Persistence and potential causes of reduced net CH₄ consumption under elevated CO₂ in a temperate forest

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Environmental Sciences and Engineering.

Chapel Hill
2009

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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\textsubscript{4} consumption in CO\textsubscript{2} -enriched plots that suggests this is likely a sustained negative feedback to increasing atmospheric CO\textsubscript{2}.

Causitive factors for the observed decrease in net CH\textsubscript{4} consumption under elevated CO\textsubscript{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\textsubscript{4} production in CO\textsubscript{2}-enriched plots, along with transient inhibition by plant exudates and low overall soil diffusivity, begin to explain reduced rates of CH\textsubscript{4} consumption and increased rates of CH\textsubscript{4} production that result in long-term reduction in net CH\textsubscript{4} consumption in these soils.
ACKNOWLEDGEMENTS

Steve Whalen has been my mentor for seven years now. I am very grateful for all that I have learned from him including, but not limited to, how to be an honest and dedicated scientist, caring teacher, and quiet leader; how to build and fix instruments and field equipment myself; how to write more clearly and scientifically; and perhaps, most importantly, that I should lighten up a bit.

My dissertation committee has been incredibly helpful to the completion of my research and studies with their time and attention, pointed questioning, guidance, and kind words. Thank you to Drs. Emily Bernhardt, Rob Jackson, Frederic Pfaender, and Howard Weinberg.

Several other professors have also been great mentors to me during my graduate school years, especially Drs. Robert Wetzel, Donald Lauria, Joe von Fischer, and Ram Oren. I cannot thank them enough for their time, candid and interesting conversations, and inspiration.

Lance Leonhardt, an amazing biology and environmental science teacher, first lit the fire in me and gave me every reason to keep following my curiosity. I thank him for his excitement that was truly contagious.

I am grateful for soil moisture data provided by Hyun Seok-Kim and Ram Oren and root exudate data provided by Emily Bernhardt and Rich Phillips. I also thank many for field assistance over the years including Eric Fischer, Ryan Elting, Lauren Elich, Omar Monzon, Steve Artabane, William Dodge, and Robert Nettles. With their help and company, Robert
Nettles, David Cooley, and Jeff Pippen made visits to the FACE site something to look forward to. I thank Ramon Garcia who was very generous with statistics guidance. Whalen lab group members (Eric Fischer, Jeff DeBerardinis, Priscilla Benson, Brian Chalfant, Marsha Fisher, Gina Panasik, Joelene Diehl, Ken Fortino, and Dendy Lofton) have been great colleagues and I thank them for their company and conversation. David Singleton was an incredibly patient and knowledgeable teacher of molecular biology, even though our method never did work. I also am very thankful for the dedicated staff of ESE that I have interacted with on a regular basis. Jack Whaley, Donna Simmons, Deborah Williams, Robin Whitley, Jim Wallace, Melody Levy, Ann Goodwin, Elise Pohl, Rebecca Riggsbee Lloyd, and Linda Brezin have made navigating graduate school much more enjoyable and easy. I thank Glenn Walters, Randy Goodman, Cliff Burgess, and Fred Bevin of the ESE design studio, who were there to answer any question about instruments, find any part, or build anything I needed.

My family and friends have been resilient and determined in their encouragement, interest, and support of my research and studies. I would especially like to thank my parents, Barry and Dawn Dubbs, my brother, Nate, and William Dodge for their patience and constant flow of encouragement over the past few months.

This study was supported by EPA STAR Grant No. RD-83145101-0 to S.C. Whalen and R.G. Wetzel, and the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG02-95ER62083. The Edward R. Kuenzler award also provided financial assistance to complete my degree and for that, I am thankful.
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<td>FACE</td>
<td>free air CO₂ enrichment</td>
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<tr>
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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 CO₂ is important to predicting future climates as they can, in turn, feed back to the biogeochemical cycling of CO₂ and other greenhouse gases.

Attempts at understanding terrestrial biological and biogeochemical responses to elevated CO₂ 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 CO₂ exchange (FACE) technology has been employed to conduct ecosystem-level studies where tall vegetation and their surrounding ecosystems are exposed to elevated CO₂ 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 CO₂, which may not fully represent how ecosystem components will react to the contemporary monotonic increase in the concentration of CO₂ 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 CO₂ 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

However, observations (McMurtrie and Comins 1996) and ecosystem models (Newton et al. 2001) indicate that biological responses to elevated CO₂ and biogeochemical feedbacks vary widely on different timescales. For instance, down-regulation of photosynthesis has been commonly reported for CO₂-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 CO₂ must be distinguished.

*Methane in the pedosphere*

Methane is another greenhouse gas that is cycled through temperate forests and thus may be affected by CO₂-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 CO₂ (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 CH₄ 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 CH₄, the only known biological sink for CH₄, and the largest natural source of CH₄ are sited in the pedosphere. The balance between rates of CH₄ production (methanogenesis) and CH₄ consumption (methanotrophy) determines whether a soil is a net source or sink for atmospheric CH₄, 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 CH₄ removal from the atmosphere annually (Ridgwell et al. 1999). This net biological sink in upland soils includes atmospheric CH₄ consumption by methanotrophic bacteria in the largely oxic soil profile, and consumption of endogenously produced CH₄ by methanogenic bacteria in anoxic microsites (reviewed by Conrad 1996).

Methanotrophic bacteria oxidize CH₄ 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 CH₄, which occurs at CH₄ 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\textsubscript{2} on CH\textsubscript{4} dynamics in soils**

Experiments examining the effect of elevated CO\textsubscript{2} on net CH\textsubscript{4} emissions from wetland soils unequivocally indicate that CH\textsubscript{4} emissions increase when wetland plants, plant communities, or ecosystems are grown under elevated CO\textsubscript{2}. Increases in CH\textsubscript{4} emissions from wetland soils ranged from 10.9% in a pot study of a rice cultivars grown under CO\textsubscript{2} at 200 mL L\textsuperscript{-1} above ambient concentrations (Lou et al. 2008) to 60% when rice fields were exposed to elevated CO\textsubscript{2} (300 mL L\textsuperscript{-1} above ambient) in open-top chambers (Ziska et al. 1998). The increase in net CH\textsubscript{4} emissions was markedly similar to the range (38 to 58%) seen in a Japanese rice paddy exposed to elevated CO\textsubscript{2} via FACE technology (Inubushi et al. 2003).

Investigations of soil-atmosphere CH\textsubscript{4} exchange in CO\textsubscript{2}-enriched ecosystems that normally function as atmospheric CH\textsubscript{4} sinks are few and show mixed results. Ambus and Robertson (1999) reported a 22% reduction in CH\textsubscript{4} consumption by soils in model *Populus tremuloides* (deciduous forest) ecosystem exposed to elevated CO\textsubscript{2}, while Phillips et al. (2001a) showed annual reductions in CH\textsubscript{4} consumption of 16% and 30% in CO\textsubscript{2} fumigated plots (200 mL L\textsuperscript{-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\textsubscript{4} uptake were three times greater in ambient CO\textsubscript{2} soils relative to CO\textsubscript{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\textsubscript{2} on atmospheric
CH₄ consumption. Further, Mosier et al. (2002) saw no impact of CO₂ level on rates of CH₄ exchange between soils and the atmosphere in a semi-arid, mixed grassland community.

If the observed CO₂-induced increases in net CH₄ emissions are extrapolated to the global scale, the wetland (natural and agricultural environments) source strength in the atmospheric CH₄ budget will increase by 29 and 160 Tg annually with a 200 to 300 mL L⁻¹ increase in atmospheric CO₂ concentration (Chen and Prinn 2005). At the same time, models suggest that the annual forest sink of 24 Tg y⁻¹ for CH₄ (Ridgwell et al. 1999) can be expected to decline from between 3.8 to 7.2 Tg as atmospheric CO₂ concentrations increase by 200 mL L⁻¹. However, more empirical data are needed before we can rely on these predictions of changes in CH₄ source and sink terms with increasing atmospheric CO₂. 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 CO₂ and the impact of those responses on CH₄ cycling.

**Ecosystem-level changes that may influence CH₄ dynamics in temperate forests**

Several CO₂-induced changes in temperate forest ecosystems may help to explain the observed decline in net CH₄ consumption under elevated CO₂. Changes in plant productivity, chemistry, and allocation of C under elevated CO₂ 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 CO₂ 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\textsubscript{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\textsubscript{2} may increase the incidence of anoxic microsites where anaerobic microbial metabolism, such as methanogenesis, is possible. Since net CH\textsubscript{4} consumption in upland soils is the net effect of CH\textsubscript{4} consumption in the oxic soil profiles and CH\textsubscript{4} production in anoxic microsites, increased incidence of anoxic loci can alter this balance, reducing rates of net CH\textsubscript{4} consumption or shifting localized areas to net CH\textsubscript{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\textsubscript{4} consumption by soils in a temperate forest enriched with elevated CO\textsubscript{2}. The cause(s) of the decline in rates of net CH\textsubscript{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\textsubscript{4} consumption by the same temperate forest soils is a sustained response to elevated CO\textsubscript{2}; and b) identify factor(s) contributing to the observed (Phillips et al. 2001a) decline in net CH\textsubscript{4} consumption under elevated CO\textsubscript{2} at the Duke Forest FACE site. Possible controls on CH\textsubscript{4} consumption resulting from elevated CO\textsubscript{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.
References


CHAPTER 2: REDUCED NET CH₄ CONSUMPTION IS A SUSTAINED RESPONSE TO ELEVATED CO₂ IN A TEMPERATE FOREST

Abstract

We compared, from 2004 through 2006, rates of soil-atmosphere CH₄ 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) CO₂ since August 1996. A total of 880 observations showed net atmospheric CH₄ 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 CH₄ 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 CH₄ consumption in control plots was higher than the mean for CO₂-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 CH₄ consumption at control plots during the three calendar years of this study were 19, 10 and 8% higher than comparable values for CO₂ 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 CO₂-enriched sites and a mixed effects model identified a significant soil moisture x CO₂ interaction on net atmospheric CH₄ consumption. Increased soil moisture at CO₂-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 µL 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 CO₂ treatment plots, with the exception of soil organic matter, which averaged 4.6% in CO₂-enriched plots, and only 3.4% in control plots. Averages for all control and elevated CO₂ 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 “CO₂-enriched”) are fumigated with CO₂ to maintain atmospheric CO₂ concentrations 200 mL L⁻¹ above ambient levels, while three additional treatment plots are fumigated with ambient air to replicate micrometeorological effects associated with CO₂ 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 CH₄ 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 CH₄ 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 CH₄ 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 N₂ carrier gas (33 mL min⁻¹). Injector and detector temperatures were set at 90 °C and 140 °C.

Soil Physicochemical Measurements

Volumetric soil moisture (mL H₂O 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 $\alpha=0.05$. 

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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 H₂O cm⁻³ soil, and averaged 0.27 mL H₂O cm⁻³ soil. Calendar year means for CO₂-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 CO₂-enriched treatment (0.28 mL H₂O cm⁻³ soil) was significantly higher than for the control treatment (0.26 mL H₂O cm⁻³ soil).

Patterns in net CH₄ flux

Net CH₄ 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 CH₄ production (flux from the soil to the atmosphere) was also observed, from 17 individual quadrants on 16 separate dates, giving 22 observations of net CH₄ production in 880 total records. Net CH₄ production was found almost twice as often in CO₂-enriched chambers as in control chambers (14 versus 8 observations). Rates of net CH₄ 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 CH₄ 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 °C 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 °C (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 CH₄ consumption southward. The average net CH₄ consumption rate of 0.39 mg CH₄ m⁻² d⁻¹ for the 12 of 68 sampling dates at soil temperatures < 10 °C was lower by 32% than the mean of 0.58 mg CH₄ m⁻² d⁻¹ at higher temperatures. Differences in the strength of the temperature-CH₄ 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 CH₄ 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 CH₄ 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 CH₄ 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 CH₄ consumption between CO₂ treatments**

We found that CO₂ enrichment resulted in a per annum decline in net atmospheric CH₄ 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 by Amthor 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
CO₂ fumigation and initial sampling (Phillips et al. 2001a), consistently lower annualized rates of net CH₄ consumption in soils from CO₂-enriched plots relative to controls, and lack of strong evidence that the magnitude of the CO₂ enrichment effect has declined with time all suggest that reduced net atmospheric CH₄ consumption is a sustained, equilibrium response of this forest soil to elevated CO₂.

**Potential reasons for reduced net CH₄ consumption under elevated CO₂**

Although reduced net atmospheric CH₄ consumption is likely a sustained negative feedback by soil to CO₂-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 CH₄ consumption are at least in part moisture-related. Several demonstrated effects of CO₂-enrichment on above- and below-ground processes within forest ecosystems feed back on soil moisture and by extension soil CH₄ cycling dynamics (Fig. 2.4).

The net soil-atmosphere CH₄ 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 CO₂ (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 CO₂ treatment). The associated insulating effect enhances
moisture conservation (Schäfer et al. 2003). The direct link between increased soil moisture and diffusion-limitation to CH₄ oxidizers is well established (Striegl 1993, Castro et al. 1995, Whalen and Reeburgh 1996). Persistently higher moisture content and reduced net atmospheric CH₄ consumption in CO₂-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 CO₂ 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 CH₄ 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 CO₂ 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 CH₄ production in intact soil cores, with stronger activity in soils from CO₂-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 CH₄ emission in CO₂-enriched versus control chambers provide confirmatory evidence for at least episodic CH₄ 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.


References


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 daily area-based data from each sampling occasion.

<table>
<thead>
<tr>
<th>Year</th>
<th>Annual net CH$_4$ consumption (mg m$^{-2}$ y$^{-1}$)</th>
<th>% Difference between treatments</th>
<th>Source</th>
<th>Volumetric soil moisture (mL H$_2$O g soil$^{-1}$)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CO2-enriched</td>
<td></td>
<td>Control</td>
<td>CO2-enriched</td>
</tr>
<tr>
<td>1998</td>
<td>183</td>
<td>156</td>
<td>16 *</td>
<td>(Phillips et al. 2001a)</td>
<td>0.24</td>
</tr>
<tr>
<td>1999</td>
<td>191</td>
<td>136</td>
<td>30 *</td>
<td>(Phillips et al. 2001a)</td>
<td>0.27</td>
</tr>
<tr>
<td>2002</td>
<td>204</td>
<td>181</td>
<td>13 *</td>
<td>(Whalen unpubl.)</td>
<td>0.22</td>
</tr>
<tr>
<td>2004</td>
<td>184</td>
<td>150</td>
<td>19 *</td>
<td>Present study</td>
<td>0.29</td>
</tr>
<tr>
<td>2005</td>
<td>196</td>
<td>175</td>
<td>10 *</td>
<td>Present study</td>
<td>0.26</td>
</tr>
<tr>
<td>2006</td>
<td>197</td>
<td>181</td>
<td>8</td>
<td>Present study</td>
<td>0.23</td>
</tr>
</tbody>
</table>

*Differences between treatments significant at $\alpha=0.05$. 
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$_2$O 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.
Figure 2.4. Conceptual model of the impact of forest ecosystem responses to elevated CO₂ that influence soil CH₄ cycling dynamics. Up and down arrows within each response function indicate a positive or negative impact, respectively, of that factor on net atmospheric CH₄ consumption. Response functions are either documented or hypothesized by the associated references.
CHAPTER 3: INHIBITION OF CH₄ CONSUMPTION BY SECONDARY CARBON COMPOUNDS IN THE TISSUES AND EXUDATES OF TEMPERATE FOREST PLANTS EXPOSED TO ELEVATED CO₂

Abstract

We previously showed a sustained reduction in net atmospheric CH₄ consumption by temperate forest soils in response to elevated CO₂ (Dubbs and Whalen submitted) and here report the influence of plant exudates on atmospheric CH₄ 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 CH₄ consumption. Plant exudates from forest plots exposed to elevated CO₂ since 1996 (~580 mL L⁻¹ CO₂) or from control plots subjected to ambient conditions and acid root exudates from loblolly pines (Pinus taeda) grown under elevated CO₂ were applied to soils. Duff leachates occasionally inhibited CH₄ consumption regardless of CO₂ treatment, and levulinic acid inhibited CH₄ 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 CH₄ consumption. While plant exudates may only assert transient and secondary control on CH₄ consumption under elevated CO₂, identification of temporal and spatial patterns of influence warrant further study because they otherwise confound the correlation between the primary drivers of CH₄ consumption and measured rates of net CH₄ consumption.
Introduction

The global atmospheric CO\textsubscript{2} concentration has more than doubled since the Industrial Revolution (Forster et al. 2007). Little is understood about how increasing atmospheric CO\textsubscript{2} will affect the biogeochemical cycling of other greenhouse gases, including CH\textsubscript{4}, but we recently reported a sustained decrease in net atmospheric CH\textsubscript{4} consumption under elevated CO\textsubscript{2} in a temperate loblolly pine (*Pinus taeda*) forest (Dubbs and Whalen submitted). Our previous work showed that a sustained CO\textsubscript{2}-induced negative feedback on forest soil CH\textsubscript{4} consumption could lead to a 15% reduction (3.6 Tg CH\textsubscript{4} yr\textsuperscript{-1}) in the current forest soil sink of 24 Tg yr\textsuperscript{-1} (Ridgwell et al. 1999). This negative feedback to increasing CO\textsubscript{2} is of concern because consumption by upland soils is the only terrestrial sink for atmospheric CH\textsubscript{4}, which is a greenhouse gas with radiative forcing second only to CO\textsubscript{2} among trace atmospheric gases (Forster et al. 2007).

The reasons for the observed decline in net atmospheric CH\textsubscript{4} consumption by these soils under elevated CO\textsubscript{2} were not entirely clear. However, we postulated several pathways by which changes in a temperate forest ecosystem exposed to elevated CO\textsubscript{2} could lead to decreased CH\textsubscript{4} consumption by methanotrophic bacteria and increased CH\textsubscript{4} production by methanogenesis (Fig. 3.1). Rates of CH\textsubscript{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\textsubscript{4} consumption accounts for the observed sink strength of upland soils in the global CH\textsubscript{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\textsubscript{4} consumption.
Ecosystem-scale elevated CO\textsubscript{2} enrichment experiments, or free air carbon exchange (FACE) experiments, indicate that temperate forest responses to elevated CO\textsubscript{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\textsubscript{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\textsubscript{2}. Norby et al. (1987) found that elevated CO\textsubscript{2} enhances root exudation of organic acids in a pot study of Pinus echinata seedlings. Elevated CO\textsubscript{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\textsubscript{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\textsubscript{4} consumption by a temperate forest soil is a sustained response to elevated CO\textsubscript{2} (Dubbs and Whalen submitted), by examining the influence of plant exudates on rates of CH\textsubscript{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 CO₂ 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 CO₂-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 CO₂-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 CO₂-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 CO₂ 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 N₂.

Gas samples were analyzed for CH₄ 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 CH₄ 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 N₂ carrier gas (33 mL min⁻¹). Injector and detector temperatures were set at 90 °C and 140 °C. Headspace measurements from replicate bottles without soil ensured that changes in headspace CH₄ concentrations did not result from gas exchange with butyl rubber stoppers.

Statistical Analysis

Soil dry mass-based rates of CH₄ consumption were calculated from the log-linear change of CH₄ concentration in jar headspaces. Rates or rate constants for CH₄ 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\textsubscript{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\textsubscript{4} consumption. A 100 µmol L\textsuperscript{-1} solution of levulinic acid reduced CH\textsubscript{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\textsubscript{4} consumption by levulinic acid revealed that CH\textsubscript{4} consumption was not significantly reduced at concentrations below 100 µmol L\textsuperscript{-1} (Fig. 3.7). Rates of CH\textsubscript{4} consumption were, however, significantly reduced at levels above 100 µmol L\textsuperscript{-1}. Further, CH\textsubscript{4} consumption was completely inhibited when levulinic acid was added to soils at concentrations of 500 µmol L\textsuperscript{-1} or 1000 µmol L\textsuperscript{-1}.

\textit{Discussion}

Plants grown under elevated CO\textsubscript{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\textsubscript{4} dynamics because they inhibit metabolism and growth by methanotrophs (Amaral and Knowles 1997, 1998). Indeed, we found evidence of transient inhibition of CH\textsubscript{4} consumption by duff collected from CO\textsubscript{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\textsubscript{2}. However, neither throughfall nor leaf leachates from the four dominant tree species at the study site affected rates of CH\textsubscript{4} consumption by forest soils, regardless of the CO\textsubscript{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 CO₂ level under which trees were grown.

Levulinic acid, a primary root exudate acid released from loblolly pines exposed to elevated CO₂, was found here to inhibit CH₄ 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 CO₂ 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 CO₂ concentration and a reduction in the forest soil sink strength for CH₄.

Despite some transient inhibition of net CH₄ consumption by forest soils by plant exudates reported here, the spatial and temporal patterns of in situ net CH₄ 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 \textit{in situ} net CH$_4$ consumption. Research in this area will help to refine models aimed predicting the upland soil sink strength for CH$_4$. 


**References**


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.

1 Gebauer et al. 1997; Peñuelas and Estiarte 1998; Wetzel and Tuchman 2005
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 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 protocateucic 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 CH₄ CONSUMPTION CAUSED BY CHANGES IN THE SOURCES AND TRANSPORT OF SOIL GASES IN A TEMPERATE FOREST EXPOSED TO ELEVATED CO₂

Abstract

We previously reported a sustained reduction in net atmospheric CH₄ consumption by temperate forest soils exposed to elevated CO₂ since 1996 (~580 mL L⁻¹ CO₂; Dubbs and Whalen submitted). Changes in the transport and supply of atmospheric gases within the soil profile under elevated CO₂, and subsequent changes in locus or activity of the CH₄ oxidizing and producing communities, may help to explain the decrease in net CH₄ consumption. We examined the depth distribution of CH₄ in the soil profile, the effective diffusivity of CH₄ through the soil, and the extent and activity of CH₄ consuming and CH₄ producing communities in CO₂-enriched and control (ambient atmospheres) plots at the same study site. High spatial and temporal variability in net CH₄ consumption and CH₄ 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 CO₂ treatments in rates of net CH₄ consumption or CH₄ production, depth profile CH₄ concentrations, or effective diffusivity. However, qualitative trends of low overall diffusivity and increased incidence and rates of CH₄ production in elevated CO₂ plots, supported by a long-term record of significantly higher soil moisture in CO₂ plots, indicate that increased soil moisture along
with increased activity of methanogens under elevated CO₂ in soils with low diffusivity at our study site contribute to the observed decline in CH₄ oxidation under elevated CO₂.

*Introduction*

The atmospheric CH₄ concentration has more than doubled from a pre-industrial level of about 750 µL L⁻¹ to a present day concentration of about 1780 µL L⁻¹ (NOAA 2008). Although CH₄ is less abundant than CO₂, additions of CH₄ to the tropospheric reservoir cause more direct warming than CO₂, 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 CH₄ 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 CH₄ include reaction with the hydroxyl radical, which removes approximately 445 Tg of CH₄ from the atmosphere annually; and mixing of tropospheric CH₄ with the stratosphere accounts for another 40 Tg of CH₄ removal annually (Forster et al. 2007). Upland soils are the only known biological sink for atmospheric CH₄, accounting for approximately 38 Tg of CH₄. This biological sink results from the net balance of CH₄ 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 CH₄ consumption by temperate forest soils under elevated CO₂ relative to plots exposed to ambient levels of CO₂ (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, methanotrophs 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
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 ≥ 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$_2$F$_2$), 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 °C and 140 °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$_2$O 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\textsubscript{4} consumption and CH\textsubscript{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\textsubscript{2} treatments. Area-based rates of net CH\textsubscript{4} consumption determined commensurate to diffusivity observations were calculated from static chamber geometry and the log-linear change in the CH\textsubscript{4} concentration in static chamber headspaces.

Soil \textsuperscript{222}Rn profiles and \textsuperscript{222}Rn chamber flux measurements were used to calculate the effective diffusivity of CH\textsubscript{4} in the soil (P\textsubscript{CH4}) according to Dörr and Münnich (1990):

\[
P_{\text{CH}_4} = \frac{D_{0,\text{CH}_4}}{D_{0,\text{Rn}}} \ast P_{\text{Rn}}
\]

where D\textsubscript{0,CH\textsubscript{4}} and D\textsubscript{0,Rn} are the diffusion coefficients of CH\textsubscript{4} (0.194 cm\textsuperscript{2} s\textsuperscript{-1} Lerman 1979) and Rn (0.1 cm\textsuperscript{2} s\textsuperscript{-1}; Tanner 1964) in air and P\textsubscript{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\textsubscript{4} was then used to calculate the flux of CH\textsubscript{4} (J\textsubscript{CH4}):

\[
J_{\text{CH}_4} = P_{\text{CH}_4} \ast \frac{\Delta C_{\text{CH}_4}}{\Delta z_{\text{CH}_4}}
\]

where \( \Delta C_{\text{CH}_4}/\Delta z_{\text{CH}_4} \) is the linear change in CH\textsubscript{4} concentration (C\textsubscript{CH4}) with depth (z\textsubscript{CH4}).

Paired t-tests were used to analyze for statistical differences between CO\textsubscript{2} treatment means for CH\textsubscript{4} concentration. Paired t-tests were likewise used to analyze for statistical differences between CO\textsubscript{2} treatment averages of rates of net CH\textsubscript{4} consumption or CH\textsubscript{4} production. Differences in diffusivity between CO\textsubscript{2} treatment plots were compared by student t-tests for each of the four observations. All statistical analyses were performed at \( \alpha=0.05 \).
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$_2$F$_2$, 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 CO₂-enriched plots from the same depth (Fig. 4.3a). In contrast, the average rates of CH₄ production in CO₂-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 CH₄ production from the same depth (Fig. 4.4a). The patterns in rates of net CH₄ consumption and CH₄ production in soils from 15 to 25 cm depths are less clear with regard to differences between CO₂ treatments. The experiment with the overall highest average rate of CH₄ production among the 15 to 25 cm depth increments showed a value for for soils from CO₂-enriched plots that exceeded that for control plots (July 2005; Fig. 4.4b). This corresponded with a higher average rate of net CH₄ consumption in control plot soils (Fig. 4.3b). The rates of CH₄ 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 CH₄ consumption, and rates in soils from CO₂-enriched plots were higher than rates in control plots. The differences in rates of net CH₄ consumption and CH₄ production between CO₂ 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 CO₂ treatments. The effective diffusivity of CH₄ 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 CH₄ ($J_{CH₄}$) was more than 25% lower than measured CH₄ 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 be 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 CH₄ consumption under elevated CO₂. The deeper duff in CO₂-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 CH₄ under elevated CO₂. 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 CH₄ consumption by temperate forest soils. Similarly, we observed an increase of 6% in net CH₄ consumption when leaf litter was removed (data not shown), suggesting that a CO₂ treatment effect on leaf litter depth may contribute to reduced rates of net CH₄ consumption under elevated CO₂. Further, a thicker duff layer can slow evaporation from the soil surface thereby causing higher soil moisture in CO₂-enriched plots (Schäfer et al. 2003). Schäfer et al. (2003) observed significantly higher soil moisture in CO₂-enriched plots at our study site through 2002, although they proposed that the difference between CO₂ treatment plots did not necessarily reflect a treatment effect. We (Dubbs and Whalen unpublished) also found higher soil moisture in CO₂-enriched plots during biweekly determination of soil-atmosphere CH₄ exchange in 2004 and 2006, and that soil moisture explained 34% of the variability in rates of net CH₄ consumption. Higher soil moisture and associated reduction in diffusivity (Suwa et al. 2004) in CO₂-enriched plots likely contributes to the observed decrease in rates of net CH₄ consumption under elevated CO₂, regardless of the cause of increased soil moisture.

Low overall effective diffusivity and high soil moisture in CO₂-enriched plots would also slow the diffusion of atmospheric O₂ 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 CH₄ 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 CH₄ augments the atmospheric supply to methanotrophs.

While reduced diffusivity of CH₄ in CO₂-enriched plots was not expressed in depth profiles of CH₄ concentrations, which showed no significant differences in CH₄ concentrations at any depth (Fig. 4.2), the depth profiles did suggest slightly higher CH₄ production in CO₂-enriched plots. Methane concentrations at depths between 10 and 20 cm in CO₂-enriched plots were slightly higher than those in control plots. Additionally, there was not any difference in CH₄ concentrations between CO₂ treatments at any depth (Fig. 4.2), nor was there a significant difference in net CH₄ oxidizing activity between CO₂ treatments at any depth (Fig. 4.3). A down-profile shift in the locus of the CH₄ oxidizing community in response to elevated CO₂ would increase the diffusional path of atmospheric CH₄ to the locus of CH₄ oxidation. Thus, this lack of difference in CH₄ concentrations between CO₂ treatments indicate that the long-term pattern of reduced net CH₄ consumption in soils exposed to elevated CO₂ (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 CH₄ oxidizing community.

High spatial variability in net CH₄ consumption and CH₄ production rates and diffusivity measurements, along with limited ability to collect soil samples hampered our ability to detect significant differences between CO₂ treatments. However, trends indicating low overall diffusivity and increased incidence and rates of CH₄ production in elevated CO₂ 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).
References


Hoosbeek, M. and G. Scarascia-Mugnozza. 2009. Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO\textsubscript{2} enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments. Ecosystems 12:220-239.


Table 4.1. Effective diffusivity ($P_{CH_4}$) and corresponding calculated net CH$_4$ flux ($J_{CH_4}$; µg m$^{-2}$ h$^{-1}$), measured net CH$_4$ flux (µg 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.

<table>
<thead>
<tr>
<th>Date</th>
<th>CO$_2$ treatment</th>
<th>$P_{CH_4}$ (cm$^2$ h$^{-1}$)</th>
<th>$J_{CH_4}$ (µg m$^{-2}$ hr$^{-1}$)</th>
<th>Measured net CH$_4$ flux (µg m$^{-2}$ hr$^{-1}$)</th>
<th>Soil moisture (mL H$_2$O cm$^{-3}$ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July '08 Control</td>
<td>2.8</td>
<td>1.3</td>
<td>1.7</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
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<td>1.1</td>
<td>1.5</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>April '08 Control</td>
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<td>0.7</td>
<td>1.6</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
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<td>0.5</td>
<td>1.6</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Oct. '08 Control</td>
<td>0.9</td>
<td>0.4</td>
<td>1.6</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
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<td>0.4</td>
<td>1.8</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Feb. '09 Control</td>
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<td>0.0</td>
<td>0.8</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
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<td>0.1</td>
<td>0.6</td>
<td>0.34</td>
<td></td>
</tr>
</tbody>
</table>
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 CH₄ production in forest soils from plots exposed to elevated CO₂ 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
CO₂. If my results can be broadly extrapolated, my research also suggests that a 200 ml L⁻¹ increase in present-day atmospheric CO₂ concentrations can be expected to reduce the forest soil sink for CH₄ of ~24 Tg y⁻¹ by approximately 15%. Further, the observed relationship between increasing soil moisture and the reduction in the forest sink for CH₄ indicates that climate forecasting models can constrain the predicted upland sink for CH₄ by relating it to soil hydrology.
References
