy under warmer temperatures [Castro *et al.*, 1995]. As a result, soil temperature appears to be relatively insignificant compared to other factors in determining methane oxidation rates in most soils [King and Adamsen, 1992;

Wickland *et al.*, 1999], including those in the FACTS-I study.

#### 4.8.2. Surface CH<sub>4</sub> Fluxes

[51] Modeling of influences on CH<sub>4</sub> fluxes into the soil showed that soil moisture again was a significant predictor and that soil CO<sub>2</sub> was a nearly significant predictor (Table 1). Soil temperature and [H<sup>+</sup>] were not significant influences. The relationship between soil moisture and surface CH<sub>4</sub> fluxes was negative, confirming that less CH<sub>4</sub> was consumed by the soils under increasingly moist conditions. As in the case of porespace CH<sub>4</sub>, the ability of moisture to promote anoxia and methanogenesis, while inhibiting methanotrophy through moisture-limited CH<sub>4</sub> diffusion, was implicated.

#### 4.8.3. Porespace CO<sub>2</sub>

[52] Additional models revealed that soil O<sub>2</sub>, moisture, temperature, and [H<sup>+</sup>] were each significant influences on soil CO<sub>2</sub> concentrations (Table 1). Soil O<sub>2</sub> was negatively correlated with soil CO<sub>2</sub> (Table 1, row 2), presumably due to the consumption of O<sub>2</sub> and production of CO<sub>2</sub> during root and microbial respiration. Soil moisture and temperature were positively correlated with soil CO<sub>2</sub> (Table 1, rows 3 and 4), illustrating the dependence of root and microbial respiration on sufficient moisture and warmth, and [H<sup>+</sup>] was also positively correlated with CO<sub>2</sub> (Table 1, row 5), possibly due to the dissociation of root-derived organic acids in soils [Larcher, 1995]. Although soil CO<sub>2</sub> might have increased soil [H<sup>+</sup>] via carbonic acid formation in situ, soils were air-dried prior to analysis, and pH effects of soil CO<sub>2</sub> itself would therefore not have been detected [Van Lierop, 1990].

#### 4.8.4. Soil [H<sup>+</sup>]

[53] Log soil CO<sub>2</sub> and soil O<sub>2</sub> each significantly predicted variability in soil [H<sup>+</sup>] ( $p = 0.0168$  and  $p = 0.0232$ , respectively). Soil CO<sub>2</sub> was positively correlated with [H<sup>+</sup>], while porespace O<sub>2</sub> was negatively correlated. These results agree with those of the porespace CO<sub>2</sub> models in suggesting that the strong correlations between porespace CO<sub>2</sub> and O<sub>2</sub> and soil pH were related to root metabolism. It is possible that the higher metabolic activity of roots in soils exposed to elevated CO<sub>2</sub> resulted in increased release of organic acids into soils [Larcher, 1995], while increased root respiration contributed to higher porespace CO<sub>2</sub> and depletion of soil O<sub>2</sub>.

#### 4.8.5. Proportion of Variance Explained

[54] For four of the response variables (soil CH<sub>4</sub>, surface CH<sub>4</sub> fluxes, log soil CO<sub>2</sub>, and soil [H<sup>+</sup>]), the best

fixed effect model was identified using a stepwise regression. Starting with a base model consisting of the categorical covariates date and, where appropriate, depth, each of the other covariates was added in a stepwise fashion and the best model was selected using Mallows's Cp statistic. "Treatment" and "ring" were not considered in these models, because the intent of the models was to reveal factors that might mediate treatment effects. The proportion of excess variance explained by the covariates added to the base model was given by  $(R_{\text{best}}^2 - R_{\text{base}}^2) / (1 - R_{\text{base}}^2)$ .

[55] The base model itself explained the majority of the variability in porespace CH<sub>4</sub>, surface CH<sub>4</sub> fluxes, and log soil CO<sub>2</sub> (Table 2), indicating that environmental influences that varied systematically by date and by soil depth were the primary controlling influences on these methane-cycling parameters. While soil moisture and temperature were obvious candidates to provide predictive value to the measurement date, they could not fully substitute for "date" as predictive covariates (data not shown), further supporting the implication that other, unquantified factors were important.

[56] Among the environmental covariates, soil moisture showed the greatest ability to explain variability in the methane-cycling parameters, accounting for over 10% of the excess variance in models of both soil CH<sub>4</sub> and surface CH<sub>4</sub> fluxes. This was a noteworthy contribution by a single factor, because a great portion of the total excess variance was likely to have resulted from measurement error, and the sum of small contributions from other covariates, both measured and unmeasured, was also likely to have been important.

[57] CO<sub>2</sub> treatment accounted for an even greater portion of the variability in surface CH<sub>4</sub> fluxes than did soil moisture, representing over 20% of the excess variance in the base + treatment model. This striking contribution showed that soil moisture, while a potentially very important intermediate between elevated CO<sub>2</sub> and surface CH<sub>4</sub> fluxes, mediated at most only half of the treatment effect. CO<sub>2</sub> treatment accounted for a far smaller proportion of variability in soil CH<sub>4</sub>, indicating that the latter parameter was more strongly influenced by other environmental factors.

[58] The best predictive model for log CO<sub>2</sub> included soil temperature, but temperature only added a small component to the model's predictive ability. Soil O<sub>2</sub> and log soil CO<sub>2</sub> were included in the best predictive model for soil [H<sup>+</sup>] and together accounted for an appreciable portion of the excess variance in this model (9.1%), especially considering the relative weakness of the base model in this case.

#### 4.9. Other Potentially Important Factors

[59] Diminished net methane consumption in the FACTS-I soils may have resulted from other unexplored factors that inhibited methanotrophy, stimulated methanogenesis, or had both effects. Nitrogen availability was one potential factor, as it has been substantially diminished in a variety of soils exposed to elevated CO<sub>2</sub> by immobilization in biomass with high C/N ratios [Curtis, 1996; Bertson and

Bazzaz, 1997, 1998; Hungate et al., 1999]. The FACTS-I site has not demonstrated this effect, however: After 3 years of fumigation, leaf litter C/N ratios, microbial biomass C/N ratios, and potential net N mineralization rates have been unaffected by elevated atmospheric CO<sub>2</sub> [Allen et al., 2000; Finzi et al., 2001], consistent with results of chamber studies with unrestricted soil volumes that have found few significant effects of elevated CO<sub>2</sub> on litter chemistry [Agren et al., 1999].

[60] A second unexplored factor was the composition of methane-cycling microbial populations. Evidence suggests that microbial populations can respond noticeably to elevated CO<sub>2</sub>, and increases have been reported in microbial biomass [Zak et al., 1993; Runion et al., 1994; Sowerby et al., 2000], numbers of nitrogen-fixing bacteria [Schortemeyer et al., 1996], and populations of mycorrhizal fungi [Runion et al., 1994; Bertson and Bazzaz, 1998] under CO<sub>2</sub> fumigation. Because methanogenic and methanotrophic bacteria are such crucial components of soil methane cycling, and because microbial populations adapt readily to changes in environmental conditions, much of the influence of elevated CO<sub>2</sub> on soil methane cycling is likely to have been mediated by changes in the activities and populations of methane-cycling bacteria.

## 5. Conclusions

[61] The results of this study showed that soil moisture and soil CO<sub>2</sub> were both significantly increased by CO<sub>2</sub> treatment, and because they were both significant predictors of variability in soil CH<sub>4</sub> and in surface CH<sub>4</sub> fluxes themselves, they have been revealed as two important mediators of atmospheric CO<sub>2</sub> effects on soil CH<sub>4</sub> cycling. Date and soil depth were also found to be significant predictors of soil porespace CH<sub>4</sub>, while porespace O<sub>2</sub>, [H<sup>+</sup>], and temperature were not significant influences. Taken together, the data presented show that elevated CO<sub>2</sub> has had a major impact on soil CH<sub>4</sub> cycling in the FACTS-I experimental site that has led to a strong inhibition of the ability of these temperate forest soils to consume atmospheric CH<sub>4</sub>. In addition, results suggest that the inhibition in net CH<sub>4</sub> consumption was mediated in part by microbial community changes in elevated CO<sub>2</sub> soils.

[62] An important debate currently surrounds the question of the influence of elevated atmospheric CO<sub>2</sub> on atmospheric CH<sub>4</sub>. One school of thought holds that increasing atmospheric CO<sub>2</sub> and accompanying warmer temperatures will lead to drier, more oxic wetland soils, simultaneously diminishing methanogenesis and increasing methanotrophy [Moore and Knowles, 1989; Whalen et al., 1990]. The other line of reasoning, supported by this study, projects that alterations in water, carbon, and O<sub>2</sub> budgets in unsaturated soils will simultaneously increase methanogenesis and diminish methanotrophy, ultimately leading to increases in atmospheric CH<sub>4</sub> [King, 1997; Ineson et al., 1998; Ambus and Robertson, 1999]. Accurate prediction of the ultimate result, corresponding to a weighted balance between the two opposing directions of

influence, will rely in part on studies like this one to elucidate the primary mechanisms governing interactions between elevated CO<sub>2</sub> and soil-based methane cycling.

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