Persistence and potential causes of reduced net CH_4 consumption under elevated CO_2 in a temperate forest

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ABSTRACT

Lindsay Laura Dubbs: Persistence and potential causes of reduced net CH_4 consumption under elevated CO_2 in a temperate forest
(Under the direction of Stephen C. Whalen)

Impacts of the projected increase in atmospheric CO_2 on other biogeochemical cycles are uncertain. In a two-year study, Phillips et al. (2001) reported a 16 to 30% decrease in net consumption of atmospheric CH_4 by soils in CO_2-enriched plots in a temperate loblolly pine (*Pinus taeda*) forest. Consumption by upland soils accounts for ~30 Tg CH_4 y^{-1} and is the only terrestrial sink for atmospheric CH_4, which is a greenhouse gas with radiative forcing second only to CO_2. However, it is uncertain whether decreased atmospheric CH_4 consumption represents a transient or sustained response of forest-soil systems to elevated CO_2.

This research focused on field observations aimed at investigating the strength and persistence of reduced atmospheric CH_4 consumption by temperate forest soils under elevated CO_2 at the same study site. It further investigates the causes of this response by CH_4 oxidizing and producing communities through field and laboratory experiments.

Rates of soil-atmosphere CH_4 exchange were repeatedly measured over 3 y from permanently established sampling sites at the Free Air Carbon Dioxide (FACE) site in the Duke Forest, where CO_2-enriched plots of a loblolly pine forest are maintained at approximately 200 mL L^{-1} above ambient concentrations (380 mL L^{-1}), while control plots are exposed to ambient atmospheres. Reduced net atmospheric CH_4 consumption persisted in CO_2-enriched plots, showing annual declines of 19, 10 and 8% relative to control plots.

This study and previous work give a nearly continuous 8 y record of reduced net atmospheric CH_4 consumption in CO_2 -enriched plots that suggests this is likely a sustained negative feedback to increasing atmospheric CO_2.

Causitive factors for the observed decrease in net CH_4 consumption under elevated CO_2 were difficult to identify because of high spatial and temporal variability in microbial activity and limited ability to collect soil samples. However, higher soil moisture and increased incidence and rates of CH_4 production in CO_2-enriched plots, along with transient inhibition by plant exudates and low overall soil diffusivity, begin to explain reduced rates of CH_4 consumption and increased rates of CH_4 production that result in long-term reduction in net CH_4 consumption in these soils.

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FACE	free air CO_2 enrichment
k	rate constant
α	p-value acceptance threshold
$D_{0,CH4}$	diffusion coefficients of CH_4
$D_{0,Rn}$	diffusion coefficients of Rn
P_{Rn}	permeability of Rn
J_{CH4}	flux of CH_4
ΔC_{CH4}	linear change in CH_4 concentration
Δz_{CH4}	linear change in depth

CHAPTER 1: INTRODUCTION

Elevated CO_2 and temperate forests

The present-day atmospheric CO_2 concentration of approximately 380 mL L^{-1} (NOAA 2008), exceeds the highest concentration measured in ice core samples from before the Industrial Revolution by almost 100 mL L^{-1} (Barnola et al. 2003). The atmospheric concentration of CO_2 is expected to continue to increase, mainly as a result of fossil fuel emissions and destruction of vegetation (Forster et al. 2007). Models project that atmospheric CO_2 concentrations, by the end of the present century, will exceed the pre-industrial concentration by up to 270% (Friedlingstein et al. 2006). A rising atmospheric CO_2 concentration is of concern because it is a long-lived greenhouse gas with a radiative forcing of 1.66 W m^{-2}, exceeding the radiative forcing of all other trace atmospheric gases that control climate (Forster et al. 2007). Increasing atmospheric CO_2 is also of significance because it is continuously exchanged between the atmosphere, the ocean, and the terrestrial biosphere through biogenic processes such as photosynthesis and respiration (Schlesinger 1997). Rates of photosynthesis and respiration are further controlled by temperature and water availability, and changes in the concentrations of CO_2 and other greenhouse gases are expected to elicit changes in air temperature and the hydrologic cycle that may vary regionally (Denman, K. L. et al. 2007). While the atmospheric concentration and radiative forcing of CO_2 are well understood (Forster et al. 2007), the impacts of the CO_2-induced changes in atmospheric composition and climate on whole ecosystems and their components

are less clear. An understanding of ecosystem responses at all levels to elevated CO_2 is important to predicting future climates as they can, in turn, feed back to the biogeochemical cycling of CO_2 and other greenhouse gases.

Attempts at understanding terrestrial biological and biogeochemical responses to elevated CO_2 have ranged in size and complexity from individual potted plants, to open-top chambers containing a community of plants, to large scale manipulations of intact ecosystems designed to embrace the entire suite of interactions and feedbacks among plants, microbial communities and elemental cycles. Each of these approaches has associated strengths and weaknesses. Physiological studies conducted in small and simple modeled ecosystems have been ineffective at capturing the complexity of ecosystem component interactions and feedbacks. On the other end of the spectrum, free-air CO_2 exchange (FACE) technology has been employed to conduct ecosystem-level studies where tall vegetation and their surrounding ecosystems are exposed to elevated CO_2 with minimal alterations of surrounding microenvironments (Hendrey et al. 1999b). The primary criticism of FACE experiments is that they are initiated by exposing an ecosystem to an abrupt increase in atmospheric CO_2, which may not fully represent how ecosystem components will react to the contemporary monotonic increase in the concentration of CO_2 in the Earth's atmosphere (Klironomos et al. 2005).

Nonetheless, FACE studies have proven to be useful in predicting ecosystem level changes in a range of terrestrial environments, among them, temperate forests. Overall, temperate forests exposed to elevated CO_2 using FACE technology show increases in tree growth and net primary production (DeLucia et al. 1999, Finzi et al. 2002, Hamilton et al. 2002, DeLucia, E.H. et al. 2005, Norby et al. 2005, Finzi et al. 2006a), increased delivery of

C to roots, the forest floor and soils with a small increase in soil C storage (Allen et al. 2000, Matamala and Schlesinger 2000, Schlesinger and Lichter 2001, Jastrow et al. 2005, Lichter et al. 2005, Lichter et al. 2008, Pritchard et al. 2008, Hoosbeek and Scarascia-Mugnozza 2009), increased soil respiration (King et al. 2004, Bernhardt et al. 2006, Taneva et al. 2006), and variable changes in N cycling (Billings and Ziegler 2005, DeLucia 2005, Finzi et al. 2006a) and soil community composition (Larson et al. 2002, Billings and Ziegler 2005, Billings and Ziegler 2008). The initial increase in net primary production in response to elevated CO_2 is predicted to slow with time as ecosystems become more N-limited (Finzi et al. 2006b), although N-limitation has yet to appear in temperate forests after 6 y of CO_2-enrichment using FACE technology (Finzi et al. 2006a).

However, observations (McMurtrie and Comins 1996) and ecosystem models (Newton et al. 2001) indicate that biological responses to elevated CO_2 and biogeochemical feedbacks vary widely on different timescales. For instance, down-regulation of photosynthesis has been commonly reported for CO_2-fertilized model and intact forest ecosystems after as little as two years (reviewed by Amthor 1995, Leakey et al. 2009). Over longer time trajectories, initial response functions of all ecosystem components from trees to microbes can be expected to adjust physiologically and demographically on different time scales through modification of biogeochemical feedbacks (Korner 2000). Thus short-and long-term responses to elevated atmospheric CO_2 must be distinguished.

Methane in the pedosphere

Methane is another greenhouse gas that is cycled through temperate forests and thus may be affected by CO_2-induced changes to the ecosystem. Methane is the simplest, most

reduced hydrocarbon, and a long-lived (9 to 15 y) greenhouse gas directly and indirectly contributing more than half of the radiative forcing of CO_2 (0.9 and 1.6 W m^{-2}, respectively; Schindell et al. 2005), through warming of the troposphere and its participation in the stratospheric chemistry of ozone and water vapor formation (Wuebbles and Hayhoe 2002). The global atmospheric CH_4 concentration has more than doubled since the Industrial Revolution to reach a present-day average concentration of ~1780 µL L^{-1} (NOAA 2008). Methane is spatially and temporally variable in the troposphere, with a higher concentration in the Northern Hemisphere where emissions are higher, and a minima corresponding to increased photochemical destruction during summer months.

While destruction by the hydroxyl radical in the atmosphere is the largest sink for CH_4, the only known biological sink for CH_4, and the largest natural source of CH_4 are sited in the pedosphere. The balance between rates of CH_4 production (methanogenesis) and CH_4 consumption (methanotrophy) determines whether a soil is a net source or sink for atmospheric CH_4, and the strength of that source/sink. Methane production usually exceeds consumption in wetland environments, accounting for about 69% of emissions to the atmosphere from natural sources (Wuebbles and Hayhoe 2002). Conversely, upland soils account for approximately 38 Tg of CH_4 removal from the atmosphere annually (Ridgwell et al. 1999). This net biological sink in upland soils includes atmospheric CH_4 consumption by methanotrophic bacteria in the largely oxic soil profile, and consumption of endogenously produced CH_4 by methanogenic bacteria in anoxic microsites (reviewed by Conrad 1996).

Methanotrophic bacteria oxidize CH_4 for energy and as their sole source of carbon (C) for biosynthesis (Hanson and Hanson 1996). Methanotrophs are responsible for both 'high affinity oxidation' of CH_4, which occurs at CH_4 concentrations close to atmospheric

concentrations (< 12 mL L^{-1}), such as in upland soils, and 'low affinity oxidation', which occurs at CH_4 concentrations > 40 mL L^{-1}, such as in the oxic zone of wetlands (Le Mer and Roger 2001). Known controls on CH_4 consumption by low affinity methanotrophs are water table position, which dictates the size of the oxic zone necessary for methanotrophy, pH, and temperature (reviewed by Whalen 2005). Demonstrated controls on atmospheric CH_4 consumption by high affinity methanotrophs in upland soils include temperature (Crill 1991, Castro et al. 1995, Phillips et al. 2001a, Steinkamp et al. 2001), soils nitrogen (Schnell and King 1994, 1995), and rate of supply of CH_4 to the subsurface aerobic zone of oxidation (King and Adamsen 1992, Dörr et al. 1993, King 1997).

Methane is produced by methanogenic Archaea through two different anaerobic metabolic processes, acetate splitting and CO_2 reduction. Of all metabolic pathways, methanogenesis yields the least free energy and methanogenic Archaea are typically out-competed by microbes with alternative metabolic pathways (Schlesinger 1997), except when the redox potential is very low, such as in persistently anoxic wetlands. The absence of oxygen, which is related to soil moisture, the availability of labile organic precursors, temperature, and pH are known controls on CH_4 production. Accordingly, wetlands and freshwater sediments, with low redox potentials and high levels of organic matter, provide natural environments favorable to methanogenesis. Low redox environments with high availability of labile organic matter have also been observed in aggregates of clay-rich forest soils (Sexstone, A.J. et al. 1985, Ramakrishnan et al. 2000). Independent reports of anoxic microzones (Sexstone, Alan J. et al. 1985, Zausig et al. 1993) and methanogenic activity in macroscopically oxygenated soils (Yavitt et al. 1995, Saari et al. 1997, von Fischer and Hedin 2002, Teh et al. 2005) indicate that simultaneous CH_4 production and consumption are

occurring in some well-drained upland soils. Waterlogged aggregates support localized zones of methanogenesis and oxic sites support methanotrophy.

Observed effects of elevated CO_2 on CH_4 dynamics in soils

Experiments examining the effect of elevated CO_2 on net CH_4 emissions from wetland soils unequivocally indicate that CH_4 emissions increase when wetland plants, plant communities, or ecosystems are grown under elevated CO_2. Increases in CH_4 emissions from wetland soils ranged from 10.9% in a pot study of a rice cultivars grown under CO_2 at 200 mL L^{-1} above ambient concentrations (Lou et al. 2008) to 60% when rice fields were exposed to elevated CO_2 (300 mL L^{-1} above ambient) in open-top chambers (Ziska et al. 1998). The increase in net CH_4 emissions was markedly similar to the range (38 to 58%) seen in a Japanese rice paddy exposed to elevated CO_2 via FACE technology (Inubushi et al. 2003).

Investigations of soil-atmosphere CH_4 exchange in CO_2-enriched ecosystems that normally function as atmospheric CH_4 sinks are few and show mixed results. Ambus and Robertson (1999) reported a 22% reduction in CH_4 consumption by soils in model *Populus tremuloides* (deciduous forest) ecosystem exposed to elevated CO_2, while Phillips et al. (2001a) showed annual reductions in CH_4 consumption of 16% and 30% in CO_2 fumigated plots (200 mL L^{-1}) relative to plots exposed ambient atmospheres in a 2 y study in a temperate forest. In grasslands, Ineson et al. (1998) observed that rates of atmospheric CH_4 uptake were three times greater in ambient CO_2 soils relative to CO_2-enriched plots in an N-fertilized sward of *Lolium perenne*, but a subsequent investigation (Baggs and Blum 2004) found a significant interaction between N fertilizer application rate and CO_2 on atmospheric

CH_4 consumption. Further, Mosier et al. (2002) saw no impact of CO_2 level on rates of CH_4 exchange between soils and the atmosphere in a semi-arid, mixed grassland community.

If the observed CO_2-induced increases in net CH_4 emissions are extrapolated to the global scale, the wetland (natural and agricultural environments) source strength in the atmospheric CH_4 budget will increase by 29 and 160 Tg annually with a 200 to 300 mL L^{-1} increase in atmospheric CO_2 concentration (Chen and Prinn 2005). At the same time, models suggest that the annual forest sink of 24 Tg y^{-1} for CH_4 (Ridgwell et al. 1999) can be expected to decline from between 3.8 to 7.2 Tg as atmospheric CO_2 concentrations increase by 200 mL L^{-1}. However, more empirical data are needed before we can rely on these predictions of changes in CH_4 source and sink terms with increasing atmospheric CO_2. The few extant observational records of < 2 y in forest ecosystems and < 3 y in wetlands are insufficient to distinguish between transient and equilibrium responses of forest and wetland ecosystems to elevated CO_2 and the impact of those responses on CH_4 cycling.

Ecosystem-level changes that may influence CH_4 dynamics in temperate forests

Several CO_2-induced changes in temperate forest ecosystems may help to explain the observed decline in net CH_4 consumption under elevated CO_2. Changes in plant productivity, chemistry, and allocation of C under elevated CO_2 impacts the quantity and quality of C in the ecosystem, and the supply and availability of C to soil organisms. Some C compounds, such as phenolics, tannins and terpenes inhibit metabolism and growth by some soil microorganisms. Examples of enhanced delivery of C to the soil under elevated CO_2 in FACE studies include increased labile dissolved organic C in throughfall, (Lichter et al. 2000b), a small increase in the storage of C in forest soils (Matamala and Schlesinger 2000,

Lichter et al. 2008) and increased root productivity and mortality (Pritchard et al. 2008). Enhanced root exudation of organic acids has been observed in a pot study of *Pinus echinata* seedlings (Norby et al. 1987), while greater litter fall in both FACE and microcosm studies (Allen et al. 2000, Lichter et al. 2005, Lichter et al. 2008, Liu et al. 2009) has been reported under elevated CO_2. Further, several researchers have seen changes in the abundance of secondary C compounds in tissues and root exudates between plants grown under elevated and ambient CO_2 (Peñuelas and Estiarte 1998, Verburg et al. 1999, Tuchman et al. 2002, Billings and Ziegler 2005, Wetzel and Tuchman 2005a). Secondary C compounds, such as phenolics and terpenes, inhibit metabolism and growth by broad groups of soil bacteria (Souto et al. 2000), and specifically, methanotrophs (Amaral and Knowles 1997, 1998).

Reduced net CH_4 consumption under elevated CO_2 in temperate forests may also be the result of higher soil moisture and the associated reduction in diffusion of atmospheric gases. Reduced gas diffusivity has been demonstrated (Dörr et al. 1993) to control rates of CH_4 supply to the usual subsurface locus of CH_4 oxidation (e.g. Whalen and Reeburgh 1992), which is itself substrate-limited in well-drained forest soils, based on kinetic considerations (Bradford et al. 2001). Thicker leaf litter in forests exposed to elevated CO_2 (Allen et al. 2000, Lichter et al. 2005, Lichter et al. 2008, Liu et al. 2009) can result in higher soil moisture because of reduced evaporation from the soil surface. Increased soil moisture in turn slows the transport of gases within the soil matrix (Suwa et al. 2004). In fact, a direct link between increased soil moisture and diffusion-limitation of substrate to CH_4 oxidizers is well established (Striegl 1993, Castro et al. 1995, Whalen and Reeburgh 1996). The excess of litterfall under elevated CO_2 additionally directly adds to diffusional resistance in soils, and experimental litter removal has been shown to increase rates of net atmospheric CH_4

consumption in forest soils by as much as 43% (Dong et al. 1998, Brumme and Borken 1999).

Finally, reduced diffusion of atmospheric O_2, because of thicker leaf litter and higher soil moisture, along with higher soil respiration (Bernhardt et al. 2006, Taneva et al. 2006), and increased soil aggregation (Hoosbeek and Scarascia-Mugnozza 2009) under elevated CO_2 may increase the incidence of anoxic microsites where anaerobic microbial metabolism, such as methanogenesis, is possible. Since net CH_4 consumption in upland soils is the net effect of CH_4 consumption in the oxic soil profiles and CH_4 production in anoxic microsites, increased incidence of anoxic loci can alter this balance, reducing rates of net CH_4 consumption or shifting localized areas to net CH_4 sources. Horn and Smucker (2005) found when soil aggregates were saturated with water, the redox potential decreased rapidly, making these soil aggregates transiently anoxic within an otherwise oxic profile.

Research objectives

This is a follow-up study to previous research reported by Phillips et al. (2001a), who saw 16 and 30% annual reductions in rates of net CH_4 consumption by soils in a temperate forest enriched with elevated CO_2. The cause(s) of the decline in rates of net CH_4 consumption were not identified and the persistence of such a reduction beyond 2 y was not determined. Therefore, the purpose of this dissertation is to a) determine if reduced net CH_4 consumption by the same temperate forest soils is a sustained response to elevated CO_2; and b) identify factor(s) contributing to the observed (Phillips et al. 2001a) decline in net CH_4 consumption under elevated CO_2 at the Duke Forest FACE site. Possible controls on CH_4 consumption resulting from elevated CO_2 concentrations include negative impacts of altered

organic compounds from the surrounding forest ecosystem on CH_4 oxidizing communities, higher soil moisture and an associated reduction in the supply of CH_4 to the zone of CH_4 oxidation, or a shift in the rates of consumption and production by CH_4 oxidizing and producing communities, respectively.

Model projections of future climates are strongly dependent on atmospheric concentrations of radiatively and chemically important trace gases, such as CH_4. Therefore, my intention is for this research to be used to improve model projections of future climates, with special attention to the feedbacks of elevated CO_2 on ecosystem components that control CH_4 dynamics within forest soils.

Dissertation structure

This dissertation has been written as 5 chapters. Chapters 2 through 4 were written with the intention of submitting each chapter as individual manuscripts. Chapters 1 and 5 introduce and conclude, respectively, the body of work. Chapter 2 shows an extension of the previous 2 y record of soil-atmosphere exchange of CH_4 in CO_2-enriched and free-air (control) plots to establish the long-term response of atmospheric CH_4 consumption under elevated CO_2. In Chapter 2, I also investigate if treatment-wise differences or interactions in environmental measures (soil moisture and temperature) account for reduced atmospheric CH_4 consumption in CO_2-enriched plots. Chapter 3 investigates the possibility of plant exudate control on CH_4 consumption in soils from the same study site. Chapter 4 evaluates the depth distribution of CH_4 in the soil profile, the effective diffusivity of CH_4 through the soil, as well as the extent and activity of CH_4 consuming and producing communities at the study site. This structure may result in some repetition in introductory material and

discussion of results.

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CHAPTER 2: REDUCED NET CH_4 CONSUMPTION IS A SUSTAINED RESPONSE TO ELEVATED CO_2 IN A TEMPERATE FOREST

Abstract

We compared, from 2004 through 2006, rates of soil-atmosphere CH_4 exchange at permanently established sampling sites in a temperate forest exposed to ambient (control plots; ~380 mL L^{-1}) or elevated (ambient + 200 mL L^{-1}) CO_2 since August 1996. A total of 880 observations showed net atmospheric CH_4 consumption (flux from the atmosphere to the soil) from all static chambers most of the time at rates varying from 0.02 mg m^{-2} d^{-1} to 4.5 mg m^{-2} d^{-1}. However, we infrequently found net CH_4 production (flux from the soil to the atmosphere) at lower rates, 0.01 mg m^{-2} d^{-1} to 0.08 mg m^{-2} d^{-1}. For the entire study, the mean rate of net CH_4 consumption in control plots was higher than the mean for CO_2-enriched plots, 0.55 (± 0.03 SEM) versus 0.51 (± 0.03 SEM) mg m^{-2} d^{-1}. Annual rates of 184, 196 and 197 mg m^{-2} for net CH_4 consumption at control plots during the three calendar years of this study were 19, 10 and 8% higher than comparable values for CO_2 enriched plots. Differences between treatments were significant ($p < 0.05$) in 2004 and 2005 and nearly significant ($p = 0.10$) in 2006. Volumetric soil water content was consistently higher at CO_2-enriched sites and a mixed effects model identified a significant soil moisture x CO_2 interaction on net atmospheric CH_4 consumption. Increased soil moisture at CO_2-enriched sites likely increases diffusional resistance of surface soils and the frequency of anaerobic microsites supporting methanogenesis,

resulting in reduced rates of net atmospheric CH_4 consumption. Our study extends previous observations of reduced net atmospheric CH_4 consumption at CO_2-enriched plots at this site to nearly 8 continuous years, suggesting that this is likely a sustained negative feedback to increasing atmospheric CO_2.

Introduction

The atmospheric concentration of CH_4 has more than doubled since the Industrial Revolution to a present-day value of ~1782 $\mu L\ L^{-1}$ (Forster et al. 2007). This generalized increase is of concern because CH_4 is second only to CO_2 among trace atmospheric constituents with respect to radiative forcing and is also chemically active in the atmosphere, playing an important role in stratospheric and tropospheric ozone chemistry (Denman et al. 2007).

The atmospheric concentration of CO_2 has increased parallel to that of CH_4, and is projected to reach 730 $mL\ L^{-1}$ by 2100, a level that exceeds the preindustrial concentration by 260% (Forster et al. 2007). Although the unprecedented rate of change in the atmospheric concentrations of CO_2 and CH_4 over the last 250 y and the influences on climate are well documented, the reasons for changing abundances are not entirely clear. Model projections of future climate are strongly dependent on atmospheric concentrations of radiatively and chemically important trace gases. Cycling of long-lived greenhouse gases such as CO_2 and CH_4 are dominated or supported by a biospheric component responsible for the production and consumption of these gases, and for modulating or mediating gas exchange between the pedosphere or hydrosphere and atmosphere. However, improvements to current models require a comprehensive

understanding of the linkage between biogeochemical processes and the troposphere with respect to trace atmospheric constituents that influence climate, and further identification of the interactions between biogeochemical cycles that impact exchange of trace gases between soil or water and the atmosphere.

The balance between rates of CH_4 production (methanogenesis) and consumption (methanotrophy) determines whether a soil is a net source or sink for atmospheric CH_4. Methane production usually exceeds consumption in wetland environments, accounting for about 69% of emissions to the atmosphere from natural sources (Wuebbles and Hayhoe 2002). In contrast, well-drained soils generally display net consumption of CH_4, and constitute the only biological loss term in the atmospheric CH_4 budget. Little is understood about the effect of elevated CO_2 on biogeochemical processes affecting CH_4 cycling dynamics. However, Phillips et al. (2001a) previously showed, in a short term (2 y) study, annual reductions in CH_4 consumption of 16% and 30% in CO_2-fumigated plots relative to plots exposed to ambient atmospheres in an upland temperate forest. Similar investigations on shorter time scales (weeks to 2 mo) report reduced atmospheric CH_4 consumption under elevated CO_2 in a deciduous forest (Ambus and Robertson 1999), and give mixed results for a grassland (Ineson et al. 1998, Baggs and Blum 2004), although results in these studies include nitrogen x CO_2 fertilization interactions. In contrast, Mosier et al. (2002) reported no CO_2-induced response in atmospheric CH_4 consumption in a shortgrass steppe over 4 y.

A negative feedback on forest soil CH_4 consumption by rising CO_2 has important implications for the atmospheric CH_4 budget. Sink strength estimates for upland soils center around 30 Tg y^{-1}, or about 75% of the stratospheric sink of 40 Tg y^{-1} (Denman et

al. 2007). The few extant observational records of < 2 y, however, are insufficient to distinguish between transient and equilibrium responses of forest ecosystems to elevated CO_2 or to determine whether the observed response will be sustained. Ecosystem models indicate that plant/community responses to elevated CO_2 and biogeochemical feedbacks can change over time (Newton et al. 2001). It is therefore critical to identify the long-term trajectory of the sign and magnitude of change.

Our study was conducted in an aggrading temperate forest where experimental plots had been continuously fumigated with CO_2. Our objectives were to: (a) extend a previous 2 y record of soil-atmosphere exchange of CH_4 in CO_2-enriched and free-air (control) plots (Phillips et al. 2001a) to establish the long term response of atmospheric CH_4 consumption under elevated CO_2; and (b) relate environmental measures (soil moisture and temperature) to rates of gas exchange to determine if treatment-wise differences or interactions in these well known controls on soil methanotrophy may account for reduced atmospheric CH_4 consumption in CO_2 enriched plots. A firmer understanding of the feedback between increasing atmospheric CO_2 and the rates and controls on CH_4 oxidation in forest soils will aid in the refinement of process-based models that contribute to larger efforts directed at predicting future climates.

Methods

Field site

Field measurements were conducted at the Duke Forest (North Carolina; USA) Free-Air CO_2 Enrichment (FACE) experiment sited in an even-aged stand of loblolly pine (*Pinus taeda* L.) planted in 1983. Soils are clay loam, Ultic Hapludalf's of the Enon

Series (Oh and Richter 2005). Average normal air temperature ranges from 3.6 ° C in January to 25.3 ° C in July and annual precipitation averages 1209 mm (State Climate Office of North State Climate Office of North Carolina 2003-2009). Soil physical characteristics are similar between CO_2 treatment plots, with the exception of soil organic matter, which averaged 4.6% in CO_2-enriched plots, and only 3.4% in control plots. Averages for all control and elevated CO_2 plots (0 to 20 cm depth zone) for soil particle density, bulk density, and pH were 2.5 g cm^{-3}, 1.2 g cm^{-3}, and 5.7 units, respectively. Soil texture was 9% clay, 42% silt, and 49% sand.

Site characteristics are fully documented in Hendrey et al. (1999a) and briefly described here. The experiment consists of eight circular 30-m diameter plots. Four treatment plots (referred to as "CO_2-enriched") are fumigated with CO_2 to maintain atmospheric CO_2 concentrations 200 mL L^{-1} above ambient levels, while three additional treatment plots are fumigated with ambient air to replicate micrometeorological effects associated with CO_2 addition. A fourth is subjected to ambient air without fumigation. The latter four plots are referred to as "controls". Continuous (24 h d^{-1}) fumigation was initiated in August 1996, but was reduced to daylight hours only from 2003 to present.

Gas Flux Measurements

Each plot is partitioned into four quadrants for a total of 24 (2004 and 2005) and 32 (2006) individual sectors. Methane flux determinations within each sector were made ~biweekly in the 2004, 2005, and 2006 calendar years using the static chamber technique (Whalen et al. 1992), yielding 12 (2004 and 2005) and 16 (2006) measurements in both control and enriched plots on each sampling date. The polyvinyl chloride collars (20 cm

diameter x 11 cm height) permanently deployed in three plots of each treatment at the conception (1999) of our initial investigation remained intact and were revisited for this study. Collars of similar design were deployed in each quadrant of two additional plots (one plot, each treatment) in 2006. Polyvinyl chloride covers fitted with a sampling port and capillary bleed were emplaced on soil collars for CH_4 flux determinations. Headspace samples were withdrawn into 10 mL gastight glass syringes at zero time and at 0.5 h intervals thereafter to 2 h. Collars were open to litterfall and rainfall between sampling sessions.

Gas samples were analyzed for CH_4 by flame ionization detection gas chromatography (Shimadzu model GC 8 A; precision expressed as the coefficient of variation for 10 replicate injections of a 0.94 mL CH_4 L^{-1} standard was < 3%) within 10 h of collection, well within our predetermined holding time of 24 h. Sample separation was accomplished on a 1-m length x 0.32 cm diameter molecular sieve 5A column with an ultrahigh purity N_2 carrier gas (33 mL min^{-1}). Injector and detector temperatures were set at 90 ^{o}C and 140 ^{o}C.

Soil Physicochemical Measurements

Volumetric soil moisture (mL H_2O cm^{-3} soil) was continuously measured by time domain reflectometry using Campbell Scientific Model CS616 probes. Probes were located randomly in each quadrant of each plot in calendar years 2004 and 2005 (Hyun unpublished) and within 30 cm of each soil collar in 2006. The soil moisture probes integrate volumetric soil moisture from the soil surface to 30 cm depth at 30 s intervals, and average values over 24 h are recorded on Campbell Scientific Model CR200

dataloggers. In conjunction with CH_4 flux determinations, soil temperature was measured at 3 cm intervals from 1 cm to 19 cm depth with a multithermistor temperature probe.

Calculations and statistics

Area-based rates of net CH_4 consumption were calculated from chamber geometry and the time-linear change of CH_4 concentration in chamber headspaces. Annual rates of net CH_4 consumption were determined by integrating for the calendar year daily, area-based data from each sampling occasion. Average soil temperature was calculated as the mean of equally spaced observations taken to 19 cm.

We analyzed for differences in CH_4 flux between CO_2 treatments with the same statistical model used in a previous study (Phillips et al. 2001a). The mixed effects linear model considered CO_2 treatment as the main effect, with soil moisture, temperature and time (continuous) as covariates. The model was a nested, hierarchical design with plot nested inside CO_2 and quadrant nested within plot. Unequal sampling intervals required the use of a time-series covariance structure, where correlations decline as a function of time. Only significant interactions remained in the model. The same model with a different nesting structure was used to analyze overall net CH_4 flux and environmental variables, in which quadrant was nested within plot and CO_2 was simply an effect.

Student t-tests were used to analyze for statistical differences between CO_2 treatment averages for environmental variables. Treatment-wise differences between annual rates of net CH_4 consumption were determined by confidence interval overlap. All statistical analyses were performed at α=0.05.

Results

Environmental variables

Air temperatures averaged 15 °C or 16 °C annually for each calendar year. Soil temperatures ranged from 4 °C to 25 °C (Fig. 2.1), closely tracking air temperatures (not shown) and showed an average of 16 °C for the entire study. Overall, soil moisture varied from 0.16 to 0.49 mL H_2O cm^{-3} soil, and averaged 0.27 mL H_2O cm^{-3} soil. Calendar year means for CO_2-enriched plots were consistently higher than means for control plots (Table 2.1). Differences between treatment means for soil moisture were significant in 2004 and 2005, but not 2006. Over the entire study (n = 68), the average soil moisture for the CO_2-enriched treatment (0.28 mL H_2O cm^{-3} soil) was significantly higher than for the control treatment (0.26 mL H_2O cm^{-3} soil).

Patterns in net CH_4 flux

Net CH_4 consumption (flux from the atmosphere to the soil) was generally found at all individual soil chambers and was always calculated for each plot if fluxes from all four quadrants were averaged. However, net CH_4 production (flux from the soil to the atmosphere) was also observed, from 17 individual quadrants on 16 separate dates, giving 22 observations of net CH_4 production in 880 total records. Net CH_4 production was found almost twice as often in CO_2-enriched chambers as in control chambers (14 versus 8 observations). Rates of net CH_4 production from individual chambers varied from 0.01 mg m^{-2} d^{-1} to 0.08 mg m^{-2} d^{-1} while rates of net CH_4 consumption from individual chambers were much higher, varying from 0.02 mg m^{-2} d^{-1} to 4.5 mg m^{-2} d^{-1}. Chamber-wise analysis showed no pattern with respect to magnitude of flux, as no chamber

showed consistently high or low values. Although there was no clear seasonal pattern, rates of net CH_4 consumption were frequently higher in the summer than the winter months (Fig. 2.1).

There was a strong inverse relationship between soil moisture and plot-averaged rates of net CH_4 consumption (Fig. 2.2). At the chamber level, the mixed-effects model used to test the factors contributing to overall net CH_4 fluxes showed that soil moisture was significantly related to net CH_4 flux in 2006, when soil moisture probes were installed proximal to chamber collars, but not in 2004 and 2005 when probes were randomly located within quadrants. Overall, net CH_4 consumption decreased with increasing soil moisture. The model showed no relationship between soil temperature and net CH_4 flux.

Differences in net CH_4 consumption between CO_2 treatments

When the entire data were considered (880 observations; each treatment), the mean net rate of CH_4 consumption in control chambers was 7.5% higher than in CO_2-enriched chambers, 0.55 (± 0.03 SEM) versus 0.51 (± 0.03) mg m^{-2} d^{-1}. The difference was significant. The disparity in net CH_4 consumption rates between treatments showed interannual variability. Mean rates for controls in 2004, 2005 and 2006 were 0.53 (± 0.06), 0.54 (± 0.06) and 0.56 (± 0.05) mg CH_4 m^{-2} d^{-1}. These values were higher by 10, 4 and 9% than corresponding annual averages for CO_2-enriched chambers. There was no seasonal pattern in the relative difference in net CH_4 consumption rates between treatments.

The mixed-effects model used to test the factors contributing to the variability in net CH_4 consumption between treatments indicated that CO_2 significantly interacted with soil moisture. Soils from CO_2-enriched plots consumed less CH_4 than soils from control plots, and the difference between CO_2 treatments increased with increasing soil moisture. Soil temperature had no effect.

The time-integrated rates of net CH_4 consumption in control plots were 184, 196 and 197 mg m^{-2} y^{-1} in 2004, 2005, and 2006 (Fig. 2.3). Comparable values for CO_2-enriched plots were lower by 19, 10, and 8% at 150, 175 and 181 mg m^{-2} y^{-1}. Differences between treatments were significant in 2004 and 2005 and nearly significant in 2006 ($p=0.10$).

Discussion

Overall patterns of net CH_4 consumption and environmental correlates

Consumption of atmospheric CH_4 by well-drained forest soils is a common observation in all climatic zones of the world. The mean net CH_4 consumption rate of 0.54 mg m^{-2} d^{-1} in the present study is consistent with the value of 0.6 mg m^{-2} d^{-1} reported by both our group (Phillips et al. 2001a) and others (McLain et al. 2002) for studies conducted roughly 6 y previously. Mean rates of net CH_4 consumption for this site falls toward the low end of worldwide reports for aerated temperate forest soils, which show averages ranging from 0.2 to 5.0 mg CH_4 m^{-2} d^{-1} and center around 1 mg CH_4 m^{-2} d^{-1} (summarized by Smith et al. 2000a, Butterbach-Bahl et al. 2002). The average annual rate of net CH_4 consumption in control plots (192 mg m^{-2}) was markedly similar to the average (187 mg m^{-2}) for a previous study (Phillips et al. 2001a). As with our average

day rate estimates of net CH_4 consumption, these annual values are at the low end of estimates for North American temperate forests, which largely fall between 320 and 2560 mg m^{-2} y^{-1}, but have a strong New England bias (Smith et al. 2000b). Gas diffusivity has been demonstrated (Dörr et al. 1993) to control rates of CH_4 supply to the usual subsurface locus of CH_4 oxidation (e.g. Whalen et al. 1992), which is itself substrate-limited in well-drained forest soils, based on kinetic considerations (Bradford et al. 2001). Soil texture influences diffusivity (Ball et al. 1997), with clay soils showing net CH_4 consumption rates an order of magnitude lower than sandy soils (Dörr et al. 1993). It is likely that the fine texture of soil at our study site limits transport of atmospheric CH_4 down-profile, resulting in comparatively low area-based rated of net CH_4 consumption.

Net CH_4 consumption showed no relationship with soil temperature when the entire data over the temperature range 4 to 25 ^oC were considered, in agreement with the general lack of seasonality in flux (Fig. 2.1). However, this is at odds with the previous report of a significant, but weak temperature effect on net CH_4 consumption at this site (Phillips et al. 2001a). Other studies have frequently shown no or low influence of temperature on atmospheric CH_4 consumption in forest soils (e.g. Borken and Brumme 1997, Butterbach-Bahl and Papen 2002), an observation consistent with the dominance of diffusion limitation (substrate supply) over enzymatic limitation of methanotrophy that can be expected at typical atmospheric CH_4 concentrations (King and Adamsen 1992). However, some north temperate forest soils show an increased influence of temperature on atmospheric CH_4 consumption at values < 10 ^oC (Crill 1991, Castro et al. 1995, Steinkamp et al. 2001). Examination of our data with respect to this threshold extends

the observations of the influence of low temperatures on CH_4 consumption southward. The average net CH_4 consumption rate of 0.39 mg CH_4 m^{-2} d^{-1} for the 12 of 68 sampling dates at soil temperatures < 10 ^oC was lower by 32% than the mean of 0.58 mg CH_4 m^{-2} d^{-1} at higher temperatures. Differences in the strength of the temperature-CH_4 flux relationship at low temperatures between the past (Phillips et al. 2001a) and present investigations may have accounted for the disparity in the observed relationship between these variables when the entire data from each study were considered.

In contrast to temperature, we observed a strong (inverse) linear relationship between net CH_4 consumption and soil moisture (Fig. 2.2), which explained 34% of the variability in the entire data, and proved significant in the mixed-effects model for the 2006 data when moisture probes were sited in proximity to soil collars. This confirms previous observations of reduced net CH_4 consumption with increasing soil moisture at this site (Phillips et al. 2001a, McLain et al. 2002) and is consistent with other *in situ* seasonal studies in forest soils. However, our site is apparently less sensitive than many others to changes in soil moisture, as this factor explained 59 to 88% of the variability in net CH_4 consumption across a range of forest ecosystem types (Castro et al. 1994, Lessard et al. 1994, Steinkamp et al. 2001, Butterbach-Bahl and Papen 2002, Price et al. 2004).

Differences in net CH_4 consumption between CO_2 treatments

We found that CO_2 enrichment resulted in a per annum decline in net atmospheric CH_4 consumption of 8 to 19% relative to unamended controls, in accord with previous reports for this site (Phillips et al. 2001a, McLain et al. 2002). Moreover, there is no

compelling evidence for a temporal decline in the magnitude of the reduction in net CH_4 consumption in CO_2-enriched plots compared with controls when our entire data are considered (Table 1). Investigations of soil-atmosphere CH_4 exchange in CO_2-enriched ecosystems that normally function as an atmospheric CH_4 sink are few. In the most directly comparable study to our own, Ambus and Robertson (1999) reported a 22% reduction in CH_4 consumption by soils in model *Populus tremuloides* ecosystems exposed to elevated CO_2. Ineson et al. (1998) observed that rates of net atmospheric CH_4 uptake were three times greater in ambient CO_2 soils relative to CO_2-enriched plots in an N-fertilized sward of *Lolium perenne*. However, a subsequent investigation (Baggs and Blum 2004) found a significant interaction between N fertilizer application rate and CO_2 on net atmospheric CH_4 consumption. Mosier et al. (2002) saw no impact of CO_2 level on rates of CH_4 exchange between soils and the atmosphere in a semi-arid, mixed grassland community.

This study adds to previous efforts (Phillips et al. 2001a, Whalen unpublished) to uniquely provide a nearly continuous 8 y record of reduced atmospheric CH_4 consumption under elevated CO_2 at the same permanently installed soil collars in a representative southern forest. Short-and long-term responses to elevated atmospheric CO_2 must be distinguished. For instance, down-regulation of photosynthesis has been commonly reported for CO_2-fertilized model and intact forest ecosystems after as little as two years (reviewed byAmthor 1995). Over longer time trajectories, initial response functions of all ecosystem components from trees to microbes can be expected to adjust physiologically and demographically on different time scales through modification of biogeochemical feedbacks (Korner 2000). The lag of nearly 2 y between the initiation of

CO_2 fumigation and initial sampling (Phillips et al. 2001a), consistently lower annualized rates of net CH_4 consumption in soils from CO_2-enriched plots relative to controls, and lack of strong evidence that the magnitude of the CO_2 enrichment effect has declined with time all suggest that reduced net atmospheric CH_4 consumption is a sustained, equilibrium response of this forest soil to elevated CO_2.

Potential reasons for reduced net CH_4 consumption under elevated CO_2

Although reduced net atmospheric CH_4 consumption is likely a sustained negative feedback by soil to CO_2-enrichment at our study site, causative factors are difficult to identify, as the destructive sampling necessary for process-level investigations is limited to maintain ecosystem integrity. However, the significant moisture x treatment interaction in our mixed effects model indicates that site-wise differences in net CH_4 consumption are at least in part moisture-related. Several demonstrated effects of CO_2-enrichment on above- and below-ground processes within forest ecosystems feed back on soil moisture and by extension soil CH_4 cycling dynamics (Fig. 2.4).

The net soil-atmosphere CH_4 flux represents the balance between methanogenesis and methanotrophy, and changes in soil moisture elicit offsetting responses in these two microbial processes. Increased net primary production under elevated CO_2 (Fig. 2.4, pathway B) at our site (DeLucia et al. 1999, Hamilton et al. 2002, DeLucia, E.H. et al. 2005, Norby et al. 2005, Finzi et al. 2006a) is responsible for a continuous ~17% greater annual increment of litterfall since fumigation (Allen et al. 2000, Lichter et al. 2005, Lichter et al. 2008; with exception of the 2 y following a 2002 ice storm where litter fall increased regardless of CO_2 treatment). The associated insulating effect enhances

moisture conservation (Schäfer et al. 2003). The direct link between increased soil moisture and diffusion-limitation to CH_4 oxidizers is well established (Striegl 1993, Castro et al. 1995, Whalen and Reeburgh 1996). Persistently higher moisture content and reduced net atmospheric CH_4 consumption in CO_2-enriched plots relative to controls (Table 2) is consistent with a reduction in substrate supply to methanotrophs (Fig. 2.4; pathway B). The excess of litterfall under elevated CO_2 also directly adds to diffusional resistance in soils within these plots. Experimental litter removal has been shown to increase rates on net atmospheric consumption in forest soils by as much as 43% (Dong et al. 1998, Brumme and Borken 1999).

Independent reports of anoxic microzones (Sexstone et al. 1985, Zausig et al. 1993) and methanogenic activity in macroscopically oxygenated soils (Yavitt et al. 1995, Saari et al. 1997, von Fischer and Hedin 2002, Teh et al. 2005) indicate that simultaneous CH_4 production and consumption are occurring in some well-drained upland soils with anoxic aggregates supporting localized zones of methanogenesis and oxic sites supporting methanotrophy. Increased soil moisture under elevated CO_2 likely favored development of additional microsites supporting methanogenesis (Fig. 2.4; pathway B). Previously we found no evidence of methanogenic activity in sieved soils from this site (Phillips et al. 2001b), but a subsequent investigation (McLain and Ahmann 2008) reported CH_4 production in intact soil cores, with stronger activity in soils from CO_2-enriched plots. It was suggested (McLain and Ahmann 2008) that sieving in our earlier study destroyed anaerobic microsites. In the present study, our more frequent observations of net CH_4 emission in CO_2-enriched versus control chambers provide confirmatory evidence for at least episodic CH_4 production under both treatments and

higher rates in CO_2-enriched plots. Increased respiration in CO_2-enriched plots (Bernhardt et al. 2006) may have also directly provided additional substrate for methanogens, as the pathway in anoxic aggregates of forest soils appears to be CO_2 reduction rather than acetate cleavage (Teh et al. 2005).

Other feedbacks to CO_2-enrichment beyond plant-mediated changes in soil moisture may also have impacted CH_4 cycling dynamics in these soils. Hoosbeek et al. (2009) reported an increase in soil macro-aggregation (250–2000 µm) under elevated CO_2 in a temperate *Populus x euramericana* plantation. The soil aggregates contained higher concentrations of C and N, providing loci of microbial activity. Enhanced respiratory O_2 consumption by microbes may increase the incidence of anoxic microsites favorable for methanogenesis (Fig. 2.4, pathway C). Elevated CO_2 also induces increased concentrations of secondary compounds such as phenolic and tannins in plant tissues (Gebauer et al. 1997, Peñuelas and Estiarte 1998, Wetzel and Tuchman 2005b; Fig. 2.4; pathway A) and enhances root exudation of organic acids (Norby et al. 1987). Methanotrophs characteristically localized in upper mineral layers of forest soils (Whalen et al. 1992) show a high sensitivity to phenolics, monoterpenes and bulk organics from the overlying O horizon at environmentally relevant levels (Amaral and Knowles 1997, 1998). Enhanced production of inhibitory chemicals delivered in a larger mass of litterfall and subsequently leached to upper mineral layers could have reduced methanotrophic activity in enriched CO_2 plots.

It is unclear if this sustained reduction in net atmospheric CH_4 consumption can be broadly extrapolated to other forested ecosystems. Atmospheric CO_2 enrichment experiments have demonstrated significant increases in net primary production of forest

vegetation (DeLucia et al. 1999, Hamilton et al. 2002, DeLucia et al. 2005, Norby et al. 2005, Finzi et al. 2006a; Fig. 2.4; pathway B). Any attendant increase in soil moisture could effect a decrease in net atmospheric CH_4 consumption as observed here. A process-based model of atmospheric CH_4 consumption by soils indicates an aggregated forest sink of 24 Tg CH_4 y^{-1} (Ridgwell et al. 1999). A decline in soil CH_4 consumption of the magnitude observed here (~15%; Table 2.1) across all forest biomes gives a decrease of 3.6 Tg CH_4 y^{-1}, a value that is not inconsequential as it represents 10% of the model estimate (Ridgwell et al. 1999) of 38 Tg CH_4 y^{-1} for the total soil sink.

Our field study of the relationship between CH_4 flux, CO_2 enrichment and soil moisture suggests that moisture sensitivity of net atmospheric CH_4 results from diffusion limitation to methanotrophs and the availability of anaerobic microsites supporting methanogenic activity, although it yields no insights into the relative importance of these microbial processes or other potential controls on CH_4 exchange at the air-soil interface. Improvement of mechanistic models of global consumption of CH_4 by soils will require field and process-oriented laboratory studies across representative forest biomes and soil types to fully identify and quantify coupling mechanisms of net CH_4 oxidation to CO_2 enrichment and plant metabolism.

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Table 2.1. Annual time-integrated rates of net CH_4 consumption and volumetric soil moisture for six nearly consecutive years in forest plots exposed to ambient or elevated levels of CO_2. Annual time-integrated rates of net CH_4 consumption were determined by integrating for the calendar year daily, area-based data from each sampling occasion.

Year	Annual net CH_4 consumption (mg m^{-2} y^{-1})		% Difference between treatments	Source	Volumetric soil moisture (mL H_2O g $soil^{-1}$)		Source
	Control	CO2-enriched			Control	CO2-enriched	
1998	183	156	16 *	(Phillips et al. 2001a)	0.24	0.26 *	(Schäfer et al. 2003)
1999	191	136	30 *	(Phillips et al. 2001a)	0.27	0.34 *	(Schäfer et al. 2003)
2002	204	181	13 *	(Whalen unpubl.)	0.22	0.23 *	(Hyun unpubl.)
2004	184	150	19 *	Present study	0.29	0.31 *	(Hyun unpubl.)
2005	196	175	10 *	Present study	0.26	0.30 *	(Hyun unpubl.)
2006	197	181	8	Present study	0.23	0.24	Present study

*Differences between treatments significant at α=0.05.

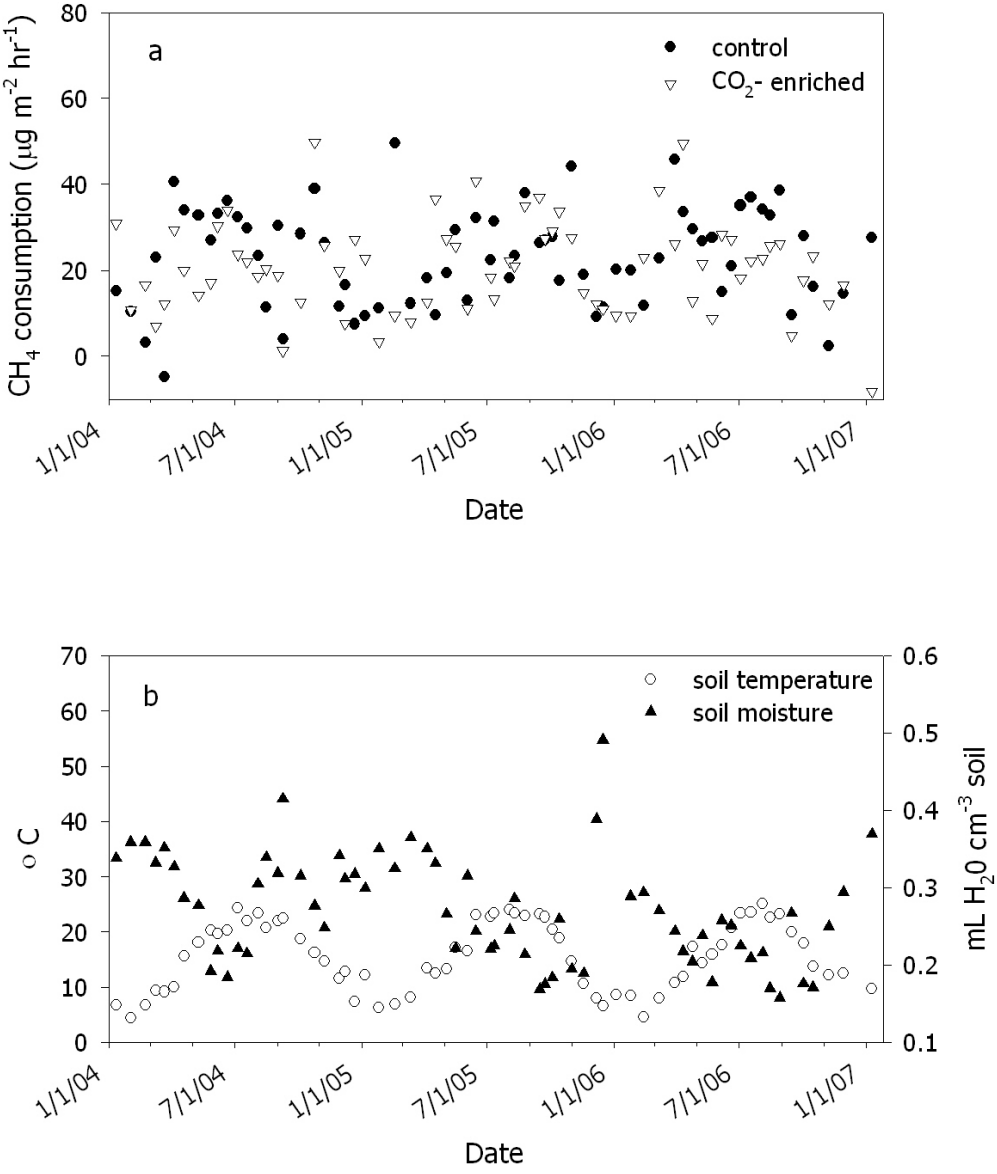


Figure 2.1. (a) Time series for rates of net atmospheric CH_4 consumption by forest soils under CO_2-enriched and ambient atmospheres (control). Each datum point represents the mean of 12 or 16 individual static chamber flux determinations for each treatment; (b) Time series for changes in mean soil temperature (1 to 19 cm depth interval) and mean volumetric soil moisture to 30 cm (mL H_2O cm^{-3} soil). In all cases error bars are eliminated for clarity.

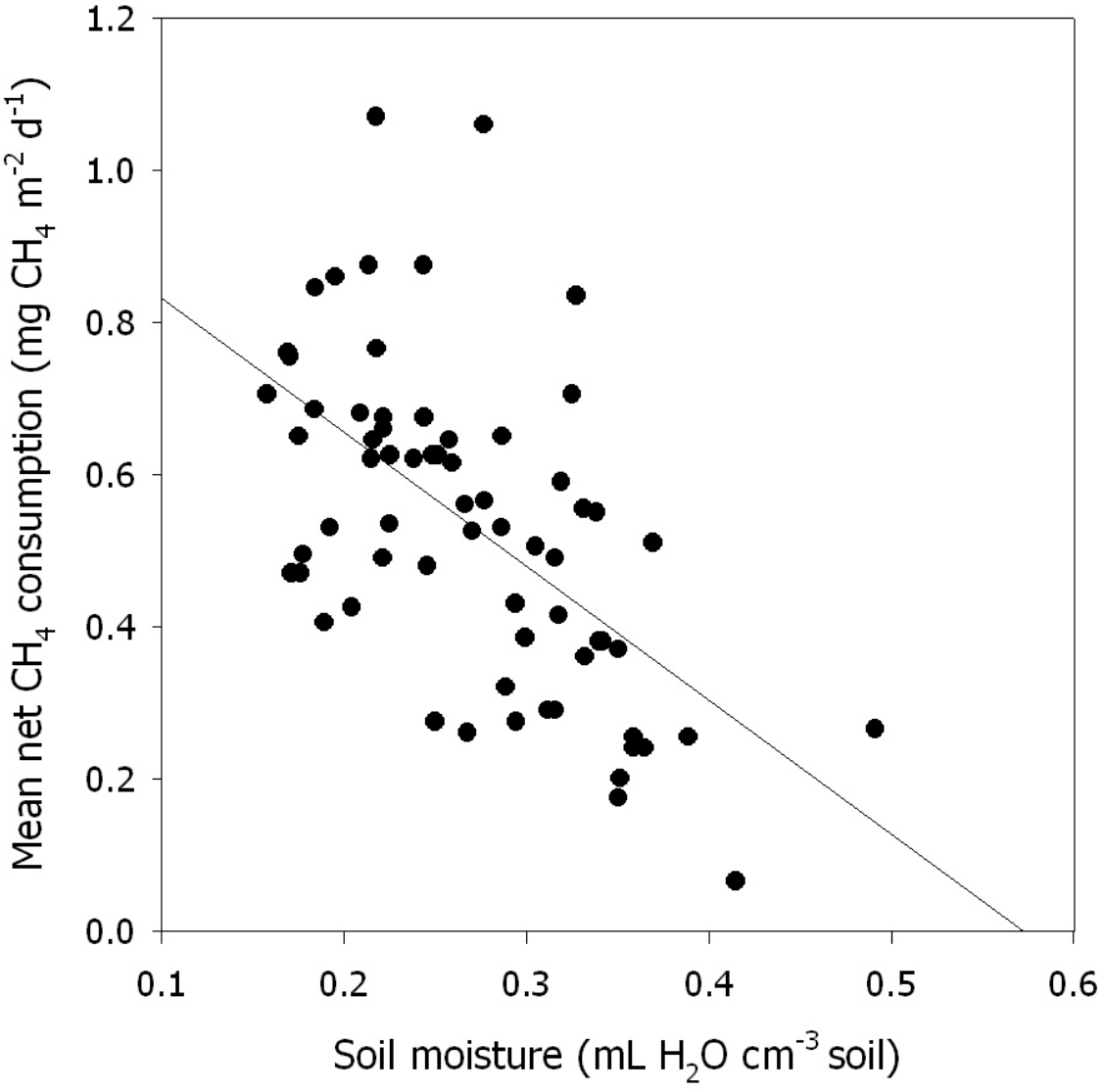


Figure 2.2. Relationship between net atmospheric CH_4 consumption and volumetric soil moisture to 30 cm depth for the entire study ($r^2 = 0.340$). Each datum point represents the mean of 24 (2004 and 2005) or 32 (2006) observations for a sampling date.

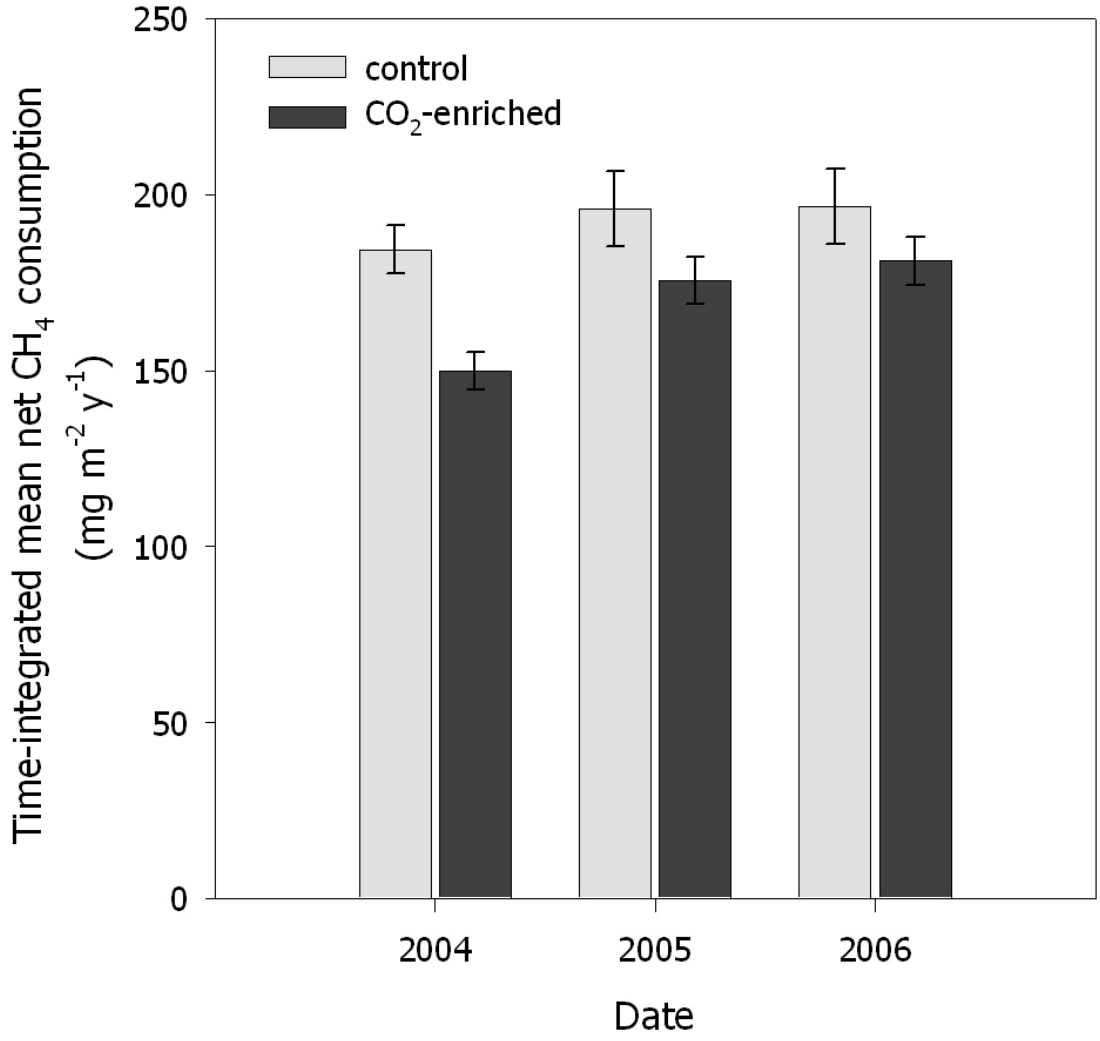
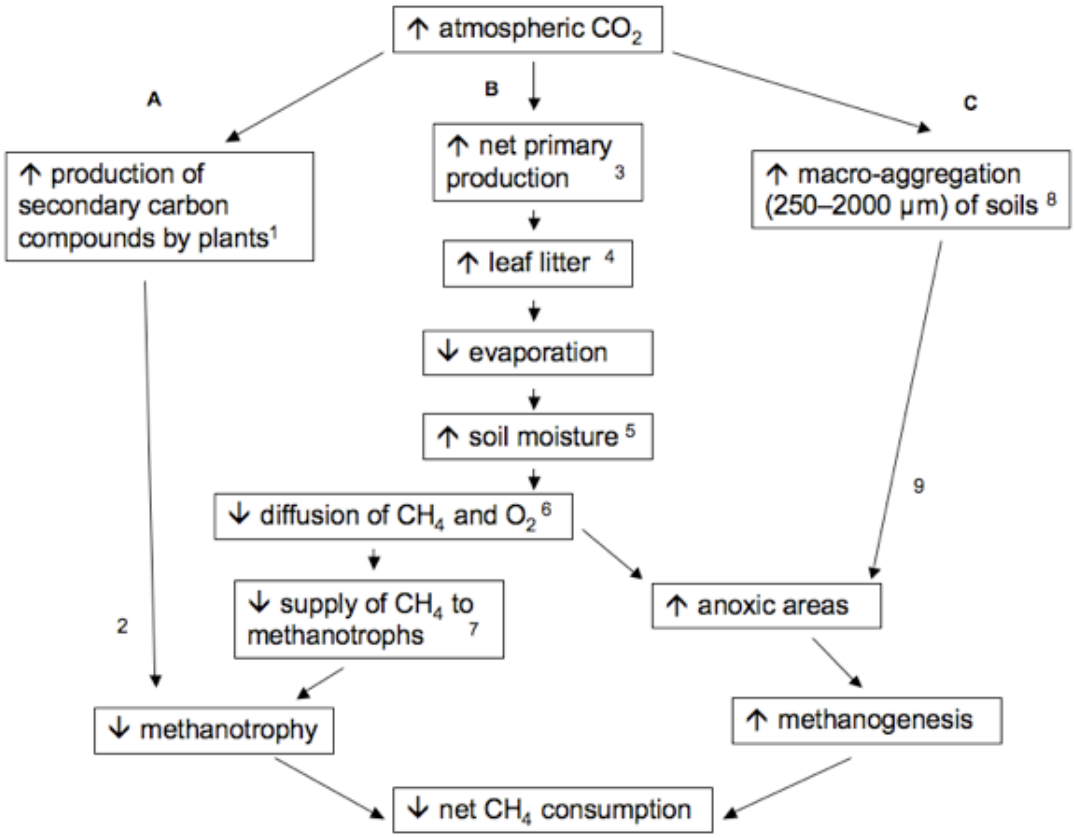


Figure 2.3. Annual time-integrated net CH_4 consumption by temperate forest soils at the Duke FACE site under ambient (control) and elevated (CO_2-enriched) concentrations of CO_2 for 2004 through 2006. Annual time-integrated rates of net CH_4 consumption were determined by integrating for the calendar year daily, area-based data from each sampling occasion (n = 23). The differences between CO_2 treatments were 19%, 10% and 8% for 2004, 2005, and 2006, respectively. Differences were significant for 2004 and 2005. Error bars represent one standard error of the mean.



[1] Gebauer et al.1997; Peñuelas and Estiarte 1998; Wetzel and Tuchman 2005
[2] Amaral and Knowles 1997, 1998
[3] DeLucia et al. 1999; Hamilton et al. 2002; DeLucia et al. 2005; Norby et al. 2005; Finzi et al. 2006
[4] Allen et al. 2000; Schlesinger and Lichter 2001
[5] Schäfer et al. 2002
[6] Suwa et al. 2004
[7] Striegl 1993; Castro et al. 1995; Whalen and Reeburgh 1996
[8] Hoosbeek and Scarascia-Mugnozza 2009
[9] Sextone et al.1985; Zausig et al. 1993

Figure 2.4. Conceptual model of the impact of forest ecosystem responses to elevated CO_2 that influence soil CH_4 cycling dynamics. Up and down arrows within each response function indicate a positive or negative impact, respectively, of that factor on net atmospheric CH_4 consumption. Response functions are either documented or hypothesized by the associated references.

CHAPTER 3: INHIBITION OF CH_4 CONSUMPTION BY SECONDARY CARBON COMPOUNDS IN THE TISSUES AND EXUDATES OF TEMPERATE FOREST PLANTS EXPOSED TO ELEVATED CO_2

Abstract

We previously showed a sustained reduction in net atmospheric CH_4 consumption by temperate forest soils in response to elevated CO_2 (Dubbs and Whalen submitted) and here report the influence of plant exudates on atmospheric CH_4 consumption in soils from the same study site. We examine the effect of root exudate acids and primary or secondary metabolites from plant exudates (throughfall, duff and leaf leachates) on CH_4 consumption. Plant exudates from forest plots exposed to elevated CO_2 since 1996 (~580 mL L^{-1} CO_2) or from control plots subjected to ambient conditions and acid root exudates from loblolly pines (*Pinus taeda*) grown under elevated CO_2 were applied to soils. Duff leachates occasionally inhibited CH_4 consumption regardless of CO_2 treatment, and levulinic acid inhibited CH_4 consumption at a concentration of 100 µmol L^{-1}, but not at 50 µmol L^{-1}. All other tested exudates had no effect on rates of CH_4 consumption. While plant exudates may only assert transient and secondary control on CH_4 consumption under elevated CO_2, identification of temporal and spatial patterns of influence warrant further study because they otherwise confound the correlation between the primary drivers of CH_4 consumption and measured rates of net CH_4 consumption.

Introduction

The global atmospheric CO_2 concentration has more than doubled since the Industrial Revolution (Forster et al. 2007). Little is understood about how increasing atmospheric CO_2 will affect the biogeochemical cycling of other greenhouse gases, including CH_4, but we recently reported a sustained decrease in net atmospheric CH_4 consumption under elevated CO_2 in a temperate loblolly pine (*Pinus taeda*) forest (Dubbs and Whalen submitted). Our previous work showed that a sustained CO_2-induced negative feedback on forest soil CH_4 consumption could lead to a 15% reduction (3.6 Tg CH_4 yr^{-1}) in the current forest soil sink of 24 Tg yr^{-1}(Ridgwell et al. 1999). This negative feedback to increasing CO_2 is of concern because consumption by upland soils is the only terrestrial sink for atmospheric CH_4, which is a greenhouse gas with radiative forcing second only to CO_2 among trace atmospheric gases (Forster et al. 2007).

The reasons for the observed decline in net atmospheric CH_4 consumption by these soils under elevated CO_2 were not entirely clear. However, we postulated several pathways by which changes in a temperate forest ecosystem exposed to elevated CO_2 could lead to decreased CH_4 consumption by methanotrophic bacteria and increased CH_4 production by methanogenesis (Fig. 3.1). Rates of CH_4 exchange between upland soils and the atmosphere are dependent upon the balance between methanotrophy in largely oxic soils and methanogenesis in anoxic microzones. The resultant net CH_4 consumption accounts for the observed sink strength of upland soils in the global CH_4 budget (Forster et al. 2007).

Pathway A (Fig. 3.1) depicts a mechanism by which changes in the chemistry of forest plant tissues may contribute to the observed reduction in net CH_4 consumption.

Ecosystem-scale elevated CO_2 enrichment experiments, or free air carbon exchange (FACE) experiments, indicate that temperate forest responses to elevated CO_2 include increased net primary production (DeLucia et al. 1999, Hamilton et al. 2002, DeLucia et al. 2005, Norby et al. 2005, Finzi et al. 2006a), litter fall (Allen et al. 2000, Lichter et al. 2005, Liu et al. 2005, Lichter et al. 2008), and fine-root production (Norby et al. 2004). Changes in plant productivity, tissue chemistry, C allocation and plant-microbe interactions under elevated CO_2 in turn impact the quantity and quality of C in the ecosystem. For example, Lichter et al. (2000a) observed an increase in labile dissolved organic C in throughfall, and Matamala and Schlesinger (2000) observed a 5.6% increase in the storage of C in forest soils under elevated CO_2. Norby et al. (1987) found that elevated CO_2 enhances root exudation of organic acids in a pot study of *Pinus echinata* seedlings. Elevated CO_2-induced changes in plants also increase the abundance of secondary C compounds in tissues and root exudates relative to plants exposed to ambient CO_2 (Peñuelas and Estiarte 1998, Verburg et al. 1999, Tuchman et al. 2002, Billings and Ziegler 2005, Wetzel and Tuchman 2005a). Secondary C compounds, such as phenolics and terpenes, inhibit metabolism and growth by broad groups of soil bacteria (Souto et al. 2000), and specifically, methanotrophs (Amaral and Knowles 1997, 1998).

Here we extend previous research, which indicated that reduced net CH_4 consumption by a temperate forest soil is a sustained response to elevated CO_2 (Dubbs and Whalen submitted), by examining the influence of plant exudates on rates of CH_4 consumption in soils from the same study site (Fig. 3.1, Pathway A). We investigate the effect of organic acids, found to be the most abundant organic component of photosynthates released from roots in the rhizosphere (Smith 1976), and the effects of

primary or secondary metabolites from exudates (throughfall, duff and leaf leachates) on CH_4 consumption. Plant exudates were collected from forest plots exposed to elevated CO_2 since 1996 or from plots subjected to ambient conditions (~380 mL L^{-1} CO_2; control), and applied to soils. Additionally, organic acids identified to be major components of root exudates (Phillips and Bernhardt unpublished) from loblolly pine laboratory-grown under elevated CO_2 were applied to soils individually and in mixed cocktails to evaluate the effects of these root exudate acids on CH_4 consumption. Effects of plant exudates on rates of CH_4 production were also determined and deemed negligible, and thus, are not discussed further in this manuscript.

Methods

Field site

Field measurements were conducted at the Duke Forest (North Carolina; USA) Free-Air CO_2 Enrichment (FACE) experiment sited in an even-aged stand of loblolly pine (*Pinus taeda* L.) planted in 1983. Soils are clay loam, Ultic Hapludalf's of the Enon Series (Oh and Richter 2005). Average normal air temperature ranges from 3.6 ° C in January to 25.3 ° C in July and annual precipitation averages 1209 mm (State Climate Office of North State Climate Office of North Carolina 2003-2009).

Site characteristics are fully documented in Hendrey et al. (1999a) and briefly described here. The experiment consists of eight circular 30-m diameter plots. Four treatment plots (referred to as "CO_2-enriched") are fumigated with CO_2 to maintain atmospheric CO_2 concentrations 200 mL L^{-1} above ambient levels, while three additional treatment plots are fumigated with ambient air to replicate micrometeorological effects

associated with CO_2 addition. A fourth is subjected to ambient air without fumigation. The latter four plots are referred to as "controls". Continuous (24 h d^{-1}) fumigation was initiated in August 1996, but was reduced to daylight hours only from 2003 to present.

Plant exudate collection

Throughfall

Throughfall collectors consisted of 4 L amber acid-washed glass bottles. The necks of the bottles were plugged by rubber stoppers, which were penetrated by acid-washed glass funnels (60° angle bowl and 100 mm stem). The funnel stems were stuffed with Pyrex glass wool to exclude large particles. One throughfall collector was randomly placed within each of the experimental plots (n=4 for each CO_2-enriched and control treatments) within 48 h of a predicted precipitation event in June and November of 2004, and June of 2005. Throughfall samples were transferred to amber HDPE wide-mouth bottles within 6 h of the conclusion of each discrete rainfall, and the contents were frozen at -10 °C. Samples were thawed and applied to soils within 10 d of collection.

Fresh leaf litter and duff collection and leaching

Approximately 5 g (wet weight) of freshly fallen *Acer rubrum* (red maple), *Liquidambar styraciflua* (sweetgum), *Pinus taeda* (loblolly pine), and *Ulmus alata* (winged elm) leaves were collected from each CO_2-enriched and control plot (n=3 for each treatment) in June and October of 2005. These species were chosen because there was at least one individual tree of each of these species in each experimental plot. Freshly fallen leaves were identified as green leaves lying on the boardwalks that divide

six of the eight experimental plots into quadrants. Upon returning to the laboratory (~1 h after collection), wet mass was determined and leaves were placed in acid-washed 30 mL glass vials. Leaves (by species) were submerged in 20 mL deionized water (DIW) while a vial filled with DIW served as a control throughout the leaching process and soil incubation. Vials were covered by parafilm and leaves were allowed to leach in the dark at ~24 °C for 24 h (Mann and Wetzel 1996).

Duff was randomly collected from the forest floor of CO_2-enriched and control plots (n=4 for each treatment) in October of 2006, and October, November, and December of 2007. Duff is identified as partially decaying plant material on the forest floor surface. Upon returning to the laboratory (~1 h after collection), 10 g of duff (wet mass) from each plot was placed in a 118 mL acid-washed glass jar. Duff was submerged in DIW (60 mL), and one jar without duff was filled with 60 mL of DIW, to serve as a control throughout the leaching process and soil incubation. Jars were covered by parafilm, and duff was allowed to leach in the dark at ~24 °C for 24 h (Mann and Wetzel 1996).

Root exudate preparation

Several organic acids were identified as primary root exudates from loblolly pine trees grown under elevated CO_2 in a glass bead rooting substitute by the Bernhardt lab at Duke University. The primary root acid exudates that were identified included citric, malic, oxalic, maleic, fumaric, levulinic, succinic, shikimic, and protocatecuic acids. Solutions of individual acids and a cocktail of all acids were prepared at 100 µmol L^{-1} concentrations in DIW used for incubation experiments in April and October of 2006.

Selected organic acid exudates were additionally prepared at 10 µmol L^{-1}, 50 µmol L^{-1}, 100 µmol L^{-1}, 500 µmol L^{-1}, and 1000 µmol $L^{-1}$and used in a companion experiment intended to identify a threshold concentration for inhibition of CH_4 consumption.

Soil assays and incubation

Soils from the 0 to 20 cm depth interval were collected at the research site from outside of the experimental plots with a hand trowel 1 d prior to initiation of experimentation. Soils were collected from outside of experimental plots because destructive sampling within experimental plots is highly restricted. Upon return to the lab, soils were immediately homogenized by sieving (4.75 mm mesh), and 10 g subsamples of field moist soil were placed into 120 mL glass serum bottles. Twice the number of soil-filled bottles were prepared as were needed (n=3 for each treatment in leaf leachate and root exudates experiments, n=4 in throughfall and duff leachate experiments). Bottles were allowed to equilibrate with laboratory air (~1.8 mL L^{-1} CH_4) for 1 h before being capped with butyl rubber stoppers and crimp-sealed. Headspace CH_4 concentrations were determined immediately upon sealing and 12 h later by removing 3 mL of headspace gas with 5 mL plastic syringes. The jars in which CH_4 was consumed at the most similar rates were used for further experimentation.

Throughfall, leaf and duff leachates, and the organic acids identified to be primary root exudates were administered to chosen soil aliquots. The amount of liquid added to soils depended upon extant soil moisture as liquid additions were intended to achieve a water holding capacity of approximately 50%. An equal volume of DIW as added to soil samples as a control in all experiments. Following liquid addition, soils were allowed to

equilibrate at the lab atmosphere for 12 to 15 h before jars were sealed and sampled as described above, except that at least 5 samples were collected at evenly-spaced intervals over 48 h incubation periods. Headspace pressure was maintained at 1 atm by replacing removed headspace gas with an equivalent volume of ultrapure N_2.

Gas samples were analyzed for CH_4 by flame ionization detection gas chromatography (Shimadzu model GC 8 A; precision expressed as the coefficient of variation for 10 replicate injections of a 0.94 mL CH_4 L^{-1} standard was < 3%) within 10 h of collection, well within our predetermined holding time of 24 h. Sample separation was accomplished on a 1-m length x 0.32 cm diameter molecular sieve 5A column with an ultrahigh purity N_2 carrier gas (33 mL min^{-1}). Injector and detector temperatures were set at 90 ^oC and 140 ^oC. Headspace measurements from replicate bottles without soil ensured that changes in headspace CH_4 concentrations did not result from gas exchange with butyl rubber stoppers.

Statistical Analysis

Soil dry mass-based rates of CH_4 consumption were calculated from the log-linear change of CH_4 concentration in jar headspaces. Rates or rate constants for CH_4 consumption were compared by paired t-tests. A significance level of α=0.05 was used for all statistical comparisons.

Results

Throughfall and leaf leachates

Rate constants (k; d^{-1}) for CH_4 consumption by soils following application of throughfall or leaf leachates from several individual species of trees collected from control or CO_2-enriched plots were not significantly different on any of the three dates tested (Fig. 3.2). Similarly, values of k for CH_4 consumption by soils to which plant exudates were added also were not significantly different from those of soils to which DIW was applied on either of the two dates tested (Fig. 3.3).

Duff leachates

Application of duff leachates from either CO_2-enriched or control plots in fall of 2006 significantly reduced rates of CH_4 consumption, by 34% (CO_2-enriched) and 38% (control), relative to rates of CH_4 consumption by soils to which DIW was added (Fig. 3.4). The rates of CH_4 consumption for CO_2-enriched and control plot treatments were not significantly different from each other. This pattern of reduced CH_4 consumption by soils to which duff leachate from both CO_2-enriched and control treatment plots was added was not repeatable, however, in similar experiments conducted three times in the fall and winter of 2007 (Fig. 3.5). In all cases, rates of CH_4 consumption were not significantly different for any treatment in soils amended with DIW or duff leachate from control or CO_2-enriched plots.

Organic acids from root exudates

In general, rates of CH_4 consumption in soils following the addition of individual organic acids or a cocktail of organic acids identified as primary components of root

exudates from loblolly pine trees grown under elevated CO_2 were not significantly different from that of soils to which DIW was added (Fig. 3.6). Levulinic acid was the exception as it significantly inhibited rates of CH_4 consumption. A 100 µmol L^{-1} solution of levulinic acid reduced CH_4 consumption in soils by 63% and 91% relative to DIW-treated soils in October and April 2006 trials, respectively. An experiment conducted to identify the concentration threshold for inhibition of CH_4 consumption by levulinic acid revealed that CH_4 consumption was not significantly reduced at concentrations below 100 µmol L^{-1} (Fig. 3.7). Rates of CH_4 consumption were, however, significantly reduced at levels above 100 µmol L^{-1}. Further, CH_4 consumption was completely inhibited when levulinic acid was added to soils at concentrations of 500 µmol L^{-1} or 1000 µmol L^{-1}.

Discussion

Plants grown under elevated CO_2 contain increased tissue concentrations of secondary C compounds (Gebauer et al. 1997, Peñuelas and Estiarte 1998, Wetzel and Tuchman 2005b; Fig. 3.1, pathway A), which have the potential to impact CH_4 dynamics because they inhibit metabolism and growth by methanotrophs (Amaral and Knowles 1997, 1998). Indeed, we found evidence of transient inhibition of CH_4 consumption by duff collected from CO_2-enriched plots, as well as from control plots, and from an organic acid identified to be a primary root exudate of loblolly pine trees grown under elevated CO_2. However, neither throughfall nor leaf leachates from the four dominant tree species at the study site affected rates of CH_4 consumption by forest soils, regardless of the CO_2 treatment origins of the plant exudates.

The inhibition of CH_4 consumption by duff leachate from both CO_2-enriched and control treatments on one occasion suggests that some chemical(s) released from fresh autumnal duff inhibits methanotrophy, but the inhibitory substances are independent of CO_2 level. While we leached the same wet mass of duff from both CO_2 treatments to conduct our leaching experiment, there is actually greater litter fall under elevated CO_2 at our site (Allen et al. 2000, Lichter et al. 2005, Lichter et al. 2008), thus, perhaps higher concentrations of the inhibitory substances are leached from the larger mass of duff to the mineral soil in CO_2-enriched plots, which would result in a stronger inhibitory affect.

There are several possible reasons why duff collected on one occasion in the fall of 2006 was inhibitory to CH_4 consumption, while that collected on other occasions in the fall and winter of 2007 was not. For instance, losses of secondary C compounds from leaf litter occurs rapidly (Yavitt and Fahey 1986, Amaral and Knowles 1997, Schofield et al. 1998, Kainulainen and Holopainen 2002), and the concentrations of secondary C compounds leached from leaf litter are influenced by environmental conditions (Harris and Safford 1996). Yavitt and Fahey (1986) found that > 80% of the soluble phenolics and carbohydrates were lost from leaf litter in a lodgepole pine ecosystem in less than a year, and Amaral and Knowles (1997) reported that forest soil extracts only inhibited CH_4 consumption for 3 to 5 d. We may have collected duff in 2006 soon enough after leaf fall that inhibitory compounds in leachates were sufficiently concentrated to significantly reduce CH_4 consumption, yet we may have missed the window between leaf fall and leachate losses in the 2007 experiments. Timing of freeze/thaw cycles may have influenced the availability of inhibitory substances. Harris and Safford (1996) observed that repeated freeze/thaw cycles pre- and post- leaf fall, among other factors, increased

the amount of water-soluble carbon leached from fallen leaves from a temperate forest. Indeed, the first freeze/thaw cycle at our study site in 2006 occurred the day before duff collection, whereas the first freeze/thaw cycle in 2007 did not occur until 2 d after duff collection for the November 2007 experiment and 25 d before duff collection for the December 2007 experiment. This suggests that duff collection may have coincided with the maximum potential for leaching of inhibitory compounds in 2006, but not in subsequent experiments. Nonetheless, the degree of inhibition was apparently independent of the CO_2 level under which trees were grown.

Levulinic acid, a primary root exudate acid released from loblolly pines exposed to elevated CO_2, was found here to inhibit CH_4 consumption. The threshold concentration for inhibition by levulinic acid lies within the range of 50 to 100 μmol L^{-1}, which far exceeds the concentration of 0.5 μmol L^{-1} for all phenolic compounds found in pore water from centrifuged samples of the top 25 cm of soil from a coniferous forest (Gallet and Pellissier 1997). Little is known about the presence and persistence of levulinic acid in forest soils. However, its increased release by tree roots under elevated CO_2 and significant and even complete inhibition of methanotrophy at concentrations between 50 and 100 μmol L^{-1} suggest that this compound could inhibit methanotrophy in the rhizosphere, and warrants further attention in attempts to understand the feedback between an increasing atmospheric CO_2 concentration and a reduction in the forest soil sink strength for CH_4.

Despite some transient inhibition of net CH_4 consumption by forest soils by plant exudates reported here, the spatial and temporal patterns of *in situ* net CH_4 consumption observed in our previous study indicate that the secondary compounds in plant exudates

produced under elevated CO_2 are not the primary reason for the observed decrease in net CH_4 consumption by temperate forest soils under elevated CO_2 (Dubbs and Whalen submitted). The quantity and quality of plant exudates vary among plant species (Smith 1976, Ström et al. 1994). Likewise, the quantity and chemistry of plant exudates from roots or leaf litter are typically seasonal (Kuzyakov and Cheng 2001, Muscolo and Sidari 2006, Phillips et al. 2008). Saerte and Bååth (2000) reported "spatial patterns of the microbial community to be related to the positions of trees" in a mixed Norway spruce-birch stand in Finland. We previously reported high spatial and temporal variability in net CH_4 consumption at permanently established sampling locations in a temperate forest where there was not any specific site that consistently exhibited higher or lower rates of net CH_4 consumption relative to other sites (Dubbs and Whalen submitted). Since the patterns in net CH_4 consumption in the temperate forest at our study site do not correspond to specific locations or periods of time, it is only reasonable to conclude that then plant exudates do not exert the primary control on methanotrophy or methanogenesis. Consequently, our previous and present research indicates that despite transient inhibition of net CH_4 consumption in forest soils by plant exudates, Pathway A (Fig. 1) is not the primary driver for reduced net CH_4 consumption in soils under elevated CO_2. However, it does deserve further consideration since the transient influences of chemical inhibitors may weaken the correlation between standard influences on CH_4 consumption (soil moisture) and measured rates of net CH_4 consumption.

Future work should focus on identifying inhibitory compounds in bulk leachates that show enhanced production by plants under elevated CO_2 by high performance liquid chromatography. Focus should also be placed on identifying the temporal and spatial

patterns of influence of these compounds on *in situ* net CH_4 consumption. Research in this area will help to refine models aimed predicting the upland soil sink strength for CH_4.

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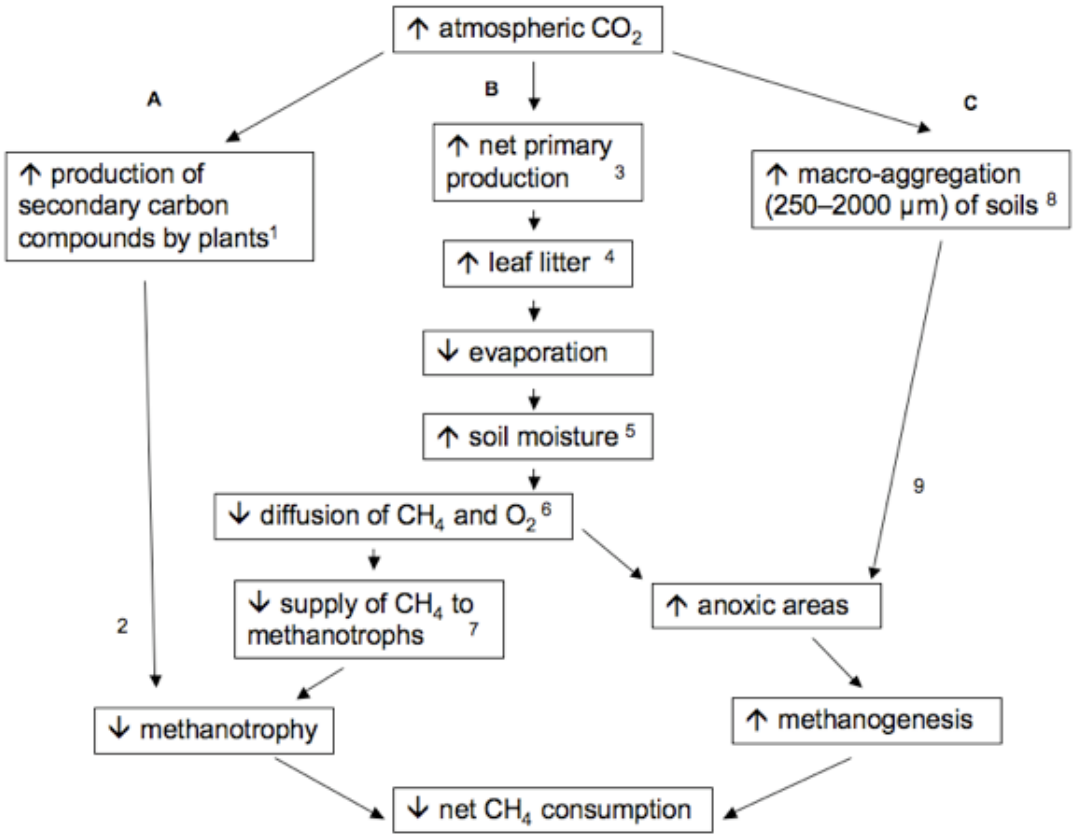

[1] Gebauer et al.1997; Peñuelas and Estiarte 1998; Wetzel and Tuchman 2005
[2] Amaral and Knowles 1997, 1998
[3] DeLucia et al. 1999; Hamilton et al. 2002; DeLucia et al. 2005; Norby et al. 2005; Finzi et al. 2006
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[9] Sextone et al.1985; Zausig et al. 1993

Figure 3.1. Conceptual model of the impact of forest ecosystem responses to elevated CO_2 that influence soil CH_4 cycling dynamics. Up and down arrows within each response function indicate a positive or negative impact, respectively, of that factor on net atmospheric CH_4 consumption. Response functions are either documented or hypothesized by the associated references.

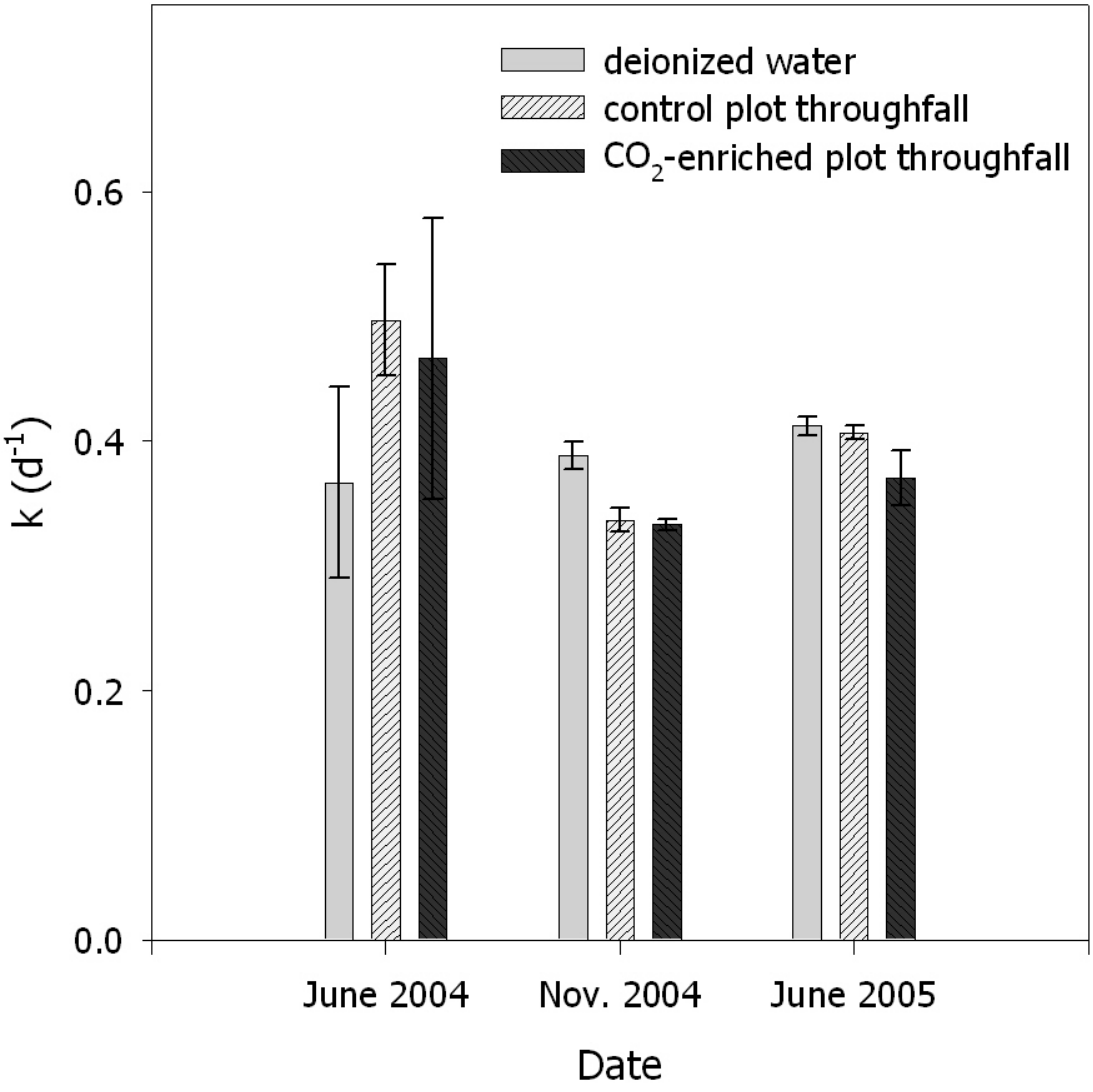

Figure 3.2. Mean first order rate constants (k; d^{-1}) for CH_4 consumption in temperate forest soils amended with deionized water or throughfall from CO_2-enriched (n=3) or control (n=3) plots. Error bars represent one standard error of the mean.

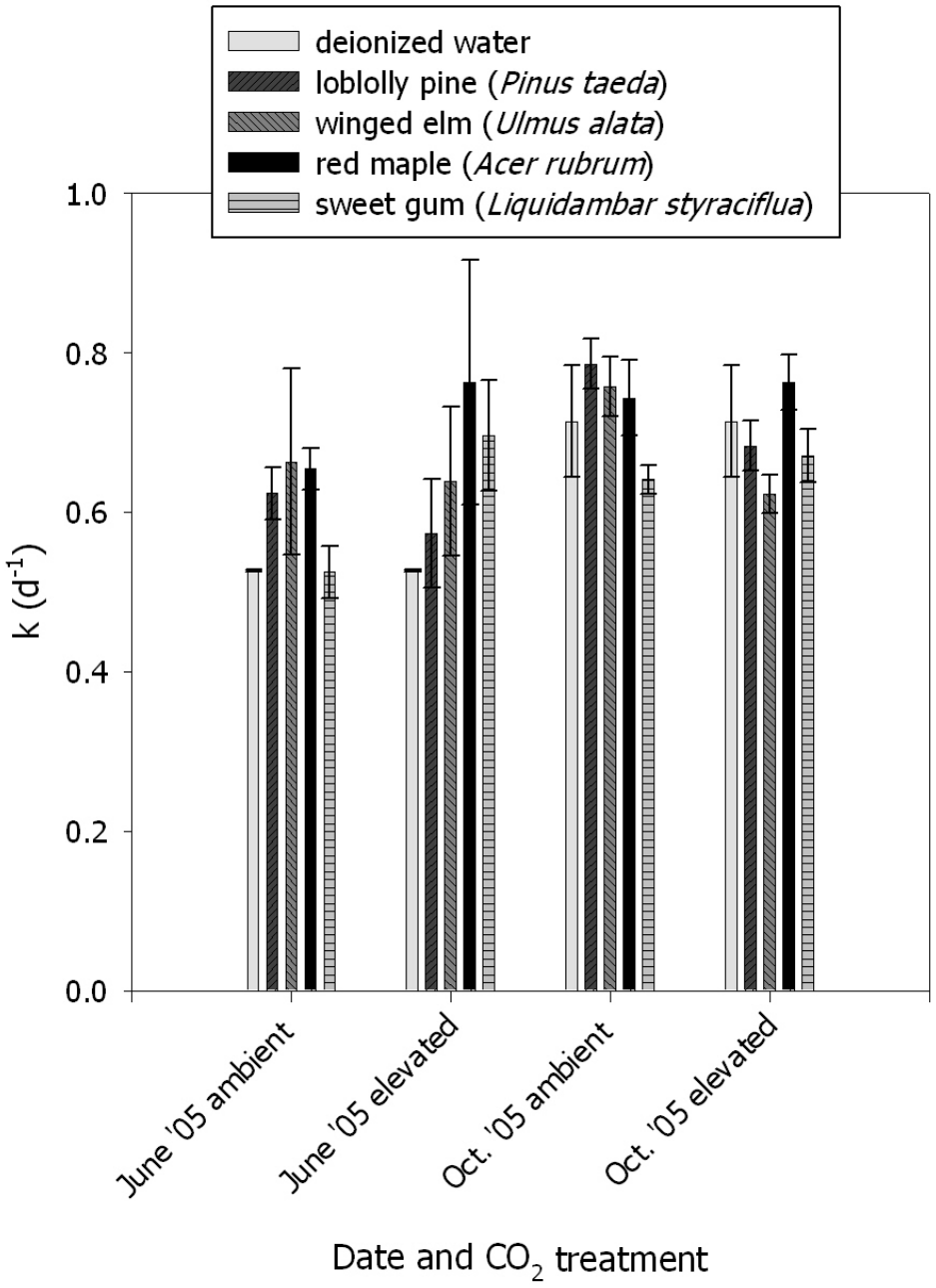

Figure 3.3. Mean first order rate constants (k; d^{-1}) for CH_4 consumption by temperate forest soils amended with leaf leachate from the four most dominant trees within the control (n=3) and CO_2-enriched (n=3) plots. Error bars represent one standard error of the mean.

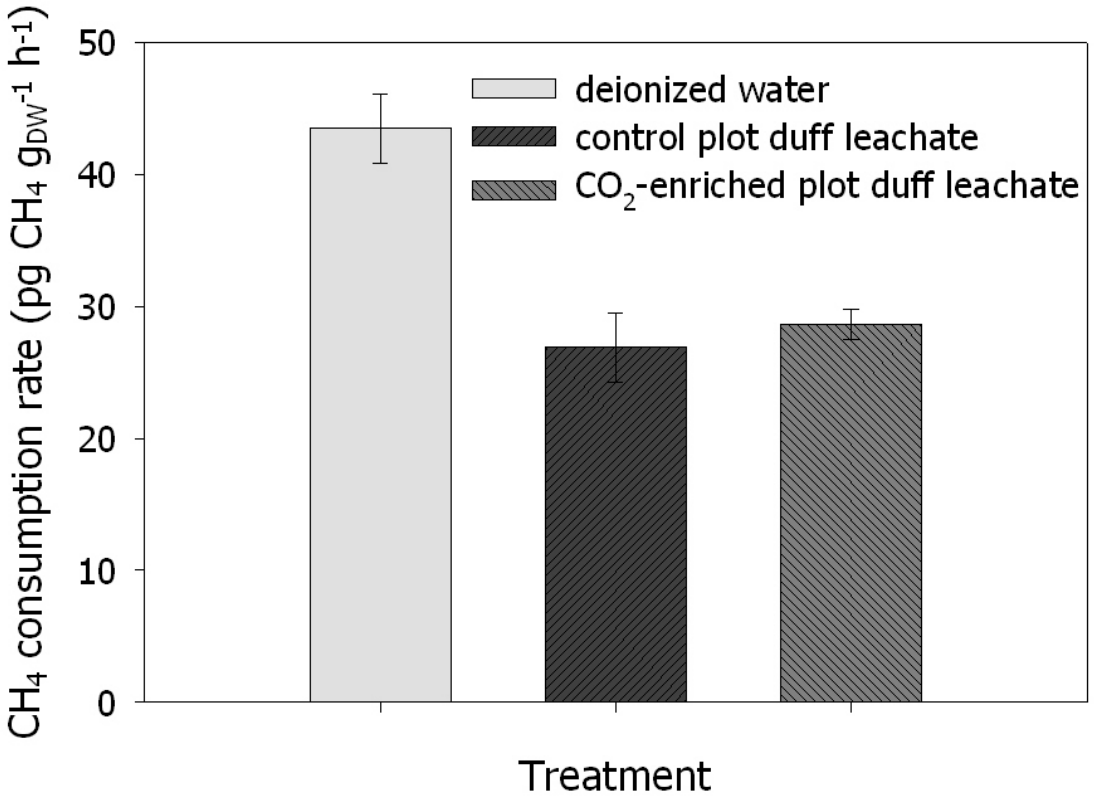

Figure 3.4. Rates of CH_4 consumption by forest soils amended with deionized water or duff leachates from CO_2-enriched (n=4) or control (n=4) plots. Error bars represent one standard error of the mean.

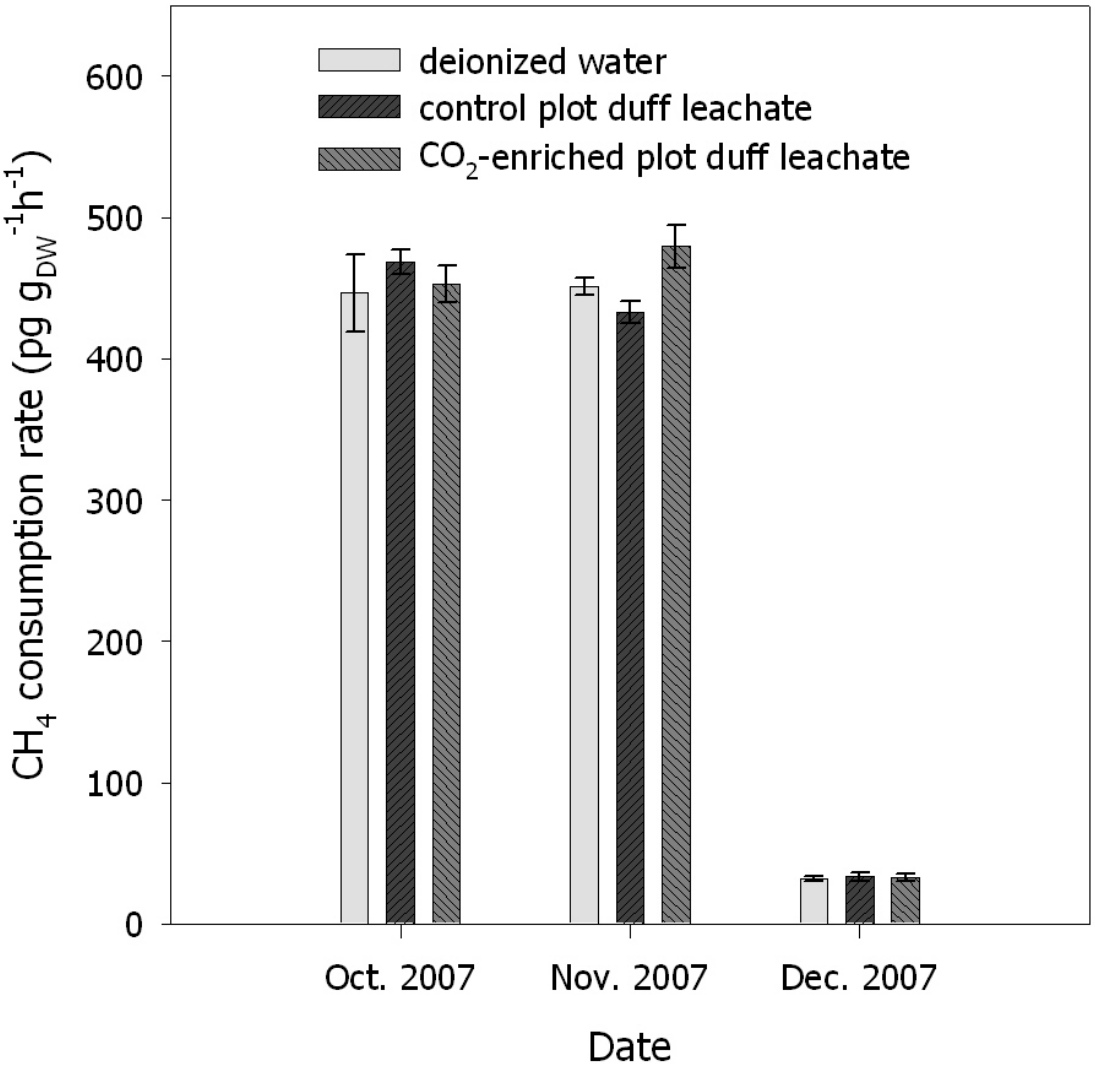

Figure 3.5. Rates of CH_4 consumption by forest soils amended with duff leachates or deionized water. Error bars represent one standard error of the mean (n=3).

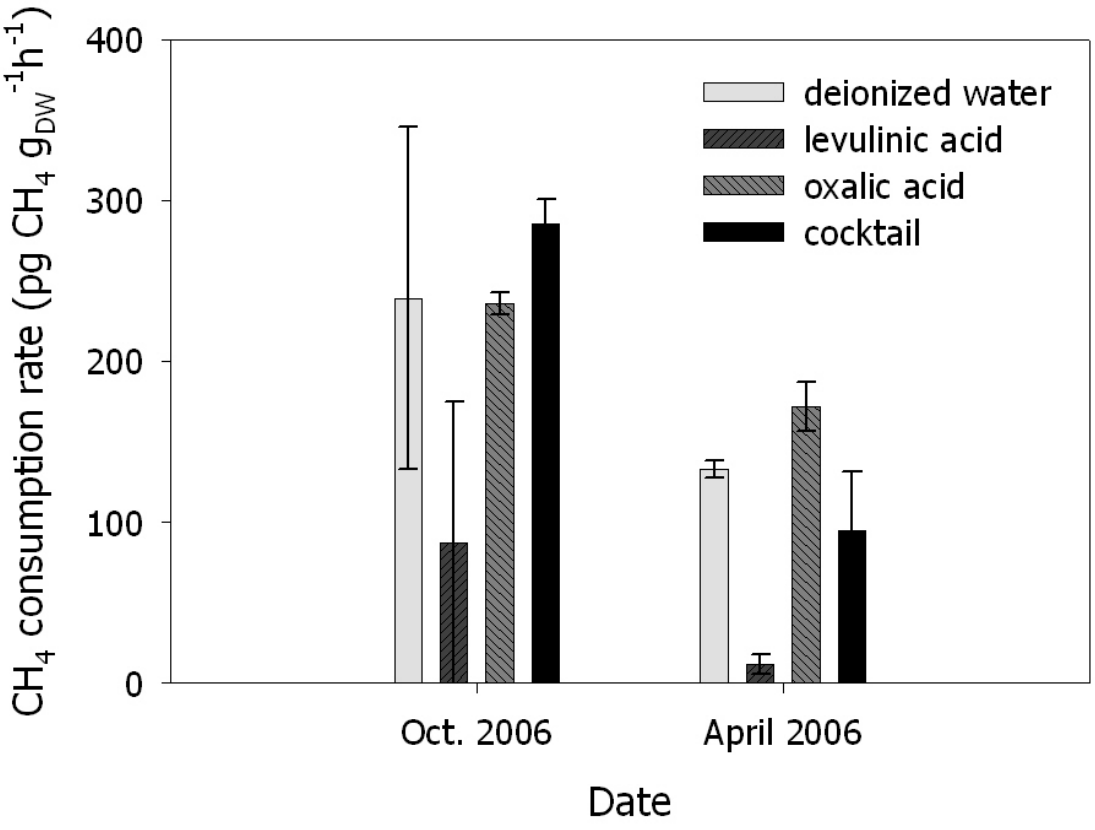

Figure 3.6. Rates of CH_4 consumption by forest soils amended with deionized water or representative organic acids (100 µmol L^{-1}) determined to be primary root exudates from loblolly pine (*Pinus taeda*) trees grown under elevated CO_2. Rates of CH_4 consumption by soils to which individual organic acids not shown here (citric, malic, maleic, fumaric, succinic, shikimic, and protocatecuic acids) was similar to that of soils to which oxalic acid and the cocktail of organic acids was added. Error bars represent one standard error of the mean (n=3).

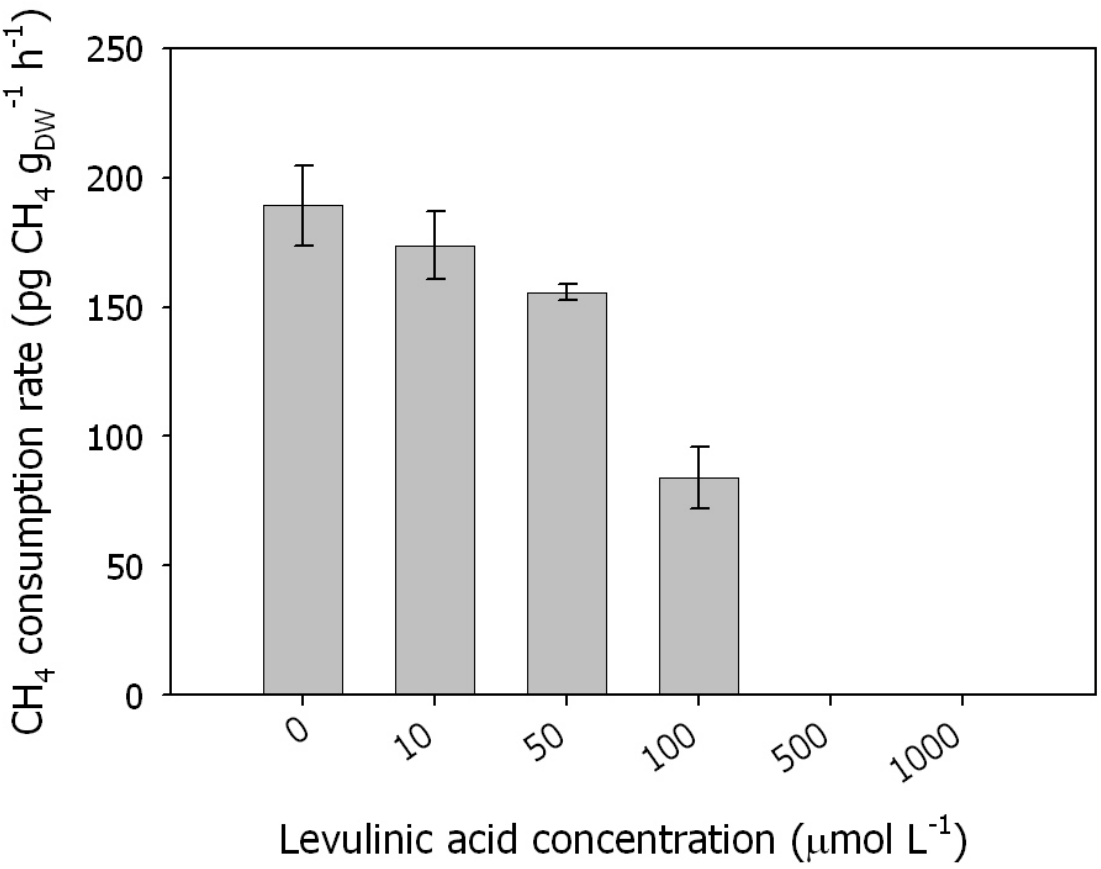

Figure 3.7. Rates of CH_4 consumption by forest soils amended with deionized water or levulinic acid, an organic acid determined to be a primary root exudate from loblolly pine (*Pinus taeda*) trees grown under elevated CO_2 for a February 2009 experiment. Error bars represent one standard error of the mean (n=3).

CHAPTER 4: REDUCED NET CH_4 CONSUMPTION CAUSED BY CHANGES IN THE SOURCES AND TRANSPORT OF SOIL GASES IN A TEMPERATE FOREST EXPOSED TO ELEVATED CO_2

Abstract

We previously reported a sustained reduction in net atmospheric CH_4 consumption by temperate forest soils exposed to elevated CO_2 since 1996 (~580 mL L^{-1} CO_2; Dubbs and Whalen submitted). Changes in the transport and supply of atmospheric gases within the soil profile under elevated CO_2, and subsequent changes in locus or activity of the CH_4 oxidizing and producing communities, may help to explain the decrease in net CH_4 consumption. We examined the depth distribution of CH_4 in the soil profile, the effective diffusivity of CH_4 through the soil, and the extent and activity of CH_4 consuming and CH_4 producing communities in CO_2-enriched and control (ambient atmospheres) plots at the same study site. High spatial and temporal variability in net CH_4 consumption and CH_4 production rates and high error in diffusivity measurements, along with limited ability to collect soil samples, largely resulted in the inability to detect significant differences between CO_2 treatments in rates of net CH_4 consumption or CH_4 production, depth profile CH_4 concentrations, or effective diffusivity. However, qualitative trends of low overall diffusivity and increased incidence and rates of CH_4 production in elevated CO_2 plots, supported by a long-term record of significantly higher soil moisture in CO_2 plots, indicate that increased soil moisture along

with increased activity of methanogens under elevated CO_2 in soils with low diffusivity at our study site contribute to the observed decline in CH_4 oxidation under elevated CO_2.

Introduction

The atmospheric CH_4 concentration has more than doubled from a pre-industrial level of about 750 $\mu L\ L^{-1}$ to a present day concentration of about 1780 $\mu L\ L^{-1}$ (NOAA 2008). Although CH_4 is less abundant than CO_2, additions of CH_4 to the tropospheric reservoir cause more direct warming than CO_2, both on a per molecule and a mass basis (Wuebbles and Hayhoe 2002). Methane also indirectly contributes to global warming because of its role in the stratospheric chemistry of ozone and water vapor formation (Wuebbles and Hayhoe 2002). Thus, a complete understanding of the CH_4 cycle as well as the feedbacks and interactions with other biogeochemical cycles are important to the accurate prediction of future climates.

Known sinks for tropospheric CH_4 include reaction with the hydroxyl radical, which removes approximately 445 Tg of CH_4 from the atmosphere annually; and mixing of tropospheric CH_4 with the stratosphere accounts for another 40 Tg of CH_4 removal annually (Forster et al. 2007). Upland soils are the only known biological sink for atmospheric CH_4, accounting for approximately 38 Tg of CH_4. This biological sink results from the net balance of CH_4 consumption by methanotrophic bacteria in the largely oxic soil profile, and production by methanogenic bacteria in anoxic microsites (reviewed by Conrad 1996).

We recently reported a sustained reduction of ~15% in net atmospheric CH_4 consumption by temperate forest soils under elevated CO_2 relative to plots exposed to ambient levels of CO_2 (Dubbs and Whalen submitted). We also proposed several pathways

whereby changes in other aspects of forest ecosystem function in response to elevated CO_2 could impact net CH_4 consumption by these soils (Fig. 4.1). Reduced gas diffusivity has been demonstrated (Dörr et al. 1993) to control rates of CH_4 supply to the usual subsurface locus of CH_4 oxidation (e.g. Whalen and Reeburgh 1992), which is itself substrate-limited in well-drained forest soils, based on kinetic considerations (Bradford et al. 2001). Thus, factors that introduce diffusion resistance or increase the diffusional path will reduce rates of atmospheric CH_4 consumption (Fig. 4.1, pathway B). Similarly, reduced gas diffusivity slows the transport of O_2 from the atmosphere and, paired with respiratory consumption of O_2 within the soil matrix, may result in the formation of anoxic microsites, supporting methanogenesis (Fig. 4.1; pathway C). In this circumstance, methantrophs are supported not only by atmospheric CH_4, but also by endogenously produced substrate.

Soil moisture increases diffusional resistance because gases diffuse 10^3 to 10^4 times more slowly through water than air. Schäfer et al. (2003) and more recently, we (Dubbs and Whalen submitted) reported higher soil moisture in CO_2-enriched plots at a temperate forest study site, relative to plots exposed to ambient atmospheres. Schäfer et al. (2003) attributed the higher soil moisture in elevated CO_2 plots to increased leaf litter depth (Allen et al. 2000, Schlesinger and Lichter 2001) and topographic convergence. Increased leaf litter depth is a manifestation of increased net primary production in temperate forests in response to elevated CO_2 (DeLucia et al. 1999, Hamilton et al. 2002, DeLucia et al. 2005, Norby et al. 2005, Finzi et al. 2006a) that ultimately inhibits evaporation from the soil surface and results in higher soil moisture (Fig. 4.1, pathway B) while topographic convergence is an inherent difference in the lateral flow of soil pore water unrelated to CO_2 treatment.

Here we examine the transport of atmospheric CH_4 in the soil profile, the effective diffusivity of CH_4 through the soil, as well as the extent and activity of CH_4 consuming and producing communities in a temperate forest exposed to elevated CO_2. Changes in the transport and source of atmospheric gases within the soil profile under elevated CO_2, or a change in the locus of the CH_4 oxidizing community, may help to explain the observed persistent decrease in net CH_4 consumption under elevated CO_2 and provide information useful to modeling efforts aimed at forecasting future climates.

Methods

Field site

Field measurements were conducted at the Duke Forest (North Carolina) Free-Air CO_2 Enrichment (FACE) experiment sited in an even-aged stand of loblolly pine (*Pinus taeda* L.) planted in 1983. Soils are clay loam, Ultic Hapludalf's of the Enon Series (Oh and Richter 2005). Average air temperature ranges from 3.6 °C in January to 25.3 °C in July and annual precipitation averages 1209 mm (State Climate Office of North State Climate Office of North Carolina 2003-2009).

Site characteristics are fully documented in Hendrey et al. (1999a) and briefly described here. The experiment consists of eight circular 30-m diameter plots. Four treatment plots (referred to as "CO_2-enriched") are fumigated with CO_2 to maintain atmospheric CO_2 concentrations 200 mL L^{-1} above ambient levels, while three additional treatment plots are fumigated with ambient air to replicate micrometeorological effects associated with CO_2 addition. A fourth is subjected to ambient air without fumigation. The latter four plots are referred to as "controls". Each plot is divided into quadrats by a

boardwalk that minimizes the impact of foot traffic during sampling. Continuous (24 h d^{-1}) fumigation was initiated in August 1996, but was reduced to daylight hours only from 2003 to present.

Soil physical characteristics are similar between CO_2 treatment plots, with the exception of soil organic matter, which averaged 4.6% in CO_2-enriched plots, and only 3.4% in control plots. Averages for all control and elevated CO_2 plots (0 to 20 cm depth zone) for soil particle density, bulk density, and pH were 2.5 g cm^{-3}, 1.2 g cm^{-3}, and 5.7 units, respectively. Soil texture was 9% clay, 42% silt, and 49% sand.

Soil gas sampling

Sets of soil gas wells were installed in 2005 within 30 cm of permanently emplaced static chambers utilized for soil-atmosphere CH_4 exchange determinations made in another aspect of this research (Dubbs and Whalen submitted). Wells were located at 5 cm depth intervals from 5 to 25 cm below the soil surface. There were a total of five wells per set located in two quadrants of each of the eight FACE plots for a total of 16 well sets per treatment. Each well consisted of 1 cm ID stainless steel tube, open and perforated at the bottom, and topped with Swagelock reducing union fitted with a septum for syringe sampling. The sampling wells were installed vertically such that the open and perforated bottom allowed diffusion of gases only from the prescribed depth.

Soil gas wells were sampled 31 times between July 2005 and July 2007. On each sampling date, wells were initially evacuated with a hand-operated vacuum pump (Handivac) and then allowed to equilibrate with soil air for approximately 0.5 to 1 h before sampling. Headspace samples were collected from each soil gas well in 10 ml glass syringes.

The atmosphere above the soil surface adjacent to wells was additionally sampled with similar syringes on each date.

Soil cores

Two soil cores (5.5 cm diameter by 25 cm length) were collected randomly from within each of the eight experimental plots in July 2005, September 2006, and April 2007 using a stainless steel soil core sampler (AMS, Inc) fitted with a slide hammer (AMS, Inc) and stainless steel liners (AMS, Inc). For each core, soil was extracted from the liner and divided into 2 depth increments from 0 to 15 cm, and from 15 to 25 cm, in the field. Soil core sections were then transported to the laboratory (<1 h) in Ziploc bags, sieved (4.75 mm mesh), and mixed.

One 10 g field moist aliquot of homogenized soil from each depth increment of each core was placed into a 120 mL glass serum bottle and allowed to equilibrate with laboratory air for 1 h. Serum bottles were then sealed with butyl rubber stoppers, crimp sealed, and incubated in the dark at approximately 20 °C. Headspace samples for net CH_4 consumption measurements were withdrawn into 10 mL gastight glass syringes at zero time and every 2 to 4 h interval thereafter for up to 3 d ($n \geq 4$). Atmospheric pressure was maintained in the serum bottles by replacing removed headspace gas with an equivalent volume of ultrapure N_2. Replicate bottles were also sealed without soil and sampled in conjunction with experimental vessels to ensure that changes in headspace CH_4 concentrations did not result from exchange with butyl rubber stopper. Upon completion of net CH_4 consumption measurements, rates of CH_4 production were determined on the same samples. This was accomplished by addition of 50 Pa of difluoromethane (CH_2F_2), an inhibitor of

methanotrophy (Miller et al. 1998), to serum bottles, following the time course for CH_4 consumption in the serum vial headspace as described above.

Methane sample measurements

All gas samples were analyzed for CH_4 by flame ionization detection gas chromatography (Shimadzu model GC 8 A; precision expressed as the coefficient of variation for 10 replicate injections of a 0.94 mL CH_4 L^{-1} standard was < 3%) within 10 h of collection, well within our predetermined holding time of 24 h. Sample separation was accomplished on a 1-m length x 0.32 cm diameter molecular sieve 5A column with an ultrahigh purity N_2 carrier gas (33 mL min^{-1}). Injector and detector temperatures were set at 90 ^{o}C and 140 ^{o}C.

Diffusivity

We employed a ^{222}Rn-based method (Born et al. 1990) to estimate effective diffusivity at soil collars within each of the eight experimental plots that we have used in previous research (Phillips et al. 2001a, Dubbs and Whalen unpublished). The ^{222}Rn-based method involves the simultaneous measurement of ^{222}Rn flux from soil collars, using the static chamber method (Whalen et al. 1992), and measurement of soil air ^{222}Rn and CH_4 concentrations at the soil surface and at a depth of 25 cm from our gas sampling wells. Two static chamber and well sets from each of the eight experimental plot were used for October 2008 and February 2009 diffusivity experiments while one static chamber and well set from each plot was used for April 2008 and July 2008 diffusivity experiments.

To initiate ^{222}Rn and CH_4 flux determinations, polyvinyl chloride covers fitted with a sampling port and capillary bleed were emplaced on soil collars. Radon-222 samples were withdrawn into 50 mL syringes from the soil surface, the 25 cm gas sampling well, and the static chamber. These samples were then used to fill evacuated 170 mL (volume) counting cells (Lucas) through quick connect fittings equipped with Teflon septa. Immediately following ^{222}Rn sample collection, a 10 mL chamber headspace sample and a 5 mL sample from each gas sampling well, from 5 cm to 25 cm depths, were also withdrawn into 10 mL glass syringes for CH_4 analysis. Additional static chamber headspace samples were similarly collected ~24 h later for ^{222}Rn and CH_4 analysis. Radon-222 activity was determined by scintillation counting of gas samples contained in Lucas cells using a portable radon monitor (Pylon Model AB-5). Gas samples were analyzed for CH_4 as described above.

Environmental measurements

Soil temperature and soil moisture were measured on each sampling date. Soil temperature was measured at 3 cm intervals from 1 cm to 19 cm depth with a multithermistor temperature probe. Volumetric soil moisture (mL H_2O cm^{-3} soil) was measured by time domain reflectometry on each sampling occasion using a hand-held portable soil reflectometry sensor (Campbell Scientific 620 with 20 cm-long probe rods).

Calculations and Statistical Analysis

Rates of net CH_4 consumption and CH_4 production of core sections were calculated from the headspace volume of the bottles and log-linear or time-linear changes of CH_4

concentrations, respectively. Rates of net CH_4 consumption and CH_4 production in core sections from the soil surface to 15 cm, and those from 15 cm to 25 cm below the soil surface, were averaged for comparison of CO_2 treatments. Area-based rates of net CH_4 consumption determined commensurate to diffusivity observations were calculated from static chamber geometry and the log-linear change in the CH_4 concentration in static chamber headspaces.

Soil ^{222}Rn profiles and ^{222}Rn chamber flux measurements were used to calculate the effective diffusivity of CH_4 in the soil (P_{CH4}) according to Dörr and Münnich (1990):

$$P_{CH4}=D_{0,CH4}/D_{0,Rn} * P_{Rn}$$

where $D_{0,CH4}$ and $D_{0,Rn}$ are the diffusion coefficients of CH_4 (0.194 $cm^2\ s^{-1}$ Lerman 1979) and Rn (0.1 $cm^2\ s^{-1}$; Tanner 1964) in air and P_{Rn} is the permeability of Rn, which is the quotient of Rn flux divided by the concentration gradient of Rn in the soil profile. The effective diffusivity of CH_4 was then used to calculate the flux of CH_4 (J_{CH4}):

$$J_{CH4}= P_{CH4}* \Delta C_{CH4}/\Delta z_{CH4}$$

where $\Delta C_{CH4}/\Delta z_{CH4}$ is the linear change in CH_4 concentration (C_{CH4}) with depth (z_{CH4}).

Paired t-tests were used to analyze for statistical differences between CO_2 treatment means for CH_4 concentration. Paired t-tests were likewise used to analyze for statistical differences between CO_2 treatment averages of rates of net CH_4 consumption or CH_4 production. Differences in diffusivity between CO_2 treatment plots were compared by student t-tests for each of the four observations. All statistical analyses were performed at α=0.05.

Results

Depth profiles of CH_4 concentrations

Soil CH_4 concentrations decreased sharply with depth from 5 to 20 cm below the soil surface. The rate of decline in CH_4 concentrations with depth decreased from 20 to 25 cm (Fig. 4.2). The depth profiles of average CH_4 concentrations in control and CO_2-enriched plots were similar. The average CH_4 concentration in control plots was slightly higher (0.1 mL L^{-1}) than in CO_2-enriched plots at 5 cm, but the average CH_4 concentration was slightly higher (0.01 to 0.05 mL L^{-1}) in CO_2-enriched plots relative to control plots at all other depths. However, there was not any significant difference in CH_4 concentrations between CO_2 treatments at any depth, nor for the whole soil profile, for any sampling date or for the entire data.

Depth profiles of net CH_4 consumption and CH_4 production

Net CH_4 consumption was observed in all core sections before addition of the CH_2F_2, at which point CH_4 production or zero flux of CH_4 was observed for the remainder of the observational period. Rates of net CH_4 consumption were similar in soils from both CO_2 treatments at each depth increment, ranging from 150 to 300 pg g_{DW}^{-1} h^{-1}, (Fig. 4.3a and b). Methane production was more variable, spanning almost three orders of magnitude in soils from both the 0 to 15 cm, (Fig. 4.4a; 0.5 to 450 pg g_{DW}^{-1} h^{-1}) and 15 to 25 cm depth intervals (Fig. 4.4b; 0 to 880 pg g_{DW}^{-1} h^{-1}).

The average rates of net CH_4 consumption in control plot soils from 0 to 15 cm were up to 14% higher than, or nearly equivalent to, the average rates of net CH_4 consumption in

soils from the CO_2-enriched plots from the same depth (Fig. 4.3a). In contrast, the average rates of CH_4 production in CO_2-enriched plots from 0 to 15 cm depths were up to two orders of magnitude higher than, or nearly equivalent to, the average rates of CH_4 production from the same depth (Fig. 4.4a). The patterns in rates of net CH_4 consumption and CH_4 production in soils from 15 to 25 cm depths are less clear with regard to differences between CO_2 treatments. The experiment with the overall highest average rate of CH_4 production among the 15 to 25 cm depth increments showed a value for for soils from CO_2-enriched plots that exceeded that for control plots (July 2005; Fig. 4.4b). This corresponded with a higher average rate of net CH_4 consumption in control plot soils (Fig. 4.3b). The rates of CH_4 production in soils from 15 to 25 cm core sections on both other sampling dates (Oct. 2006 and April 2007; Fig. 4.4b) were between 40 and 100% lower than the corresponding rates of net CH_4 consumption, and rates in soils from CO_2-enriched plots were higher than rates in control plots. The differences in rates of net CH_4 consumption and CH_4 production between CO_2 treatments were not significant for either depth interval or for the whole core on any date.

Effective diffusivity

There was not any consistent or significant difference in P_{CH4} between CO_2 treatments. The effective diffusivity of CH_4 was higher in control plots on two of four dates (Table 4.1), which also corresponded with higher overall P_{CH4} values. There was not a clear relationship between P_{CH4} and soil moisture, or between P_{CH4} and J_{CH4} (Table 4.1). The calculated flux of CH_4 (J_{CH4}) was more than 25% lower than measured CH_4 flux for three out of four observations (Table 4.1). The exception was in April 2008 when predicted (J_{CH4}) and

measured CH_4 fluxes were only different by 20 and 24 % in CO_2-enriched and control plots, respectively. Relatively good agreement between measured and predicted rates of net CH_4 flux in April corresponded with the highest P_{CH4} value. Soil moisture was higher in control plots relative to CO_2-enriched plots on each date when diffusivity measurements were made (Table 4.1).

Discussion

Methane concentrations at depth within the soil profile are determined by relative rates of diffusion from the atmosphere, consumption by methanotrophs, and/or production by methanogens. Our values for effective diffusivity are on the low end of reported values for a multitude of European (Born et al. 1990; Dörr et al. 1993) and boreal forest soils (Whalen et al. 1992), which range from 2 to 1504 $cm^2\ h^{-1}$. The low effective diffusivity may in part by due to the clay loam texture of our soils as soil texture influences diffusivity (Ball et al. 1997). For example, clay soils show net CH_4 consumption rates an order of magnitude lower than sandy soils (Dörr et al. 1993). The low diffusivity of our soils was evident during the collection and measurement of ^{222}Rn samples, as it was difficult to flush sample wells and subsequently collect sufficient sample for ^{222}Rn analysis. Further, soils produced ^{222}Rn at such low rates that accurate zero time ^{222}Rn determination ($t_{1/2}$ = 3.85 d) was problematic for stored, synoptically collected samples after the first few samples had been assayed because each assay required 6 h of counting. Therefore, the error associated with diffusivity measurements may overwhelm any treatment effect.

Nonetheless, decreased soil diffusivity, as a result of increased leaf litter depth and/or higher soil moisture, would reduce the substrate supply for methanotrophs, and could explain

the observed decline in net CH_4 consumption under elevated CO_2. The deeper duff in CO_2-enriched plots at our study site (Allen et al. 2000, Schlesinger and Lichter 2001, Lichter et al. 2005, Lichter et al. 2008; Fig. 4.1; pathway B) may be responsible for the observed reduction in net consumption of CH_4 under elevated CO_2. Duff has been shown to reduce diffusion of atmospheric gases to the mineral soil occupied by methanotrophs (Borken and Brumme 1997, Saari et al. 1997, Dong et al. 1998). Dong et al. (1998) observed that the removal of the leaves and humus layer from the soil surface resulted in 17% higher rates of CH_4 consumption by temperate forest soils. Similarly, we observed an increase of 6% in net CH_4 consumption when leaf litter was removed (data not shown), suggesting that a CO_2 treatment effect on leaf litter depth may contribute to reduced rates of net CH_4 consumption under elevated CO_2. Further, a thicker duff layer can slow evaporation from the soil surface thereby causing higher soil moisture in CO_2-enriched plots (Schäfer et al. 2003). Schäfer et al. (2003) observed significantly higher soil moisture in CO_2-enriched plots at our study site through 2002, although they proposed that the difference between CO_2 treatment plots did not necessarily reflect a treatment effect. We (Dubbs and Whalen unpublished) also found higher soil moisture in CO_2-enriched plots during biweekly determination of soil-atmosphere CH_4 exchange in 2004 and 2006, and that soil moisture explained 34% of the variability in rates of net CH_4 consumption. Higher soil moisture and associated reduction in diffusivity (Suwa et al. 2004) in CO_2-enriched plots likely contributes to the observed decrease in rates of net CH_4 consumption under elevated CO_2, regardless of the cause of increased soil moisture.

Low overall effective diffusivity and high soil moisture in CO_2-enriched plots would also slow the diffusion of atmospheric O_2 into and within the mineral soil. This reduced

supply of O_2, paired with increased respiratory O_2 consumption within the soil matrix under elevated CO_2 (Bernhardt et al. 2006) may result in the formation of anoxic microsites. Independent reports of anoxic microzones (Sexstone et al. 1985, Zausig et al. 1993) and methanogenic activity in macroscopically oxygenated soils (Yavitt et al. 1995, Saari et al. 1997, von Fischer and Hedin 2002, Teh et al. 2005) indicate that simultaneous CH_4 production and consumption are occurring in well-drained upland soils, with anoxic soil aggregates supporting localized zones of methanogenesis and oxic sites supporting methanotrophy. In fact, Hoosbeek and Scarascia-Munozza (2009) saw increased macro-aggregation (250–2000 µm) of soils under elevated CO_2 in a temperate *Populus x euramericana* plantation (Fig. 4.4; pathway C), and further found that the soil aggregates contained higher concentrations of C and N. Horn and Smucker (2005) found that the redox potential decreased rapidly, and thus the propensity for anoxia increased, when such soil aggregates were saturated by water. While not explicitly determined here, such loci of microbial activity where respiratory consumption of O_2 is enhanced and the development of anaerobic conditions are stimulated may explain the qualitative trend of increased CH_4 production under elevated CO_2 observed here. We previously reported episodic net CH_4 efflux from the soil (indicated net CH_4 production) under both CO_2 treatments, with nearly double the observations in CO_2-enriched plots (Dubbs and Whalen unpublished). We additionally measured gross CH_4 production in all soils from a 0 to 15 cm depth increment in laboratory experiments where an inhibitor of methanotrophy was administered (Fig. 4.4). Further, in the 15 to 25 cm depth increment, high rates of CH_4 production were clearly manifested by reduced rates of net CH_4 consumption (July 2005; Figs. 4.3 and 4.4). Finally, the depth profiles (Fig. 4.2) provide further evidence of methanogenesis at depth since there

were only 15 occasions out of 2480 observations, where the CH_4 concentration was drawn below the widely acknowledged threshold of about 0.2 µl L^{-1} for high affinity methanotrophs (Bender and Conrad 1995), indicating a soil source of CH_4 augments the atmospheric supply to methanotrophs.

While reduced diffusivity of CH_4 in CO_2-enriched plots was not expressed in depth profiles of CH_4 concentrations, which showed no significant differences in CH_4 concentrations at any depth (Fig. 4.2), the depth profiles did suggest slightly higher CH_4 production in CO_2-enriched plots. Methane concentrations at depths between 10 and 20 cm in CO_2-enriched plots were slightly higher than those in control plots. Additionally, there was not any difference in CH_4 concentrations between CO_2 treatments at any depth (Fig. 4.2), nor was there a significant difference in net CH_4 oxidizing activity between CO_2 treatments at any depth (Fig. 4.3). A down-profile shift in the locus of the CH_4 oxidizing community in response to elevated CO_2 would increase the diffusional path of atmospheric CH_4 to the locus of CH_4 oxidation. Thus, this lack of difference in CH_4 concentrations between CO_2 treatments indicate that the long-term pattern of reduced net CH_4 consumption in soils exposed to elevated CO_2 (Phillips et al. 2001a, b, McLain et al. 2002, Whalen unpublished, Dubbs and Whalen unpublished) is not the result of such a downprofile shift in the CH_4 oxidizing community.

High spatial variability in net CH_4 consumption and CH_4 production rates and diffusivity measurements, along with limited ability to collect soil samples hampered our ability to detect significant differences between CO_2 treatments. However, trends indicating low overall diffusivity and increased incidence and rates of CH_4 production in elevated CO_2 plots relative to control plots. This conclusion is supported by long-term repeated field

measures of soil moisture and net atmospheric CH_4 consumption where significant, quantitative differences between CO_2 treatments were observed with CO_2-enriched plots showing higher soil moisture and lower rates of net atmospheric CH_4 consumption (Dubbs and Whalen, submitted). Thus, global changes that impact soil hydrology directly or through biological feedbacks (Denman et al. 2007) are useful predictors of the direction and rates of CH_4 flux in upland soils. Factors that increase soil aggregation can also be expected to influence CH_4 dynamics, although more research is needed regarding this pathway (Fig. 4.1, Pathway C).

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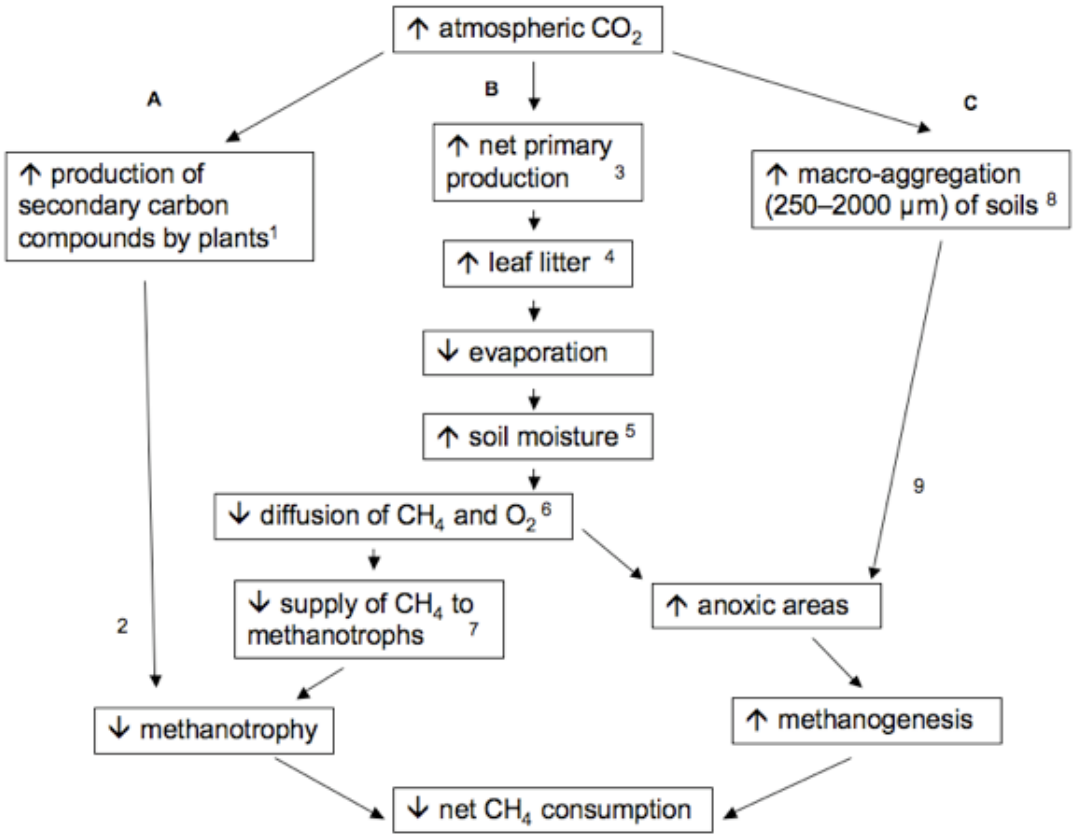
Table 4.1. Effective diffusivity (P_{CH4}) and corresponding calculated net CH_4 flux (J_{CH4}; $\mu g\ m^{-2}\ h^{-1}$), measured net CH_4 flux ($\mu g\ m^{-2}\ h^{-1}$), and soil moisture in control and CO_2 enriched plots (n=3, each treatment for July 2008 and April 2008; n=6, each treatment for Oct.2008 and Feb. 2009) at the Duke FACE site.

Date	CO_2 treatment	P_{CH4} ($cm^2\ h^{-1}$)	J_{CH4} ($\mu g\ m^{-2}\ hr^{-1}$)	Measured net CH_4 flux ($\mu g\ m^{-2}\ hr^{-1}$)	Soil moisture (mL H_20 cm^{-3} soil)
July '08	Control	2.8	1.3	1.7	0.32
	Enriched	2.4	1.1	1.5	0.27
April '08	Control	1.8	0.7	1.6	0.33
	Enriched	1.3	0.5	1.6	0.31
Oct. '08	Control	0.9	0.4	1.6	0.24
	Enriched	1.1	0.4	1.8	0.24
Feb. '09	Control	0.0	0.0	0.8	0.39
	Enriched	0.5	0.1	0.6	0.34



[1] Gebauer et al.1997; Peñuelas and Estiarte 1998; Wetzel and Tuchman 2005
[2] Amaral and Knowles 1997, 1998
[3] DeLucia et al. 1999; Hamilton et al. 2002; DeLucia et al. 2005; Norby et al. 2005; Finzi et al. 2006
[4] Allen et al. 2000; Schlesinger and Lichter 2001
[5] Schäfer et al. 2002
[6] Suwa et al. 2004
[7] Striegl 1993; Castro et al. 1995; Whalen and Reeburgh 1996
[8] Hoosbeek and Scarascia-Mugnozza 2009
[9] Sextone et al.1985; Zausig et al. 1993

Figure 4.1. Conceptual model of the impact of forest ecosystem responses to elevated CO_2 that influence soil CH_4 cycling dynamics. Up and down arrows within each response function indicate a positive or negative impact, respectively, of that factor on net atmospheric CH_4 consumption. Response functions are either documented or hypothesized by the associated references.

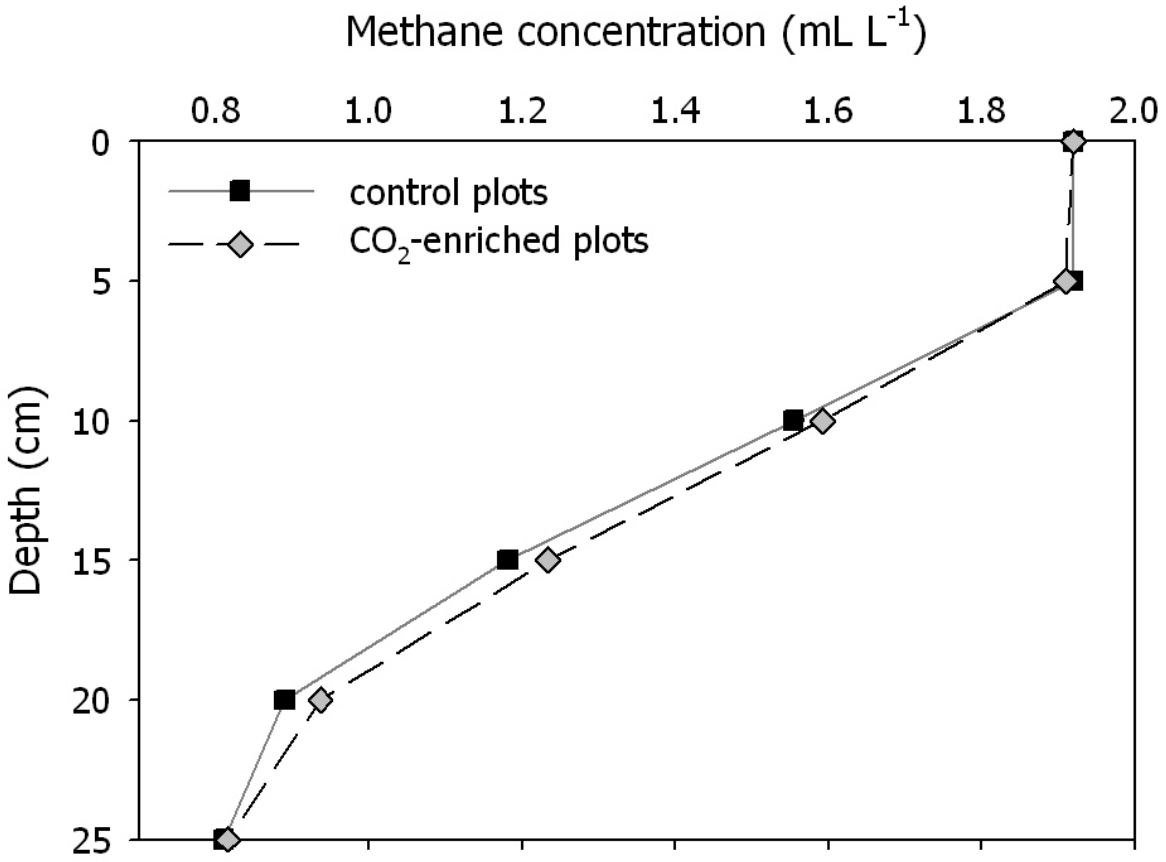


Figure 4.2. Composite depth profiles of CH_4 in soils in forest plots exposed to elevated CO_2 or the ambient atmosphere (control). Data for each depth represent the mean from 8 soil gas wells for each treatment over 31 dates. Error bars are eliminated for clarity

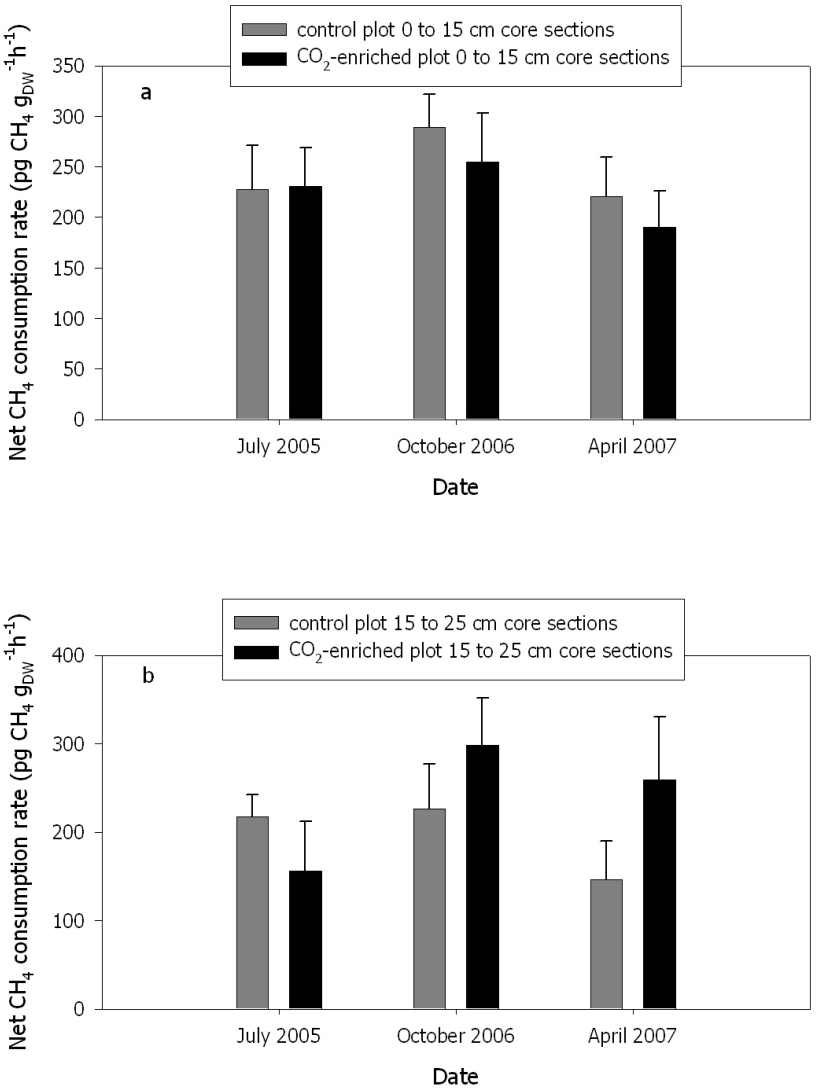


Figure 4.3. Mean rates of net CH_4 consumption in forest soils from plots exposed to elevated CO_2 or the ambient atmosphere (control). Data are mean rates for: a) 0 to 15 cm; and b) 15 to 25 cm depth increments from 16 cores. Error bars represent 1 standard error of the mean (n=8).

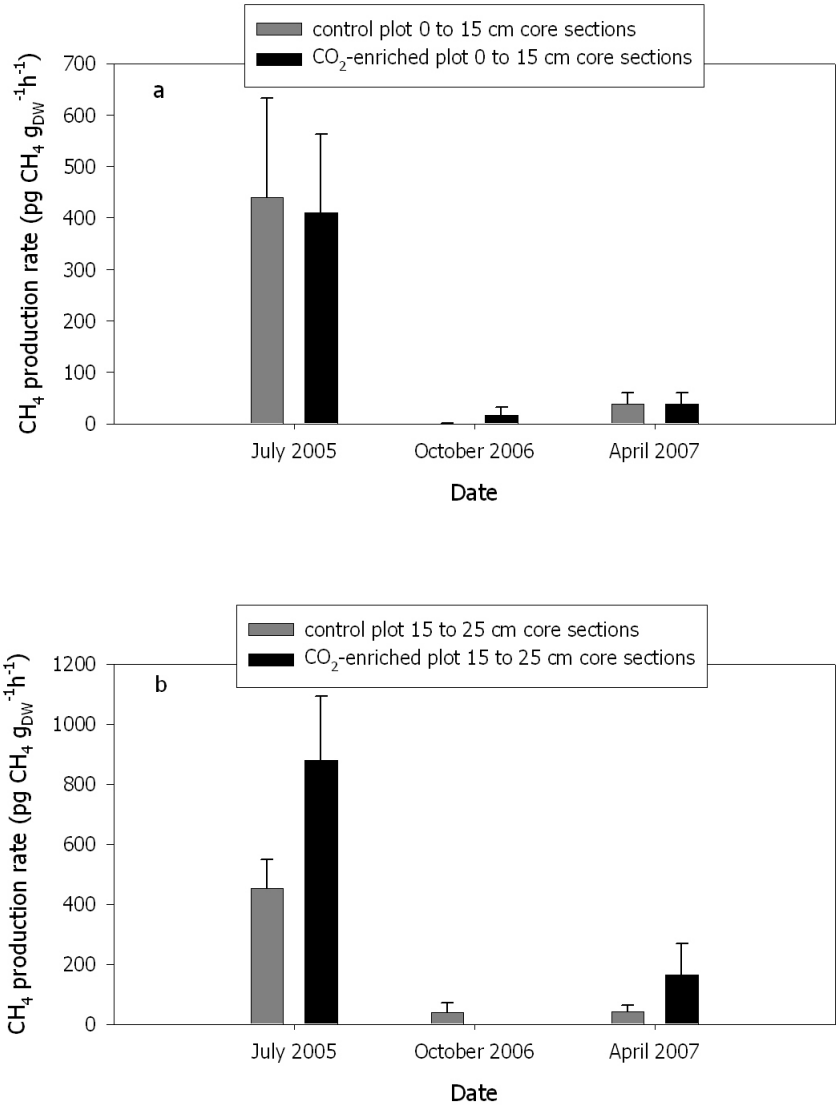


Figure 4.4. Mean rates of CH_4 production in forest soils from plots exposed to elevated CO_2 or the ambient atmosphere (control). Data are mean rates for: a) 0 to 15 cm; and b) 15 to 25 cm depth increments from 16 cores. Error bars represent 1 standard error of the mean (n=8).

CHAPTER 5: CONCLUSION

My research suggests that reduced net CH_4 consumption is a sustained, equilibrium response of this temperate forest soil to elevated CO_2. I observed lower annualized rates of net CH_4 consumption in soils from CO_2-enriched plots relative to controls for all 3 y of my study, extending the record from permanently emplaced soil collars at my study site (Phillips *et al.* 2001; Whalen and Fischer unpublished) to 8 nearly continuous years. The average decrease in net CH_4 consumption under elevated CO_2 for all annual observations was ~15% and there was not any consistent change in the magnitude of the CO_2 enrichment effect on the CH_4 sink strength over the extended record.

A decline in soil CH_4 consumption of the magnitude observed here (~15%) across all forest biomes with an estimated aggregated sink of 24 Tg CH_4 y^{-1} (Ridgwell et al. 1999), gives a decrease of 3.6 Tg CH_4 y^{-1}. This reduction represents 10% of the model estimate (Ridgwell et al. 1999) of 38 Tg CH_4 y^{-1} for the total soil sink.

Causative factors for the observed decrease in net CH_4 consumption under elevated CO_2 are difficult to identify, as the destructive sampling necessary for process-level investigations is limited to maintain ecosystem integrity at the Duke FACE site. However, the modeled soil moisture x CO_2 treatment interaction for 3 y of field measurements of net CH_4 flux and corresponding environmental variables was significant, indicating that site-wise differences in net CH_4 consumption are at least in part moisture-related. The observation that soil moisture explains 34% of the variability in net CH_4 measurements further supports soil

moisture control of net CH_4 consumption where soil moisture is higher in elevated CO_2 plots. However, soil moisture does not appear to be the only driver of the observed decline in net CH_4 consumption under elevated CO_2.

I found that some plant exudates from this forest ecosystem inhibit CH_4 consumption, including levulinic acid, an organic acid that is released from plant roots in greater quantities under elevated CO_2; and duff leachates from the duff of both CO_2 treatment plots, but which is thicker under elevated CO_2. These leachates do not exert consistent control over rates of atmospheric CH_4 consumption. However, their temporal and spatial influence on net CH_4 consumption under elevated CO_2 deserve further consideration since their transient influences may weaken the correlation between well-studied influences on CH_4 consumption and measured rates of net CH_4 consumption.

While high spatial variability and high error, along with limited ability to collect soil samples largely resulted in the inability to detect significant differences in rates of CH_4 consumption and CH_4 production and soil diffusivity between CO_2 treatments, qualitative trends showed low overall effective diffusivity of these soils and increased incidence and rates of CH_4 production in elevated CO_2 plots. When these trends are viewed together with the contributions of soil moisture to explaining reduced net CH_4 consumption under elevated CO_2, it is apparent that increased activity of methanogens under elevated CO_2 contribute to the observed decline in CH_4 oxidation at this study site.

My research has identified several research needs. These include further investigation of the spatial and temporal inhibitory influences of plant compounds produced under elevated CO_2 that may influence rates of CH_4 consumption, as well as determination of the factors that contribute to formation of anoxic microsites in upland soils under elevated

CO_2. If my results can be broadly extrapolated, my research also suggests that a 200 ml L^{-1} increase in present-day atmospheric CO_2 concentrations can be expected to reduce the forest soil sink for CH_4 of ~24 Tg y^{-1} by approximately 15%. Further, the observed relationship between increasing soil moisture and the reduction in the forest sink for CH_4 indicates that climate forecasting models can constrain the predicted upland sink for CH_4 by relating it to soil hydrology.

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