METHANE OXIDATION IN TERRESTRIAL ECOSYSTEMS: PATTERNS AND EFFECTS OF DISTURBANCE

By

Pongthep Suwanwaree

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

2003

ABSTRACT

METHANE OXIDATION IN TERRESTRIAL ECOSYSTEMS:
PATTERNS AND EFFECTS OF DISTURBANCE

By

Pongthep Suwanwaree

Methane oxidation in aerobic upland soils is an important sink for annually increasing atmospheric methane. The methane oxidation capacity of soil may be declining due to the conversion of natural forest to other uses. I investigate methane oxidation along a gradient of soil disturbance from mature old-growth forest to agricultural fields. I found that soils in the mature deciduous forest oxidize substantially more CH_4 than mid-successional forest and no-till agricultural fields.

A single application of 100 kg N fertilizer per hectare significantly decreased CH_4 oxidation rates in both mature deciduous forest and mid-successional communities but had no effects in agricultural fields, which already had low oxidation rates. In contrast, a 10 cm depth plowing did not show a significant detectable effect on soil CH_4 consumption in any soils. Additionally, lower levels of N fertilization (30 kg N ha^{-1}) significantly affected CH_4 oxidation in coniferous forest, but not in deciduous forest nor in mid-successional communities.

Methane oxidation significantly differed among soil depths. In deciduous forest soil cores incubated in the laboratory, CH_4 uptake was highest at 5-10 cm depth whereas there were no significant differences with depth (0-5, 5-10, 10-20, and 20-30 cm) in coniferous forest, in a mid-successional community, nor in no-till agricultural soils.

Organic and conventional corn-corn-soybean-wheat management systems also had similar average soil CH_4 uptake rates. Nevertheless, CH_4 oxidation significantly differed among crops, such that organic corn had the highest rates of oxidation while organic soybean and wheat had the lowest rates. Rates in conventionally managed corn, soybean, and wheat crops were similar and intermediate.

Soil methane oxidation was not significantly correlated with CO_2 emission. Soil nitrate and ammonium were weak indicators of changes of CH_4 oxidation among land use types and soil depths. Nitrification potential was also a good indicator of CH_4 oxidation rates.

Results suggest a major effect of agricultural management on CH_4 oxidation in terrestrial ecosystems, with reductions in oxidation capacity related more to changes in nitrogen availability than physical soil disturbance itself. Low-level nitrogen additions (10 kg N ha^{-1}), similar to rates of N-deposition, however, were not sufficient to reduce oxidation in undisturbed forests or mid-successional communities.

ACKNOWLEDGMENTS

I consider the four years at KBS and six years in the US as one of the most extraordinary journeys in my life. I value this time so dearly and mainly attribute this to all the support I received and to people I had the great fortune of meeting. My first deepest appreciation goes to my advisor – Prof. G.P. Robertson for his "hands off" mentoring, advice, encouragement, and untiring editing of my dissertation. I am heartily thankful for his untiring support in this endeavor to push my abilities to the test in order to pursue my degree.

I would also like to extend my deepest appreciation to my committee members – Prof. Alvin J. Smucker, Prof. Michael J. Klug, and Prof. Bernard Knezek for their support and guidance in my research.

This dissertation could not be successfully operated without the assistance of Claire McSwiney for the backbreaking gas and soil sampling in the hot and humid fields and forests, Star Shelton and Christine Easley for gas and soil analysis and lab assistance, Barb Fox for administrative work, Andrew Corbin, Joe Simmons, Greg Parker for field maintenances, and Brady West from Center for Statistical Consultation and Research, University of Michigan for Statistics help.

I also greatly appreciate the campus and KBS faculty members, staff, and friends who have remained helpful and supportive as always. Special thanks for my fellow students – Tim Bergsma, Skip Vanbloem, Richard Smith, Natalie Dubious, Laura Broughton, Ann-Marie Fortuna, Stuart Grandy, Terry Loecke, and Sven Bohm for their unfading friendships and support.

In pursuing my educational goals, I am genuinely thankful to the Michigan State University, KBS, NSF as well as to the Royal Thai Government for providing me with support and opportunities.

Last, but not the least, I am sincerely grateful to my parents for their love, support, and patience for my being far away for years. This dissertation is dedicated to them.

TABLE OF CONTENT

LIST OF TABLES

CHAPTER 1

CHAPTER 2

LIST OF FIGURES

CHAPTER 3

Chapter 1

Methane Oxidation in Arable Soils

Introduction

Methane (CH_4) is the most abundant greenhouse gas in the atmosphere, after water vapor and carbon dioxide (CO_2). It has an average atmospheric lifetime of 12 years. Methane today contributes around 20% of radiative forcing by greenhouse gases since 1750 (IPCC 2001) and has a global warming potential 62 times that of CO_2 over a 20 year time horizon. The current (1998) concentration of CH_4 in the atmosphere is 1,745 ppb, approximately 2.5 fold higher than the 700 ppb concentration of preindustrial times based on ice-core studies (Blunier et al. 1993). This increase is due principally to the increased use of fossil fuels and the expansion of animal and lowland rice agriculture. The current annual rate of increase is 7.0 ppb (IPCC 2001), which is slightly lower than a decade ago (Dlugokencky et al. 1994) for still unknown reasons.

Saturated soils in wetlands are the primary natural sources of CH_4 emission, responsible for approximately 21% of global emissions, while over half of global emissions are from anthropogenic sources such as agriculture, natural gas activities, and landfills (Table 1.1). More than 80% of CH_4 produced is removed from the atmosphere by reaction with hydroxyl radicals (OH). Of the remainder, about half remains in the atmosphere to contribute to the 7.0 ppb annual increase, and the rest appears to be removed by biological methane oxidation in soils. Microbial oxidation in aerobic soils is the only biological sink for atmospheric CH_4. Globally some 30 Tg are oxidized

annually, close to the 37 Tg annual CH_4 atmospheric increase. Though small relative to total global emissions of 598 Tg y^{-1}, this sink could significantly affect atmospheric CH_4 concentrations and thus global warming. Understanding methane oxidation in soil, carried out by methanotrophic bacteria, is therefore important for understanding CH_4 mitigation potentials.

Methanotrophic Bacteria

Methane-oxidizing bacteria (methanotrophs) are obligately aerobic, Gram-negative bacteria that utilize methane as their sole source of carbon and energy. Some can also use methanol but none grow on multicarbon compounds. The first methanotroph to be isolated, by Söhngen in 1906, was named *Bacillus methanicus* (Söhngen 1906), later renamed *Methylomonas methanica*. Prior to 1970 only a few more had been isolated.

In 1970, after studying more than 100 methanotroph isolates, Whittenbury and colleagues proposed five genera of methanotrophs based on cell morphology, form of the resting state, intracytoplasmic membrane structure, and other physiological characteristics (Whittenbury et al. 1970). They are *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylocystis*, and *Methylosinus*; four new genera have been added since: *Methylomicrobium*, *Methylocaldum*, *Methylosphaera* and *Methylocella* (Murrell and Radajewski 2000).

Methanotrophs have been isolated from a wide variety of environments including soils, sediments, freshwater, seawater and even more extreme environments such as hot springs, Antarctic tundra, acid peat bogs, and soda lakes (Hanson and Hanson 1996).

There are two distinct groups of methanotrophs. The low affinity methanotrophs can oxidize methane only in concentrations of methane higher than 40 ppm (Le Mer and

Roger 2001). This is the "known" group, which we can culture and study in laboratories. The other group is the high affinity methanotrophs, which can oxidize methane at much lower concentrations (<12 ppm) as in the atmosphere (1.7 ppm). Members of this group have not yet been cultivated. Most of what we know about methanotrophs is from the bacteria we can culture, which may not represent the more prevalent methanotrophs found in natural soils.

Characteristics of Methanotrophs

Methanotrophs have been classified into three types (I, II, and X) based on their cell morphology, membrane type, resting stage, pathways of carbon assimilation, physiology, and phospholipid fatty acid content (Table 1.2).

Type I methanotrophs belong to the γ-subclass of the Proteobacteria. They contain bundles of membranes (Fig. 1.1), assimilate formaldehyde produced from the oxidation of methane via the ribulose monophosphate (RMP) pathway, and contain predominantly 16-carbon (C_{16}) fatty acids. They include four genera: *Methylomonas*, *Methylomicrobium*, *Methylobacter*, and *Methylosphaera* (Table 1.3).

Type II methanotrophs belong to the α-subclass of the Proteobacteria. They generally have membranes around the periphery of the cell (Fig. 1.2), assimilate formaldehyde via the serine pathway, and contain predominately 18-carbon (C_{18}) fatty acids. They include three genera: *Methylosinus*, *Methylocystis*, and *Methylocella*.

Type X was added to accommodate methanotrophs similar to *Methylococcus capsulatus*. They belong to the γ-subclass of the Proteobacteria and assimilate formaldehyde primarily via the RMP pathway, like type I methanotrophs. However, they

also possess low levels of serine pathway enzymes, and they can fix nitrogen and carbon dioxide. Type X methanotrophs can also grow at higher temperatures than Type I and Type II methanotrophs. Two genera of Type X methanotrophs are *Methylococcus* and *Methylocaldum.*

Physiology and Biochemistry

Methane is first catalysed by methane monooxygenase (MMO) to produce methanol. The methanol is further oxidized to formaldehyde by the enzyme methanol dehydrogenase (MDH) and the formaldehyde can be assimilated into cell carbon by the RMP or serine pathway. Formaldehyde can also be further oxidized to formate by formaldehyde dehydrogenase (FADH) and then finally CO_2 by formate dehydrogenase (FDH) to provide reducing power for the initial oxidation of methane and for biosynthetic reactions (Fig. 1.3).

Methane Monooxygenase

There are two forms of methane monooxygenase; a soluble, cytoplasmic enzyme complex (sMMO), which is found in only some methanotrophs, and a membrane-bound, particulate enzyme (pMMO), which is present in all known methanotrophs.

The most extensively characterized enzyme is the sMMO, which is a non-heme iron-containing enzyme complex consisting of three components (Fig. 1.4). Protein A is a hydroxylase containing three subunits of approximately 60, 45 and 20 kDa, arranged in a $\alpha_2 \beta_2 \gamma_2$ complex. The α subunits contain a diiron centre believed to be the site of the monooxygenation reaction. Protein B, a coupling protein of 16 kDa, facilitates electron

flow and/or the interactions between Proteins A and C. Protein C (38 kDa) is the reductase component of the sMMO and catalyses the transfer of reducing equivalents from NADH to the hydroxylase (Murrell et al. 2000).

The pMMO enzyme probably consists of three membrane-associated polypeptides of around 47, 27 and 25 kDa. The first two probably contain the active site. The active enzyme contains two iron and approximately 15 copper atoms per mol. The pMMO also associates with small copper-binding polypeptides of 1,218 and 779 Da (Murrell et al. 2000).

The sMMO is not present in all methanotrophs and is found only in Type II genera *Methylosinus*, *Methylocystis*, *and Methylocella*. It is also found in some species of *Methylococcus, Methylomonas, Methylomicrobium,* and *Methylocaldum* (Table 1.3). In contrast to pMMO, sMMO has extremely broad substrate specificity and can oxidize a wide range of non-growth compounds such as alkanes, alkenes, and aromatic compounds (see more examples in Knowles, 1993 . This enzyme is expressed only under copper-deficient conditions for methanotrophs and apparently suppressed by pMMO.

The pMMO is found in all cultured methanotrophs during copper-sufficient growth conditions and appears to require copper ions for both expression and activity. It has a relatively narrow substrate specificity but will, in addition to oxidising methane, also co-oxidise a number of short chain alkanes, alkenes, and ammonia.

Ribulose Monophosphate Pathway

The ribulose monophosphate (RMP) pathway cycle occurs in Type I and X methanotrophs. This pathway consists of three parts: fixation, cleavage, and

rearrangement (Fig. 1.5). First, fixation is a result of an aldol condensation of three formaldehyde molecules and three ribulose 5-phosphate (ribulose monophosphate) molecules, forming three molecules of fructose 6-phosphate. Second, during cleavage, one molecule of fructose 6-phosphate is converted to 2-keto 3-deoxy 6-phosphogluconate, which is cleaved to yield pyruvate and glyceraldehyde 3-phosphate. Finally, the remaining two molecules of fructose 6-phosphate and glyceraldehyde 3-phosphate undergo a series of rearrangement reactions, which regenerate three molecules of ribulose 5- phosphate.

Serine Pathway

The serine pathway cycle occurs in Type II and X methanotrophic bacteria. First, formaldehyde reacts with glycerine to form serine, catalyzed by serine hydroxymethyl transferase (Fig 1.6). The serine then undergoes a series of reactions to form two molecules of 2-phosphoglycerate, one of which forms a 3-phosphoglycerate molecule. The 3-phosphogly-cerate becomes cellular material by another metabolic pathway. The remaining 2-phosphogly-cerate molecule forms Phosphoenolpyruvate, which then combines with carbon dioxide to form oxaloacetate. The oxaloacetate then proceeds through a series of reactions to finally form glycerine.

The ribulose monophosphate pathway is more efficient than the serine pathway since all carbon atoms for cell material construction are derived from formaldehyde. Additionally, the formaldehyde is at the same oxidation level as cellular biomass, so there is no need for more reducing power.

Environmental Factors Influencing Soil Methane Oxidation

The factors affecting CH_4 oxidation in soil generally divide into two categories: chemical and biological factors, which will be discussed below.

Chemical Factors

Methane oxidation is affected by chemical factors such as methane concentrations, oxygen, water, temperature, pH, nitrogen, and other carbon compounds.

Methane Availability

Methane is the primary source of carbon and energy for methanotrophs, so any factors that affect the availability of methane will affect the growth and function of methanotrophic bacteria. The minimum threshold for CH_4 oxidation (<0.1 to 0.4 ppm) in upland soil is much lower than that in sediments (2–3 ppm) (Born et al. 1990, Whalen and Reeburgh 1990, Whalen et al. 1990, Yavitt et al. 1990). This means that aerobic soils can consume CH_4 at lower concentrations, as in atmosphere, than sediment soil in water, which get CH_4 from anaerobic fermentation at lower depths. Because CH_4 is the sole source of energy for methanotrophs, when soil is exposed to CH_4 at higher concentrations than atmospheric, the numbers of methanotrophs and CH_4 oxidation tends to (Whalen et al. 1990, Bender and Conrad 1992, 1994a, Schnell and King 1995). This also happens in soil sediment under water or soil saturated by water after precipitation.

In contrast, periodically low CH_4 concentrations do not always decrease soil CH_4 consumption capacity. For example, some taiga soils lose their CH_4 uptake capacity when incubated with low CH_4 concentration (<0.03 ppm) for a period of time, whereas

temperate forest soils do not lose the ability when incubated with similar CH_4 concentration (Schnell and King 1995, Benstead and King 1997, Gulledge et al. 1998). This might be because methanotrophic bacteria in temperate forest soil can persist in low CH_4 by either staying in a dormant state or by using carbon from other sources than CH_4, whereas the bacteria population in taiga soil perishes from a lack of energy source.

Methane availability is primarily limited by gas diffusion (Whalen et al. 1990, Nesbit and Breitenbeck 1992, Dörr et al. 1993, Koschorreck and Conrad 1993), which is usually governed by soil moisture, texture, and composition. Therefore, these factors should be considered when we try to predict CH_4 oxidation in soil.

Oxygen Availability

Oxygen is required for methane oxidation. To oxidize one mole of CH_4 to CO_2 requires two moles of O_2 (Fig. 1.3). The importance of O_2 to methane oxidation was first demonstrated in the 1970s (Smirnova 1971a, b, Nesterov et al. 1977). Methanotrophic bacteria are sensitive to O_2 concentration. Low O_2 concentration (0.2 and 2%) partially inhibits methane uptake in incubated temperate forest soil (Schnell and King 1995) and CH_4 oxidation ceases when soils are incubated under anoxic conditions (Bender and Conrad 1995). In contrast, anaerobic conditions promote methane production in soil by methanogens.

Water

The optimum water content for soil methane uptake varies depending on soil type and texture, but generally is 10-35% w/w (Bender and Conrad 1995, Schnell and King

1996, van den Pol-van Dasselaar et al. 1998), 50% water-holding capacity (Torn and Harte 1996), 40-60% water-filled pore space (Castro et al. 1995), or –0.2 MPa water potential (Schnell and King 1996). This optimum soil water content shows that CH_4 uptake is low at both low and high water content. Rates of CH_4 uptake generally decrease with increasing soil water content (Steudler et al. 1989, Crill 1991, Adamsen and King 1993) because CH_4 and oxygen diffusion and transport to methanotrophs are reduced. Water also increases CH_4 production in soil by increasing the number of anaerobic microsites in soil crumbs and aggregates, thus reducing the net uptake of CH_4 by soil (Yavitt et al. 1990). Conversely, rates of CH_4 uptake decrease at lower water contents because of the physiological response of methanotrophs to water stress (Czepiel et al. 1995, Dobbie and Smith 1996). Therefore in dry sites such as deserts, some studies have demonstrated increased rates of CH_4 consumption associated with precipitation because rain alleviated water stress for CH_4 oxidizing bacteria (Striegl et al. 1992).

Temperature

Most of the known methanotrophic bacteria are mesophilic except for *Methylomonas vinelandii*, *Methylococcus thermophilus*, and *Methylococcus capsulatus*, which can grow at 45 °C or above (Anthony 1982, Green 1992, Bowman et al. 1995). The optimum temperature for CH_4 oxidation is generally between 25-35 °C both in the laboratory (Whittenbury et al. 1970, Whalen et al. 1990, Bender and Conrad 1995) and in the field (Nesbit and Breitenbeck 1992, Dunfield et al. 1993). However, some CH_4 uptake can occur even at –2 °C in soil core experiments (Prieme and Christensen 1997) and -5 °C in winter soils (Castro et al. 1995) but rates are very low . Different soils

exhibit different CH_4 oxidation responses to temperature, indicating that different CH_4 oxidizing bacteria populations adapt to different temperatures in nature. However, the Q_{10} values of CH_4 consumption range from 1.0-2.9 (Born et al. 1990, Crill 1991, King and Adamsen 1992, Lessard et al. 1994, Prieme and Christensen 1997) indicating only a modest direct sensitivity of soil CH_4 oxidation toward seasonal temperature changes (King 1993). This may be because methanotrophs are located in subsoil layers, in which temperature is more constant and has more soil moisture than surface soil. This may also explain why atmospheric temperature changes in the field have a small effect on CH_4 flux (King and Adamsen 1992, Peterjohn et al. 1993, Crill et al. 1994).

pH

Soil pH plays a very important role in methane consumption since pH directly affects the physiology of methanotrophs and can determine the availability of toxic elements and nutrients (e.g. ammonium, aluminum, iron). Methane uptake can occur in environments with pH ranging from <4 to 9 (Born et al. 1990, King 1992, Dörr et al. 1993). But the optimum pH for CH_4 consumption and growth of methanotrophs *in vitro* is 6.0 to 7.0 for most known methanotrophic bacteria (Whittenbury et al. 1970, Dunfield et al. 1993, Bender and Conrad 1995, Amaral et al. 1998). However, reported responses of soil methane consumption to pH have varied among sites and studies (Hütsch et al. 1994, Sitaula et al. 1995, Amaral et al. 1998, Hütsch 1998a). Methane uptake was lower in low-pH (pH 4.8-5.1) grassland soils (Hütsch et al. 1994). In contrast, hardwood and pine plantations still show high CH_4 oxidation rates even at low pH 3.3 (Steudler et al. 1989) and pH 2.3 (Conrad 1996). Generally, acidification decreases methane oxidation in

forest soils (Benstead and King 2001, Sitaula et al. 2001). Different types of acid compounds also have different effects on soil CH_4 uptake. For instance, nitric acid inhibits CH_4 oxidation more than sulfuric acid (Benstead and King 2001, Bradford et al. 2001) and nitric acid affects agricultural soils more than forest soils.

Nitrogen Compounds

Although nitrogen is an essential nutrient for methanotrophs, different kinds of nitrogen compounds have different effects on CH_4 consumption. Methanotrophic bacteria prefer nitrate as a nitrogen source rather than ammonium (Whittenbury et al. 1970, Mancinelli 1995) because ammonium appears to inhibit CH_4 oxidation (Bender and Conrad 1994b, King and Schnell 1994, Hütsch 1996, Gulledge et al. 1997, King and Schnell 1998). However, in some cases, no immediate effect was observed after NH_4^+ addition (Delgado and Mosier 1996, Dobbie and Smith 1996, Gulledge et al. 1997). The mechanism of NH_4^+ inhibition is complex and thought to include both a competitive inhibition of the CH_4 monooxygenase as well as a toxic inhibition by hydroxylamine or nitrite produced via NH_4^+ oxidation (King and Schnell 1994). Additionally, the inhibition from a salt effect has also been demonstrated (MacDonald et al. 1997, Gulledge and Schimel 1998). The amount of exchangeable NH_4^+ could be another important factor for CH_4 uptake inhibition by NH_4^+(De Visscher et al. 1998).

The effect of nitrate, another product of NH_4^+ oxidation, on CH_4 oxidation is ambiguous. Some authors did not find an effect of NO_3^- (Boeckx and Van Cleemput 1996), while others did (Whalen 2000). A recent report shows a greater effect of NO_3^- than NH_4^+ on the inhibition of CH_4 oxidation in coniferous forest soil (Wang and Ineson

2003). It has also been suggested that N-turnover rates (mineralisation and gross nitrification) influence CH_4 uptake rather than the actual soil mineral N (NH_4^+, NO_3^- or NO_2^-) content (Mosier et al. 1991, Steudler et al. 1996a).

Other Carbon Compounds

It is often assumed that atmospheric CH_4 oxidizers use CH_4 as their sole carbon and energy source, but recent studies have indicated that methanotrophs can also utilize non- CH_4 substrates, specifically methanol, formate (Roslev et al. 1997, Benstead et al. 1998, Jensen et al. 1998), and acetate (West and Schmidt 1999). Moreover, CO_2 is also required for the growth and function of methanotrophs in bioreactors (Acha et al. 2002), although likely to never be limiting in aerobic soils.

Biological Factors

The interaction of methanotrophic bacteria with other organisms in soil can, by effects on methanotroph population size, affect soil CH_4 uptake capacity.

Competition

Although no direct competition studies have been reported for methanotrophs in soil, methanotrophs compete with other soil inhabitants for some nutrients. For example, nitrogen, phosphate, and oxygen are in finite supply, and various soil organisms compete for these resources. An active population of methanotrophs has been shown to inhibit the ammonium oxidation activity of nitrifiers due to competition for O_2 (Megraw and Knowles 1987). The plant-growth promoting rhizopseudomonad strains, *Pseudomonas*

aeruginosa 7NSK2 and *P. fluorescens* ANP15, transiently reduced CH_4 oxidation by 20-30% during a *in vitro* 5 day incubation (Arif et al. 1996). Among the same methanotrophs, *Methylomonas trichosporium* OB3b (a Type II methanotroph) outgrows *Methylobactor albus* BG8 (a Type I methanotroph) in bioreactors under copper and nitrate limiting conditions, due to the *M. trichosporium*'s ability to express sMMO under copper limitation and nitrogenase under nitrate deficiency (Graham et al. 1993).

Predation

Nearly all bacteria in soil are susceptible to predation by other soil microorganisms. The populations of predator and prey are dependent and fluctuate but normally are in balance. Protozoa are the major predators of bacteria, consuming approximately 150-900 g bacteria m^{-2} y^{-1} in arable soil (Clarholm 1981). Soil bacteria are also consumed by other soil bacteria (McInerney 1986, Casida 1988). However, no direct studies of predation on methanotrophic bacteria have been reported to date yet. Predation nonetheless may potentially limit the consumption of available methane in soil by methanotrophs (Mancinelli 1995).

Other Methane Oxidizing Organisms

Methane oxidation also occurs in anoxic environments such as ocean sediments, soda lakes, and peat by a consortium of CH_4 oxidizing archaea and sulfate reducing bacteria (Hanson and Hanson 1996, Valentine and Reeburgh 2000, Kotelnikova 2002).

Some yeasts such as *Rhodotorula glutinis*, *Rhodotorula rubra*, *Sporobolomyces gracilis*, *and Sporobolomyces roseus* can grow very slowly on methane (Wolf 1981).

Methanotrophic bacteria can also form symbioses with some marine invertebrates such as deep-sea mytilid mussels and the tubeworm *Siboglinum poseidoni* (Cavanaugh 1993).

Methane Oxidation in Aerobic Soils

The uptake of methane by soils was first demonstrated in swamp soils in Virginia, USA, when the water table dropped below the sediment surface (Harriss et al. 1982). Two years later it was observed in tropical soils (Seiler et al. 1984). Since then, soil methane oxidation has been measured in many ecosystems in both tropical and temperate zones, including temperate forests (Steudler et al. 1989, Adamsen and King 1993, Ishizuka et al. 2000, Robertson et al. 2000), tropical forests (Keller et al. 1986, Keller and Reiners 1994, Prieme and Christensen 1999), grasslands (Whalen and Reeburgh 1990, Mosier et al. 1993, Tate and Striegl 1993, Mosier et al. 1997b), and deserts (Striegl et al. 1992).

Oxidation Rates in Different Ecosystems

Methane uptake rates in different ecosystems in various parts of the world are presented in Table 1.4. From these data, published from 1986 until 2000, it is clear that CH_4 oxidation is higher in forest soils than grassland and arable soils. The average of the minimum and maximum values of CH_4 uptake are in Table 1.5

These summary data show a reduction of CH_4 consumption in arable soils of 59% from grassland soils and 77% from forest soils, consistent with 71% reduction in European studies (Smith et al. 2000) and a 63% decrease in Ghana studies (Prieme and Christensen 1999).

It is apparent that, irrespective of fertilization type or level, tillage system, soil texture and structure, and climate conditions, conversion of forests or grasslands to agriculture results in a reduced capacity for soil CH_4 uptake.

Global Methane Sinks Related to Soil and Land Use

Table 1.6 shows the soils and land use related CH_4 sink calculated from CH_4 uptake rates in various land uses (Reeburgh et al. 1993) and land use data (Bouwman 1990). Methane oxidation rates vary from 0.023 mg CH_4 m^{-2} h^{-1} in taiga soils (Whalen et al. 1991) to 0.028 mg CH_4 m^{-2} h^{-1} in desert soils (Striegl et al. 1992) to 0.16 mg CH_4 m^{-2} h^{-1} in temperate forest soils (Steudler et al. 1989). Temperate forest soils have the highest CH_4 consumption rates, approximately 28% of total global uptake, surprisingly followed by desert and semidesert soils at 19%, and tropical rain forest soils at 12%. The cultivated lands consume only 6% of global CH_4 uptake while constituting 10% of global land area.

From Table 1.6, the total global annual CH_4 sink is 43.7 Tg CH_4, higher than the IPCC calculation of 30 Tg CH_4 (IPCC 1995), 29 Tg CH_4 (Smith et al. 2000), 38 Tg CH_4 (Ridgwell et al. 1999), and a model value of 17 Tg CH_4 (Potter et al. 1996).

Agricultural Effects

Agricultural practices reduce soil CH_4 oxidation capacity compared with undisturbed soils in forests and grasslands. Fertilization, tillage, and the application of pesticides and herbicides, and lime can potentially affect CH_4 oxidation (Mosier et al. 1991, Clymo et al. 1995, Goulding et al. 1995, Arif et al. 1996, Mosier et al. 1997a, Powlson et al. 1997, Robertson et al. 2000, Hütsch 2001); each of these is discussed

briefly below.

Nitrogen Fertilizer

Nitrogen fertilization appears to be the number one factor inhibiting CH_4 oxidation in agriculture. Numerous studies have confirmed the reduction of soil CH_4 uptake after N fertilization in forest, grassland, arable, and landfill soils (Steudler et al. 1989, Mosier et al. 1991, Hansen et al. 1993, Hütsch et al. 1993, Bronson and Mosier 1994, Crill et al. 1994, Hütsch et al. 1994, Castro et al. 1995, Willison et al. 1995b, Hütsch 1996, Tlustos et al. 1998, Hilger et al. 2000). However, N fertilizer had no effect on CH_4 oxidation in a regularly fertilized wheat field (Mosier and Schimel 1991), in irrigated crops in Colorado (Bronson and Mosier 1993), and in acid oxisol sites in Puerto Rico (Mosier et al. 1998). Different forms of nitrogen have different effects on soil CH_4 consumption (see section 3.1.5). Nitrogen fertilizer can immediately interfere with methane monooxygenase or on a longer-term basis can cause changes in microbial populations (Adamsen and King 1993).

Tillage

Tillage alone reduced CH_4 oxidation in a continuous wheat plot compared with woodland over 150 years (Hütsch et al. 1994, Willison et al. 1995b). In grassland, tillage reduced CH_4 consumption by 35% immediately and the effect persisted for more than 3 years (Mosier et al. 1997b). Disking after plowing further reduced CH_4 consumption another 60-70% (Kessavalou et al. 1998a). However, plowing had no apparent effect on CH_4 uptake in tropical savanna (Sanhueza et al. 1994), Piedmont cornfields (Burke et al.

1999), nor in an acid oxisol in Puerto Rico (Mosier et al. 1998). On the other hand, plowing a plot in Denmark heathland increased CH_4 consumption to seven times that of native heathland (Kruse and Iversen 1995). In general, it might be expected that reduced tillage is beneficial for CH_4 consumption in agriculture since methanotrophs are sensitive to soil disturbance. Tillage reduction can restore CH_4 oxidation in some soils. For instance, no-till management showed higher CH_4 oxidation rates than the plowed treatments of wheat-fallow rotation in Nebraska (Kessavalou et al. 1998b), and also in continuous spring barley in Alaska (Cochran et al. 1997). Fifteen years of direct-drilling resulted in 4.5-11 times the CH_4 oxidation rates of continuous plowed treatments (Hütsch 1998b). On the other hand, Robertson et al. (2000) found no difference in oxidation between tilled and no till in a 10-year Michigan study. The vertical profile of soil CH_4 oxidation also reveals different zonation in plowed, direct-drilled, set aside treatments, and forest soil. Methane oxidation showed the highest activity below 25 cm in the plowed and set aside treatments, while under direct-drilling maximum rates were at 5-15 cm and under forest soil at 5-10 cm (Hütsch 1998b).

Pesticides

Few studies have investigated the inhibitory effects of pesticides, herbicides, and insecticides on soil CH_4 oxidation. For example, the herbicide dichlorophenoxy acetic acid (2,4-D) had a strong negative effect on CH_4 oxidation in sandy Belgium soils *in vitro* (Arif et al. 1996). The herbicides Lenacil and Mikado and the fungicide oxadixyl significantly reduced CH_4 uptake in sandy soils, while atrazine, Mikado and dimethenamid decreased CH_4 consumption in clayey soils in Germany (Boeckx et al.

1998). Among 30 agrochemicals tested, only the herbicide bromoxynil and the insecticide methomyl effectively decreased CH_4 oxidation in Canadian soils (Topp et al. 1999). The insecticide Dimethoat 40 EC, the herbicide Tolkan and the fungicides Tilt 250 EC, Tilt Top, and Corbel drastically reduced atmospheric CH_4 uptake in slurries from a Danish forest soil (Prieme and Ekelund 2001). In the tropics, the insecticides HCH and Carbuforan inhibited CH_4 oxidation in flooded rice soils (Kumaraswamy et al. 1997, Kumaraswamy et al. 1998). Pesticide utilization in agriculture is thus also a potential inhibitor for soil CH_4 oxidation.

Liming

Soil acidification tends to decrease CH_4 oxidation in soil, and therefore liming might be expected to increase soil CH_4 uptake by alleviating soil acidity. However, results are mixed. Liming had an insignificant effect on CH_4 oxidation in hardwood forest in the Adirondack region of New York after 2 years of application, but it significantly reduced CH_4 uptake in incubated forest soils (Yavitt et al. 1993) and in German spruce forest soils four years after application (Butterbach-Bahl and Papen 2002). In contrast, dolomite slightly increased CH_4 oxidation in Sweden spruce forests after seven years of liming (Klemedtsson and Klemedtsson 1997) and in an acid oxisol in western Puerto Rico after several years (Mosier et al. 1998). Liming also significantly increased CH_4 uptake rates in four out of five deciduous and spruce forest plots in Germany (Borken and Brumme 1997), in Dutch forests (Saari et al. 1997), and in landfill soils (Hilger et al. 2000). Therefore, the effect of liming is varied depending on certain soil conditions.

Vegetation and Crop Management

Until recently, the effects of plants on CH_4 oxidation have been studied exclusively in wetlands and paddy soils. Only limited data are available for aerobic arable soils. Arif et al. (1996) found no differences in CH_4 oxidation between corn and an unplanted control in sandy soil incubation studies. Additionally, in a wheat-fallow rotation, CH_4 oxidation rates were not different between wheat and the fallow period (Kessavalou et al. 1998b). The plant itself thus does not appears to be the primary factor affecting CH_4 oxidation; rather CH_4 oxidation seems more affected by secondary direct factors such as pesticide treatments, pH changes, and tillage. An exception appears to be the rice in India dryland soils (Singh et al. 1999), where differences appear related to methanotrophs being more abundant in the rice rhizosphere than in either bulk cropped soil or bare fallow soil (Dubey and Singh 2001).

The removal of crop surface residue decreased CH_4 uptake in spring barley due to the reduction of soil moisture (Cochran et al. 1997). However, the application of crop residues after harvest has different effects. Fresh sugar beet leaves mixed with a loamy soil reduced CH_4 oxidation by 20%, while wheat straw had no effect (Hütsch 1998a).

In ley and legume crop rotations in Norway, the tractor traffic alone reduced the accumulated CH_4 uptake by 52% while its combination with fertilizer decreased CH_4 consumption by 78% (Hansen et al. 1993, Sitaula et al. 2000); the effect persists even after soils are sieved before incubation (Sitaula et al. 2000).

Soil Methane Oxidation Recovery

Soils in the temperate zone generally take more than 100 years to recover a CH_4 consumption capacity as high as the undisturbed soils nearby after abandonment to grasslands or forests (Smith et al. 2000). It takes more than 200 years for European soils (Prieme et al. 1997) and longer than this for soils in North America (Ojima et al. 1993, Hudgens and Yavitt 1997, Mosier et al. 1997b, Robertson et al. 2000). However, it is possibly much faster in the tropics as; perhaps as little as 50 years (Keller and Reiners 1994).

Summary

Methane oxidation by upland soils is an important process for alleviating global warming potentials. Although the net annual oxidation rate is small it rivals the annual increase of atmospheric CH_4. Therefore, if we can promote this soil sink we can help to keep atmospheric CH_4 stable.

However, the expansion of deforestation and urbanization decrease the capacity of soil to consume CH_4. Agricultural practice, especially nitrogen addition, is the major soil CH_4 oxidation inhibitor. Many studies have been conducted to try to understand the mechanisms of this inhibition but much is still unknown. Because agriculture is important to us, it is necessary to find ways to improve agricultural productivity and reduce greenhouse effects simultaneously. Reducing soil disturbances and N application or finding more appropriate N fertilizers might be key for developing mitigation strategies.

References

Acha, V., J. Alba, and F. Thalasso. 2002. The absolute requirement for carbon dioxide for aerobic methane oxidation by a methanotrophic-heterotrophic soil community of bacteria. Biotechnology Letters **24**:675-679.

Adamsen, A. P. S., and G. M. King. 1993. Methane consumption in temperate and sub-arctic forest soils - rates, vertical zonation, and responses to water and nitrogen. Applied and Environmental Microbiology **59**:485-490.

Amaral, J. A., T. Ren, and R. Knowles. 1998. Atmospheric methane consumption by forest soils and extracted bacteria at different pH values. Applied and Environmental Microbiology **64**:2397-2402.

Ambus, P., and S. Christensen. 1995. Spatial and seasonal nitrous oxide and methane fluxes in Danish forest ecosystems, grassland-ecosystems, and agroecosystems. Journal of Environmental Quality **24**:993-1001.

Andersen, I. C., and M. Poth. 1998. Controls on fluxes of trace gases from Brazilian cerrado soils. Journal of Environmental Quality **27**:1117-1124.

Anthony, C. 1982. Biochemistry of Methylotrophs. Academic Press Ltd., London.

Arif, M. A. S., F. Houwen, and W. Verstraete. 1996. Agricultural factors affecting methane oxidation in arable soil. Biology and Fertility of Soils **21**:95-102.

Bender, M., and R. Conrad. 1992. Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH_4 mixing ratios. Fems Microbiology Ecology **101**:261-270.

Bender, M., and R. Conrad. 1994a. Methane oxidation activity in various soils and fresh-water sediments - occurrence, characteristics, vertical profiles, and distribution on grain-size fractions. Journal of Geophysical Research-Atmospheres **99**:16531-16540.

Bender, M., and R. Conrad. 1994b. Microbial oxidation of methane, ammonium and carbon monoxide, and turnover of nitrous oxide and nitric oxide in soils. Biogeochemistry **27**:97-112.

Bender, M., and R. Conrad. 1995. Effect of CH_4 concentrations and soil conditions on the induction of CH_4 oxidation activity. Soil Biology & Biochemistry **27**:1517-1527.

Benstead, J., and G. M. King. 1997. Response of methanotrophic activity in forest soil to methane availability. Fems Microbiology Ecology **23**:333-340.

Benstead, J., and G. M. King. 2001. The effect of soil acidification on atmospheric methane uptake by a Maine forest soil. Fems Microbiology Ecology **34**:207-212.

Benstead, J., G. M. King, and H. G. Williams. 1998. Methanol promotes atmospheric methane oxidation by methanotrophic cultures and soils. Applied and Environmental Microbiology **64**:1091-1098.

Blunier, T., J. Chappellaz, J. Schwander, J. Barnola, T. Deperts, B. Stauffer, and D. Raynaud. 1993. Atmospheric methane record from a Greenland ice core over the past 1000 years. Geophysical Research Letters **20**:2219-2222.

Boeckx, P., and O. Van Cleemput. 1996. Methane oxidation in a neutral landfill cover soil: influence of moisture content, temperature and nitrogen-turnover. Journal of Environmental Quality **25**:178-183.

Boeckx, P., and O. van Cleemput. 2001. Estimates of N_2O and CH_4 fluxes from agricultural lands in various regions in Europe. Nutrient Cycling in Agroecosystems **60**:35-47.

Boeckx, P., O. Van Cleemput, and T. Meyer. 1998. The influence of land use and pesticides on methane oxidation in some Belgian soils. Biology and Fertility of Soils **27**:293-298.

Borken, W., and R. Brumme. 1997. Liming practice in temperate forest ecosystems and the effects on CO_2, N_2O and CH_4 fluxes. Soil Use and Management **13**:251-257.

Born, M., H. Dörr, and H. Levin. 1990. Methane consumption in aerated soils of the temperate zone. Tellus B **42**:2-8.

Bouwman, A. F. 1990. Exchange of greenhouse gases between terrestrial ecosystems and atmosphere. Pages 61-127 *in* A. F. Bouwman, editor. Soils and the Greenhouse Effect. John Wiley & Sons, New York.

Bowman, J. P., L. I. Sly, and E. Stackebrandt. 1995. The phylogenetic position of the family *Methylococcacea*. International Journal of Systematic and Evolutionary Microbiology **45**:182-185.

Bradford, M. A., P. A. Wookey, P. Ineson, and H. M. Lappin-Scott. 2001. Controlling factors and effects of chronic nitrogen and sulphur deposition on methane oxidation in a temperate forest soil. Soil Biology & Biochemistry **33**:93-102.

Bronson, K., and A. Mosier. 1993. Nitrous oxide emissions and methane consumption in wheat and corn-cropped systems in northeastern Colorado. Pages 133-144 *in* L. A. Harper, A. Mosier, J. Duxbury, and D. E. Rolston, editors. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. American Society of Agronomy, Madison.

Bronson, K. F., and A. R. Mosier. 1994. Suppression of methane oxidation in aerobic soil by nitrogen fertilizers, nitrification inhibitors, and urease inhibitors. Biology and Fertility of Soils **17**:263-268.

Brumme, R., and W. Borken. 1999. Site variation in methane oxidation as affected by atmospheric deposition and type of temperate forest ecosystem. Global Biogeochemical Cycles **13**:493-501.

Burke, R. A., J. L. Meyer, J. M. Cruse, K. M. Birkhead, and M. J. Paul. 1999. Soil-atmosphere exchange of methane in adjacent cultivated and floodplain forest soils. Journal of Geophysical Research-Atmospheres **104**:8161-8171.

Butterbach-Bahl, K., R. Gasche, C. H. Huber, K. Kreutzer, and H. Papen. 1998. Impact of N-input by wet deposition on N-trace gas fluxes and CH_4-oxidation in spruce forest ecosystems of the temperate zone in Europe. Atmospheric Environment **32**:559-564.

Butterbach-Bahl, K., and H. Papen. 2002. Four years continuous record of CH_4-exchange between the atmosphere and untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany. Plant and Soil **240**:77-90.

Casida, L. E. 1988. Minireview: nonobligate bacteria predation of bacteria in soil. Microbial Ecology **15**:1-8.

Castro, M. S., J. M. Melillo, P. A. Steudler, and J. W. Chapman. 1994. Soil-moisture as a predictor of methane uptake by temperate forest soils. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **24**:1805-1810.

Castro, M. S., P. A. Steudler, J. M. Melillo, J. D. Aber, and R. D. Bowden. 1995. Factors controlling atmospheric methane consumption by temperate forest soils. Global Biogeochemical Cycles **9**:1-10.

Cavanaugh, C. M. 1993. Methanotroph-invertebrate symbioses in the marine environment: ultrastructural, biochemical and molecular studies. *in* J. C. Murrell and D. P. Kelley, editors. Microbial Growth on C_1 Compounds. Intercept Ltd., Andover, UK.

Clarholm, M. 1981. Protozoan grazing of bacteria in soil-impact and importance. Microbial Ecology **7**:343-350.

Clymo, R. S., K. W. T. Goulding, K. A. Smith, and M. G. R. Cannell. 1995. The effect of agriculture on methane oxidation in soil - discussion. Philosophical Transactions of the Royal Society of London Series a-Mathematical Physical and Engineering Sciences **351**:324-325.

Cochran, V. L., E. B. Sparrow, S. F. Schlentner, and C. W. Knight. 1997. Long-term tillage and crop residue management in the subarctic: fluxes of methane and nitrous oxide. Canadian Journal of Soil Science **77**:565-570.

Conrad, R. 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiological Reviews **60**:609-&.

Crill, P. M. 1991. Seasonal patterns of methane uptake and carbon dioxide release by a temperate woodland soil. Global Biogeochemical Cycles **5**:319-334.

Crill, P. M., P. J. Martikainen, H. Nykanen, and J. Silvola. 1994. Temperature and N-fertilization effects on methane oxidation in a drained peatland soil. Soil Biology & Biochemistry **26**:1331-1339.

Czepiel, P. M., P. M. Crill, and R. C. Harriss. 1995. Environmental-factors influencing the variability of methane oxidation in temperate zone soils. Journal of Geophysical Research-Atmospheres **100**:9359-9364.

Dalton, H. 1992. Methane oxidation by methanotrophs. Pages 85-114 *in* J. C. Murrell and H. Dalton, editors. Methane and Methanol Utilizer. Plenum Press, New York.

De Visscher, A., P. Boeckx, and O. Van Cleemput. 1998. Interaction between nitrous oxide formation and methane oxidation in soils: Influence of cation exchange phenomena. Journal of Environmental Quality **27**:679-687.

Delgado, J. A., and A. Mosier. 1996. Mitigation alternatives to decrease nitrous oxides emissions and urea-nitrogen loss and their effect on methane flux. Journal of Environmental Quality **25**:1105-1111.

Delmas, R., A. Marenco, J. P. Tathy, B. Cros, and J. G. R. Baudet. 1991. Sources and sinks of methane in the African savanna; CH_4 emissions from biomass burning. Journal of Geophysical Research-Atmospheres **96**:7287-7299.

Delmas, R., J. Servant, J. P. Tathy, B. Cros, and M. Labat. 1992. Sources and sinks of methane and carbon dioxide exchanges in mountain forest in equatorial Africa. Journal of Geophysical Research-Atmospheres **97**:6169-6179.

Dobbie, K. E., and K. A. Smith. 1996. Comparison of CH_4 oxidation rates in woodland, arable and set aside soils. Soil Biology & Biochemistry **28**:1357-1365.

Dobbie, K. E., K. A. Smith, A. Prieme, S. Christensen, A. Degorska, and P. Orlanski. 1996. Effect of land use on the rate of methane uptake by surface soils in northern Europe. Atmospheric Environment **30**:1005-1011.

Dörr, H., L. Katruff, and I. Levin. 1993. Soil texture parameterization of the methane uptake in aerated soils. Chemosphere **26**:697-713.

Dubey, S. K., and J. S. Singh. 2001. Plant-induced spatial variations in the size of methanotrophic population in dryland and flooded rice agroecosystems. Nutrient Cycling in Agroecosystems **59**:161-167.

Dunfield, P., R. Knowles, R. Dumont, and T. Moore. 1993. Methane production and consumption in temperate and subarctic peat soils - response to temperature and pH. Soil Biology & Biochemistry **25**:321-326.

Flessa, H., W. Pfau, P. Dörsch, and F. Beese. 1996. The influence of nitrate and ammonium fertilization on N_2O release and CH_4 uptake of a well-drained topsoil demonstrated by a soil microcosm experiment. Z. Pflanzenernähr. Bodenkd. **159**:499-503.

Goldman, M. B., P. M. Groffman, R. V. Pouyat, M. J. McDonnell, and S. T. A. Pickett. 1995. CH_4 uptake and N availability in forest soils along an urban to rural gradient. Soil Biology & Biochemistry **27**:281-286.

Goreau, T. J., and W. Z. de Mello. 1988. Tropical deforestation: some effects on atmospheric chemistry. Ambio **17**:275-281.

Goulding, K., T. Willison, C. Webster, and D. Powlson. 1996. Methane fluxes in aerobic soils. Environment Monitoring Assessment **42**:175-187.

Goulding, K. W. T., B. W. Hütsch, C. P. Webster, T. W. Willison, and D. S. Powlson. 1995. The effect of agriculture on methane oxidation in soil. Philosophical Transactions of the Royal Society of London Series a-Mathematical Physical and Engineering Sciences **351**:313-324.

Graham, D. W., J. A. Chaudhary, R. S. Hanson, and R. G. Arnold. 1993. Factors affecting competition between type I and type II methanotrophs in two-organism, continuous-flow reactors. Microbial Ecology **25**:1-17.

Green, P. N. 1992. Taxonomy of methylotrophic bacteria. Pages 23-84 *in* J. C. Murrell and H. Dalton, editors. Methane and Methanol Utilizer. Plenum Press, New York.

Gulledge, J., A. P. Doyle, and J. P. Schimel. 1997. Different NH_4^+-inhibition patterns of soil CH_4 consumption: A result of distinct CH_4-oxidizer populations across sites? Soil Biology & Biochemistry **29**:13-21.

Gulledge, J., and J. P. Schimel. 1998. Low-concentration kinetics of atmospheric CH_4 oxidation in soil and mechanism of NH_4^+ inhibition. Applied and Environmental Microbiology **64**:4291-4298.

Gulledge, J., P. A. Steudler, and J. P. Schimel. 1998. Effect of CH_4-starvation on atmospheric CH_4 oxidizers in taiga and temperate forest soils. Soil Biology & Biochemistry **30**:1463-1467.

Hansen, S., J. E. Maehlum, and L. R. Bakken. 1993. N_2O and CH_4 fluxes in soil influenced by fertilization and tractor traffic. Soil Biology & Biochemistry **25**:621-630.

Hanson, R. S., and T. E. Hanson. 1996. Methanotrophic bacteria. Microbiological Reviews **60**:439-471.

Harriss, R. C., D. I. Sebacher, and F. P. J. Day. 1982. Methane flux in the Great Dismal Swamp. Nature **297**:673-674.

Hilger, H. A., A. G. Wollum, and M. A. Barlaz. 2000. Landfill methane oxidation response to vegetation, fertilization, and liming. Journal of Environmental Quality **29**:324-334.

Hudgens, D. E., and J. B. Yavitt. 1997. Land-use effects on soil methane and carbon dioxide fluxes in forests near Ithaca, New York. Ecoscience **4**:214-222.

Hütsch, B. W. 1996. Methane oxidation in soils of two long-term fertilization experiments in Germany. Soil Biology & Biochemistry **28**:773-782.

Hütsch, B. W. 1998a. Methane oxidation in arable soil as inhibited by ammonium, nitrite, and organic manure with respect to soil pH. Biology and Fertility of Soils **28**:27-35.

Hütsch, B. W. 1998b. Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. Biology and Fertility of Soils **27**:284-292.

Hütsch, B. W. 2001. Methane oxidation in non-flooded soils as affected by crop production. European Journal of Agronomy **14**:237-260.

Hütsch, B. W., C. P. Webster, and D. S. Powlson. 1993. Long-term effects of nitrogen-fertilization on methane oxidation in soil of the Broadbalk Wheat Experiment. Soil Biology & Biochemistry **25**:1307-1315.

Hütsch, B. W., C. P. Webster, and D. S. Powlson. 1994. Methane oxidation in soil as affected by land-use, soil-pH and N-fertilization. Soil Biology & Biochemistry **26**:1613-1622.

IPCC. 1995. Climate Change 1994; Radiative Forcing of Climate Change. Cambridge University Press, Cambridge.

IPCC. 2001. Climate Change 2001; the Scientific Basis. Cambridge University Press, Cambridge.

Ishizuka, S., T. Sakata, and K. Ishizuka. 2000. Methane oxidation in Japanese forest soils. Soil Biology & Biochemistry **32**:769-777.

Jensen, S., A. Prieme, and L. Bakken. 1998. Methanol improves methane uptake in starved methanotrophic microorganisms. Applied and Environmental Microbiology **64**:1143-1146.

Keller, M., W. A. Kaplan, and S. C. Wofsy. 1986. Emissions of N_2O, CH_4 and CO_2 from tropical forest soils. Journal of Geophysical Research-Atmospheres **91**:1791-1802.

Keller, M., M. E. Mitre, and R. F. Stallard. 1990. Consumption of atmospheric methane in soils of central Panama: effects of agricultural development. Global Biogeochemical Cycles **4**:21-27.

Keller, M., and W. A. Reiners. 1994. Soil atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. Global Biogeochemical Cycles **8**:399-409.

Kessavalou, A., J. W. Doran, A. Mosier, and R. A. Drijber. 1998a. Greenhouse gas fluxes following tillage and wetting in a wheat-fallow cropping system. Journal of Environmental Quality **27**:1105-1116.

Kessavalou, A., A. Mosier, J. W. Doran, R. A. Drijber, D. J. Lyon, and O. Heinemeyer. 1998b. Fluxes of carbon dioxide, nitrous oxide, and methane in grass sod and winter weat-fallow tillage management. Journal of Environmental Quality **27**:1094-1104.

King, G. M. 1992. Ecological aspects of methane oxidation, a key determinant of global methane dynamics. Advances in Microbial Ecology **12**:431-468.

King, G. M. 1993. Ecophysiological characteristics of obligate methanotrophic bacteria and methane oxidation *in situ*. Pages 303-313 *in* J. C. Murrell and D. P. Kelley, editors. Microbial Growth on C_1 Compounds. Intercept Ltd., Andover, UK.

King, G. M., and A. P. S. Adamsen. 1992. Effects of temperature on methane consumption in a forest soil and in pure cultures of the methanotroph *Methylomonas rubra*. Applied and Environmental Microbiology **58**:2758-2763.

King, G. M., and S. Schnell. 1994. Ammonium and nitrite inhibition of methane oxidation by *Methylobacter albus* BG8 and *Methylosinus trichosporium* OB3b at low methane concentrations. Applied and Environmental Microbiology **60**:3508-3513.

King, G. M., and S. Schnell. 1998. Effects of ammonium and non-ammonium salt additions on methane oxidation by *Methylosinus trichosporium* OB3b and Maine forest soils. Applied and Environmental Microbiology **64**:253-257.

Klemedtsson, A. K., and L. Klemedtsson. 1997. Methane uptake in Swedish forest soil in relation to liming and extra N-deposition. Biology and Fertility of Soils **25**:296-301.

Knowles, R. 1993. Methane: processes of production and consumption. Pages 145-156 *in* L. A. Harper, A. R. Mosier, J. M. Duxbury, and D. E. Rolston, editors. Agricultural Ecosystem Effect on Trace Gases and Global Climate Change. American Society of Agronomy, Madison, Wis.

Koschorreck, M., and R. Conrad. 1993. Oxidation of atmospheric methane in soil - Measurements in the field, in soil cores and in soil samples. Global Biogeochemical Cycles **7**:109-121.

Kotelnikova, S. 2002. Microbial production and oxidation of methane in deep subsurface. Earth-Science Reviews **58**:367-395.

Kruse, C. W., and N. Iversen. 1995. Effect of plant succession, plowing, and fertilization on the microbiological oxidation of atmospheric methane in a heathland soil. Fems Microbiology Ecology **18**:121-128.

Kumaraswamy, S., A. K. Rath, K. Bharati, B. Ramakrishnan, and N. Sethunathan. 1997. Influence of pesticides on methane oxidation in a flooded tropical rice soil. Bulletin of Environmental Contamination and Toxicology **59**:222-229.

Kumaraswamy, S., A. K. Rath, A. N. Satpathy, B. Ramakrishnan, T. K. Adhya, and N. Sethunathan. 1998. Influence of the insecticide carbofuran on the production and oxidation of methane in a flooded rice soil. Biology and Fertility of Soils **26**:362-366.

Le Mer, J., and P. Roger. 2001. Production, oxidation, emission and consumption of methane by soils: A review. European Journal of Soil Biology **37**:25-50.

Lessard, R., P. Rochette, E. Topp, E. Pattey, R. L. Desjardins, and G. Beaumont. 1994. Methane and carbon dioxide fluxes from poorly drained adjacent cultivated and forest sites. Canadian Journal of Soil Science **74**:139-146.

MacDonald, J. A., P. Eggleton, D. E. Bignell, F. Forzi, and D. Fowler. 1998. Methane emission by termites and oxidation by soils, across a forest disturbance gradient in the Mbalmayo Forest Reserve, Cameroon. Global Change Biology **4**:409-418.

MacDonald, J. A., D. Jeeva, P. Eggleton, R. Davies, D. E. Bignell, D. Fowler, J. Lawton, and M. Maryati. 1999. The effect of termite biomass and anthropogenic disturbance on the CH4 budgets of tropical forests in Cameroon and Borneo. Global Change Biology **5**:869-879.

MacDonald, J. A., U. Skiba, L. J. Sheppard, B. Ball, J. D. Roberts, K. A. Smith, and D. Fowler. 1997. The effect of nitrogen deposition and seasonal variability on methane oxidation and nitrous oxide emission rates in an upland spruce plantation and moorland. Atmospheric Environment **31**:3693-3706.

MacDonald, J. A., U. Skiba, L. J. Sheppard, K. J. Hargreaves, K. A. Smith, and D. Fowler. 1996. Soil environmental variables affecting the flux of methane from a range of forest, moorland and agricultural soils. Biogeochemistry **34**:113-132.

Mancinelli, R. L. 1995. The regulation of methane oxidation in soil. Annual Review of Microbiology **49**:581-605.

McInerney, M. J. 1986. Transient and persistent associations among prokaryotes. *in* J. S. Poindexter and E. R. Leadbetter, editors. Bacteria in Nature. Plenum, New York.

Megraw, S. R., and R. Knowles. 1987. Methane production and consumption in a cultivated humisol. Biology and Fertility of Soils **5**:56-60.

Minami, K., J. Goudriaan, E. A. Lantinga, and T. Kimaru. 1993. The significance of grasslands in emission and absorption of greenhouse gases. Pages 1211-1238 *in* Proceedings 17th International Grassland Congress.

Mosier, A., J. A. Delgado, and M. Keller. 1998. Methane and nitrous oxide fluxes in an acid oxisol in western Puerto Rico; effects of tillage, liming, and fertilization. Soil Biology & Biochemistry **30**:2087-2098.

Mosier, A., and D. Schimel. 1991. Influence of agricultural nitrogen on atmospheric methane and nitrous oxide. Chemical Industry **24**:334-336.

Mosier, A., D. Schimel, D. Valentine, K. Bronson, and W. Parton. 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature **350**:330-332.

Mosier, A. R., J. A. Delgado, V. L. Cochran, D. W. Valentine, and W. J. Parton. 1997a. Impact of agriculture on soil consumption of atmospheric CH_4 and a comparison of CH_4 and N_2O flux in subarctic, temperate, and tropical grasslands. Nutrient Cycling in Agroecosystems **49**:71-83.

Mosier, A. R., L. K. Klemedtsson, R. A. Sommerfeld, and R. C. Musselman. 1993. Methane and nitrous oxide flux in a Wyoming sub-alpine meadow. Global Biogeochemical Cycles **7**:771-784.

Mosier, A. R., W. J. Parton, D. W. Valentine, D. S. Ojima, D. S. Schimel, and O. Heinemeyer. 1997b. CH_4 and N_2O fluxes in the Colorado shortgrass steppe .2. Long- term impact of land use change. Global Biogeochemical Cycles **11**:29-42.

Murrell, J. C., B. Gilbert, and I. R. McDonald. 2000. Molecular biology and regulation of methane monooxygenase. Archives of Microbiology **173**:325-332.

Murrell, J. C., I. R. McDonald, and D. G. Bourne. 1998. Molecular methods for the study of methanotroph ecology. Fems Microbiology Ecology **27**:103-114.

Murrell, J. C., and S. Radajewski. 2000. Cultivation-independent techniques for studying methanotroph ecology. Research in Microbiology **151**:807-814.

Nesbit, S. P., and G. A. Breitenbeck. 1992. A laboratory study of factors influencing methane uptake by soils. Agriculture Ecosystems & Environment **41**:39-54.

Nesterov, A. I., Y. Mshensky, V. F. Gachenko, B. B. Namsaraev, and V. Ilchenko. 1977. A comparative study on parameters of growth of methanotrophic bacteria. Mikrobiologiya **46**:10-14.

Ojima, D. S., D. W. Valentine, A. R. Mosier, W. J. Parton, and D. S. Schimel. 1993. Effect of land-use change on methane oxidation in temperate forest and grassland soils. Chemosphere **26**:675-685.

Paustian, K., G. P. Robertson, and E. T. Elliott. 1995. Management impacts on carbon storage and gas fluxes (CO_2, CH_4) in mid-latitude cropland. Pages 69-83 *in* R. Lal, J. Kimble, E. Levine, and J. Stewart, editors. Soil Management and the Greenhouse Effect. CRC Press, Boca Raton, FL.

Peterjohn, W. T., J. M. Melillo, and F. P. Bowles. 1993. Soil warming and trace gas fluxes - experimental design and preliminary flux results. Oecologia **93**:18-24.

Poth, M., I. C. Anderson, H. S. Miranda, A. C. Miranda, and P. J. Riggan. 1995. The magnitude and persistence of soil NO, N_2O, CH_4, and CO, fluxes from burned tropical savanna in Brazil. Global Biogeochemical Cycles **9**:503-513.

Potter, C. S., E. A. Davidson, and L. V. Verchot. 1996. Estimation of global biogeochemical controls and seasonality in soil methane consumption. Chemosphere **32**:2219-2246.

Powlson, D. S., K. W. T. Goulding, T. W. Willison, C. P. Webster, and B. W. Hütsch. 1997. The effect of agriculture on methane oxidation in soil. Nutrient Cycling in Agroecosystems **49**:59-70.

Prieme, A., and S. Christensen. 1997. Seasonal and spatial variation of methane oxidation in a Danish spruce forest. Soil Biology & Biochemistry **29**:1165-1172.

Prieme, A., and S. Christensen. 1999. Methane uptake by a selection of soils in Ghana with different land use. Journal of Geophysical Research-Atmospheres **104**:23,617-623,622.

Prieme, A., S. Christensen, K. E. Dobbie, and K. A. Smith. 1997. Slow increase in rate of methane oxidation in soils with time following land use change from arable agriculture to woodland. Soil Biology & Biochemistry **29**:1269-1273.

Prieme, A., and F. Ekelund. 2001. Five pesticides decreased oxidation of atmospheric methane in a forest soil. Soil Biology & Biochemistry **33**:831-835.

Reeburgh, W. S., S. C. Whalen, and M. J. Alpern. 1993. The role of methylotrophy in the global methane budget. Pages 1-14 *in* J. C. Murrell and D. P. Kelly, editors. Microbial Growth on C_1 Compounds. Intercept Ltd., Andover.

Ridgwell, A. J., S. J. Marshall, and K. Gregson. 1999. Consumption of atmospheric methane by soils: A process-based model. Global Biogeochemical Cycles **13**:59-70.

Robertson, G. P., E. A. Paul, and R. R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science **289**:1922-1925.

Roslev, P., N. Iversen, and K. Henriksen. 1997. Oxidation and assimilation of atmospheric methane by soil methane oxidizers. Applied and Environmental Microbiology **63**:874-880.

Ruser, R., H. Flessa, R. Schilling, H. Steindl, and F. Beese. 1998. Soil compaction and fertilization effects on nitrous oxide and methane fluxes in potato fields. Soil Science Society of America Journal **62**:1587-1595.

Saari, A., P. J. Martikainen, A. Ferm, J. Ruuskanen, W. De Boer, S. R. Troelstra, and H. J. Laanbroek. 1997. Methane oxidation in soil profiles of Dutch and Finnish coniferous forests with different soil texture and atmospheric nitrogen deposition. Soil Biology & Biochemistry **29**:1625-1632.

Sanhueza, E., L. Cardenas, L. Donoso, and M. Santana. 1994. Effect of plowing on CO_2, CO, CH_4, N_2O, and NO fluxes from tropical savannah soils. Journal of Geophysical Research-Atmospheres **99**:16,429-416,434.

Scharffe, D., W. M. Hao, L. Donoso, P. J. Crutzen, and E. Sanhueza. 1990. Soil fluxes and atmospheric concentration of CO_2 and CH_4 in the northern part of the Guayana shield, Venezuela. Journal of Geophysical Research-Atmospheres **95**:22,475-422,480.

Schnell, S., and G. M. King. 1995. Stability of methane oxidation capacity to variations in methane and nutrient concentrations. Fems Microbiology Ecology **17**:285-294.

Schnell, S., and G. M. King. 1996. Responses of methanotrophic activity in soils and cultures to water stress. Applied and Environmental Microbiology **62**:3203-3209.

Seiler, W., R. Conrad, and D. Scharffe. 1984. Field studies of methane emission from termite nests into the atmosphere and measurements of methane uptake by tropical soils. Journal of Atmospheric Chemistry **1**:171-186.

Singh, J. S., S. Singh, A. S. Raghubanshi, A. K. Kashyap, and V. S. Reddy. 1997. Effect of soil nitrogen, carbon and moisture on methane uptake by dry tropical forest soils. Plant and Soil **196**:115-121.

Singh, S., J. S. Singh, and A. K. Kashyap. 1999. Methane consumption by soils of dryland rice agriculture: Influence of varieties and N-fertilization. Chemosphere **38**:175-189.

Sitaula, B. K., L. R. Bakken, and G. Abrahamsen. 1995. CH_4 uptake by temperate forest soil - Effect of N input and soil acidification. Soil Biology & Biochemistry **27**:871-880.

Sitaula, B. K., S. Hansen, J. I. B. Sitaula, and L. R. Bakken. 2000. Methane oxidation potentials and fluxes in agricultural soil: Effects of fertilisation and soil compaction. Biogeochemistry **48**:323-339.

Sitaula, B. K., J. I. B. Sitaula, A. Aakraz, and L. R. Bakken. 2001. Nitrification and methane oxidation in forest soil: Acid deposition, nitrogen input, and plant effects. Water Air and Soil Pollution **130**:1061-1066.

Smirnova, Z. S. 1971a. Efficiency of the utilization of free energy by methane oxidizing bacteria. Mikrobiologiya **40**:5-7.

Smirnova, Z. S. 1971b. The material balance of methane oxidation by microorganisms. Dokl. Akad. Nauk. SSSR Ser. Biol. **3**:423-427.

Smith, K. A., K. E. Dobbie, B. C. Ball, L. R. Bakken, B. K. Sitaula, S. Hansen, R. Brumme, W. Borken, S. Christensen, A. Prieme, D. Fowler, J. A. Macdonald, U. Skiba, L. Klemedtsson, A. Kasimir-Klemedtsson, A. Degorska, and P. Orlanski. 2000. Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. Global Change Biology **6**:791-803.

Söhngen, N. L. 1906. Uber bakterien, welche methan ab kohlenstoffnahrung and energiequelle gebrauchen. Parasitenkd. Infectionskr. Abt. 2 **15**:513-517.

Steudler, P. A., R. D. Bowden, J. M. Melillo, and J. D. Aber. 1989. Influence of nitrogen fertilization on methane uptake in temperate forest soil. Nature **341**:314-316.

Steudler, P. A., R. D. Jones, M. S. Castro, J. M. Melillo, and D. Lewis. 1996a. Microbial controls of methane oxidation in temperate forest and agricultural soils. Pages 69-84 *in* J. C. Murrell and D. P. Kelly, editors. The Microbiology of Atmospheric Trace Gases. Springer, Berlin.

Steudler, P. A., J. M. Melillo, R. D. Bowden, M. S. Castro, and A. E. Lugo. 1991. The effects of natural and human disturbances on soil nitrogen dynamics and trace gas fluxes in a Puerto Rican wet forest. Biotropica **23**:356-363.

Steudler, P. A., J. M. Melillo, B. J. Feigl, C. Neill, M. C. Piccolo, and C. C. Cerri. 1996b. Consequences of forest-to-pasture conversion on CH_4 fluxes in the Brazilian Amazon basin. Journal of Geophysical Research-Atmospheres **101**:18547-18554.

Striegl, R. G., T. A. McConnaughey, D. C. Thorstenson, E. P. Weeks, and J. C. Woodward. 1992. Consumption of atmospheric methane by desert soils. Nature **357**:145-147.

Tate, C. M., and R. G. Striegl. 1993. Methane consumption and carbon dioxide emission in tallgrass prairie - Effects of biomass burning and conversion to agriculture. Global Biogeochemical Cycles **7**:735-748.

Tlustos, P., T. W. Willison, J. C. Baker, D. V. Murphy, D. Pavlikova, K. W. T. Goulding, and D. S. Powlson. 1998. Short-term effects of nitrogen on methane oxidation in soils. Biology and Fertility of Soils **28**:64-70.

Topp, E. M., M. P. Maila, M. Clerinx, J. Goris, P. De Vos, and W. Verstraete. 1999. Methane oxidation as a method to evaluate the removal of 2,4-dichlorophenoxyacetic acid (2,4-D) from soil by plasmid- mediated bioaugmentation. Fems Microbiology Ecology **28**:203-213.

Torn, M. S., and J. Harte. 1996. Methane consumption by montane soils: Implications for positive and negative feedback with climatic change. Biogeochemistry **32**:53-67.

Valentine, D. L., and W. S. Reeburgh. 2000. New perspectives on anaerobic methane oxidation. Environmental Microbiology **2**:477-484.

van den Pol-van Dasselaar, A., M. L. van Beusichem, and O. Oenema. 1998. Effects of soil moisture content and temperature on methane uptake by grasslands on sandy soils. Plant and Soil **204**:213-222.

van der Weerden, T. J., R. R. Sherlock, P. H. Williams, and K. C. Cameron. 1999. Nitrous oxide emissions and methane oxidation by soil following cultivation of two different leguminous pastures. Biology and Fertility of Soils **30**:52-60.

Wang, Z. P., and P. Ineson. 2003. Methane oxidation in a temperate coniferous forest soil: effects of inorganic N. Soil Biology & Biochemistry **35**:427-433.

West, A. E., and S. K. Schmidt. 1999. Acetate stimulates atmospheric CH_4 oxidation by an alpine tundra soil. Soil Biology & Biochemistry **31**:1649-1655.

Whalen, S. C. 2000. Influence of N and non-N salts on atmospheric methane oxidation by upland boreal forest and tundra soils. Biology and Fertility of Soils **31**:279-287.

Whalen, S. C., and W. S. Reeburgh. 1990. Consumption of atmospheric methane by tundra soils. Nature **346**:160-162.

Whalen, S. C., W. S. Reeburgh, and K. S. Kizer. 1991. Methane consumption by taiga. Global Biogeochemical Cycles **5**:261-273.

Whalen, S. C., W. S. Reeburgh, and K. A. Sandbeck. 1990. Rapid methane oxidation in a landfill cover soil. Applied and Environmental Microbiology **56**:3405-3411.

Whittenbury, R., K. C. Phillips, and J. G. Wilkinson. 1970. Enrichment, isolation, and some properties of methane utilizing bacteria. Journal of General Microbiology **61**:205-218.

Willison, T. W., K. Goulding, and D. Powlson. 1995a. Effect of land use change and methane mixing ratio on methane uptake from United Kingdom soil. Global Change Biology **1**:209-212.

Willison, T. W., C. P. Webster, K. W. T. Goulding, and D. S. Powlson. 1995b. Methane oxidation in temperate soils - Effects of land-use and the chemical form of nitrogen fertilizer. Chemosphere **30**:539-546.

Wolf, H. J. 1981. Biochemical characterization of methane-oxidizing yeasts. Pages 202-210 *in* H. Dalton, editor. Microbial Growth on C1 Compounds. Heyden & Son Ltd., London.

Yavitt, J. B., D. M. Downey, G. E. Lang, and A. J. Sexstone. 1990. Methane consumption in 2 temperate forest soils. Biogeochemistry **9**:39-52.

Yavitt, J. B., J. A. Simmons, and T. J. Fahey. 1993. Methane fluxes in a northern hardwood forest ecosystem in relation to acid precipitation. Chemosphere **26**:721-730.

Zepp, R. G., W. L. Miller, R. A. Burke, D. A. B. Parsons, and M. C. Scholes. 1996. Effects of moisture and burning on soil-atmosphere exchange of trace carbon gases in a southern Africa savanna. Journal of Geophysical Research-Atmospheres **101**:23,699-623,706.

Table 1.1 Estimated sources and sinks of methane (IPCC 1995, 2001)

	Annual release ($Tg\ CH_4$)		Range ($Tg\ CH_4$)
Natural sources			
Wetlands	115		55-150
Termites	20		10-50
Oceans	10		5-50
Others	15		10-40
Anthropogenic sources			
Enteric fermentation and animal waste	110		85-130
Energy production and use	100		70-120
Ricefields	60		20-100
Biomass burning	40		20-80
Landfills	40		20-70
Domestic sewage	25		15-80
Total sources	535	598*	410-660
Sinks			
Consumption in atmosphere	485	546*	420-520
Oxidation in upland soils	30		15-45
Total sinks	515	576*	430-600
Atmospheric increase	37	22*	35-40

*IPCC (2001) adjustment

$Tg = 10^{12}$ g

Table 1.2 General characteristics of methanotrophic bacteria (modified after King 1992 and Hanson and Hanson 1996).

Character	Type I	Type II	Type X
Cell morphology	Rods	Rods, vibroid	Coccoid
Membrane structure			
Bundles of vesicles	Yes	No	Yes
Peripheral paired membranes	No	Yes	No
Resting stages	Cysts	Exospores or cysts	Cysts
Rosettes	No	Yes	No
Carbon assimilation pathway	RMP[a]	Serine[b]	RMP/serine
Tricarboxylic acid cycle	Incomplete	Complete	Incomplete
Nitrogen fixation	No	Yes	Yes
Diagnostic fatty acid carbon length	16	18	16
Carbon dioxide fixation	No	No	Yes

[a] Incorporation of formaldehyde via the ribulose monophosphate pathway
[b] Incorporation of formaldehyde via the serine pathway

Table 1.3 Characterization of methanotrophs (modified after Murrell et al. 1998)

Genus	Phylogeny	Type	Formaldehyde assimilation pathway	Enzyme type	Mol% G+C DNA content
Methylomonas	γ	Type I	RMP	pMMO	51-59
Methylobacter	γ	Type I	RMP	pMMO	49-54
Methylomicrobium	γ	Type I	RMP	pMMO	50-60
Methylosphaera	γ	Type I	RMP	pMMO	43-46
Methylosinus	α	Type II	Serine	pMMO/sMMO	62-63
Methylocystis	α	Type II	Serine	pMMO/sMMO	62-63
Methylocella	α	Type II	Serine	pMMO/sMMO	na
Methylococcus	γ	Type X	RMP/serine	pMMO	59-66
Methylocaldum	γ	Type X	RMP/serine	pMMO/sMMO	56-58

Table 1.4. Annual methane uptake in different global ecosystems (adapted from Bouwman 1990 and Reeburgh et al. 1993).

Land cover type	Area (10^{12} m^2)	Uptake rate (mg CH_4 m^{-2} y^{-1})	System uptake (Tg CH_4 y^{-1})
Tropical rain forest	17.0	0.3	5.1
Tropical seasonal forest	7.5	0.3	2.3
Temperate forest	12.0	1.1	12.1
Boreal forest	12.0	0.2	2.4
Woodland-schrubland	8.5	0.3	2.6
Temperate grassland	9.0	0.3	2.7
Savanna	15.0	0.3	4.5
Tundra and alpine	8.0	0.1	0.8
Desert-semidesert	42.0	0.2	8.4
Cultivate land	14.0	0.2	2.8
Total	145	-	43.7

Table 1.5 Methane oxidation of aerobic soils under field condition in various locations of the world (modified after Boeckx and van Cleemput 2001; Hütsch 2001; Prieme and Christensen 1999; Smith et al. 2000).

Location	Average CH_4 oxidation rate (mg CH_4 m^{-2} d^{-1})			Literature
	Arable	Grassland	Forest	
Canada				
Ottawa	0-0.13		0.04-1.10	Lessard et al. (1994)
			1.0-3.0	Adamsen and King (1993)
Denmark	0.04-0.08	0.03-0.25	0.14-0.33	Ambus and Christensen (1995)
	0.2		0.7	Dobbie et al. (1996)
Germany			0.12-0.96	Butterbach-Bahl et al. (1998)
	0.04-0.05			Ruser et al. (1998)
Göttingen	0.11			Flessa et al. (1996)
			0.03-0.68	Brumme and Borken (1999)
Heidelberg	0.005-0.55		0.25-3.56	Born et al. (1990)
Konstanz	0-0.62		0-1.79	Koschorreck and Conrad (1993)
Ireland			1.34	Butterbach-Bahl et al. (1998)
Japan		0.6	2.1	Minami et al. (1993)
			1.8-7.6	Ishizuka et al. (2000)
New Zealand		0.04-0.05		van der Weerden et al. (1999)
Norway			0.8-1.4	Sitaula et al. (1995)
Poland	0.2		1.0	Dobbie et al. (1996)
Scotland	0.02-0.12	0.85	0.86-1.06	MacDonald et al. (1996)
	0.7		1.4	Dobbie et al. (1996)
	0.82		2.19-5.97	Dobbie and Smith (1996)
			0.19-0.36	MacDonald et al. (1997)
Sweden			0.38	Klemedtsson and Klemedtsson (1997)
			0.48	MacDonald et al. (1997)
UK				
	0.18	0.48	1.05	Willison et al. (1995a)
	0-0.13	0-0.19	0-0.24	Goulding et al. (1996)
USA				
Alaska			0.67-0.87	Goulding et al. (1997)
Colorado	0.17	0.35		Mosier et al. (1991)
	0.03-0.20	0.35-0.84		Mosier and Schimel (1991)
	0.09			Delgado and Mosier (1996)
	0.18	0.82		Ojima et al. (1993)
Durham			4.9	Crill (1991)
Massachusetts			3.20-4.16	Steudler et al. (1989)
			3.84-5.44	Castro et al. (1994)
Michigan	0.24		1.92	Paustian et al. (1995)

Table 1.5 (cont'd)

	0.24	0.73	1.22	Robertson et al. (2000)
Nebraska	0.91-1.03	1.16		Kessavalou et al. (1998b)
New York			0.60	Yavitt et al. (1993)
			2.1-6.9	Goldman et al. (1995)
	0.25		0.75	Hudgens and Yavitt (1997)
Piedmont	0.3		1.4	Burke et al. (1999)
Tropic				
Brazil			1.71	Steudler et al. (1996b)
		0.96		Andersen and Poth (1998)
		-0.72		Poth et al. (1995)
			0.38	Keller et al. (1986)
	0.35		1.15	Goreau and de Mello (1988)
Cameroon	0.41		1.8	MacDonald et al. (1998)
Congo	0.46	0.62		Delmas et al. (1991)
			1.0	Delmas et al. (1992)
Costa Rica	-0.9-(-0.2)		1.20-1.26	Keller and Reiners (1994)
Ecuador	-0.07		-0.62-0.82	Keller et al. (1986)
Ghana	0.16	0.89	0.94	Prieme and Christensen (1999)
India		11.52	8.64-13.68	Singh et al. (1997)
	6.0			Singh et al. (1999)
Malaysia			1.34	MacDonald et al. (1999)
Mali		0.072		Delmas et al. (1991)
Panama	0.032		0.58	Keller et al. (1990)
Puerto Rico			0.5	Steudler et al. (1991)
		0.19		Mosier et al. (1998)
South Africa		-1.63-0.96		Zepp et al. (1996)
		1.23		Seiler et al. (1984)
Venezuela		-0.82	1.15	Scharffe et al. (1990)
	-1.13			Sanhueza et al. (1994)

Note: a negative number means net CH_4 production

Table 1.6 Average methane oxidation in different ecosystems; a summary of data from Table 1.5

Ecosystems	Average	Minimum	Maximum
Forest	1.66	1.28	2.05
Grassland	0.89	0.85	0.88
Agriculture	0.37	0.33	0.41

Unit = mg CH_4 m^{-2} d^{-1}

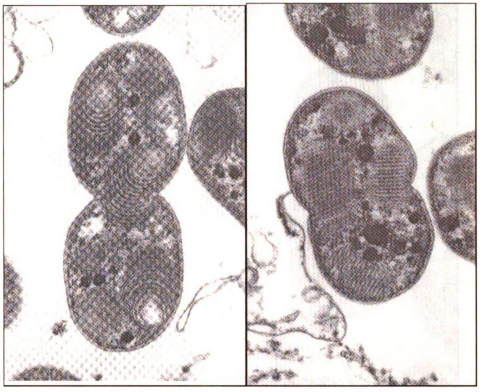


Figure 1.1 Electron micrographs showing type I membrane systems of *Methylomonas methanica* (Green 1992).

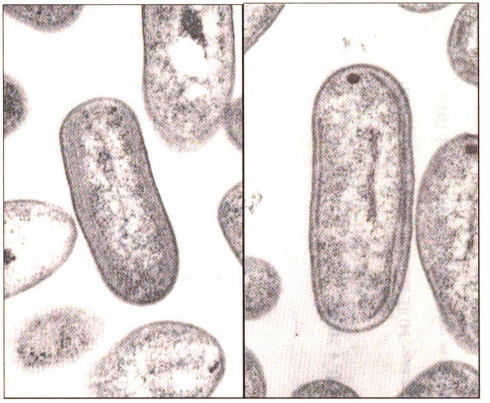


Figure 1.2 Electron micrographs showing type II membrane systems of *Methylocystis parvus* (Green 1992).

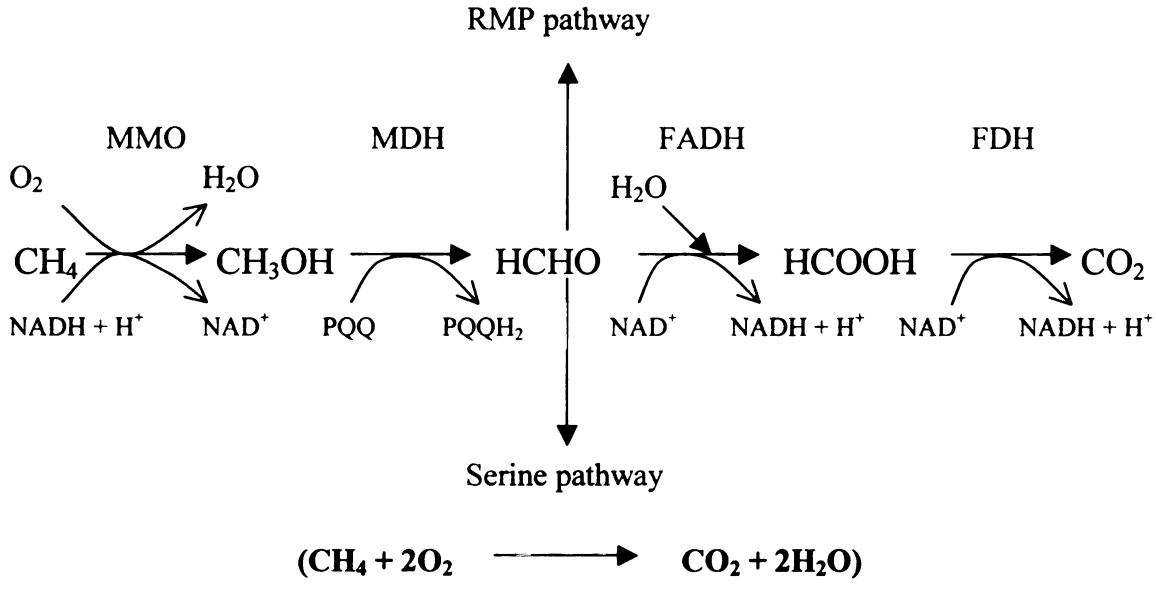


Figure 1.3 Methane oxidation in methanotrophs; MMO, methane monooxygenase; MDH, methanol dehydrogenase; FADH, formaldehyde dehydrogenase; FDH, formate dehydrogenase; PQQ, pyrroquinoline quinone (modified after Hütsch 2001).

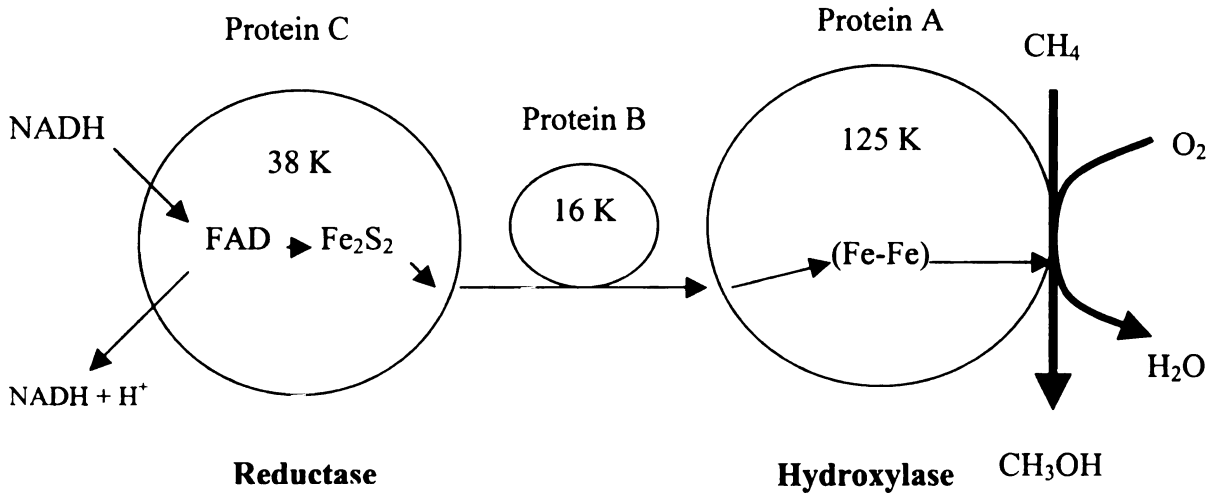


Figure 1.4 Electron transfer pathway between the three protein components of the sMMO (modified after Dalton 1992).

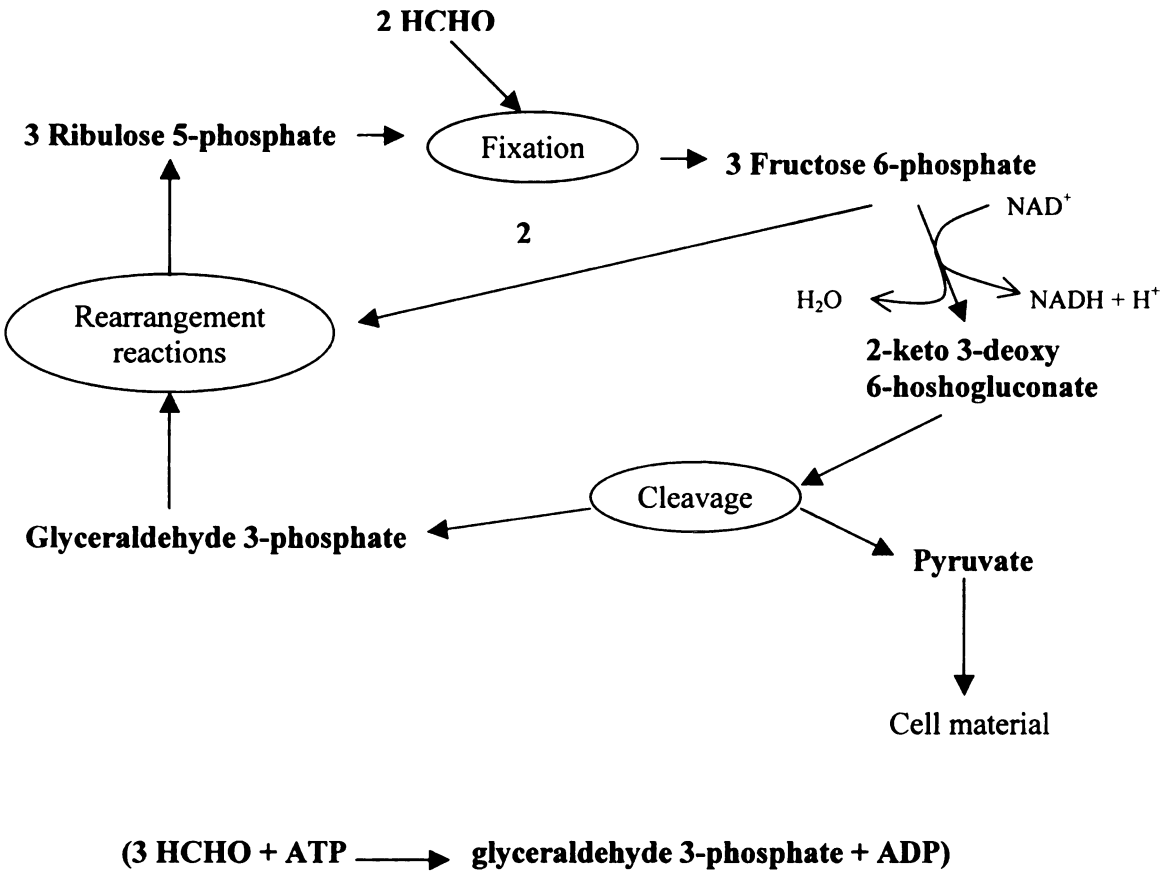
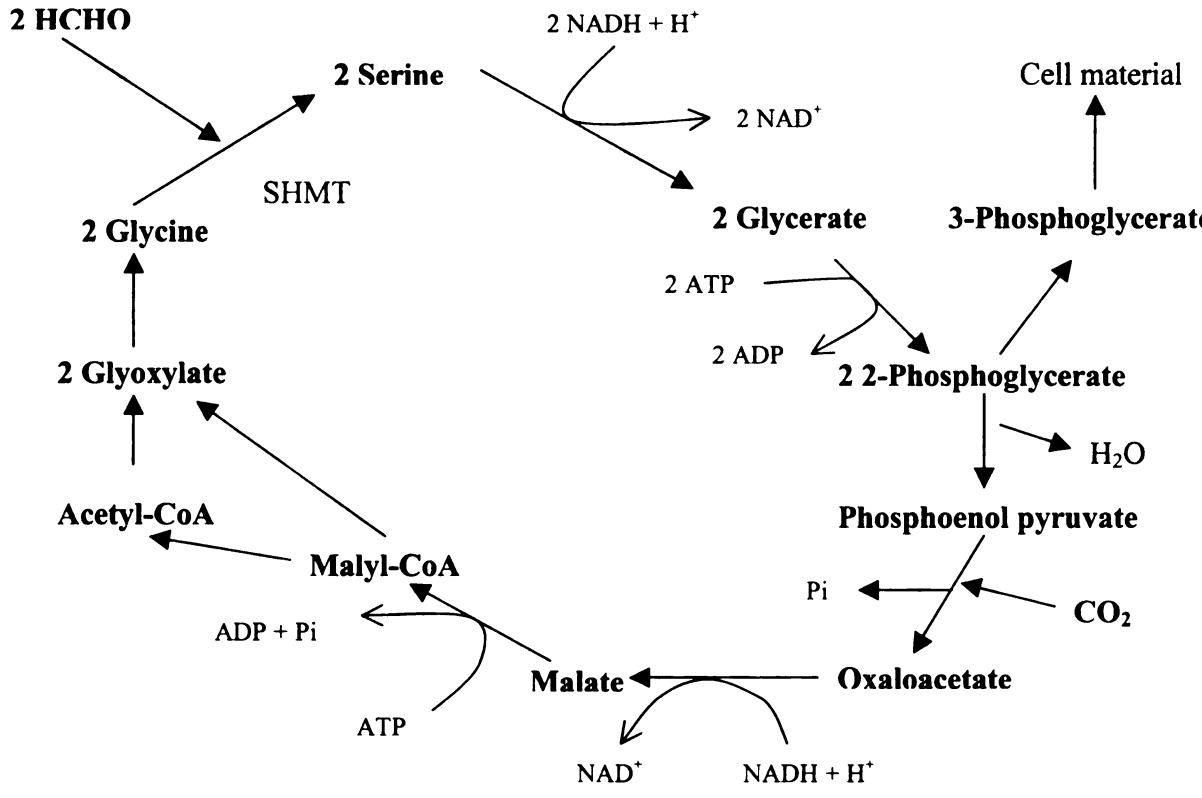


Figure 1.5 Ribulose monophosphate (RMP) pathway for formaldehyde fixation (modified after Mancinelli 1995)



(2 HCHO + CO_2 + 3 ATP + 2 NADH → 2-phosphoglycerate + 2 ADP + Pi + NAD^+)

Figure 1.6 Serine pathway for formaldehyde fixation; SHMT, serine hydroxymethyl transferase (modified after Hanson and Hanson 1996; Mancinelli 1995).

Chapter 2

Methane Oxidation in Successional and Agricultural Ecosystems: Effects of Nitrogen and Soil Disturbance

Introduction

Upland soil is an important global sink for the greenhouse gas methane, consuming about 30 Tg CH_4 y^{-1}, slightly more than the annual atmospheric loading rate of 22 Tg CH_4 y^{-1}(IPCC 2001). Increased soil methane uptake could thus help to mitigate increasing concentrations of methane in the atmosphere, now at 1,745 ppb CH_4 (IPCC 2001).

Land use and in particular agriculture has a big impact on rates of soil CH_4 oxidation: a number of studies have shown that undisturbed forest and grassland soils consume more methane than similar soils converted to agriculture (e.g. Ambus and Christensen 1995, Willison et al. 1995a, Goulding et al. 1996, MacDonald et al. 1996, Prieme and Christensen 1999, Robertson et al. 2000). Various agricultural practices including fertilization, tillage, and the use of insecticides and herbicides have been demonstrated to inhibit soil methane uptake to different degrees in a variety of different studies (Mosier and Schimel 1991, Goulding et al. 1995, Arif et al. 1996, Mosier et al. 1997a, Powlson et al. 1997, Topp et al. 1999, Hütsch 2001).

Nitrogen fertilizer has been shown most often to reduce methane oxidation in forest, grassland, arable, and landfill soils, especially when applied in the ammonium form (Steudler et al. 1989, Mosier et al. 1991, Hansen et al. 1993, Hütsch et al. 1993, Bronson and Mosier 1994, Crill et al. 1994, Hütsch et al. 1994, Castro et al. 1995,

Willison et al. 1995b, Hütsch 1996, Tlustos et al. 1998, Hilger et al. 2000). Tillage also has been shown to decrease methane oxidation in both natural and agricultural soils (Hütsch et al. 1994, Willison et al. 1995a, Cochran et al. 1997, Mosier et al. 1997b), however, it had no effect (Sanhueza et al. 1994, Mosier et al. 1998, Burke et al. 1999) and even increased (Kruse and Iversen 1995) methane uptake in some soils.

These studies of N fertilizer and tillage effects on soil methane consumption have investigated each factor alone and in a particular ecosystem. In this study I attempt to study both factors simultaneously by simulating soil disturbance (as tillage) and applying N fertilizer separately and in combination in different type of ecosystems along a 3-point disturbance gradient that includes old growth deciduous forest, mid-successional old field, and no-till agricultural sites. In this way I am able to separate the effects of tillage on N availability separate from its effects on other soil properties.

Methodology

Site Description

The study was conducted at the Long-term Ecological Research (LTER) site at the W.K. Kellogg Biological Station (KBS), Hickory Corners, Michigan (42° 24'N, 85° 24'W, elevation 288 m). Annual rainfall at KBS averages 890 mm y^{-1} with about half falling as snow; potential evapotranspiration (PET) exceeds precipitation for about 4 months of the year. Mean annual temperature is 9.7 °C. I measured methane fluxes at three replicated sites along a management intensity gradient: mature deciduous forests (DF), mid-successional fields (also called mid-successional communities, SF) abandoned

from agriculture 40-60 years ago, and a no-till corn-soybean-wheat rotation (T2) established in 1988 (see details at http://lter.kbs.msu.edu).

The major plants in the deciduous forest sites are red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marsh.), white oak (*Quercus alba* L.), northern red oak (*Quercus rubra* L.), flowering dogwood (*Cornus florida* L.), and sassafras (*Sassafras albidum* (Nutt.) Nees). The dominant plants in the mid-successional communities are Canada goldenrod (*Solidago canadensis* L.), quackgrass (*Elytrigia repens* (L.) Nevski), timothy (*Phleum pratense* L.), white hearth aster (*Aster pilosus* Willd.), Kentucky bluegrass (*Poa pratensis* L.), common yarrow (*Achillea millefolium* L.), Canada bluegrass (*Poa compressa* L.), autumn olive (*Elaeagnus umbellata* Thunb.), sassafras (*Sassafras albidum* (Nutt.) Nees), gray goldenrod (*Solidago nemoralis* Ait.), smooth brome (*Bromus inermis* Leyss.), germander speedwell (*Veronica chamaedrys* L.), orchardgrass (*Dactylis glomerata* L.), flowering spurge (*Euphorbia corollata* L.), and honeysuckle (*Lonicera* spp.).

The no-till system was planted to corn (*Zea mays* L.) during 2002, the year that this study was conducted.

All sites were replicated within the larger landscape (n=3 locations) and were on the same Kalamazoo/Oshtemo soil series (Austin 1979). The soils at these sites are Typic Hapludalfs (fine or coarse-loamy, mixed, mesic soils) derived from glacial till about 12,000 years ago (Crum and Collins 2003).

Four 0.5 x 0.5 m plots were established in each replicate site and a 2 x 2 factorial design was imposed with N fertilizer and tillage as factors. To one plot was added 100 kg N ha^{-1} ammonium nitrate (NH_4NO_3), another plot was physically disturbed by hand

shoveling to simulate soil tillage to 10 cm depth, another plot was both fertilized and tilled, and a fourth plot served as control.

In Situ Gas Sampling

I measured in situ methane oxidation rates using a static chamber technique (Hutchinson and Livingston 1993, Robertson et al. 2000). Static chambers were fashioned from 25 cm diameter PVC pipe: bases (25 cm diameter x 10 cm high x 3 cm depth) were installed in each plot and left in place except during agronomic operations. Immediately prior to sampling, a 4.5 cm high cap was placed on each base and sealed with a latex skirt wrapped with an elastic band. At 10-minute intervals, four 10 mL headspace samples were removed through rubber septa in each cap using a syringe and put into 3 mL glass sample vials pre-flushed with headspace air. Within 3 days vial contents were measured for CH_4 using a gas chromatograph (GC 5890 Series II, Hewlett Packard) equipped with a flame ionization detector (FID), and for CO_2 using an infrared gas absorption (IRGA) analyzer (EGA CO_2 Analyzer, Analytical Development CO. LTD. Hoddesdon, England).

Chambers were sampled one day before treatment and 1, 6, 16, 23, 52, 73 and 101 days after treatment.

Soil Analyses

Soil temperature was measured at 0-5 cm depth using a temperature probe. Soil samples for other analyses were taken from the top 10 cm of soil using a 2.5 cm diameter soil probe. Fresh soils were passed through a 4 mm sieve and mixed by hand, and then

sub samples were taken for moisture content and mineral N analysis. Prior to analysis, soils were stored in a refrigerator at 4°C. Soil moisture content was measured gravimetrically by drying the soil samples at 65°C for 3 days or until dry; further drying these soils at 105 °C typically removes only 0.8 g H2O 100 g $soil^{-1}$ more moisture (data not shown). Mineral N measurements were obtained by extracting 20 g of dry soil with 100 mL 1 M KCl for 24 h then filtering through 2-μm pore size glass fiber filters. The filtrates were frozen prior to analysis for NH_4^+ and NO_3^- using an Alpkem continuous flow analyzer (Alpkem 3550, OI Analytical, College Station, TX) (Bundy and Meisinger 1994).

Statistical Analyses

The data were divided into two parts: before fertilization (day 0) and after fertilization (day 1, 6, 16, 23, 52, 73, and 101). For analysis of the first part, I used SPSS version 10.0.1 (SPSS Inc. 2001) for the analysis of variance (ANOVA), analysis of covariance (ANCOVA), and correlation analysis. I used Proc Mixed of SAS program version 8.0 (SAS Institute 1999) for the ANOVA and ANCOVA for the post-fertilization data, for which I treated site and treatment as fixed effects and day and site x treatment x day as random effects. Methane and CO_2 data were natural log transformed before ANOVA and ANCOVA to homogenize variances. I used untransformed data for correlation analysis.

Results

Methane Oxidation in the Field

Methane oxidation rates were highest in the mature deciduous forest (Table 2.2), where average rates in the Control treatment were 32 (±3.2, n=3 sites x 7 sample dates) μg CH_4-C m^{-2} h^{-1} and daily rates over all treatments ranged from 0 to 73 μg CH_4-C m^{-2} h^{-1}. Added nitrogen reduced methane oxidation substantially (Figure 2.1), with the effect appearing to increase over a period of time until day 52 (Figure 2.2). In contrast, plowing had no significant effect on methane oxidation in mature forests and no-till cornfields, nor in mid-successional communities. There was also no significant fertilizer x plowing interaction (Table 2.5). Methane oxidation in both fertilized and plow x fertilized plots in the mature forests dropped sharply after treatment and started to increase after day 52, when CH_4 uptake in the plow x fertilized plots began to increase faster than in the fertilizer only plots (Figure 2.2a). In contrast, CH_4 oxidation in plowed plots was similar to control plots throughout the experiment.

Methane oxidation rates in the mid-successional sites were intermediate between rates in the forest and cropped systems (Table 2.2). Average oxidation in the control plots was 24 (±1.6) μg CH_4-C m^{-2} h^{-1} and rates over all plots ranged from 2.4 to 50 μg CH_4-C m^{-2} h^{-1}. Following treatment, N fertilizer and fertilizer x plowing reduced methane oxidation rates in these plots significantly, from 24 to 15 μg CH_4-C m^{-2} h^{-1} on average. In contrast, plowing alone had no detectable effect on oxidation. Similar to fluxes in mature forests, CH_4 oxidation in fertilized and in plow x fertilized plots exponentially decreased after treatment but started to increase at day 23 (Figure 2.2b).

In the No-till corn sites, CH_4 oxidation rates were variable and low; the average control plot rate was 4.0 (±0.7) μg CH_4-C m^{-2} h^{-1}. Rates across all treatments ranged from -8 to 17 μg CH_4-C m^{-2} h^{-1} (negative oxidation rates indicate methane production).

Neither added nitrogen nor plowing significantly affected soil methane uptake in this site (Figure 2.2c).

Carbon dioxide fluxes in the fields

In situ carbon dioxide production ranged from 20 to 335 mg CO_2-C m^{-2} h^{-1} among the different sites and treatments. Before treatment, there were significantly different ($P<0.0001$) CO_2 fluxes among sites (Table 2.3 and 2.4); CO_2 flux was highest in mature forest followed closely by mid-successional communities then no-till corn field (Figure 2.4). However, after treatment CO_2 fluxes were highest in mid-successional communities (124.9 ±13.1 mg CO_2-C m^{-2} h^{-1}) followed closely by mature deciduous forest (108.9 ± 7.7 mg CO_2-C m^{-2} h^{-1}) and no-till cornfield (104.9 ± 9.2 mg CO_2-C m^{-2} h^{-1}) in control plots (Table 2.2 and Figure 2.3).

Although treatment had overall no significant effect on CO_2 fluxes (Table 2.7), N fertilizer significantly increased rates of CO_2 production in both mid-successional communities and mature forests but not in no-till cornfield (Figure 2.3 and Table 2.2). Fertilizer x plowing also had the same effect on CO_2 production as fertilizer alone. In contrast, plowing alone did not show any significant effect on soil CO_2 fluxes in any site (Table 2.2 and Figure 2.3).

Early in the experiment, CO_2 fluxes in the mid-successional and mature forests were greater than in the no-till cornfield, but these differences were not persistent (Figure 2.4). Carbon dioxide fluxes likely differed among dates of sampling ($P=0.059$; Table 2.7), however CO_2 fluxes in mature and mid-successional communities decreased modestly after treatment and increased significantly at day 52 then declined drastically,

whereas in the no-till corn site CO_2 fluxes varied very little among the sampling dates (Figure 2.4).

Soil physical and chemical factors

Average soil moisture (0-10 cm depth) was highest in mature forests (18 g H_2O· 100 g $soil^{-1}$) followed by mid-successional communities (0.9 g H_2O·100 g $soil^{-1}$) and no-till cornfields (1.0 g H_2O·100 g $soil^{-1}$) respectively (Table 2.2). Soil moisture did not vary significantly among treatments and likely differed (P=0.053) across sample dates (Table 2.8 and Figure 2.5). It was highest after rainfall at day 16 and 52.

Soil temperature was, on average, 2-4 °C cooler in the mature forests (16°C average) than the mid-successional communities (18 °C) and no-till cornfield (20°C; Table 2.2). Temperature dropped over the course of the experiment, but the relative ranking of the sites remained unchanged (Figure 2.6). There were no treatment effects on soil temperature and it significantly varies among sample dates (Table 2.8).

Soil nitrate in control plots was significantly different among sites (Table 2.8). It was highest in no-till cornfields (30.4 ± 7.4 μg NO_3^--N g $soil^{-1}$), followed by mature forests (4.1 ± 0.7 μg NO_3^--N g $soil^{-1}$), and mid-successional communities (0.5 ± 0.1 μg NO_3^--N g $soil^{-1}$) (Table 2.2). Fertilizer also significantly increased soil nitrate (Table 2.7). Nitrate was highest in fertilized x plow plots followed by fertilized plots and control plots. Levels of soil nitrate dramatically increased after fertilizer application in every site and then declined rapidly until reaching pre-fertilization levels at day 73 for mature forests and day 52 for mid-successional communities (Figure 2.7). However, nitrate in no-till cornfield soils remained high during the sampling period.

Unlike nitrate, soil ammonium did not vary by site (Table 2.8). Average soil ammonium levels in control plots were similar for all sites (ca. 22.2 μg NH_4^+-N g $soil^{-1}$; Table 2.2). Treatment effects were significant, similar to those for nitrate (Table 2.8), with fertilized treatments having an order of magnitude more N than control and plowed treatments, which did not differ. The temporal patterns of soil ammonium were also similar to those for nitrate (Figure 2.8).

Controls on Methane and Carbon Dioxide Fluxes

Methane had a very strong and significant correlation ($r=0.70$, $P<0.01$, $n=3$ forest sites x 3 replications x 4 plots) with CO_2 initially but it disappeared after treatment (Table 2.9 and Figure 2.9).

Before treatment, temperature seemed to be a significant factor affecting the rate of soil CH_4 uptake (Table 2.4). However, after treatment no soil factor showed a significant effect on CH_4 flux (Table 2.5).

Only soil temperature had a significant effect on CO_2 fluxes before treatment (Table 2.6) but only soil moisture significantly affected CO_2 after treatment (Table 2.7). Moisture was also strongly correlated with CO_2 (Table 2.9). Even though soil temperature, nitrate, and ammonium were significantly correlated with CO_2 emission before treatment, only soil ammonium remained significantly but weakly correlated after treatment (Table 2.9).

Discussion

Methane Oxidation

Soil CH_4 oxidation was highest in mature forest, followed by mid-successional communities and no-till agriculture, respectively (Table 2.2 and Figure 2.1). Agricultural fluxes were less than 25% of those in the forests and successional sites. The rate decline along the successional gradient is similar to that observed in previous agriculture and natural vegetation comparisons (e.g. Mosier et al. 1991, Goulding et al. 1995, Arif et al. 1996, Powlson et al. 1997, Robertson et al. 2000, Hütsch 2001).

Before treatment, soil temperature appeared to be the most significant factor controlling differences in CH_4 oxidation among sites. Mature forest soils have significantly higher levels of total carbon, total nitrogen, and ammonium as compared to the no-till corn field soils, which had higher soil nitrate levels and greater bulk density (Table 2.1). The lower soil bulk density in the forest implies more gas diffusion, which has been shown to increase soil CH_4 uptake by methanotrophs in soil crumbs and soil aggregates (Ball et al. 1997a, Ball et al. 1997b). Mature deciduous forest soils were also acidic, which suggests acid-adapted CH_4 oxidizing bacteria in this forest.

However, after treatment, no physical or chemical factor had a significant effect on CH_4 uptake (Table 2.5). Across all treatments, mature forest soil still had more CH_4 oxidation than the no-till cornfield soils, while mid-successional communities soils were intermediate (Figure 2.1) and also had higher soil moisture, lower soil temperature, and less soil nitrate than the mid-successional communities and no-till cornfield (Table 2.2).

Fertilizer (100 kg N ha^{-1}) markedly inhibited soil CH_4 consumption up to 60% in mature forest soils and to 40% in mid-successional communities soils (Table 2.2). These results were similar to most previous studies (Bender and Conrad 1994, King and Schnell 1994, Hütsch 1996, Gulledge et al. 1997, King and Schnell 1998), indicating the

detrimental effect of inorganic N on CH_4 oxidation. However, added nitrogen did not further suppress CH_4 oxidation in no-till cornfield as found in some studies (Mosier and Schimel 1991, Bronson and Mosier 1993, Tate and Striegl 1993, Mosier et al. 1998), probably due to the already high soil nitrate and ammonium levels in these soils.

Soil tillage alone did not show any significant effect on soil CH_4 uptake in any of these sites, but faintly increased CH_4 oxidation in mature forest and cornfields and faintly decreased CH_4 uptake in mid-successional communities (Figure 2.1). The depth of tillage in this study was about 10 cm less than the 20 cm depth of normal tillage, and thus has a less destructive effect on soil structure than normal agricultural tillage, but nevertheless represents a strong disturbance my results were similar to those from other studies in tropical savanna (Sanhueza et al. 1994), Piedmont cornfields (Burke et al. 1999), and an acid oxisol site in Puerto Rico (Mosier et al. 1998).

Interestingly, although not statistically significant, soil tillage slightly alleviated the inhibition effect of N fertilizer in mature and mid-successional communities, especially after day 52 (Figure 2.2) when soil nitrate and ammonium had already declined markedly (Figure 2.7 and 2.8). The tillage might have increased soil aeration in these plots allowing a greater flow of CH_4 into soil micro sites and providing CH_4 oxidizing bacteria more access to the gas.

Soil CH_4 consumption in this study showed some seasonal changes ($P=0.065$) over the course of this 101-day investigation (Table 2.5). This suggests that fertilizer has a stronger effect on soil CH_4 consumption than other seasonal physical factors such as soil temperature and moisture in this study.

That CH_4 oxidation was strongly and positively related to CO_2 production before fertilization (Table 2.9) indicates that conditions were favorable for both soil respiration and CH_4 oxidation.

Carbon dioxide

Carbon dioxide fluxes differed among sites only after treatment (Table 2.6 and 2.7). Even though treatments did not significantly affect CO_2 fluxes (Table 2.7), the fertilizer and fertilizer x plow combination plots in mid-successional and mature forests had significantly higher CO_2 production, approximately 25% more than control and plow-alone plots (Table 2.2 and Figure 2.3). In no-till cornfield plow and N fertilizer treatments had no noteworthy effect on soil CO_2 emission.

Soil moisture was the most important positive factor affecting soil CO_2 emission (Table 2.7 and 2.9).

Conclusion

The mature forest soils had the highest overall methane oxidation rates, followed by mid-successional and agricultural systems, respectively. Nitrogen added to the forest and mid-successional sites substantially reduced methane oxidation within 2 weeks of nitrogen addition. In the no-till site, where oxidation rates were already low and soil nitrogen levels already high, additional nitrogen had no effect on soil CH_4 uptake. Plowing had no detectable effect on methane oxidation in any of the three sites. N fertilizer also significantly increased CO_2 emission in mature forest and mid-successional communities. The impact of agriculture on methane oxidation is likely due primarily to

greater nitrogen availability via N fertilization rather than to the disruption of soil structure or other effects of plowing. Increasing nitrogen inputs to mid-successional and mature ecosystems reduces rates of oxidation that would otherwise be relatively high.

References

Ambus, P., and S. Christensen. 1995. Spatial and seasonal nitrous oxide and methane fluxes in Danish forest ecosystems, grassland-ecosystems, and agroecosystems. Journal of Environmental Quality **24**:993-1001.

Arif, M. A. S., F. Houwen, and W. Verstraete. 1996. Agricultural factors affecting methane oxidation in arable soil. Biology and Fertility of Soils **21**:95-102.

Austin, F. R. 1979. Soil Survey of Kalamazoo County, Michigan. United States Department of Agriculture, Soil Conservation Service, in cooperation with Michigan Agricultural Experiment Station. The Soil Conservation Service, Washington.

Ball, B. C., K. E. Dobbie, J. P. Parker, and K. A. Smith. 1997a. The influence of gas transport and porosity on methane oxidation in soils. Journal of Geophysical Research-Atmospheres **102**:23301-23308.

Ball, B. C., K. A. Smith, L. Klemedtsson, R. Brumme, B. K. Sitaula, S. Hansen, A. Prieme, J. MacDonald, and G. W. Horgan. 1997b. The influence of soil gas transport properties on methane oxidation in a selection of northern European soils. Journal of Geophysical Research-Atmospheres **102**:23309-23317.

Bender, M., and R. Conrad. 1994. Microbial oxidation of methane, ammonium and carbon monoxide, and turnover of nitrous oxide and nitric oxide in soils. Biogeochemistry **27**:97-112.

Bronson, K., and A. Mosier. 1993. Nitrous oxide emissions and methane consumption in wheat and corn-cropped systems in northeastern Colorado. Pages 133-144 *in* L. A. Harper, A. Mosier, J. Duxbury, and D. E. Rolston, editors. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. American Society of Agronomy, Madison.

Bronson, K. F., and A. R. Mosier. 1994. Suppression of methane oxidation in aerobic soil by nitrogen fertilizers, nitrification inhibitors, and urease inhibitors. Biology and Fertility of Soils **17**:263-268.

Bundy, L. G., and J. J. Meisinger. 1994. Nitrogen availability indices. Pages 951-984 *in* R. W. Weaver, J. S. Angle, and B. S. Bottomley, editors. Methods of soil analysis. Part 2. Microbiological and biochemical properties. SSSA Book Series, no. 5., Madison, WI.

Burke, R. A., J. L. Meyer, J. M. Cruse, K. M. Birkhead, and M. J. Paul. 1999. Soil-atmosphere exchange of methane in adjacent cultivated and floodplain forest soils. Journal of Geophysical Research-Atmospheres **104**:8161-8171.

Castro, M. S., P. A. Steudler, J. M. Melillo, J. D. Aber, and R. D. Bowden. 1995. Factors controlling atmospheric methane consumption by temperate forest soils. Global Biogeochemical Cycles **9**:1-10.

Cochran, V. L., E. B. Sparrow, S. F. Schlentner, and C. W. Knight. 1997. Long-term tillage and crop residue management in the subarctic: fluxes of methane and nitrous oxide. Canadian Journal of Soil Science **77**:565-570.

Crill, P. M., P. J. Martikainen, H. Nykanen, and J. Silvola. 1994. Temperature and N-fertilization effects on methane oxidation in a drained peatland soil. Soil Biology & Biochemistry **26**:1331-1339.

Crum, J. R., and H. P. Collins. 2003. KBS soils *in* http://lter.kbs.msu.edu/Soil/characterization/.

Goulding, K., T. Willison, C. Webster, and D. Powlson. 1996. Methane fluxes in aerobic soils. Environment Monitoring Assessment **42**:175-187.

Goulding, K. W. T., B. W. Hütsch, C. P. Webster, T. W. Willison, and D. S. Powlson. 1995. The effect of agriculture on methane oxidation in soil. Philosophical Transactions of the Royal Society of London Series a-Mathematical Physical and Engineering Sciences **351**:313-324.

Gulledge, J., A. P. Doyle, and J. P. Schimel. 1997. Different NH_4^+-inhibition patterns of soil CH_4 consumption: A result of distinct CH_4-oxidizer populations across sites? Soil Biology & Biochemistry **29**:13-21.

Hansen, S., J. E. Maehlum, and L. R. Bakken. 1993. N_2O and CH_4 fluxes in soil influenced by fertilization and tractor traffic. Soil Biology & Biochemistry **25**:621-630.

Hilger, H. A., A. G. Wollum, and M. A. Barlaz. 2000. Landfill methane oxidation response to vegetation, fertilization, and liming. Journal of Environmental Quality **29**:324-334.

Hutchinson, G. L., and G. P. Livingston. 1993. Use of chamber systems to measure trace gas fluxes. Pages 63-78 *in* L. A. Harper, A. R. Mosier, J. M. Duxbury, and D. E.

Rolston, editors. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. American Society of Agronomy, Madison, Wisconsin.

Hütsch, B. W. 1996. Methane oxidation in soils of two long-term fertilization experiments in Germany. Soil Biology & Biochemistry **28**:773-782.

Hütsch, B. W. 2001. Methane oxidation in non-flooded soils as affected by crop production. European Journal of Agronomy **14**:237-260.

Hütsch, B. W., C. P. Webster, and D. S. Powlson. 1993. Long-term effects of nitrogen-fertilization on methane oxidation in soil of the Broadbalk Wheat Experiment. Soil Biology & Biochemistry **25**:1307-1315.

Hütsch, B. W., C. P. Webster, and D. S. Powlson. 1994. Methane oxidation in soil as affected by land-use, soil-pH and N-fertilization. Soil Biology & Biochemistry **26**:1613-1622.

IPCC. 2001. Climate Change 2001; the Scientific Basis. Cambridge University Press, Cambridge.

King, G. M., and S. Schnell. 1994. Ammonium and nitrite inhibition of methane oxidation by *Methylobacter albus* BG8 and *Methylosinus trichosporium* OB3b at low methane concentrations. Applied and Environmental Microbiology **60**:3508-3513.

King, G. M., and S. Schnell. 1998. Effects of ammonium and non-ammonium salt additions on methane oxidation by *Methylosinus trichosporium* OB3b and Maine forest soils. Applied and Environmental Microbiology **64**:253-257.

Kruse, C. W., and N. Iversen. 1995. Effect of plant succession, plowing, and fertilization on the microbiological oxidation of atmospheric methane in a heathland soil. Fems Microbiology Ecology **18**:121-128.

MacDonald, J. A., U. Skiba, L. J. Sheppard, K. J. Hargreaves, K. A. Smith, and D. Fowler. 1996. Soil environmental variables affecting the flux of methane from a range of forest, moorland and agricultural soils. Biogeochemistry **34**:113-132.

Mosier, A., J. A. Delgado, and M. Keller. 1998. Methane and nitrous oxide fluxes in an acid oxisol in western Puerto Rico; effects of tillage, liming, and fertilization. Soil Biology & Biochemistry **30**:2087-2098.

Mosier, A., and D. Schimel. 1991. Influence of agricultural nitrogen on atmospheric methane and nitrous oxide. Chemical Industry **24**:334-336.

Mosier, A., D. Schimel, D. Valentine, K. Bronson, and W. Parton. 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature **350**:330-332.

Mosier, A. R., J. A. Delgado, V. L. Cochran, D. W. Valentine, and W. J. Parton. 1997a. Impact of agriculture on soil consumption of atmospheric CH_4 and a comparison of CH_4 and N_2O flux in subarctic, temperate, and tropical grasslands. Nutrient Cycling in Agroecosystems **49**:71-83.

Mosier, A. R., W. J. Parton, D. W. Valentine, D. S. Ojima, D. S. Schimel, and O. Heinemeyer. 1997b. CH_4 and N_2O fluxes in the Colorado shortgrass steppe .2. Long- term impact of land use change. Global Biogeochemical Cycles **11**:29-42.

Powlson, D. S., K. W. T. Goulding, T. W. Willison, C. P. Webster, and B. W. Hütsch. 1997. The effect of agriculture on methane oxidation in soil. Nutrient Cycling in Agroecosystems **49**:59-70.

Prieme, A., and S. Christensen. 1999. Methane uptake by a selection of soils in Ghana with different land use. Journal of Geophysical Research-Atmospheres **104**:23,617-623,622.

Robertson, G. P., E. A. Paul, and R. R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science **289**:1922-1925.

Sanhueza, E., L. Cardenas, L. Donoso, and M. Santana. 1994. Effect of plowing on CO_2, CO, CH_4, N_2O, and NO fluxes from tropical savannah soils. Journal of Geophysical Research-Atmospheres **99**:16,429-416,434.

SAS Institute. 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc., Cary, NC.

SPSS Inc. 2001. SPSS Base 11.0 for Windows User's Guide. SPSS Inc.

Steudler, P. A., R. D. Bowden, J. M. Melillo, and J. D. Aber. 1989. Influence of nitrogen fertilization on methane uptake in temperate forest soil. Nature **341**:314-316.

Tate, C. M., and R. G. Striegl. 1993. Methane consumption and carbon dioxide emission in tallgrass prairie - Effects of biomass burning and conversion to agriculture. Global Biogeochemical Cycles **7**:735-748.

Tlustos, P., T. W. Willison, J. C. Baker, D. V. Murphy, D. Pavlikova, K. W. T. Goulding, and D. S. Powlson. 1998. Short-term effects of nitrogen on methane oxidation in soils. Biology and Fertility of Soils **28**:64-70.

Topp, E. M., M. P. Maila, M. Clerinx, J. Goris, P. De Vos, and W. Verstraete. 1999. Methane oxidation as a method to evaluate the removal of 2,4-

dichlorophenoxyacetic acid (2,4-D) from soil by plasmid- mediated bioaugmentation. Fems Microbiology Ecology **28**:203-213.

Willison, T. W., K. Goulding, and D. Powlson. 1995a. Effect of land use change and methane mixing ratio on methane uptake from United Kingdom soil. Global Change Biology **1**:209-212.

Willison, T. W., C. P. Webster, K. W. T. Goulding, and D. S. Powlson. 1995b. Methane oxidation in temperate soils - Effects of land-use and the chemical form of nitrogen fertilizer. Chemosphere **30**:539-546.

Table 2.1 Soil properties for the mature deciduous forest, coniferous forest, mid-successional communities, and no-till agricultural field at the KBS LTER site.

Soil property	Deciduous forest	Mid-successional communities	No-till cornfield
Texture	Sandy loam	Sandy loam	Sandy loam
pH[1]	5.20 ± 0.12 a	5.66 ± 0.04 b	6.45 ± 0.09 c
Bulk density (g cm^{-3})[2]	1.22 ± 0.08 a	1.40 ± 0.04 b	1.57 ± 0.06 bc
Total carbon (mg C g $soil^{-1}$)[3]	1.52 ± 0.12 a	0.97 ± 0.03 ab	0.73 ± 0.09 b
Total nitrogen (mg N g $soil^{-1}$)[3]	0.123 ± 0.09 a	0.093 ± 0.003 a	0.087 ± 0.009 b
Nitrate (μg N g $soil^{-1}$)[4]	1.93 ± 0.21 a	0.38 ± 0.05 b	2.79 ± 0.30 c
Ammonium (μg N g $soil^{-1}$)[4]	3.50 ± 0.37 ab	4.46 ± 0.58 a	2.08 ± 0.73 b

Note: Values are means (±s.e., n=varied). Data are from the KBS LTER website http://kbs.msu.edu/lter/.

[1] sampled 8 March 2000 to a depth of 10 cm.

[2] sampled 10 April 1996 to a depth of 15 cm.

[3] sampled 7 April 1999 to a depth of 25 cm.

[4] average values from 2000 season to a depth of 25 cm.

Table 2.2 The effects of tillage and N fertilizer on average methane oxidation, carbon dioxide flux, and soil properties in mature deciduous forest, mid-successional communities, and no-till agriculture at the KBS LTER site.

	Control	Tillage	Fertilizer	Fertilizer x Tillage
CH_4 ($\mu g\ CH_4$-C $m^{-2}\ h^{-1}$)				
Mature forest	31.6±3.2 Aa	33.7±3.6 Aa	12.7±1.8 ABb	14.8±2.1 Ab
Mid successional communities	23.7±1.6 Aa	22.0±2.3 Ba	14.8±1.8 Ab	16.1±1.7 Ab
No-till cornfield	4.0±0.7 B	5.0±1.1 C	4.3±1.1 B	2.3±1.3 B
CO_2 (mg CO_2-C $m^{-2}\ h^{-1}$)				
Mature forest	108.9±7.7a	118.8±9.6a	135.9±13.1 Ab	132.7±12.7 Ab
Mid successional communities	124.9±13.1a	118.2±14.8a	153.8±13.6 Ab	156.3±15.5 Ab
No-till cornfield	104.9±9.2	95.6±9.5	83.1±7.4 B	91.5±9.8 B
Moisture (g H_2O·100g $soil^{-1}$)				
Mature forest	17.6±1.5 A	17.9±1.8 A	20.1±1.6 A	18.5±1.6 A
Mid successional communities	9.1±0.9 B	9.4±1.0 B	8.4±0.8 B	7.9±0.8 B
No-till cornfield	9.7±0.6 B	10.1±0.5 B	10.8±0.5 B	10.6±0.6 B
Temperature (°C)				
Mature forest	16.3±0.6 AB	16.3±0.6 AB	16.3±0.6 AB	16.3±0.6 AB
Mid successional communities	18.6±0.5 AB	18.6±0.5 AB	18.6±0.5 AB	18.6±0.5 AB
No-till cornfield	20.4±0.5 BC	20.4±0.5 BC	20.4±0.5 BC	20.4±0.5 BC
Nitrate ($\mu g\ NO_3^-$-N $g\ soil^{-1}$)				
Mature forest	4.1±0.7 a	6.1±1.1 a	105.2±19.9ABb	154.0±33.7 b
Mid successional communities	0.5±0.1 a	1.2±0.3 a	77.3±22.9 Aab	137.7±41.6 b
No-till cornfield	30.4±7.4 a	19.7±8.2 a	176.2±25.6 Bb	181.5±17.9 b
Ammonium ($\mu g\ NH_4^+$-N $g\ soil^{-1}$)				
Mature forest	21.3±2.8 a	22.0±3.5 a	101.8±20.1 ab	181.2±38.4 b
Mid successional communities	24.9±5.4 a	26.9±4.3 a	98.2±19.6 ab	180.3±42.7 b
No-till cornfield	20.3±4.7 ab	27.2±8.2 abc	124.4±25.0 bcd	161.8±28.0 cd

Note: Values are means ± standard error of mean for four replicates.
Values followed by different higher case letters are significantly different ($P<0.05$) among sites.
Values followed by different lower case letters are significantly different ($P<0.05$) among treatments within a site.

Table 2.3 Analysis of variance to determine effects of sites on methane oxidation rate, carbon dioxide emission, and soil properties before treatment in mature deciduous forest, the mid-successional community, and the no-till agricultural field at the KBS LTER site.

		SS	df	MS	F	P
CH_4	Between Groups	8444	2	4222	36	<0.0001
	Within Groups	3812	33	115		
	Total	12257	35			
CO_2	Between Groups	87304	2	43652	43	<0.0001
	Within Groups	33162	33	1005		
	Total	120466	35			
Nitrate	Between Groups	790	2	395	59	<0.0001
	Within Groups	219	33	7		
	Total	1010	35			
Ammonium	Between Groups	3637	2	1819	11	<0.0001
	Within Groups	5235	33	159		
	Total	8873	35			
Moisture	Between Groups	1830	2	915	35	<0.0001
	Within Groups	866	33	26		
	Total	2697	35			
Temperature	Between Groups	640	2	320	124	<0.0001
	Within Groups	85	33	2		
	Total	726	35			

Table 2.4 Analysis of covariance to determine effects of site and soil properties on methane oxidation rate before treatment in mature deciduous forest, the mid-successional community, and the no-till agricultural field at the KBS LTER site.

Source	SS	df	MS	F	P
Site	1.8	2	0.88	6.2	0.008
Treatment	0.1	3	0.05	0.3	0.808
Site x treatment	0.6	6	0.09	0.7	0.676
Nitrate	0.4	1	0.36	2.5	0.127
Ammonium	0.008	1	0.008	0.06	0.810
Moisture	0.3	1	0.3	1.9	0.180
Temperature	0.8	1	0.8	5.6	0.028
Error	2.8	20	0.14		
Total	442	36			
Corrected Total	16.2	35			

Table 2.5 Analysis of variance to determine effects of site, treatment, and soil properties on methane oxidation rate after treatment in mature deciduous forest, the mid-successional community, and the no-till agricultural field at the KBS LTER site.

Variable	Num. df [a]	Den. df [a]	F	P
Site	2	230	105	<0.0001
Treatment	3	230	14.9	<0.0001
Site x Treatment	6	230	3.8	0.001
Day	6	230	1.5[b]	0.065
Site x Treatment x Day	36	230	-0.1[b]	0.916
Nitrate	1	230	0.2	0.629
Ammonium	1	230	0.1	0.808
Moisture	1	230	2.4	0.123
Temperature	1	230	0.1	0.777

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] Z value

Table 2.6 Analysis of covariance to determine effects of site, and soil properties on carbon dioxide emission before treatment in mature deciduous forest, the mid-successional community, and the no-till agricultural field at the KBS LTER site.

Source	SS	df	MS	F	P
Site	0.2	2	0.1	1.6	0.220
Treatment	0.1	3	0.04	0.6	0.623
Site x treatment	0.08	6	0.01	0.2	0.970
Nitrate	0.002	1	0.002	0.04	0.843
Ammonium	0.001	1	0.001	0.01	0.898
Moisture	0.03	1	0.03	0.46	0.504
Temperature	0.27	1	0.27	4.3	0.050
Error	1.26	20	0.06		
Total	852	36			
Corrected Total	9.2	35			

Table 2.7 Analysis of covariance to determine effects of site, treatment, and soil properties on carbon dioxide emission after treatment in mature deciduous forest, the mid-successional community, and the no-till agricultural field at the KBS LTER site.

Variable	Num. df [a]	Den. df [a]	F	P
Site	2	230	17.6	<0.0001
Treatment	3	230	0.7	0.573
Site x Treatment	6	230	2.2	0.045
Day	6	230	1.6[b]	0.059
Site x Treatment x Day	36	230	1.9[b]	0.058
Nitrate	1	230	2.6	0.109
Ammonium	1	230	3.3	0.071
Moisture	1	230	24.0	<0.0001
Temperature	1	230	1.1	0.295

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] Z value

Table 2.8 Analysis of variance to determine effects of site, treatment, and day on soil properties from mature deciduous forest, the mid-successional community, and the no-till agricultural field at the KBS LTER site.

Variable	Num. df [a]	Den. df [a]	Moisture		Nitrate		Ammonium		Temperature	
			F	P	F	P	F	P	F	P
Site	2	234	156	<0.0001	3.8	0.023	0.0	0.990	48.2	<0.0001
Treatment	3	234	0.8	0.518	23.4	<0.0001	23.7	<0.0001	0.0	1.000
Site x treatment	6	234	1.0	0.429	0.47	0.827	0.3	0.953	0.0	1.000
Day	6	234	1.6[b]	0.053	1.4[b]	0.087	1.6[b]	0.061	1.6[b]	0.050
Site x treatment x day	36	234	-2.3[b]	0.022	10.9[b]	<0.0001	7.7[b]	<0.0001	9.1[b]	<0.0001

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively
[b] Z value

Table 2.9 Relationships among methane oxidation, carbon dioxide, and other soil properties before and after experimental treatment in mature deciduous forest, the mid-successional community, and the no-till agricultural sites. Values are Pearson correlation coefficients (r). [a] n = 3 sites x 3 replications x 4 sample plots. [b] n = 3 sites x 3 replications x 4 treatments x 7 sample dates.

	CO_2	Moisture	Temperature	Nitrate	Ammonium
Before treatment[a]					
CH_4	0.701**	-0.678**	-0.168	-0.401*	0.179
CO_2	1.00	0.536*	-0.831**	-0.336*	0.512**
After treatment[b]					
CH_4	0.054	0.094	-0.339**	-0.279**	-0.216**
CO_2	1.000	0.306**	0.009	0.055	0.170**

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

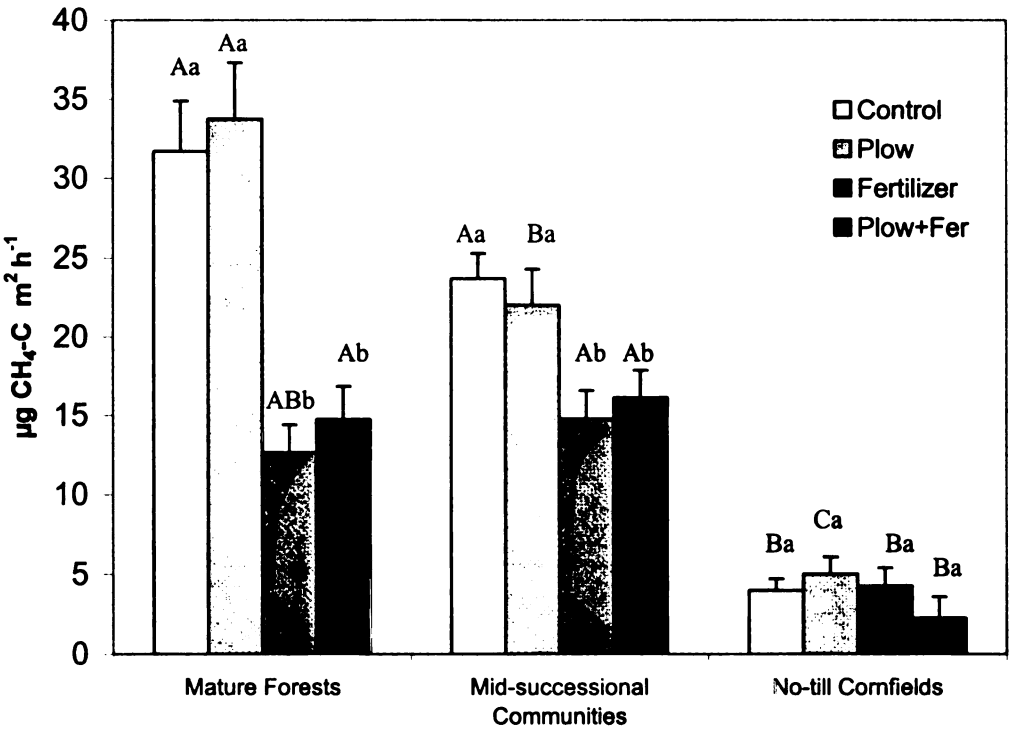


Figure 2.1 The reduction of methane oxidation due to soil disturbance and ammonium nitrate fertilizer (100 kg N ha^{-1}) in mature forests, mid-successional communities, and no-till cornfields at the KBS LTER site. Vertical bars are standard errors of mean (s.e., n = 3 sites x 7 sample dates). Different higher and lower case letters represent significant differences ($P<0.05$) of treatments among sites and within site respectively.

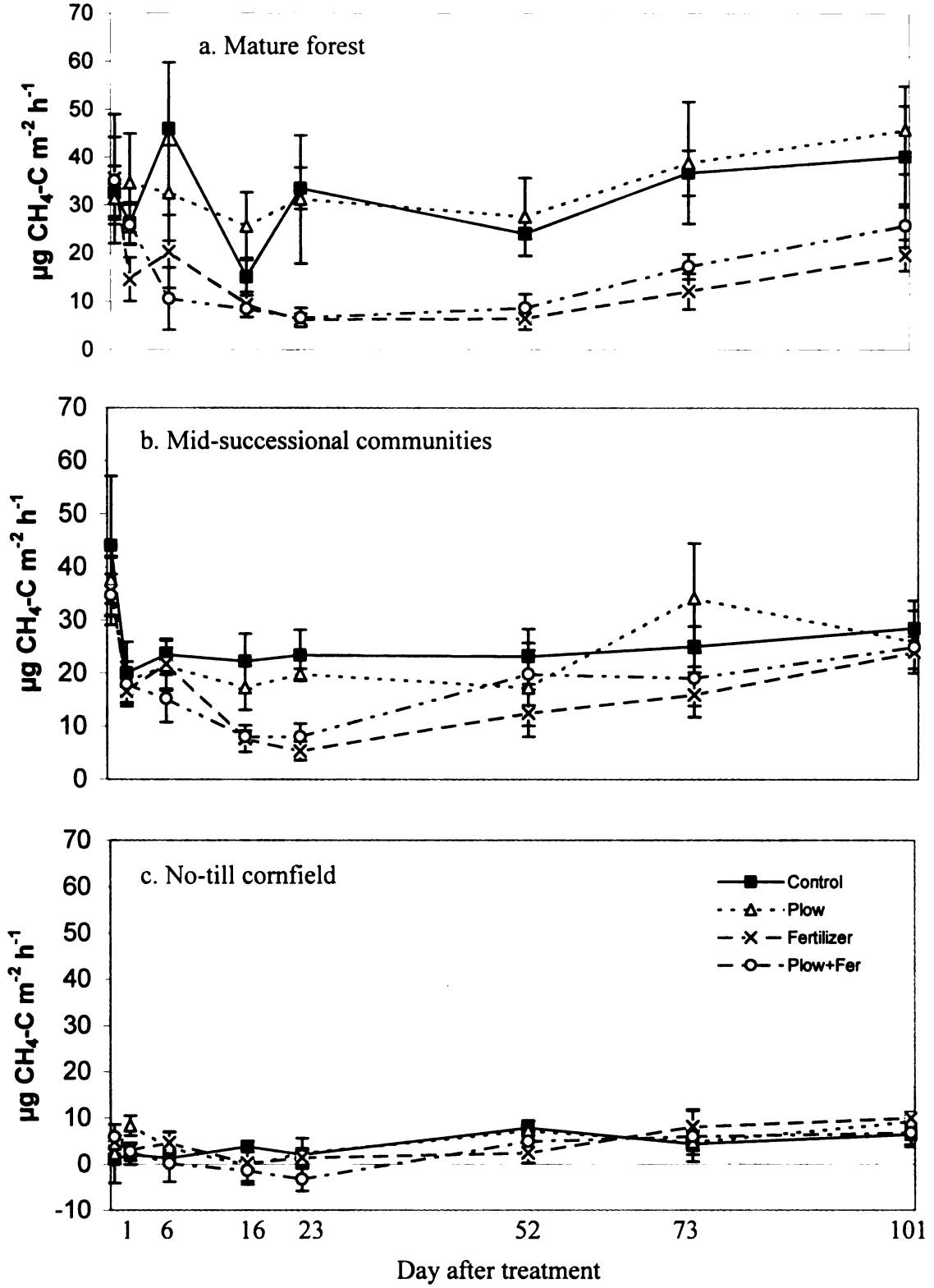


Figure 2.2 Average daily methane oxidation in the KBS LTER site: a) mature deciduous forests, b) mid-successional communities, c) no-till cornfields, with standard error bars (n=3 sites).

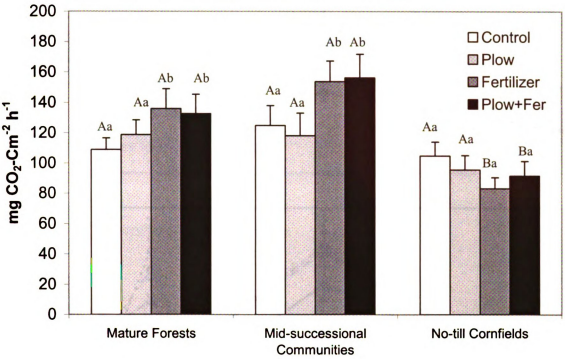


Figure 2.3 Net carbon dioxide fluxes due to soil disturbance, and ammonium nitrate fertilizer (100 kg N ha^{-1}) in mature forests, mid-successional communities, and no-till cornfields at the KBS LTER site. Vertical bars are standard errors of mean (s.e., n = 3 sites x 7 sample dates). Different higher and lower case letters represent significant differences ($P<0.05$) of the treatment among sites and among treatments within the same site, respectively.

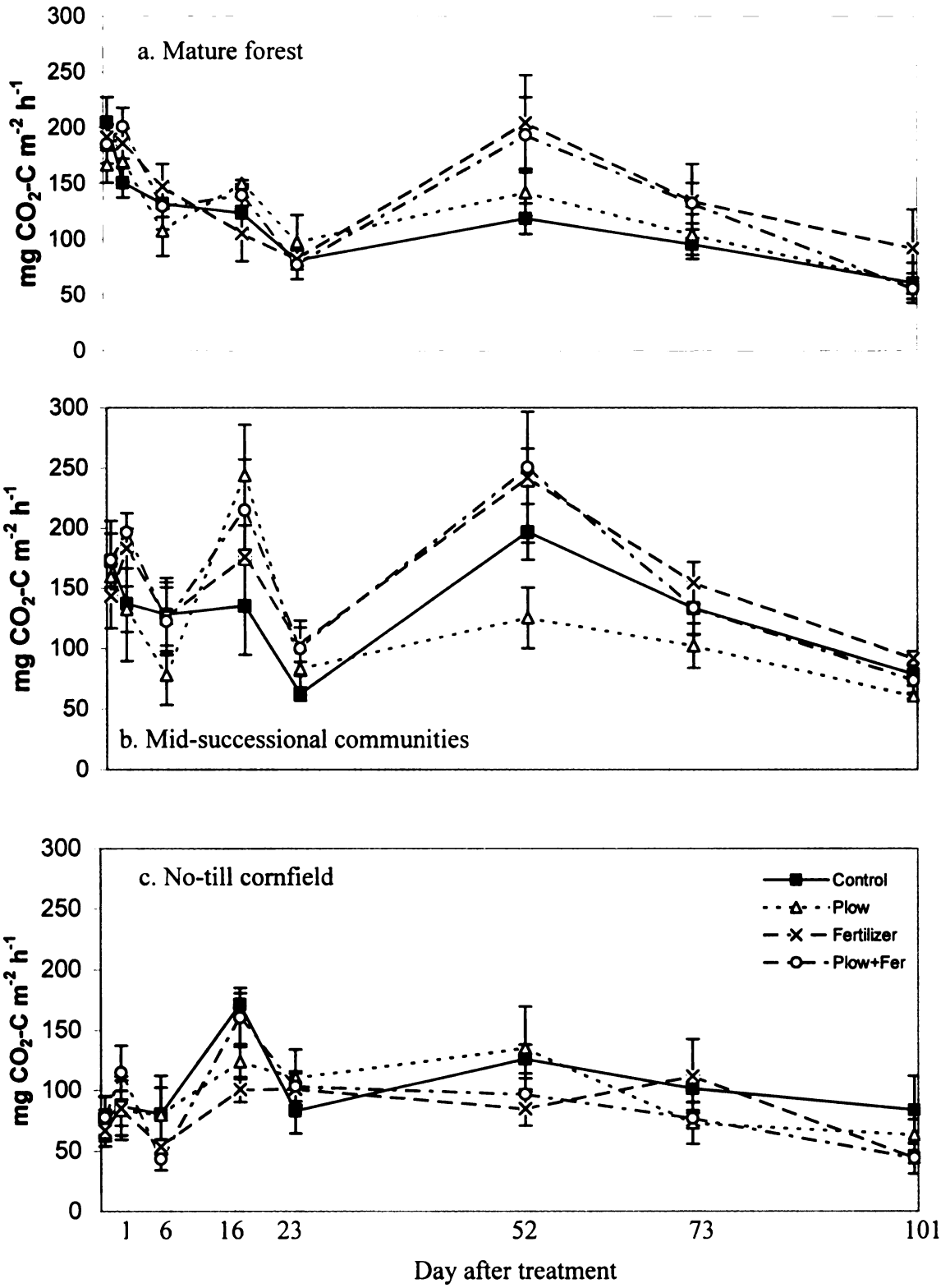


Figure 2.4 Average daily carbon dioxide fluxes in the KBS LTER site: a) mature deciduous forests, b) mid-successional communities, c) no-till cornfields, with standard error bars (n = 3 sites).

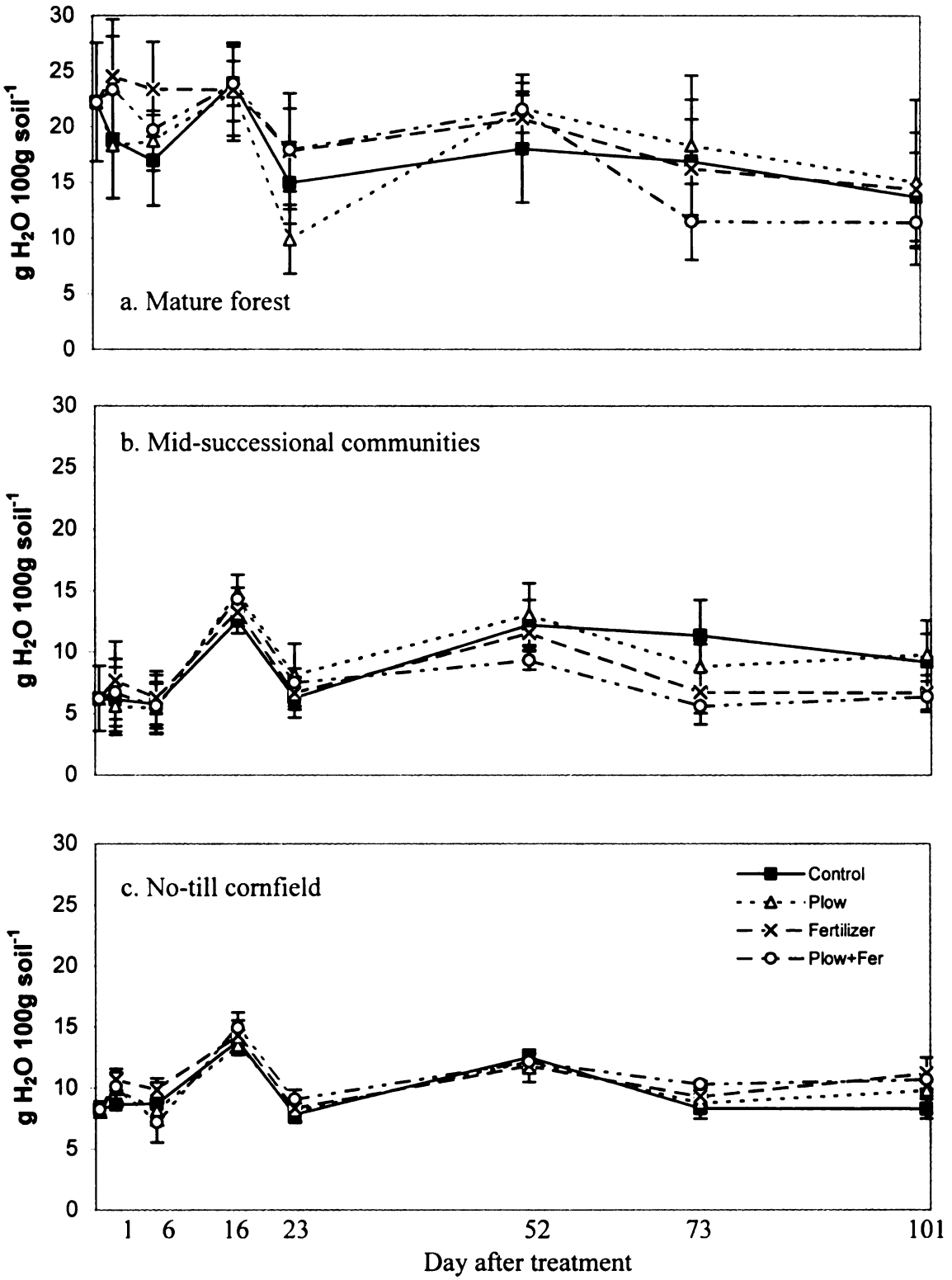


Figure 2.5 Average daily soil moisture in the KBS LTER site: a) mature deciduous forests, b) mid-successional communities, c) no-till cornfields, with standard error bars (n =3 sites).

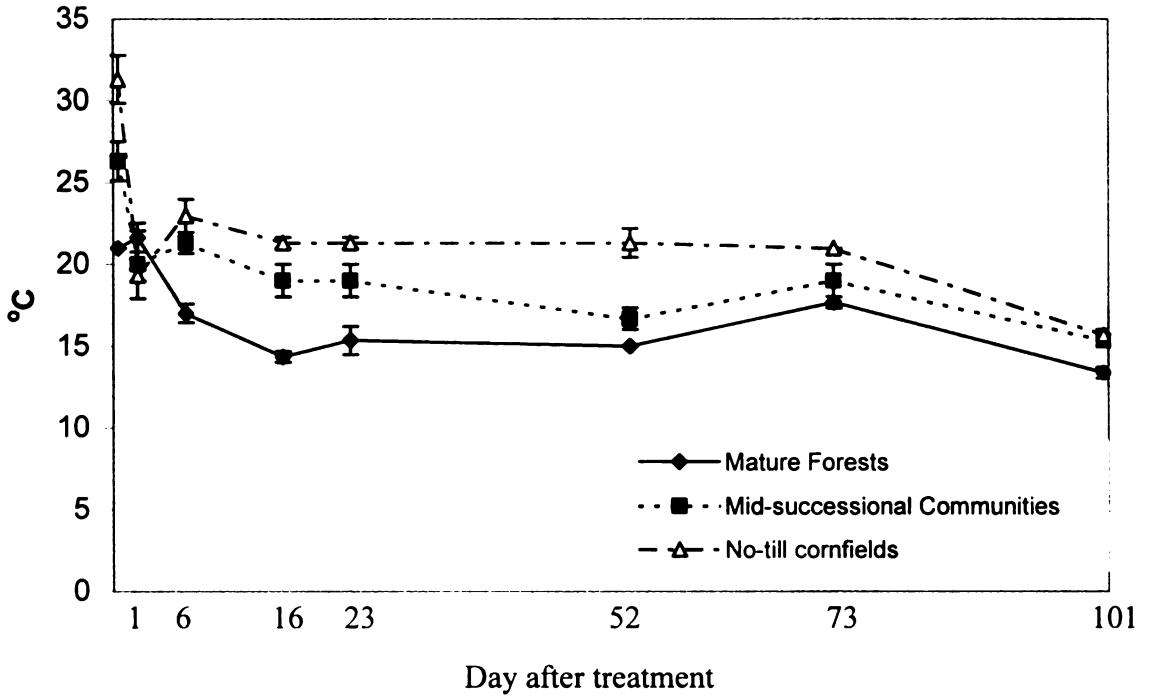


Figure 2.6 Average daily soil temperature in the KBS LTER site: a) mature deciduous forests, b) mid-successional communities, c) no-till cornfields, with standard error bars (n =3 sites).

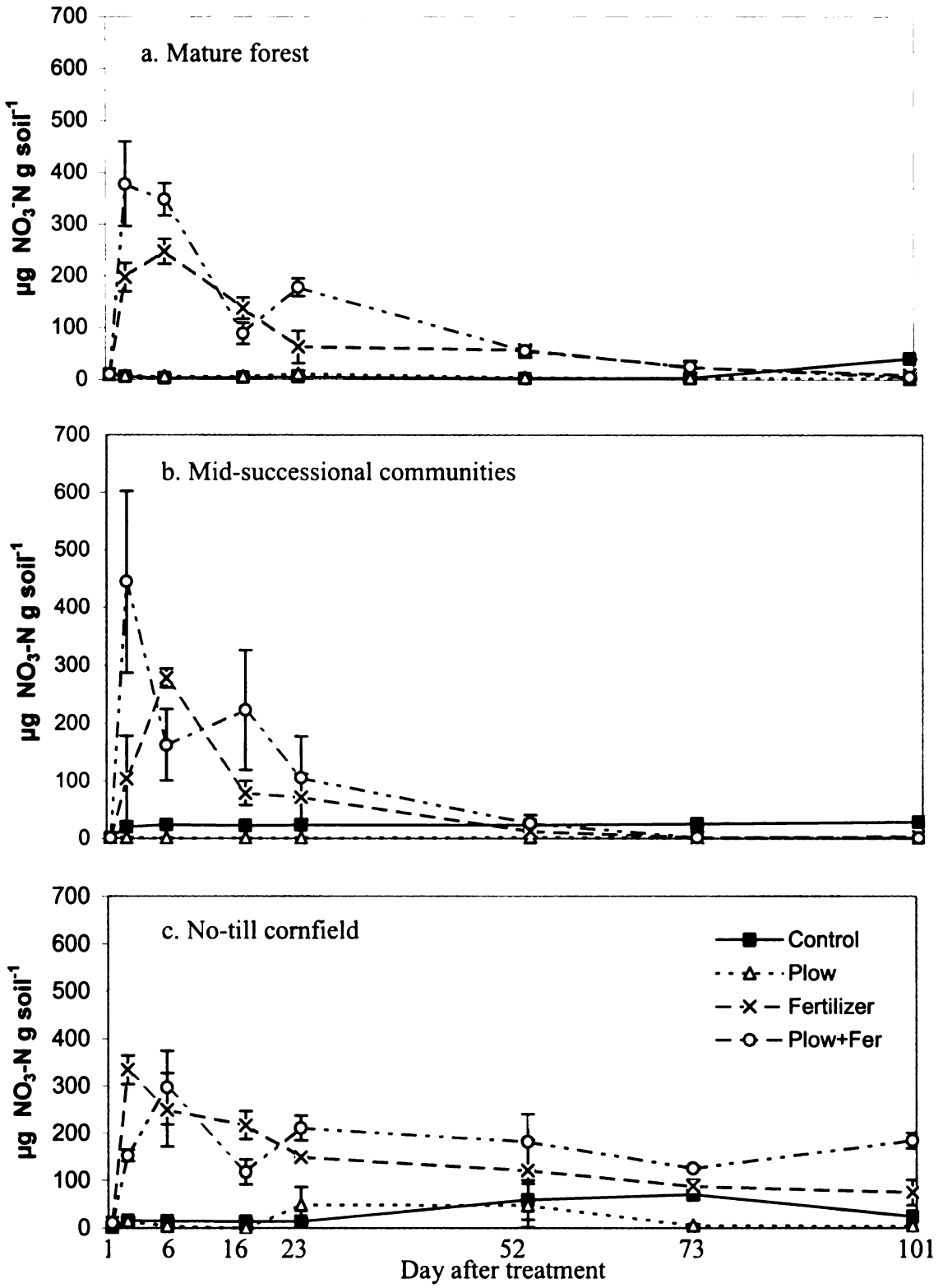


Figure 2.7 Average daily soil nitrate in the KBS LTER site: a) mature deciduous forests, b) mid-successional communities, c) no-till cornfields, with standard error bars (n = 3 sites).

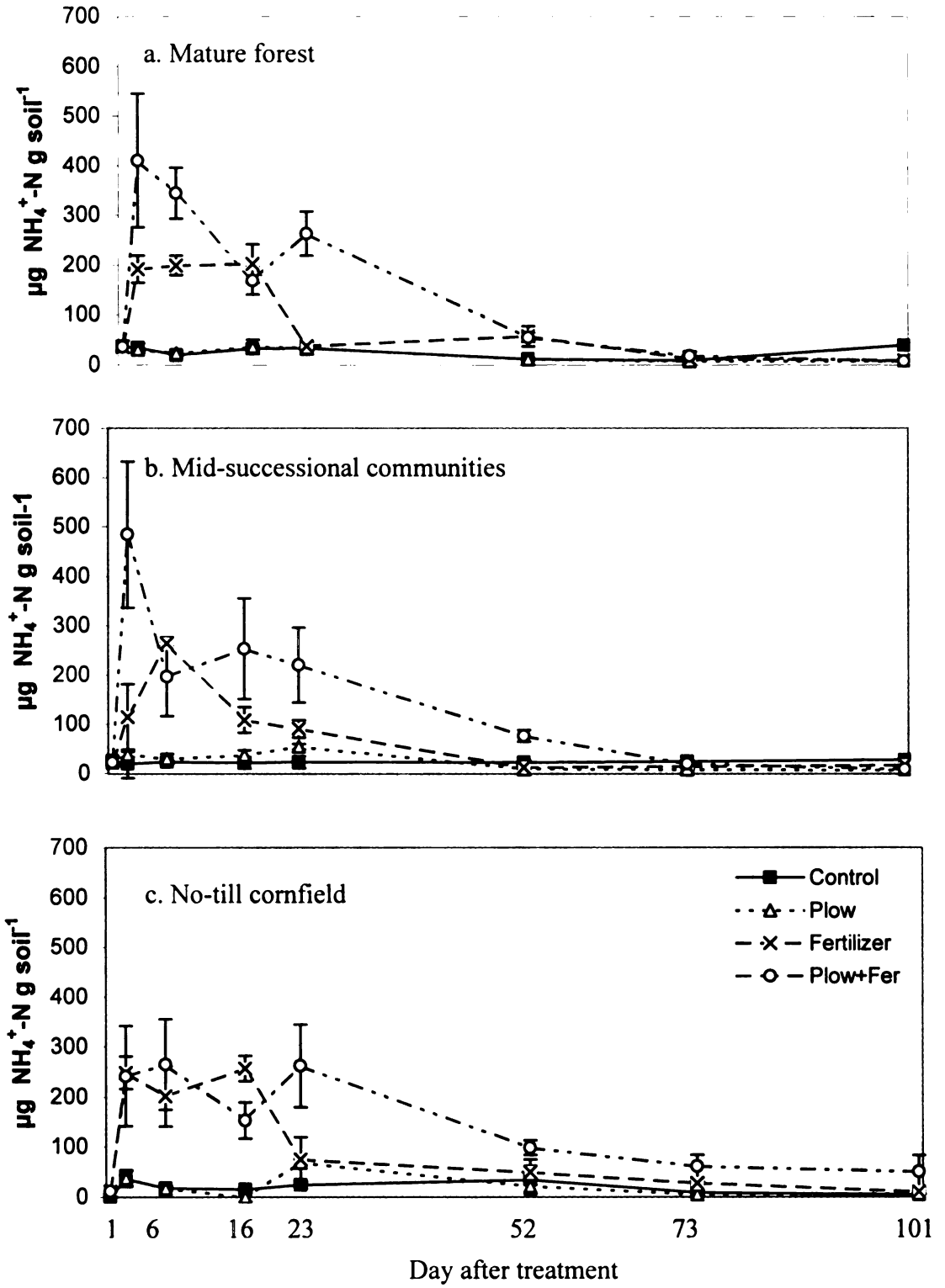


Figure 2.8 Average daily soil ammonium in the KBS LTER site: a) mature deciduous forests, b) mid-successional communities, c) no-till cornfields, with standard error bars (n=3 sites).

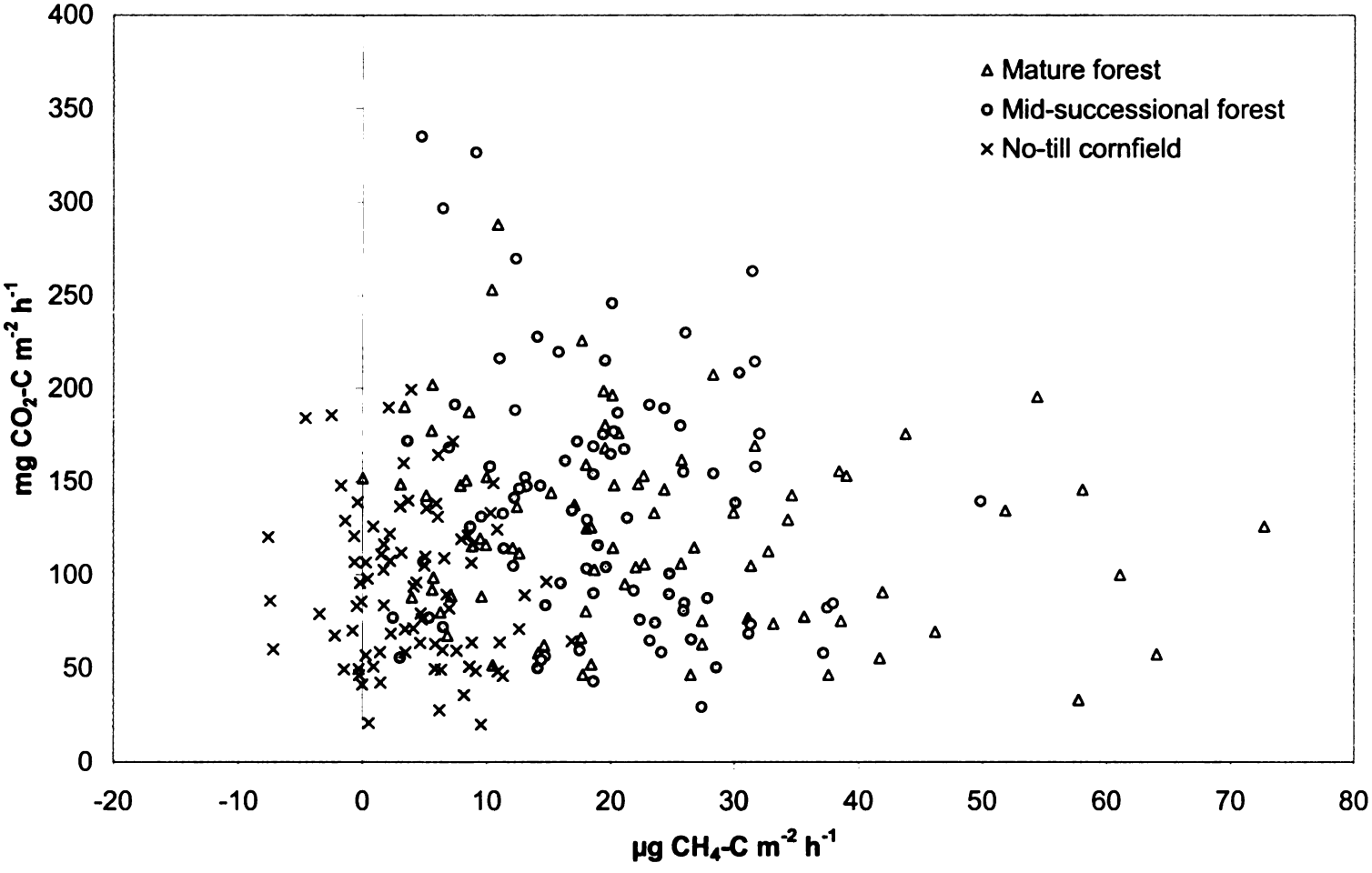


Figure 2.9 Relationship between methane oxidation and carbon dioxide flux from mature deciduous forest, the mid-successional communities, and the no-till agricultural field at the KBS LTER site.

Chapter 3

Soil Methane Oxidation in Organic vs. Conventional Agriculture

Introduction

The oxidation of methane by methanotrophs in soil is a small but significant sink of atmospheric methane. Previous studies show higher methane consumption rates in forest and prairie than in agricultural soils (Ambus and Christensen 1995, Willison et al. 1995a, Goulding et al. 1996, MacDonald et al. 1996, Prieme and Christensen 1999), suggesting a loss of soil oxidation capacity upon conversion to agriculture (Keller and Reiners 1994). However, oxidation rates have been reported only for row crops under conventional management (e.g.Willison et al. 1995b, Boeckx and van Cleemput 2001, Hütsch 2001). Fertilizer, especially nitrogen, has been demonstrated to significantly reduce soil CH_4 uptake (Hütsch 1996, Jambert et al. 1997, Hütsch 1998a, Tlustos et al. 1998). Many pesticides and herbicides also inhibit soil CH_4 oxidation to some degree (Topp 1993, Arif et al. 1996, Boeckx et al. 1998, Prieme and Ekelund 2001).

It is possible, then, that different management systems, in particular those that rely more on organic than synthetic sources for nutrients and pest control, may have a higher oxidation capacity. If so, then agricultural management for soil methane oxidation may be a practical means for helping to mitigate the buildup of atmospheric methane.

Robertson et al. (2000) found no differences between an organically managed corn-soybean-wheat system and a conventionally managed counterpart in Michigan, but they did not include compost or manure application, common in organic farming.

The aim of this study is to compare the effects of conventional vs. organic agricultural practices on methane oxidation, and within each system to examine differences across seasons and between different crops within the rotation cycle.

Methodology

Site Description

The study was conducted at the W.K. Kellogg Biological Station's (KBS) Living Field Lab (LFL), which is a set of field plots under a variety of organic and conventional crop management systems established 10 years prior to this study. It is located on Kalamazoo loam and Oshtemo sandy loam soils (Typic Hapludalfs; Table 1). I measured methane oxidation in two corn-corn (*Zea mays* L.) - soybean (*Glycine max* L.) - wheat (*Triticum aestivum* L.) cropping systems, one managed organically with compost and cover crops vs. one managed conventionally with pesticides and inorganic nitrogen applied as per best management practice (BMP).

Compost material contained ~50% dairy manure and ~50% oak leaves (*Quercus rubra*) on a dry weight basis. Materials were composted for a minimum of one year. The total amount of N applied to corn in the form of compost was 117 kg N ha^{-1} yr^{-1} or an average dry compost mass of 4480 kg ha^{-1} yr^{-1}. Compost was not applied to the soybean crop. Nitrogen was applied as urea to treatments in corn under N fertilizer management at a rate of 110 kg N ha^{-1} y^{-1}. Cumulatively, compost applied plus previous compost applications provided 40- 53 kg N ha^{-1} yr^{-1} inorganic N (Willson 1998).

The cover crops in the organic system varied with crop; crimson clover (*Trifolium incarnatum* L.) was sown into standing corn in late June and red clover (*Trifolium*

pratense L.) was frost seeded into wheat in late March (Table 3.1). Both systems were replicated four times in a split-split-plot randomized complete block design. Each plot is 4.5 x 15 m (15 x 50 ft). Soil properties and yield of these experiment plots are presented in Table 3.2.

In Situ Gas Sampling

I measured in situ methane oxidation rates using the static chamber technique (Hutchinson and Livingston 1993). Static chambers were fashioned from 25 cm diameter PVC pipe: bases (25 cm diameter x 10 cm high) were installed in each plot and left in place except during agronomic operations. Immediately prior to sampling, a 4.5 cm high cap was placed on each base and sealed with a latex skirt wrapped with an elastic band. At 15-20 minute intervals, four 10 mL headspace samples were removed through rubber septa in each cap using a syringe, and put into 3 mL glass sample vials pre-flushed with headspace air. Within 24 hours vial contents were measured for CH_4 using a gas chromatograph (GC 5890 Series II, Hewlett Packard) equipped with a flame ionization detector (FID), and measured for CO_2 using an infrared gas absorption (IRGA) analyzer (EGA CO2 Analyzer, Analytical Development CO. LTD. Hoddesdon, England).

Chambers were sampled twice per month during the growing season and once per month during other parts of the year.

Soil Analyses

Soil temperature was measured at 0-5 cm depth using a temperature probe. Soil samples for other analysis were taken from the top 10 cm of soil using a 2.5 cm diameter

soil probe. Fresh soils were passed through a 4 mm sieve and mixed by hand, and then sub samples were taken for moisture content and mineral N analysis. Prior to analysis, soils were stored in a refrigerator at 4°C for up to 4 weeks. Soil moisture content was measured gravimetrically by drying the soil samples at 65°C for 3 days or until dry. Further drying at 105°C removes an additional 0.8 g $H_2O \cdot 100g\ soil^{-1}$ moisture in these soils. Mineral N measurements were obtained by extracting 20 g of dry soil with 100 mL 1 M KCl for 24 h then filtering through 2-μm pore size fiberglass filters. The filtrates were frozen prior to analysis for NH_4^+ and NO_3^- using a continuous flow analyzer (Alpkem 3550, OI Analytical, College Station, TX) (Bundy and Meisinger, 1994).

Soil Depth Study

Soil cores (5 cm diameter x 30 cm deep) were taken from the field and stored intact at 4°C for one week, after which they were separated into four different depth increments (0-5, 5-10, 10-20, and 20-30 cm). Each soil increment was transferred to a 473 mL Mason jar for 0-5 cm and 5-10 cm increments and to a 946 mL Mason jar for 10-20 cm and 20-30 cm increments; jars were left open and allowed to pre-incubate with atmospheric methane at room temperature for one week in order to reduce soil moisture levels to ca. 15% w/w. At the end of this period, methane oxidation was measured by sealing the jar with a cap and removing 0.5 mL of headspace at four 10-minute intervals. The headspace aliquot was immediately injected into the gas chromatograph for methane analysis. After the CH_4 oxidation study, soil samples were sieved and separated for soil moisture, soil inorganic N content, and nitrification assays.

Nitrification Assay

A sieved soil sample of 10 g was placed in a 100 mL Erlenmeyer flask and combined with 50 mL of the phosphate buffer solution and ammonium sulfate (Hart et al. 1994) and sealed with Parafilm. The flasks were placed on a shaker table and shaken for 24 h. Soil slurry samples of 10 mL were taken before and another after 24 h incubation, centrifuged for 30 min and filtered through 0.2 μm fiberglass filter. The aliquots were frozen until analysis of nitrate as above. Rates of nitrate production were calculated on the basis of the difference between the initial and final nitrate concentrations.

Statistical Analyses

I used Proc Mixed of SAS program version 8.0 (SAS Institute 1999) for the analysis of variance (ANOVA) and analysis of covariance (ANCOVA) for field data, in which I treated crop nested in management as fixed effects and day and crop x management x day as random effects. I also used SPSS program version 10.0.1 (SPSS Inc. 1999) for ANOVA and ANCOVA for the soil depth study and for the correlation analysis for both field and soil depth data. Methane and CO_2 data were natural log transformed before ANOVA and ANCOVA to homogenize variances. I used untransformed data for correlation analysis.

Results

Conventional Crops vs. Organic Crops

Overall, average seasonal soil methane consumption in the conventional rotation was 3.8 (±0.3) μg CH_4-C m^{-2} h^{-1}, slightly but not significantly higher than rates in the

organic treatment at 3.3 (± 0.4) μg CH_4-C m^{-2} h^{-1} (Table 3.3 and 3.4). Mean seasonal CH_4 oxidation rates in soils under conventional corn, soybean, and wheat were similar at ca. 3.8 μg CH_4-C m^{-2} h^{-1} (Table 3.5). In contrast, analysis of variance shows significant differences in CH_4 uptake rates among crops in the organic rotation (Table 3.5 and Figure 3.1). Methane oxidation under organic corn was the highest at 4.8 (±0.6 s.e., n= 4 replicate plots x 15 sampling dates) μg CH_4-C m^{-2} h^{-1}, and oxidation rates under organic soybean and wheat were about 50% lower, at 2.5 (± 0.7) and 3.4 (± 0.5) μg CH_4-C m^{-2} h^{-1}, respectively (Table 3.5). Only dates of sampling were significant for CH_4 uptake rates (Table 3.4).

In contrast to CH_4, the average seasonal CO_2 flux from organic treatments (94.0 ± 6.3 mg CO_2-C m^{-2} h^{-1}) was 22% higher than fluxes in conventional treatments (73.5 ± 4.6 mg CO_2-C m^{-2} h^{-1}; Table 3.3 and 3.6). Crops and sample dates also significantly affected soil CO_2 emission. Soils under organic wheat produced the most CO_2 (130 ± 15 mg CO_2-C m^{-2} h^{-1} on average) followed by conventional wheat (93.7 ±10.7 mg CO_2-C m^{-2} h^{-1}; Table 3.5 and Figure 3.2). The organic corn and soybean soils produced 42% less CO_2 than organic wheat soils (ca. 75 mg CO_2-C m^{-2} h^{-1}) but 18% more CO_2 than their conventional counterparts (ca. 63 mg CO_2-C m^{-2} h^{-1}).

Seasonal Trends in Conventional Rotations

Methane oxidation in the conventional systems was generally highest in late summer and early fall, and lowest in early spring and summer (Figure 3.3a). Oxidation rates in conventional treatments ranged from –5 to 15 μg CH_4-C m^{-2} h^{-1} (a negative

oxidation rate indicates methane production). For corn the highest methane uptake rates were in late September (9.4 ± 1.0 μg CH_4-C m^{-2} h^{-1}, n = 4 replicates) and the lowest rates were in December and March (around 1.0 μg CH_4-C m^{-2} h^{-1}). For soybeans, rates were also highest in late September (8.3 ± 2.1 μg CH_4-C m^{-2} h^{-1}); lowest rates occurred in July (-0.1 ± 0.9 μg CH_4-C m^{-2} h^{-1}). For winter wheat, oxidation rates were also highest after harvest in early August (6.6 ± 3.2 μg CH_4-C m^{-2} h^{-1}) and lowest in November and June (around 1.9 μg CH_4-C m^{-2} h^{-1}).

As for CH_4 uptake, CO_2 fluxes in the conventional systems were highest in late summer and early fall, and lowest in winter and early spring (Figure 3.3a). Fluxes ranged from 3.4 to 345 mg CO_2-C m^{-2} h^{-1} in conventional treatments. For corn, the highest CO_2 production rates were in October (109 ± 11 mg CO_2-C m^{-2} h^{-1}, n=4 replicates) and the lowest rates were in January (11.5 ± 1.7 mg CO_2-C m^{-2} h^{-1}). For soybeans, rates were also highest in August (140 ± 19 mg CO_2-C m^{-2} h^{-1}); lowest rates occurred in January (7.6 ± 0.9 mg CO_2-C m^{-2} h^{-1}). For wheat, flux was highest in August (293 ± 36 mg CO_2-C m^{-2} h^{-1}) and lowest in January (6.8 ± 1.1 mg CO_2-C m^{-2} h^{-1}).

Seasonal Trends in Organic Rotations

Methane oxidation in the organic rotations also exhibited seasonal trends, with high rates of oxidation in the late growing season and lower rates in early spring except for wheat (Figure 3.3b). Oxidation rates ranged from –20 to 17 μg CH_4-C m^{-2} h^{-1}. For corn, the highest methane uptake rates were in the July – October period (9.2 ± 1.6 μg CH_4-C m^{-2} h^{-1}, n = 4 replicates, in late September) and the lowest were during winter to early spring (-4.1 ± 4.3 μg CH_4-C m^{-2} h^{-1} in November). For soybeans, rates were also

highest in late September (10.6 ±1.2 μg CH_4-C m^{-2} h^{-1}) and low in the spring, although the lowest rate followed a rain event in early September (-2.5 ± 6.0 μg CH_4-C m^{-2} h^{-1}). In winter wheat, which is harvested in late August, oxidation was highest in September - October (6.0 ± 1.1 μg CH_4-C m^{-2} h^{-1} for late September) and lowest in early September (-3.7 ± 5.0 μg CH_4-C m^{-2} h^{-1}) following crop harvest and rain. As for conventional wheat, seasonal variation in organic wheat was lower than in other crops; in particular, oxidation was moderately high in spring when for other crops oxidation rates were lowest.

Carbon dioxide production in the organic systems was similar to that in conventional systems, but generally of higher magnitude especially during the wheat part of the rotation. The flux was highest in August and lowest in January (Figure 3.4b). Daily fluxes ranged from 1.8 to 544 mg CO_2-C m^{-2} h^{-1}. For corn the highest CO_2 production rates were in October (162 ± 21 mg CO_2-C m^{-2} h^{-1}, n = 4 replicates) and the lowest rates were in January and March (around 22 mg CO_2-C m^{-2} h^{-1}). For soybean, rates were also highest in August (197 ± 39 mg CO_2-C m^{-2} h^{-1}); lowest rates occurred in January (19.1 ± 3.3 mg CO_2-C m^{-2} h^{-1}). For wheat, fluxes were highest in August (432 ± 47 mg CO_2-C m^{-2} h^{-1}) and lowest in January (6.5 ± 2.5 mg CO_2-C m^{-2} h^{-1}).

Yield

Overall, conventional rotations produced more yield than organic rotations (Table 3.3). Conventional corn and soybean yielded 21% and 43% than organic counterparts while organic and conventional wheat had similar yields.

Soil Moisture

Soil moisture was significantly different among crops, management, and sample dates (Table 3.7). Organic rotations had significantly higher average soil moisture (14.9 ± 0.4 g $H_2O \cdot 100g\ soil^{-1}$) than conventional rotations (12.5 ± 0.3 g $H_2O \cdot 100g\ soil^{-1}$, n = 4 replicates x 15 sample dates; Table 3.3). Moisture ranged from 1 to 23 g $H_2O \cdot 100g\ soil^{-1}$ and the highest average soil moisture was in organic soybean and corn (around 15 g $H_2O \cdot 100g\ soil^{-1}$; Table 3.5). Soil moisture remained constant from early October 2001 (at around 17 g H_2O $100g\ soil^{-1}$ in conventional treatments and 20 g $H_2O \cdot 100g\ soil^{-1}$ in organic treatments; Figure 3.3) to March 2002, then slightly decreased until June before exponentially decreasing to 3-8 g $H_2O \cdot 100g\ soil^{-1}$. Soil moisture increased to around 18 g $H_2O \cdot 100g\ soil^{-1}$ after rainfall in early August then fell again in early September before increasing at the end of the month (Figure 3.5).

Soil Temperature

Organic rotations also had higher average soil temperature (14.8 ± 0.4 °C) than those of conventional rotations (12.5 ± 0.3 °C; Table 3.3). Within rotations, soil temperatures were not significantly different among crops (Table 3.4); nor was there a significant crop x treatment x day interaction, but temperature did differ significantly among dates of sampling (Table 3.7). Soil temperature was highest in summer around late June (to around 30 °C in both rotations), and another peak occurred in late August (around 25 °C) (Figure 3.6). Soil temperatures were lowest in winter (from December to March), but were above freezing during the sample dates.

Soil Inorganic Nitrogen

Soil nitrate contents were similar between conventional and organic management treatments (Table 3.3). However, there were significant differences among crops (Table 3.5 and 3.7), crop x management x sampling date interactions, and among sampling dates. Average soil nitrate in organic rotations (8.88 ± 0.73 μg N g $soil^{-1}$, n = 4 replicates x 3 crops x 15 sample dates) was slightly but not significantly higher than those in conventional rotations (7.88 ± 1.15 μg N g $soil^{-1}$; Table 3.3). Soil nitrate was highest in conventional corn (12.2 ± 3.2 μg N g $soil^{-1}$) followed by organic soybean (11.2 ± 1.4 μg N g $soil^{-1}$) and organic corn (9.2 ± 1.3 μg N g $soil^{-1}$; Table 3.5). In conventional rotations, soil nitrate reached its first peak at mid January (around 16 μg N g $soil^{-1}$) and its second peak in conventional corn at mid July (44.7 ± 41.3 μg N g $soil^{-1}$; Figure 3.7a), just three weeks after fertilization and two weeks after tillage (Table 3.1). In organic rotations, soil nitrate began to increase from December 2001 to mid January 2002 then remained constant until mid June (Figure 3.7b). Nitrate levels in organic rotations were higher (approximately 20 μg N g $soil^{-1}$) than in conventional rotations (10 μg N g $soil^{-1}$) during this period. Then nitrate levels declined until they reached their lowest levels in early August. Under conventional management, soil nitrate began to decline first in wheat, followed by soybean and corn respectively, but under organic management, soil nitrate first decreased in wheat and then in corn and soybean correspondingly.

Soil ammonium differed significantly by sample date (Table 3.7) but not by management (Table 3.3) or crops (Table 3.4 and 3.7). Conventional corn had the highest average soil ammonium (21.4 ± 5.4 μg N g $soil^{-1}$) followed by the other crops at approximately 15 μg N g $soil^{-1}$ (Table 3.5). The seasonal trends for soil ammonium were

similar to those of soil nitrate. Ammonium levels reached their highest point in mid-July after fertilization and tillage and reached a second peak in mid-August (Figure 3.8a). Whereas in organic management, soil ammonium was highest in late May and mid-August, levels much lower than in conventional corn (Figure 3.8b).

Controls on Methane and Carbon Dioxide Flux in the Field

Analysis of covariance shows that CH_4 oxidation was not affected by any measured soil factor (Table 3.4). Correlation analysis also shows a weak but significant negative correlation between CH_4 oxidation and moisture ($r=-0.152$, $p<0.01$, n = 2 managements x 3 crops x 4 replicate plots x 15 sample dates; Table 3.8). In contrast, CO_2 fluxes were significantly affected by soil nitrate, ammonium, and temperature but not soil moisture (Table 3.6). Carbon dioxide fluxes were significantly correlated with soil temperature and nitrate (Table 3.8). Fluxes of CO_2 and CH_4 were not correlated (Table 3.8 and Figure 3.9).

Soil Depth Study

CH_4 oxidation in soil cores did not differ between conventional and organic systems (Table 3.9). Oxidation rates were similar at approximately 0.05 μg CH_4-C kg $soil^{-1}$ h^{-1}. Rates also did not vary among crops but did significantly vary by soil depth (Table 3.10 and 3.11), and generally uptake was highest at 10-30 cm depth (Table 3.10 and Figure 3.10). Soil CH_4 uptake rates ranged from –0.05 to 0.21 μg CH_4-C kg $soil^{-1}$ h^{-1}. Some soil cores from organic rotations showed net CH_4 production in the incubation (Table 3.10).

Soil moisture in collected cores differed significantly between management systems and among crops, but not among soil depths (Table 3.12 and Figure 3.11). Soil cores from the conventional systems had less soil moisture (10.5 ± 0.3 g $H_2O \cdot 100g\ soil^{-1}$) than those from the organic systems (11.5 ± 0.4 g $H_2O \cdot 100g\ soil^{-1}$; Table 3.9).

Soil nitrate was significantly different among crops and soil depths but not between management systems (Table 3.9 and 3.12). Nitrate was highest at the soil surface then declined along the soil profile (Figure 3.12). Organic soybean had the highest levels of soil nitrate (Table 3.10).

Like nitrate, ammonium varied significantly among crops and soil depths (Table 3.12) but not management systems (Table 3.9). There also was more ammonium at the soil surface vs. lower soil levels (Table 3.10 and Figure 3.13). Conventional and organic corn had the highest soil ammonium content among other crops (Table 10).

Soil nitrification significantly differed between management systems and among soil depths (Tables 3.9 and 3.12). Soil from organic systems had higher nitrification rates (0.31 ± 0.03 μg N $kg\ soil^{-1}\ h^{-1}$) than did soils from the conventional systems (0.20 ± 0.02 μg N $kg\ soil^{-1}\ h^{-1}$; Table 3.9). Nitrification was also highest in surface soil rather than deeper soils (Table 3.10 and Figure 3.14). Organic wheat had the highest nitrification rate followed by organic corn and soybean.

Controls on Methane Oxidation in Soil Depth

Soil moisture was the only factor almost significantly ($P=0.069$) affecting CH_4 uptake in incubated soil cores (Table 3.11); soil nitrate and ammonium did not

significantly affect oxidation. Nevertheless, all three factors were negatively and significantly correlated with CH_4 oxidation (r=-0.23 to –0.51; Table 3.13).

Discussion

Field Study

Methane Oxidation

Soil methane oxidation in organic cropping systems was overall 15% lower than in the conventional systems but this difference was not statistically significant. Mean CH_4 oxidation rates were similar among crops in the conventional system (ca. 3.8 μg CH_4-C m^{-2} h^{-1}). In the organic system oxidation rates were 50% lower in the wheat and soybean rotations (ca. 2.4 μg CH_4-C m^{-2} h^{-1}) than in the corn, which exhibited rates similar to those in the conventional counterparts. The lower CH_4 consumption rate in organic soybean and wheat was mainly due to high net CH_4 emission in November and early September while there was lower CH_4 production in conventional systems on the same dates. The November CH_4 emission in the organic soybean and wheat might be a result of high soil moisture at that time, combined with compost application from late October in organic wheat plots. However, CH_4 production occurred in early September even in low soil moisture at 0-10 cm depth. Therefore, the source of CH_4 production is likely in a deeper soil horizon, perhaps due to a higher soil water table caused by high rainfall two weeks before.

Most crops showed a similar seasonal pattern of methane oxidation. In general, methane oxidation was highest in late summer and fall, and lowest in early spring.

Oxidation in the winter wheat rotations was less seasonally variable than in the other crops, with lowest rates immediately following harvest in July.

Net CH_4 production occurred on several sample dates and more frequently in organic systems.

Carbon dioxide

Unlike CH_4, overall CO_2 fluxes in the organic rotation were 22% higher than in the conventional rotation; largely because of higher soil CO_2 emission in organic wheat. Corn and soybean had similar soil CO_2 production rates while wheat had the highest CO_2 flux in both management systems, from 33% higher in conventional to 43% higher in organic rotations. This might be in part from the compost application into organic wheat, which increased available carbon in soil. However, organic corn, which also received compost, did not show any increase in CO_2 emissions. Another factor might be the clover crops in the organic wheat, but organic corn, which also had a cover crop, had CO_2 fluxes no different from non-cover crop organic soybeans. Additionally, the organic system had more total carbon, total nitrate, and higher soil pH as compared to the conventional treatment (Table 3.2).

Although soil moisture was not a statistically significant predictor of CO_2 flux, CO_2 emission during summer sampling dates were correlated with soil moisture especially at the peak of both CO_2 production and soil moisture content in late August. Overall soil moisture content in the organic treatment was also higher than in the conventional treatment for every crop.

Both soil nitrate and ammonium reached their peak approximately three weeks after fertilization in conventional corn, the highest among other treatments. However, conventional corn had overall soil nitrate no different than organic corn and soybean. Although not statistically different, soil ammonium in conventional corn was approximately 30% higher than in other plots.

Soil Depth Study

In the laboratory soil core study, CH_4 consumption (either overall or at various depths) did not differ between conventional and organic systems, nor among crops within systems.

Only soil depth significantly affected soil CH_4 uptake in the laboratory study, as occurred in with previous studies (Adamsen and King 1993, Koschorreck and Conrad 1993, Czepiel et al. 1995). However, the highest oxidation region was 20-30 cm depth, deeper than in other studies; others have found highest oxidation at 3-6 cm in temperate soil cores (Czepiel et al. 1995), 10-12 cm in German forest soils (Koschorreck and Conrad 1993), 9-12 cm in subarctic spruce-lichen woodland in Canada, and 4-8 cm in temperate mixed forest in Maine (Adamsen and King 1993), and 5-15 cm in no-till agricultural soils in UK (Hütsch 1998b).

One possible reason for higher oxidation at this depth is that CH_4 might be produced at an adjacent level, providing CH_4 for a larger oxidizing population at 20-30 cm as the CH_4 diffuses upward or downward. Methane production still occurred at 5-10 cm depths even after a week of drying. Further experiments to verify this possibility are needed.

Soil moisture was a significant predictor of CH_4 oxidation in the field but it was not a good predictor for differences in CH_4 oxidation among soil depths because moisture did not differ among depths. In contrast, soil nitrate and ammonium were not significant predictors for CH_4 uptake in both field and laboratory studies even though both declined with soil depth, supporting the hypothesis that higher soil N leads to less soil CH_4 oxidation as has been found in other studies. Soil nitrification also declined with depth, suggesting that nitrifying bacteria were not responsible for the CH_4 oxidation trends in these soils. In fact, soil CH_4 consumption was strongly negative correlated with soil nitrification, ammonium, nitrate, and moisture.

From this study, organic and conventional systems are too simplistic a division to define differences in soil CH_4 oxidation. Each had similar net average soil CH_4 consumption. The organic corn had the highest CH_4 consumption, perhaps because it had less inorganic nitrogen than those of the conventional plots but had higher soil moisture and soil carbon from compost. The organic soybean and wheat had the lowest CH_4 oxidation, perhaps from the decomposition and N mineralization of the remaining compost in organic wheat and N fixation from soybean. Soil from organic management tends to produce CH_4 easily when wet. The high soil carbon and organic matter might contribute to this phenomenon.

It appears to be very difficult to increase CH_4 oxidation in agricultural soils. First, the inorganic nitrogen is very high. Second, tillage destroys soil structure and disrupts the colonization of soil microorganisms. Third, some pesticides are CH_4 oxidation inhibitors. Forth, soil becomes compacted from traffic in the field. Nevertheless, the best available ways to increase CH_4 oxidation appear to be related to reducing tillage and N

fertilization. Organic N should slowly release N to soil microbes thus decrease its toxicity to methanotroph. But organic matter addition might increase CH_4 production when soil wet.

Conclusion

Soil methane oxidation in organic cropping systems was overall 12% lower than in the conventional system primarily due to low oxidation rates in organic wheat and soybean crops. Mean annual oxidation rates were similar among crops in the conventional system. In the organic system oxidation rates were lower in the wheat and soybean rotations than in the corn, which exhibited rates higher than those in the conventional system. Most crops showed a similar seasonal pattern of methane oxidation. In general, methane oxidation was highest in late summer and fall, and lowest in early spring. Oxidation in the winter wheat rotations was less seasonally variable than in the other crops, with lowest rates following harvest in July. Net methane production occurred on several sample dates and more frequently in organic systems.

References

Adamsen, A. P. S., and G. M. King. 1993. Methane consumption in temperate and sub-arctic forest soils - rates, vertical zonation, and responses to water and nitrogen. Applied and Environmental Microbiology **59**:485-490.

Ambus, P., and S. Christensen. 1995. Spatial and seasonal nitrous oxide and methane fluxes in Danish forest ecosystems, grassland-ecosystems, and agroecosystems. Journal of Environmental Quality **24**:993-1001.

Arif, M. A. S., F. Houwen, and W. Verstraete. 1996. Agricultural factors affecting methane oxidation in arable soil. Biology and Fertility of Soils **21**:95-102.

Boeckx, P., and O. van Cleemput. 2001. Estimates of N_2O and CH_4 fluxes from agricultural lands in various regions in Europe. Nutrient Cycling in Agroecosystems **60**:35-47.

Boeckx, P., O. Van Cleemput, and T. Meyer. 1998. The influence of land use and pesticides on methane oxidation in some Belgian soils. Biology and Fertility of Soils **27**:293-298.

Bundy, L. G., and J. J. Meisinger. 1994. Nitrogen availability indices. Pages 951-984 *in* R. W. Weaver, J. S. Angle, and B. S. Bottomley, editors. Methods of soil analysis. Part 2. Microbiological and biochemical properties. SSSA Book Series, no. 5., Madison, WI.

Czepiel, P. M., P. M. Crill, and R. C. Harriss. 1995. Environmental-factors influencing the variability of methane oxidation in temperate zone soils. Journal of Geophysical Research-Atmospheres **100**:9359-9364.

Goulding, K., T. Willison, C. Webster, and D. Powlson. 1996. Methane fluxes in aerobic soils. Environment Monitoring Assessment **42**:175-187.

Hart, S. C., J. M. Stark, E. A. Davidson, and M. K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. Pages 985-1018 *in* R. W. Weaver, J. S. Angle, and B. S. Bottomley, editors. Methods of soil analysis. Part 2. Microbiological and biochemical properties. SSSA Book Series, no. 5., Madison, WI.

Hutchinson, G. L., and G. P. Livingston. 1993. Use of chamber systems to measure trace gas fluxes. Pages 63-78 *in* L. A. Harper, A. R. Mosier, J. M. Duxbury, and D. E. Rolston, editors. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. American Society of Agronomy, Madison, Wisconsin.

Hütsch, B. W. 1996. Methane oxidation in soils of two long-term fertilization experiments in Germany. Soil Biology & Biochemistry **28**:773-782.

Hütsch, B. W. 1998a. Methane oxidation in arable soil as inhibited by ammonium, nitrite, and organic manure with respect to soil pH. Biology and Fertility of Soils **28**:27-35.

Hütsch, B. W. 1998b. Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. Biology and Fertility of Soils **27**:284-292.

Hütsch, B. W. 2001. Methane oxidation in non-flooded soils as affected by crop production. European Journal of Agronomy **14**:237-260.

Jambert, C., R. Delmas, D. Serca, L. Thouron, L. Labroue, and L. Delprat. 1997. N_2O and CH_4 emissions from fertilized agricultural soils in southwest France. Nutrient Cycling in Agroecosystems **48**:105-114.

Keller, M., and W. A. Reiners. 1994. Soil atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. Global Biogeochemical Cycles **8**:399-409.

Koschorreck, M., and R. Conrad. 1993. Oxidation of atmospheric methane in soil - Measurements in the field, in soil cores and in soil samples. Global Biogeochemical Cycles **7**:109-121.

MacDonald, J. A., U. Skiba, L. J. Sheppard, K. J. Hargreaves, K. A. Smith, and D. Fowler. 1996. Soil environmental variables affecting the flux of methane from a range of forest, moorland and agricultural soils. Biogeochemistry **34**:113-132.

Prieme, A., and S. Christensen. 1999. Methane uptake by a selection of soils in Ghana with different land use. Journal of Geophysical Research-Atmospheres **104**:23,617-623,622.

Prieme, A., and F. Ekelund. 2001. Five pesticides decreased oxidation of atmospheric methane in a forest soil. Soil Biology & Biochemistry **33**:831-835.

Robertson, G. P., E. A. Paul, and R. R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science **289**:1922-1925.

SAS Institute. 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc., Cary, NC.

SPSS Inc. 1999. SPSS. SPSS Inc.

Tlustos, P., T. W. Willison, J. C. Baker, D. V. Murphy, D. Pavlikova, K. W. T. Goulding, and D. S. Powlson. 1998. Short-term effects of nitrogen on methane oxidation in soils. Biology and Fertility of Soils **28**:64-70.

Topp, E. 1993. Effects of selected agrochemicals on methane oxidation by an organic agricultural soil. Canadian Journal of Soil Science **73**:287-291.

Willison, T. W., K. Goulding, and D. Powlson. 1995a. Effect of land use change and methane mixing ratio on methane uptake from United Kingdom soil. Global Change Biology **1**:209-212.

Willison, T. W., C. P. Webster, K. W. T. Goulding, and D. S. Powlson. 1995b. Methane oxidation in temperate soils - Effects of land-use and the chemical form of nitrogen fertilizer. Chemosphere **30**:539-546.

Willson, T. C. 1998. Managing nitrogen mineralization and biologically active organic matter fractions in agricultural soil. Ph.D. Michigan State University, East Lansing, MI.

Table 3.1 Agronomic field activities for the sites studied. Data are from the KBS LTER website http://kbs.msu.edu/lter/.

Date	Field activity
10/30/2001	Apply compost 409 kg plot^{-1} in organic wheat
10/31/2001	Plant wheat Pioneer 25R49 2.5 bu/a
3/19/2002	Plant red clover 7 kg acre^{-1} in organic wheat
4/25/2002	Apply compost 350 kg plot^{-1} in organic corn
4/25/2002	Fertilize 46-0-0 N 18 kg acre^{-1} in conventional wheat
5/2/2002	Chisel in conventional corn and soybean
5/20/2002	Field cultivation in conventional corn and soybean
5/22/2002	Plant corn Pioneer 37M34 28,000 seeds/a
5/22/2002	Fertilize 28% N 1.3 kg acre^{-1} in conventional corn
5/24/2002	Plant soybean 150,000 seeds/a
5/28/2002	Broadcast herbicide mix 7 in conventional corn
5/28/2002	Broadcast herbicide Duall II in conventional soybean
6/7/2002	Rotary hoe in organic corn and soybean
6/8/2002	Rotary hoe in organic corn and soybean
6/13/2002	Rotary hoe in organic corn and soybean
6/17/2002	Row cultivation in organic corn and soybean
6/27/2002	Fertilize 28% N 50 kg acre^{-1} in conventional corn
6/28/2002	Row cultivation in organic corn and soybean
7/8/2002	Row cultivation in both conventional and organic corn and soybean
7/8/2002	Plant crimson clover 5.4 kg acre^{-1} in organic corn
7/15/2002	Harvest wheat
10/8/2002	Chisel in both conventional and organic soybean
10/8/2002	Harvest soybean
10/17/2002	Harvest corn
10/23/2002	Field cultivation both conventional and organic soybean
10/23/2002	Plant wheat Pioneer 25R26 red winter 2.5 bu/a
10/23/2002	Apply compost 300 kg plot^{-1} in organic wheat

Table 3.2 Soil properties of conventional and organic management systems at the KBS LFL site. Values are means ± standard error of mean for four replicates. Data are from the KBS LTER website http://kbs.msu.edu/lter/. Values followed by different higher case letters are significantly different ($P<0.05$) of the same crop between treatments. Values followed by different lower case letters are significantly different ($P<0.05$) among crops within treatments.

Soil property	Conventional treatment			Organic treatment		
	Corn	Soybean	Wheat	Corn	Soybean	Wheat
pH[1]	6.14 ± 0.17	6.27 ± 0.09	5.97 ± 0.19 A	6.67 ± 0.07	6.75 ± 0.06	6.63 ± 0.14 B
Total carbon (mg C g soil^{-1})[2]	0.55 ± 0.05	0.61 ± 0.14	0.57 ± 0.09	0.86 ± 0.11	0.75 ± 0.10	0.91 ± 0.03
Total nitrogen (mg N g soil^{-1})[2]	0.046 ± 0.009	0.052 ± 0.02	0.050 ± 0.01	0.075 ± 0.02	0.065 ± 0.014	0.079 ± 0.007
C/N ratio[2]	12.04 ± 1.96	11.75 ± 1.70	11.48 ± 1.39	11.49 ± 1.13	11.37 ± 0.89	11.42 ± 0.58
Nitrate (μg N g soil^{-1})[2]	9.38 ± 2.12 a	3.52 ± 0.77 ab	3.83 ± 0.54 Ab	8.62 ± 1.13	7.46 ± 1.33	5.88 ± 0.50
Yield (MT ha^{-1})	7.66 ± 0.13A	2.24 ± 0.10	2.1212 ± 0.07	6.06 ± 0.67B	1.28 ± 0.08	2.21 ± 0.10

[1] sampled 24 April 2002 to a depth of 30 cm.

[2] sampled 28 April 2002 to a depth of 30 cm.

Table 3.3 Net average methane oxidation and carbon dioxide emission rates and other soil properties in conventional and organic systems at the KBS LFL site. Values are means ± 1 SE for 4 replications x 15 sampling dates; significance values are based on *t* tests.

Factor	Conventional Agriculture	Organic Agriculture	*t*-test[a]
CH_4 ($\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$)	3.80 ± 0.28	3.25 ± 0.37	ns
CO_2 ($mg\ CO_2\text{-}C\ m^{-2}\ h^{-1}$)	73.5 ± 4.6	94.0 ± 6.4	*
Moisture ($g\ H_2O \cdot 100g\ soil^{-1}$)	12.5 ± 0.3	14.9 ± 0.4	***
Temperature (°C)	12.5 ± 0.3	14.8 ± 0.4	**
Nitrate ($\mu g\ N\ g\ soil^{-1}$)	7.88 ± 1.15	8.88 ± 0.73	ns
Ammonium ($\mu g\ N\ g\ soil^{-1}$)	16.7 ± 2.0	15.2 ± 0.9	ns
Yield ($MT\ ha^{-1}$)	4.01 ± 0.8	3.18 ± 0.7	ns

[a] ns, not significant, P>0.1; * P<0.05; **P<0.01; ***P<0.0001

Table 3.4 Analysis of covariance to determine effects of treatment, crop, day, and soil properties on methane oxidation rates from conventional and organic agriculture at the KBS LFL site.

Variable	Num. Df [a]	Den. Df [a]	F	P
Management	1	84	2.13	0.149
Crop	2	84	2.56	0.083
Crop x Management	2	84	2.08	0.131
Crop x Management x Day	28	266	0.06[b]	0.477
Day	14	266	1.79[b]	0.037
Nitrate	1	266	0.00	0.969
Ammonium	1	266	0.15	0.696
Moisture	1	266	0.11	0.742
Temperature	1	266	0.09	0.760

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively.

[b] Z value

Table 3.5 Average soil methane oxidation and carbon dioxide fluxes and soil properties among crops in conventional and organic agriculture systems. Values are means ± standard error of mean for four replicates. Values followed by different upper case letters are significantly different ($P<0.05$) between management systems; values followed by different lower case letters are significantly different ($P<0.05$) among crops within systems.

Variable	Conventional Agriculture			Organic Agriculture		
	Corn	Soybean	Wheat	Corn	Soybean	Wheat
CH_4 ($\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$)	3.99 ± 0.49	3.89 ± 0.53	3.52 ± 0.44	4.83 ± 0.56 a	2.49 ± 0.73 b	2.42 ± 0.55 b
CO_2 ($mg\ CO_2\text{-}C\ m^{-2}\ h^{-1}$)	63.8 ± 5.6	62.8 ± 6.2	93.7 ± 10.7 A	76.9 ± 6.4 a	74.8 ± 7.7 a	130.2 ± 15.4 Bb
Moisture ($g\ H_2O \cdot 100g\ soil^{-1}$)	12.3 ± 0.6 A	13.3 ± 0.5	12.0 ± 0.6	15.1 ± 0.7 B	15.3 ± 0.7	14.2± 0.8
Temperature (°C)	15.2 ± 1.2	15.6 ± 1.2	15.7 ± 1.2	15.7 ± 1.1	16.1 ± 1.1	16.1 ± 1.1
Nitrate ($\mu g\ NO_3^-\text{-}N\ g\ soil^{-1}$)	12.2 ± 3.2 a	7.0 ± 0.8 ab	4.5 ± 0.7 b	9.2 ± 1.3	11.2 ± 1.4	6.3 ± 1.0
Ammonium ($\mu g\ NH_4^+\text{-}N\ g\ soil^{-1}$)	21.4 ± 5.4	15.5 ± 1.8	13.3 ± 1.4	15.1 ± 1.6	15.0 ± 1.5	15.6 ± 1.4

Table 3.6 Analysis of covariance to determine effects of treatment, crop, day, and soil properties on carbon dioxide emission rates from conventional and organic agriculture at the KBS LFL site.

Variable	Num. df [a]	Den. df [a]	F	P
Management	1	84	3.9	0.051
Crop	2	84	4.3	0.017
Crop x Management	2	84	0.0	0.992
Crop x Management x Day	28	266	4.5[b]	<0.0001
Day	14	266	2.2[b]	0.013
Nitrate	1	266	7.2	0.008
Ammonium	1	266	6.3	0.013
Moisture	1	266	0.3	0.554
Temperature	1	266	15.5	0.0001

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively.

[b] Z value

Table 3.7 Analysis of variance to determine effects of site, treatment, and day on soil properties from conventional and organic agriculture at the KBS LFL site.

Variable	Num. df [a]	Den. df [a]	Moisture		Temperature		Nitrate		Ammonium	
			F	P	F	P	F	P	F	P
Management	1	84	74.1	<0.0001	3.3	0.074	0.5	0.473	0.6	0.433
Crop	2	84	6.1	0.003	1.2	0.301	5.2	0.007	1.5	0.231
Crop x Management	2	84	0.9	0.411	0.0	0.973	2.4	0.096	1.7	0.180
Crop x Management x Day	28	266	2.1[b]	0.019	0.7[b]	0.232	1.7[b]	0.048	0.7[b]	0.233
Day	14	266	2.6[b]	0.005	2.6[b]	0.004	2.1[b]	0.017	2.3[b]	0.012

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] Z value

Table 3.8 Relationships among methane oxidation, carbon dioxide and other soil properties in conventional and organic treatments at the KBS LFL site. Values are Pearson correlation coefficients (r). n = 2 management systems x 3 crops x 4 replicates x 15 sample dates.

	CO_2	Moisture	Temperature	Nitrate	Ammonium
CH_4	0.053	-0.152*	0.083	-0.034	0.011
CO_2	1.000	-0.094	0.411*	-0.201*	0.090

* Correlation is significant at the 0.01 level (2-tailed)

Table 3.9 Differences in average soil methane oxidation rates and other properties among soil cores from conventional and organic systems at the KBS LFL site. Methane fluxes were measured in the laboratory; values are means ± 1 SE for 4 replications x 3 crops x 4 depths; significance values are based on *t* tests.

Factor	Conventional Agriculture	Organic Agriculture	*t*-test[a]
CH_4 ($\mu g\ CH_4\text{-}C\ kg\ soil^{-1}\ h^{-1}$)	0.049 ± 0.007	0.051 ± 0.008	ns
Moisture ($g\ H_2O \cdot 100g\ soil^{-1}$)	10.5 ± 0.3	11.5 ± 0.4	*
Nitrate ($\mu g\ N\ g\ soil^{-1}$)	5.07 ± 0.59	5.50 ± 0.69	ns
Ammonium ($\mu g\ N\ g\ soil^{-1}$)	3.85 ± 0.23	4.21 ± 0.22	ns
Nitrification ($\mu g\ N\ kg\ soil^{-1}\ h^{-1}$)	0.20 ± 0.02	0.31 ± 0.03	**

[a] ns not significant ($P>0.1$); * $P<0.05$; ** $P<0.01$.

Table 3.10 Average methane oxidation, soil properties, and nitrification rates among crops in conventional and organic agriculture along soil profile at the KBS LFL site. Values are means ± standard error of mean for four replicates. Values followed by different lower case letters are significantly different ($P<0.05$) between treatments within site; values followed by different higher case letters are significantly different ($P<0.05$) among soil depths within the same site and treatment.

	Corn		Soybean		Wheat	
	Conventional	Organic	Conventional	Organic	Conventional	Organic
CH_4 ($\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$)						
0-5 cm	0.02 ± 0.01 A	0.02 ± 0.01 A	0.03 ± 0.01 AB	0.04 ± 0.01 A	0.03 ± 0.01A	0.03 ± 0.01AB
5-10 cm	0.01 ± 0.01 A	0.00 ± 0.02 A	0.00 ± 0.01 A	0.02 ± 0.01 A	0.01 ± 0.01A	0.00 ± 0.01AB
10-20 cm	0.06 ± 0.01 A	0.03 ± 0.00 AB	0.06 ± 0.02 AB	0.04 ± 0.01 A	0.08 ± 0.02AB	0.11 ± 0.04 B
20-30 cm	0.13 ± 0.02 B	0.09 ± 0.01 B	0.07 ± 0.01 B	0.13 ± 0.02 B	0.09 ± 0.02B	0.11 ± 0.02 B
Moisture ($g\ H_2O \cdot 100g\ soil^{-1}$)						
0-5 cm	11.6 ± 2.0	14.3 ± 0.8	10.9 ± 0.7	11.2 ± 0.9	10.2 ± 0.3	10.3 ± 0.1
5-10 cm	11.1 ± 1.2	12.1 ± 1.1	9.6 ± 0.6	12.3 ± 1.3	10.1 ± 0.6	9.7 ± 0.6
10-20 cm	10.9 ± 1.6	14.3 ± 2.0	11.2 ± 0.5	12.7 ± 0.9	9.7 ± 1.1	8.4 ± 0.3
20-30 cm	10.3 ± 2.0	13.7 ± 0.9	10.1 ± 0.7	11.1 ± 1.1	9.6 ± 1.6	7.6 ± 0.7
Nitrate ($\mu g\ NO_3^-\text{-}N\ g\ soil^{-1}$)						
0-5 cm	5.74 ± 0.90	7.66 ± 0.68	8.96 ± 2.26 a	16.22± 1.34 Ab	5.28 ± 1.65 A	8.60 ± 0.99 A
5-10 cm	9.35 ± 2.93	4.44 ± 0.77	6.47 ± 1.43	12.11 ± 1.02 A	1.82 ± 0.26 B	4.36 ± 0.17 BC
10-20 cm	6.62 ± 2.28	2.54 ± 0.55	5.39 ± 1.96	3.94 ± 0.10 B	1.04 ± 0.23 B	2.15 ± 0.23 BCD
20-30 cm	5.40 ± 2.66	0.84 ± 0.18	4.18 ± 1.16	2.22 ± 0.68 B	0.59 ± 0.10 B	0.90 ± 0.16 CD
Ammonium ($\mu g\ NH_4^+\text{-}N\ g\ soil^{-1}$)						
0-5 cm	6.65 ± 1.43 AB	6.62 ± 0.33 A	4.70 ± 0.33 A	5.21 ± 0.71 AB	4.57 ± 0.67	5.73 ± 0.37 A
5-10 cm	5.54 ± 0.58 ABC	4.52 ± 0.28 AB	3.46 ± 0.24 A	4.57 ± 0.38 ABC	4.03 ± 0.45	5.31 ± 0.24 A
10-20 cm	3.33 ± 0.25 BCD	3.85 ± 0.83 AB	2.88 ± 0.37 A	2.97 ± 0.36 BC	3.04 ± 0.20	4.23 ± 0.25 AB
20-30 cm	2.26 ± 0.29 CD	2.82 ± 0.64 B	2.52 ± 0.42 B	2.21 ± 0.25 CD	3.25 ± 0.65	2.51 ± 0.39 B
Nitrification ($\mu g\ N\ g\ soil^{-1}\ h^{-1}$)						
0-5 cm	0.33 ± 0.08	0.54 ± 0.05 A	0.30 ± 0.04 A	0.52 ± 0.05 AB	0.26 ± 0.05 a	0.70 ± 0.05 Ab
5-10 cm	0.35 ± 0.09	0.30 ± 0.08 AB	0.24 ± 0.06 A	0.39 ± 0.09 ABC	0.23 ± 0.06	0.45 ± 0.06 BC
10-20 cm	0.29 ± 0.05	0.20 ± 0.09 B	0.08 ± 0.01 A	0.26 ± 0.04 BC	0.18 ± 0.06	0.25 ± 0.06 BCD
20-30 cm	0.08 ± 0.03	0.03 ± 0.01 B	0.03 ± 0.00 B	0.07 ± 0.08 CD	0.05 ± 0.01	0.05 ± 0.09 CD

Table 3.11 Analysis of covariance for methane oxidation in laboratory soil cores as affected by management, crops, soil depth, and soil properties at the KBS LFL site.

Source	SS	df	MS	F	P
Crop	$1.0x10^{-5}$	2	$5.3x10^{-6}$	0.5	0.600
Management	$9.8x10^{-6}$	1	$9.8x10^{-6}$	1.0	0.331
Depth	$4.5x10^{-4}$	3	$1.5x10^{-4}$	14.5	0.000
Crop x Management	$5.6x10^{-5}$	2	$2.8x10^{-5}$	2.7	0.072
Crop x Depth	$1.1x10^{-4}$	6	$1.9x10^{-5}$	1.8	0.111
Management x Depth	$2.1x10^{-5}$	3	$7.0x10^{-6}$	0.7	0.564
Crop x Management x Depth	$1.0x10^{-4}$	6	$1.7x10^{-5}$	1.7	0.139
Nitrate	$2.0x10^{-5}$	1	$2.0x10^{-5}$	2.0	0.163
Ammonium	$1.4x10^{-5}$	1	$1.4x10^{-5}$	1.4	0.243
Moisture	$3.2x10^{-5}$	1	$3.2x10^{-5}$	3.1	0.082
Nitrification	$1.1x10^{-5}$	1	$1.1x10^{-5}$	1.1	0.301
Error	$7.0x10^{-4}$	68	$1.0x10^{-5}$		
Total	511	96			
Corrected Total	$2.4x10^{-3}$	95			

Table 3.12 Analysis of variance to determine effects of crop, treatment, and depth on properties of soil cores from conventional and organic agriculture at the KBS LFL site.

Source	df	Moisture		Temperature		Nitrate		Ammonium	
		F	P	F	P	F	P	F	P
Crop	2	12.8	0.000	21.2	0.000	5.7	0.005	0.9	0.419
Management	1	5.0	0.029	0.6	0.434	2.8	0.100	24.9	0.000
Depth	3	1.0	0.412	27.7	0.000	37.3	0.000	58.0	0.000
Crop x Management	2	5.1	0.009	9.5	0.000	0.9	0.397	6.0	0.004
Crop x Depth	6	0.5	0.802	2.6	0.023	1.3	0.266	0.3	0.913
Management x Depth	3	0.0	0.994	6.8	0.000	0.7	0.573	8.3	0.000
Crop x Management x Depth	6	0.6	0.715	1.5	0.190	1.3	0.250	1.2	0.341

Table 3.13 The relationships among methane oxidation and other soil properties in soil cores from different depths at the KBS LFL site. Values are Pearson correlation coefficients (r). n = 2 management systems x 3 crops x 4 replicates.

	Moisture	Nitrate	Ammonium	Nitrification
CH_4	-0.230*	-0.351**	-0.454**	-0.505**

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

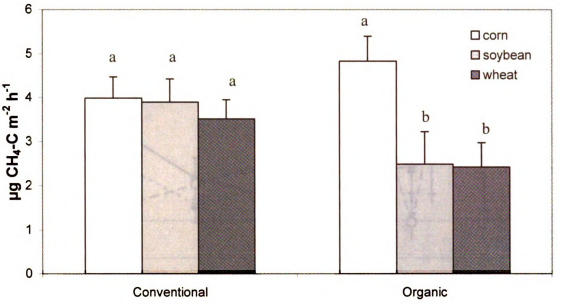


Figure 3.1 Net methane oxidation of different crops in conventional and organic agriculture at the KBS LFL site. Vertical bars represent standard errors (n = 4 replicate plots x 13 sample dates). Different higher and lower case letters represent significant differences ($P<0.05$) of crops within management systems and between management systems respectively.

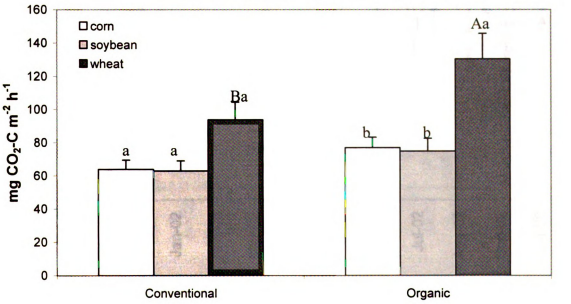


Figure 3.2 Net soil carbon dioxide fluxes of different crops in conventional and organic agriculture at the KBS LFL site. Vertical bars represent standard error (n = 4 replicate plots x 13 sample dates). Different higher and lower case letters represent significant differences ($P<0.05$) of crops within management systems and between management systems respectively.

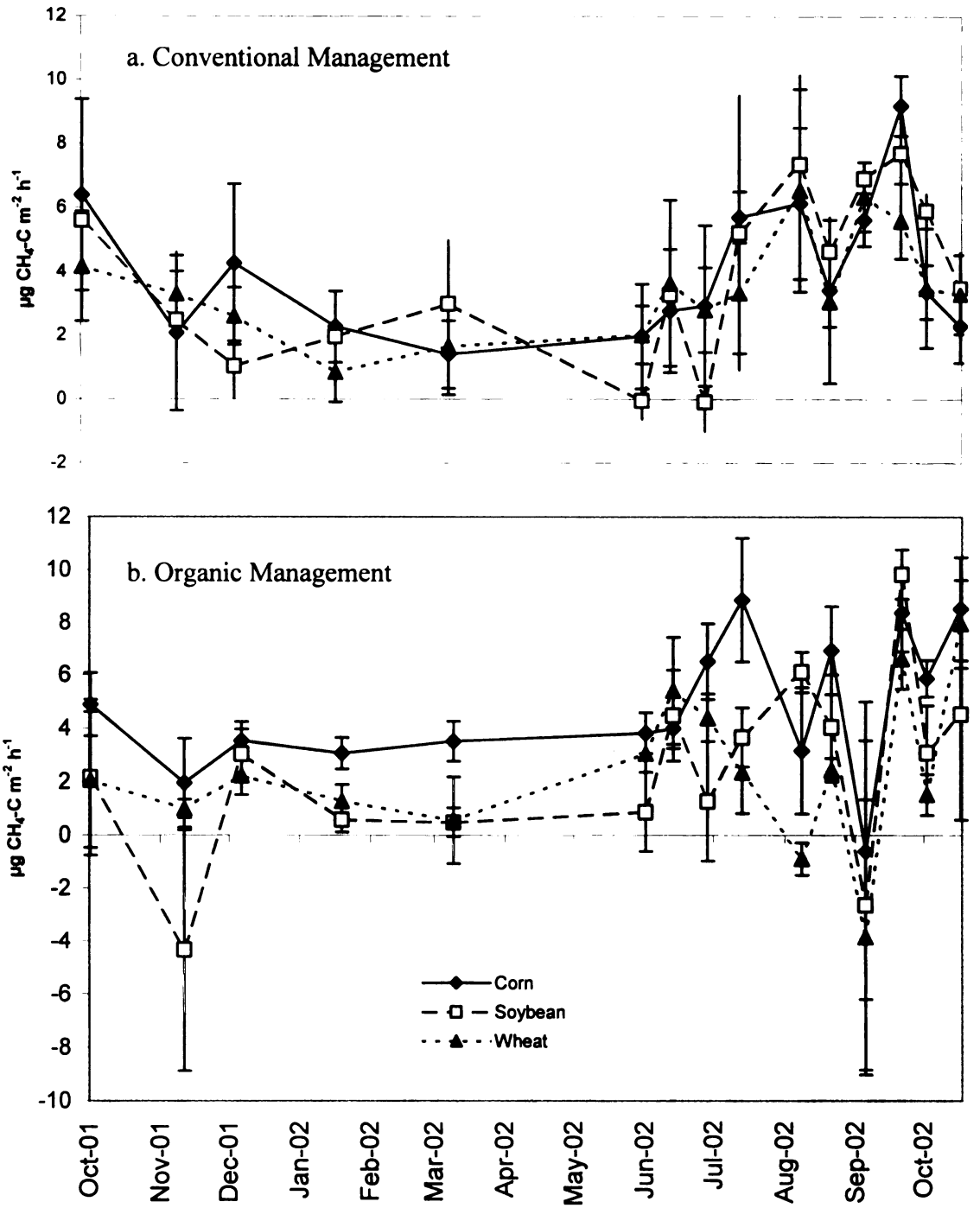


Figure 3.3 Average daily soil methane oxidation under different crops from conventional (top) and organic management systems (bottom) at the KBS LFL site in 2001-2002. Vertical bars represent standard errors (n = 4 replicate plots).

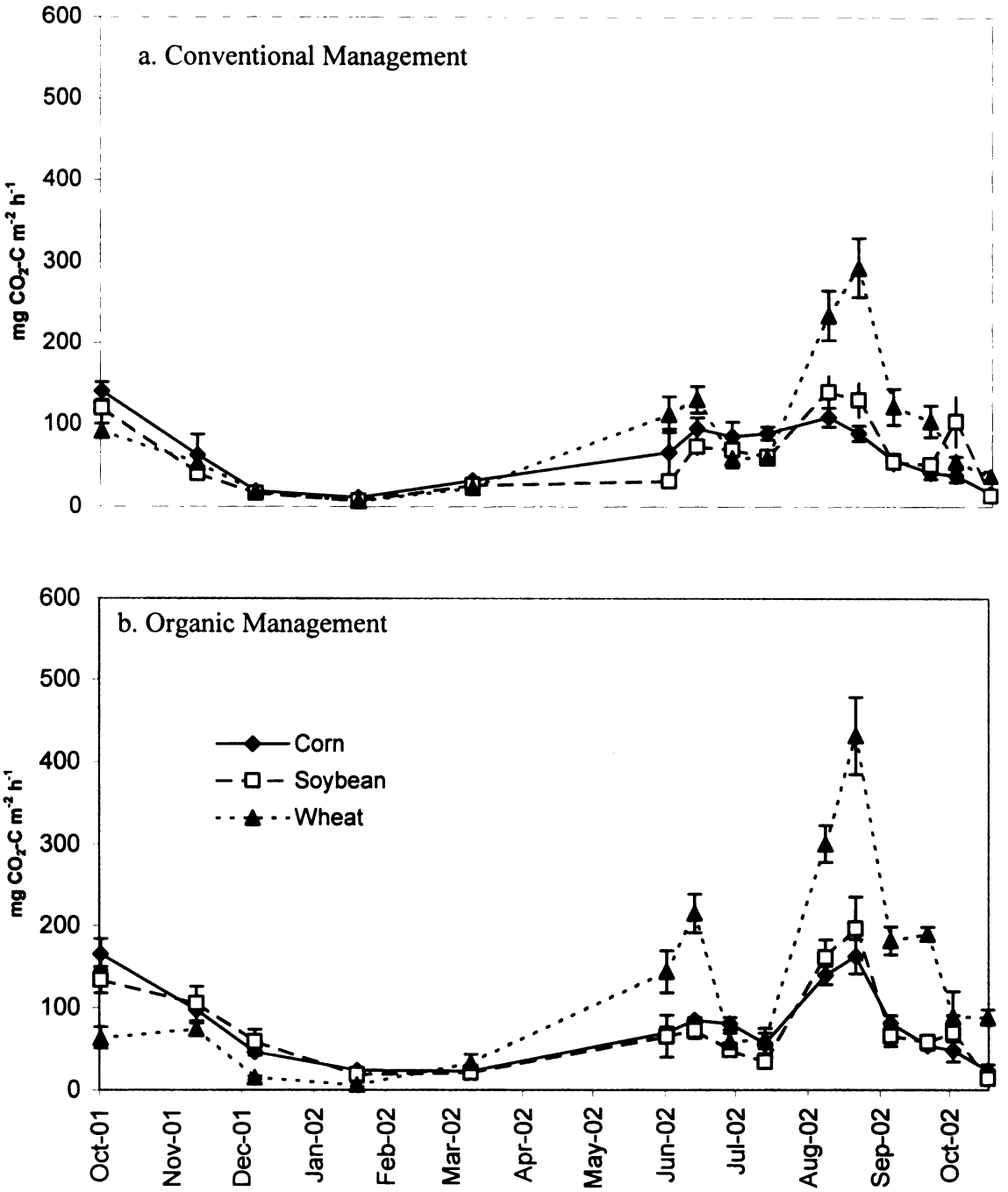


Figure 3.4 Average daily soil carbon dioxide emissions under different crops from conventional (top) and organic management systems (bottom) at the KBS LFL site in 2001-2002. Vertical bars represent standard errors (n = 4 replicate plots).

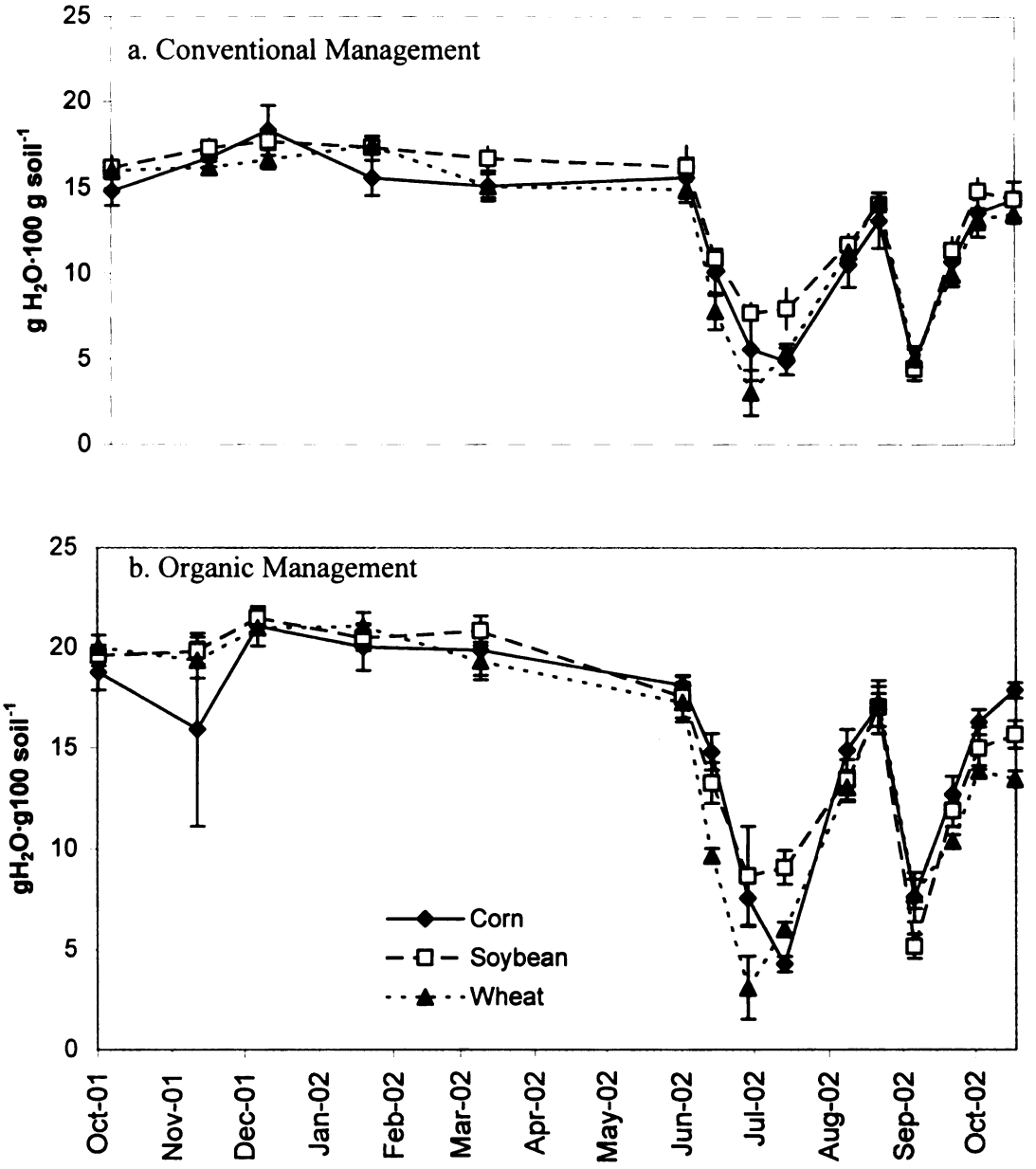


Figure 3.5 Average daily soil moisture in different crops under conventional (top) and organic management systems (bottom) at the KBS LFL site in 2001-2002. Vertical bars represent standard errors (n = 4 replicate plots).

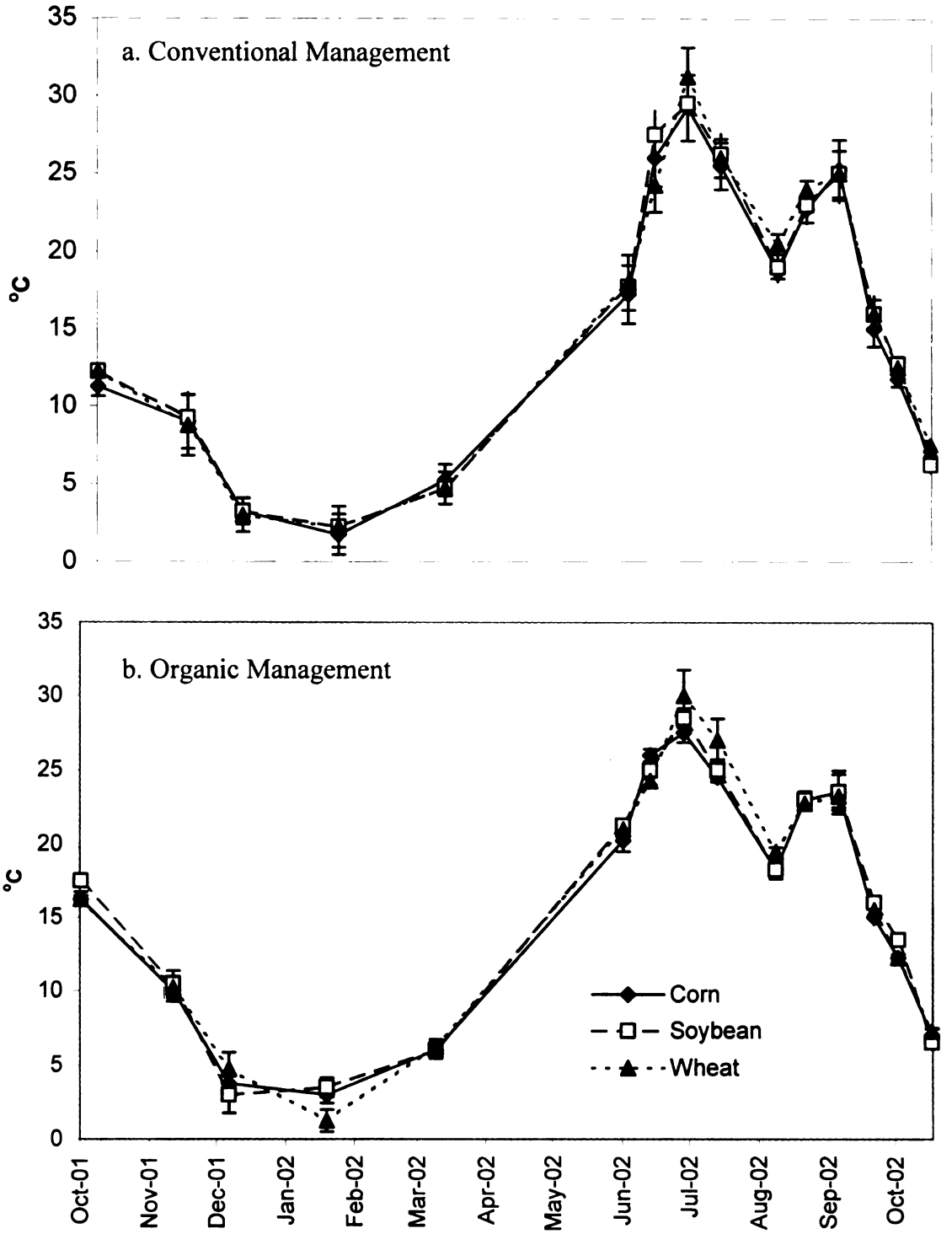


Figure 3.6 Average daily soil temperature under different crops in conventional (top) and organic management systems (bottom) at the KBS LFL site in 2001-2002. Vertical bars represent standard errors (n = 4 replicate plots)

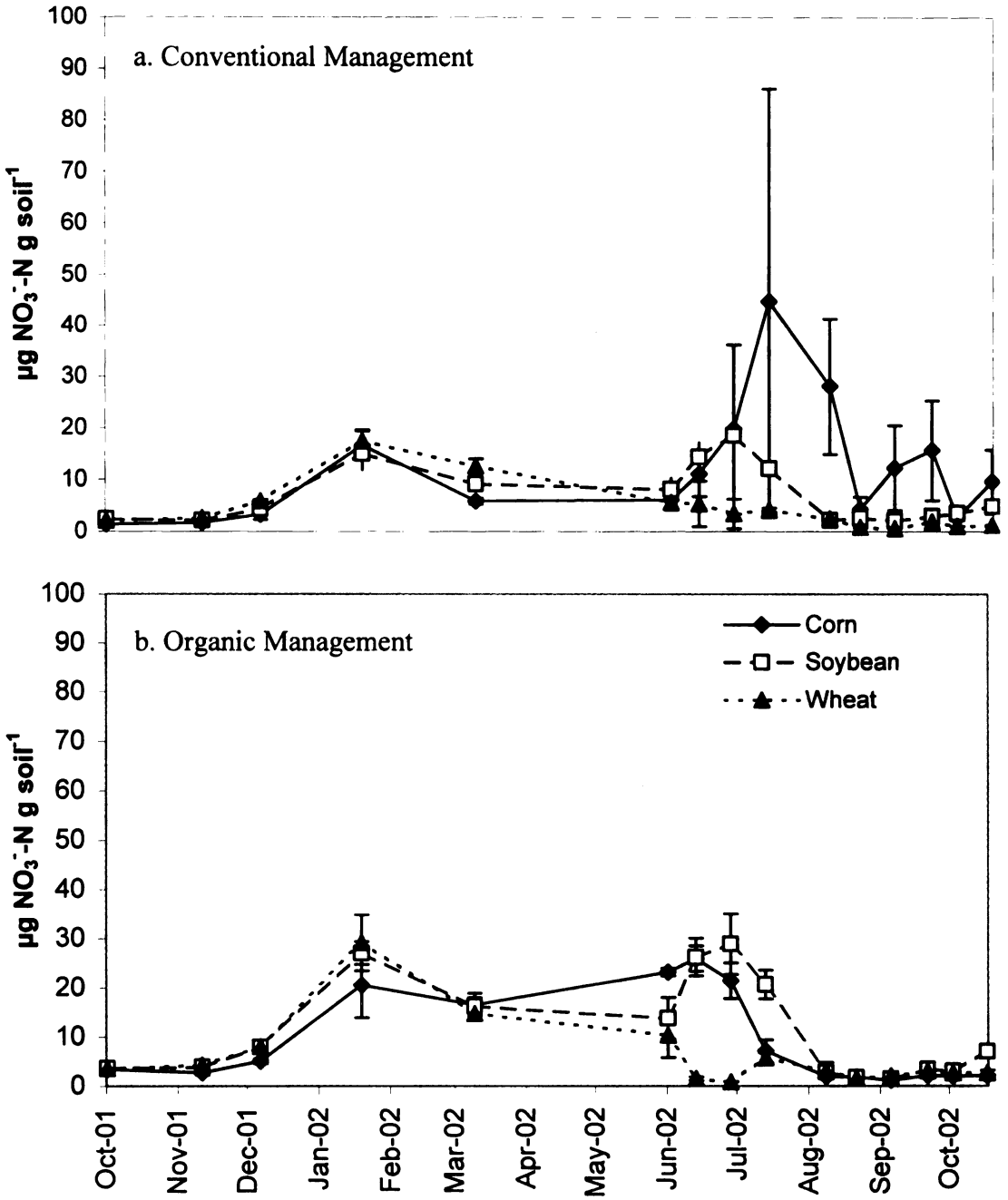


Figure 3.7 Average daily soil nitrate levels under different crops in conventional (top) and organic management systems (bottom) at the KBS LFL site in 2001-2002. Vertical bars represent standard errors (n=4 replicate plots).

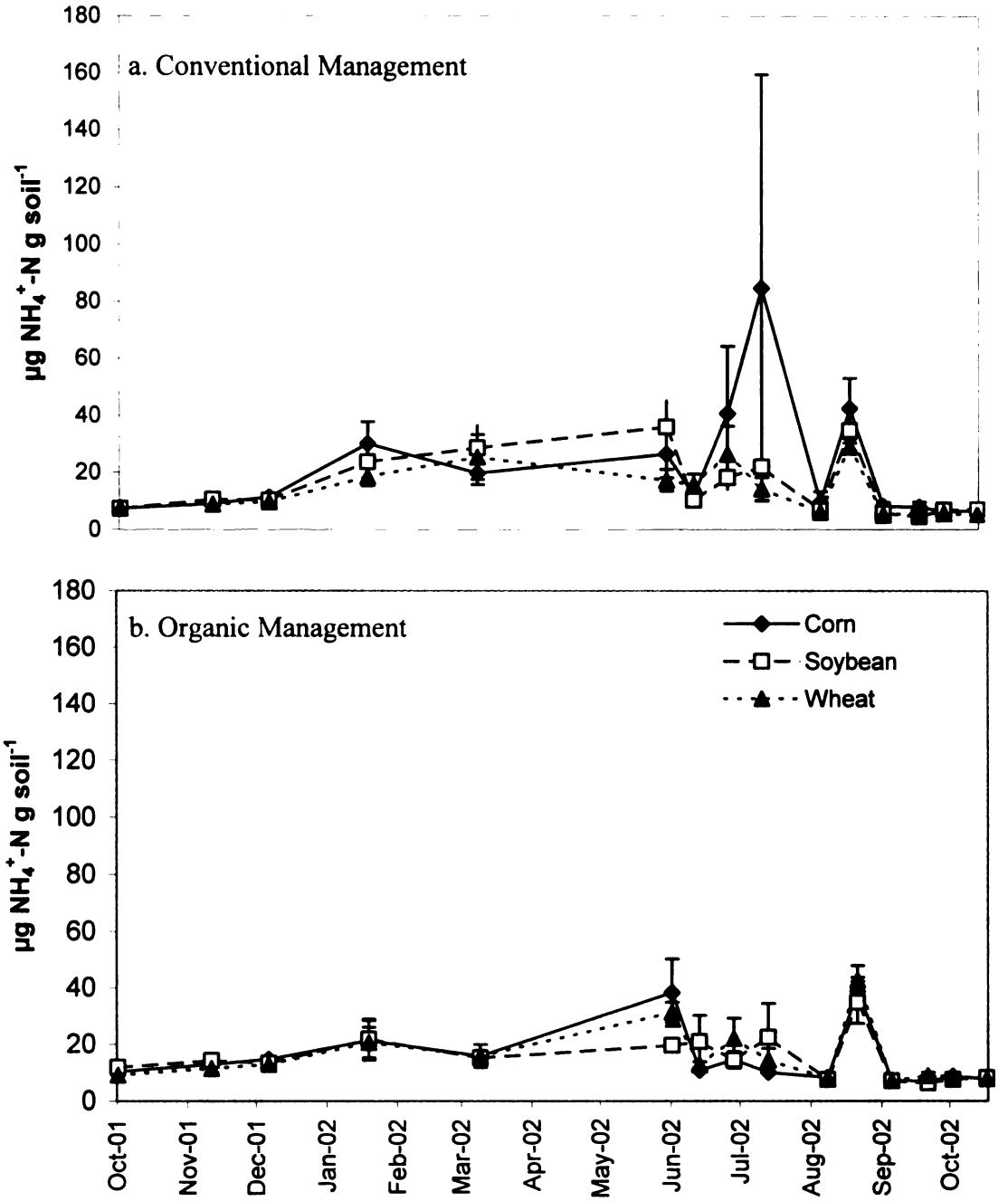


Figure 3.8 Average daily soil ammonium levels in different crops under conventional (top) and organic management systems (bottom) at the KBS LFL site in 2001-2002. Vertical bars represent standard errors (n=4 replicate plots).

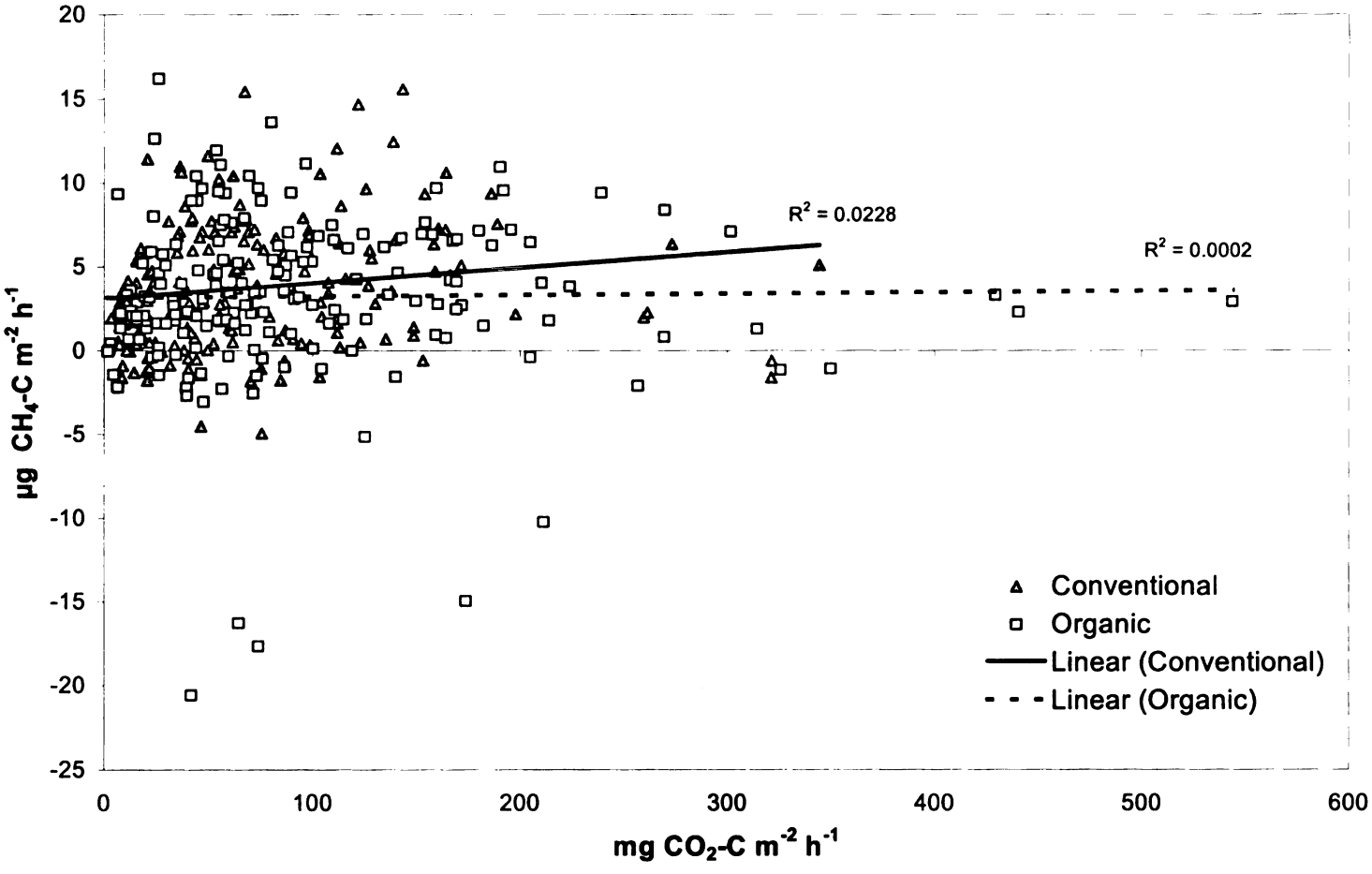


Figure 3.9 Relationships between methane oxidation and carbon dioxide fluxes in conventional and organic agriculture at the KBS LFL site.

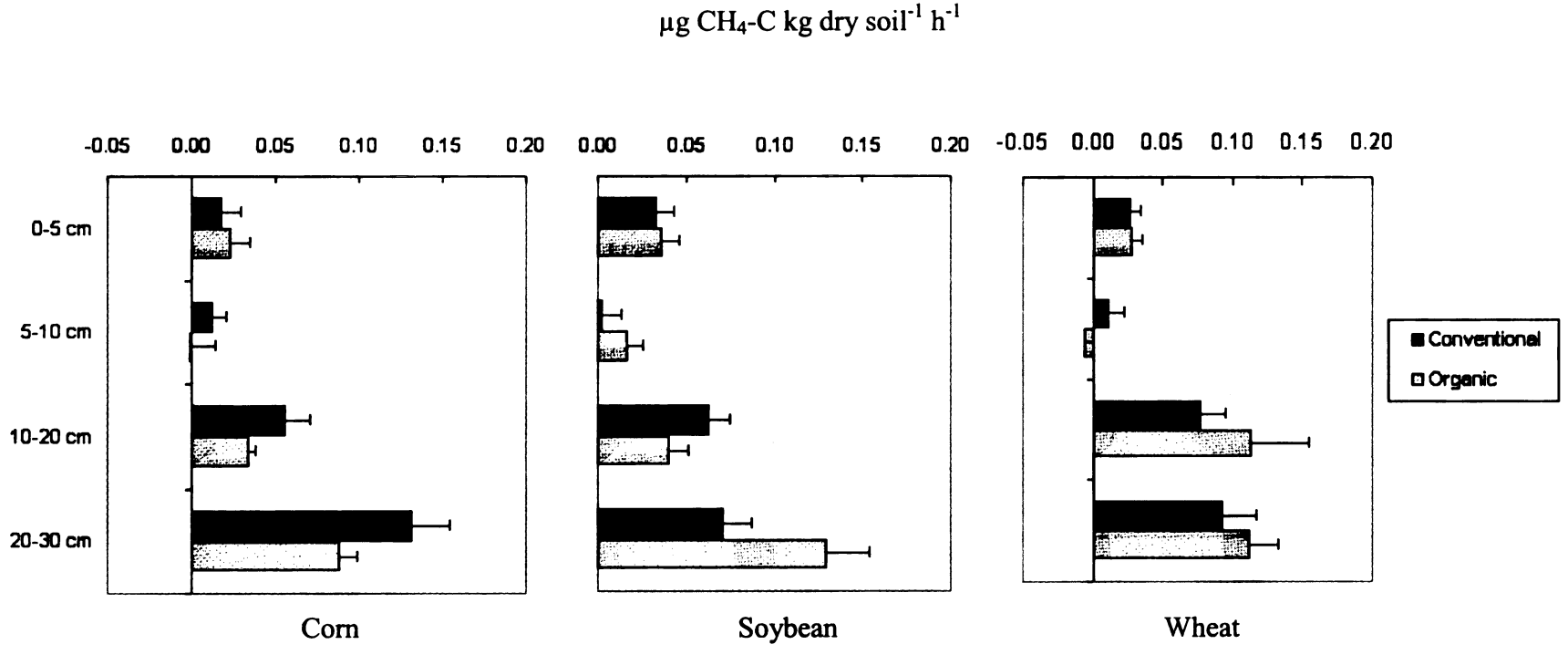


Figure 3.10 Soil methane oxidation with depth in soil cores from corn, soybean, and wheat rotations under conventional and organic management at the KBS LFL site. Bars represent mean rates (± standard error, n=4 cores).

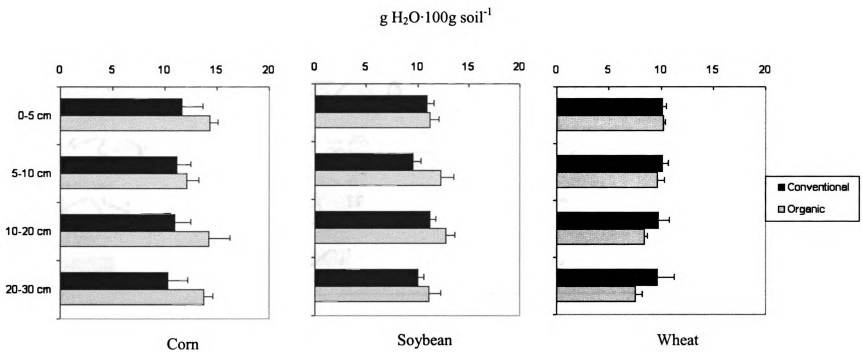


Figure 3.11 Soil moisture with depth in soil cores from corn, soybean, and wheat rotations under conventional and organic management at the KBS LFL site. Bars represent mean rates (± standard error, n=4 cores).

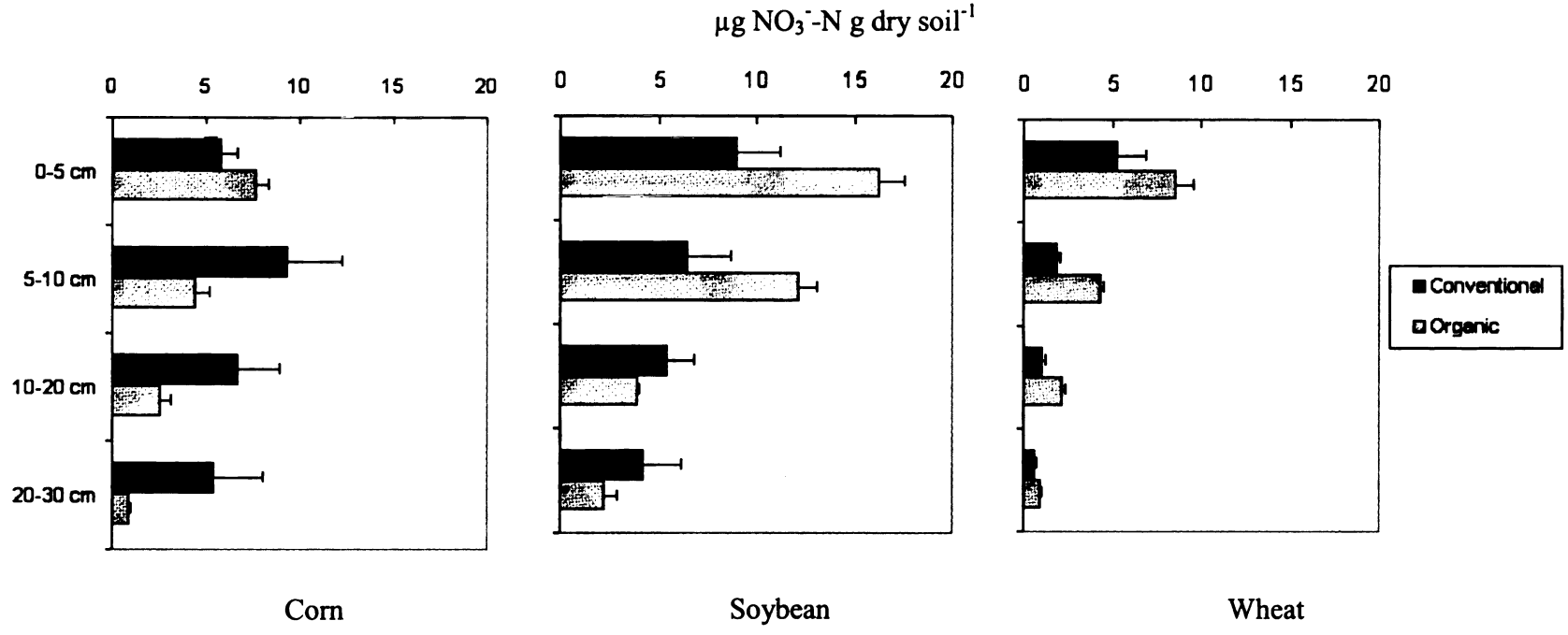


Figure 3.12 Soil nitrate with depth in soil cores from corn, soybean, and wheat rotations under conventional and organic management at the KBS LFL site. Bars represent mean rates (± standard error, n=4 cores).

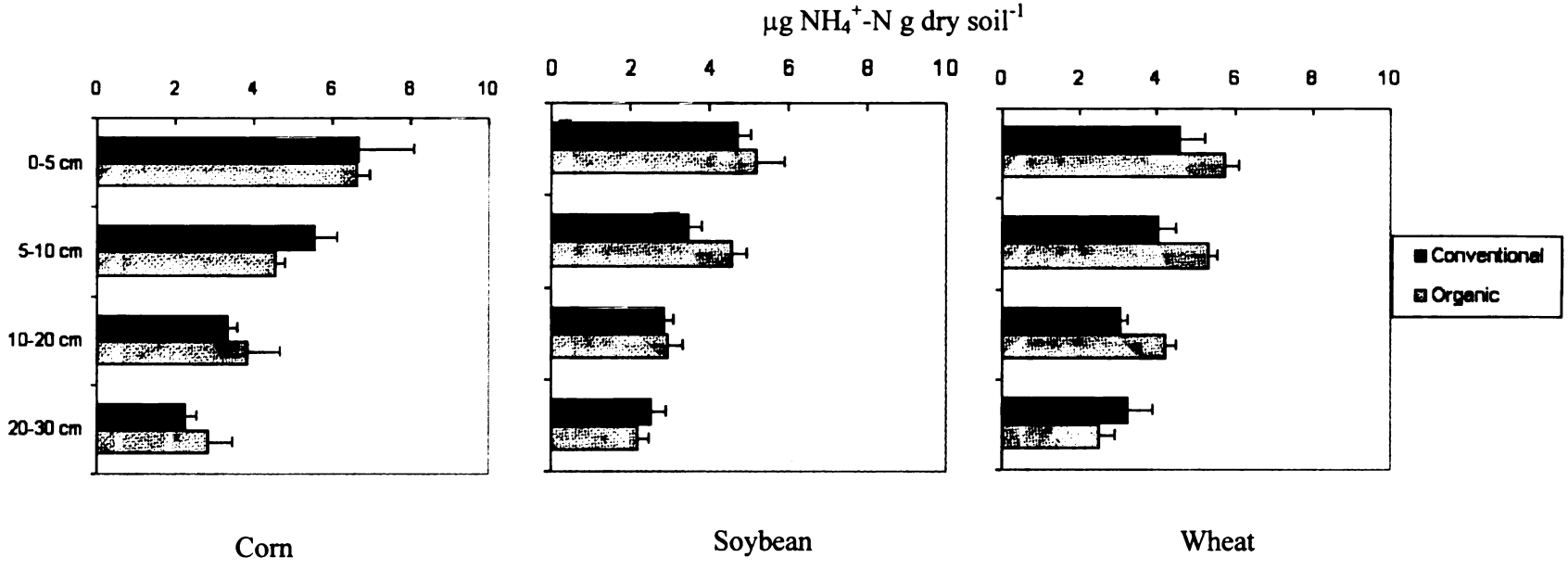


Figure 3.13 Soil ammonium with depth in soil cores from corn, soybean, and wheat rotations under conventional and organic management at the KBS LFL site. Bars represent mean rates (± standard error, n=4 cores).

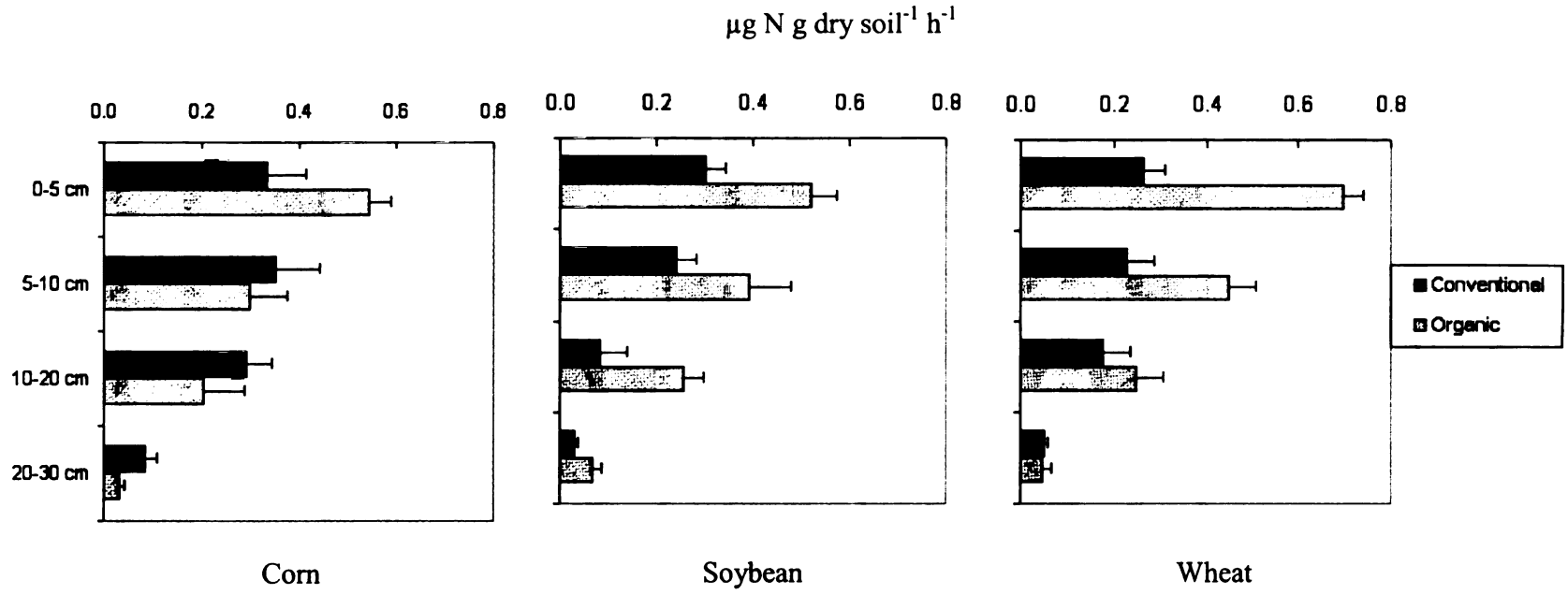


Figure 3.14 Soil nitrification with depth in soil cores from corn, soybean, and wheat rotations under conventional and organic management at the KBS LFL site. Bars represent mean rates (± standard error, n=4 cores).

Chapter 4

Methane Oxidation in Mature and Successional Forests: Effects of Nitrogen Deposition

Introduction

Atmospheric nitrogen deposition from air pollution has increased substantially with the industrial revolution and urbanization (Holland et al. 1999). This extra input of N into soil can alter both natural terrestrial and aquatic ecosystems (Holland et al. 1997). Results from several studies in which high rates of added N depresses CH_4 oxidation in soil suggest that anthropogenic N inputs might decrease the soil sink for atmospheric CH_4 in forests (Castro et al. 1992, Klemedtsson and Klemedtsson 1997, Saari et al. 1997, Butterbach-Bahl et al. 1998, Sitaula et al. 2001, Butterbach-Bahl et al. 2002, Wang and Ineson 2003). Circumstantial evidence also suggests a link. For example, a high latitude coniferous forest in the U.K. receives more N deposition and has lower rates of soil CH_4 oxidation than a lower latitude forest (MacDonald et al. 1997). Low N deposition coniferous soils in Finland have CH_4 uptake rates three times higher than high N deposition Dutch forest soils (Saari et al. 1997).

However, not all studies support this linkage. Steinkamp et al. (2001) found identical CH_4 oxidation rates between fertilized and unfertilized German Black forest soils, and Bradford et al. (2001) found that the chronic deposition of nitric acid and ammonium sulphate had no significant effect on soil CH_4 uptake in deciduous woodland in southwest England. Additionally, Borjesson and Nohrstedt (2000) found only transient

inhibition of CH_4 oxidation after N fertilization in Swedish forest soils during two years of investigation.

There are also conflicting results for the effects of ammonium vs. nitrate on forest soil CH_4 consumption (Schnell and King 1994). The different patterns of reduction can be explained partly by differences in the associated counter-ions of the ammonium or nitrate salts (King and Schnell 1998).

A problem with many of the studies that have directly tested the effect of added N on CH_4 oxidation is that N-levels have been much higher than the levels added in acid deposition, ca. 5-10 kg N ha^{-1} y^{-1}, and usually have been added for a much shorter period (ca. 1-3 years). The present study was designed to assess the impact of N deposition on soil CH_4 consumption in different types of temperate forest with N added at rates close to those added by deposition, and for longer periods.

Methodology

Site Description

The study was conducted at the Long-Term Ecological Research (LTER) sites at the W.K. Kellogg Biological Station (KBS), Hickory Corners, Michigan (42° 24'N, 85° 24'W, elevation 288 m). Annual rainfall at KBS averages 890 mm y^{-1} with about half falling as snow; potential evapotranspiration (PET) exceeds precipitation for about 4 months of the year. Mean annual temperature is 9.7 °C.

I measured methane fluxes at three sites along a management intensity gradient: mature deciduous forests (DF), 40-60 years old conifer plantations (CF), and mid-successional communities (SF) abandoned from agriculture 40-60 years ago (see details

at http://lter.kbs.msu.edu). All sites were replicated within the larger landscape (n=3 locations) and were on the same Kalamazoo/Oshtemo soil series (Austin 1979). The soils at these sites are Typic Hapludalfs (fine or coarse-loamy, mixed, mesic soils) derived from glacial till about 12,000 years ago (Crum and Collins 2003).

The major plants in the deciduous forest sites are red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marsh.), white oak (*Quercus alba* L.), northern red oak (*Quercus rubra* L.), flowering dogwood (*Cornus florida* L.), and sassafras (*Sassafras albidum* (Nutt.) Nees). The prevailing plants in coniferous forests are red pine (*Pinus resinosa* Aiton), white pine (*Pinus strobus* L.), pignut hickory (*Carya glabra* (Miller) Sweet), Norway spruce (*Picea abies* (L.) Karsten), wild black cherry (*Prunus serotina* Ehrh.), and big-toothed aspen (*Populus grandidentata* Michx.).

The dominant plants in the mid-successional communities are Canada goldenrod (*Solidago canadensis* L.), quackgrass (*Elytrigia repens* (L.) Nevski), timothy (*Phleum pratense* L.), white hearth aster (*Aster pilosus* Willd.), Kentucky bluegrass (*Poa pratensis* L.), common yarrow (*Achillea millefolium* L.), Canada bluegrass (*Poa compressa* L.), autumn olive (*Elaeagnus umbellata* Thunb.), sassafras (*Sassafras albidum* (Nutt.) Nees), gray goldenrod (*Solidago nemoralis* Ait.), smooth brome (*Bromus inermis* Leyss.), germander speedwell (*Veronica chamaedrys* L.), orchardgrass (*Dactylis glomerata* L.), flowering spurge (*Euphorbia corollata* L.), and honeysuckle (*Lonicera* spp.).

The fertilized plots were establish in 1993, nine years prior to the start of this study. Two levels of ammonium nitrate (NH_4NO_3) are applied annually in three equal aliquots per year during April to November. At each replicated site, a 10 x 10 m fertilized (F) plot receives 10 kg N ha^{-1} y^{-1} and a 2 x 2 m highly fertilized (HF) plot receives 30 kg

N ha^{-1} y^{-1}. Average N deposition in this area is ca. 10 kg N ha^{-1} y^{-1}(National Atmospheric Deposition Program website http://nadp.sws.uiuc.edu/). During the 2002 study year, the fertilized dates were March 28, June 27, and September 17. From a 20 x 20 cm microplot in the highly fertilized plot, forest litter was regularly remove from soil surface beginning in May.

In Situ Gas Sampling

I measured in situ methane oxidation rates using the static chamber technique (Hutchinson and Livingston 1993). Static chambers were fashioned from 25 cm diameter PVC pipe: bases (25 cm diameter x 10 cm high) were installed in each plot and left in place except during agronomic operations. Immediately prior to sampling, a 4.5 cm high cap was placed on each base and sealed with a latex skirt wrapped with an elastic band. At 10-minute intervals, four 10 mL headspace samples were removed through a rubber septa in each cap using a syringe and put into 3 mL glass sample vials pre-flushed with headspace air. Within 3 days vial contents were measured for CH_4 using a gas chromatograph (GC 5890 Series II, Hewlett Packard) equipped with a flame ionization detector (FID), and for CO_2 using an infrared gas absorption (IRGA) analyzer (EGA CO_2 Analyzer, Analytical Development CO. LTD. Hoddesdon, England).

Chambers were sampled twice per month during the growing season from late May to late September. All chambers were sampled on the same day.

Soil Analyses

Soil temperature was measured at 0-5 cm depth using a temperature probe. Soil

samples for other analysis were taken from the top 10 cm of soil using a 2.5 cm diameter soil probe. Fresh soils were passed through a 4 mm sieve and mixed by hand, and then subsamples were taken for moisture content and mineral N analyses. Prior to analysis, soils were stored in a refrigerator at 4°C for up to 4 weeks. Soil moisture content was measured gravimetrically by drying the soil samples at 65°C for 3 days or until dry. Further drying at 105°C removes an additional 0.8 g H_2O 100g $soil^{-1}$ moisture in these soils. Mineral N measurements were obtained by extracting 20 g of dry soil with 100 mL 1 M KCl for 24 h and then filtering the extract through 2-μm pore size fiberglass filters. The filtrates were frozen prior to analysis for NH_4^+ and NO_3^-, which was performed using an Alpkem continuous flow analyzer (Alpkem 3550, 01 Analytical, College Station, TX) (Bundy and Meisinger 1994).

Soil Depth Study

Soil cores (5 cm diameter x 30 cm deep) were taken from the coniferous forest, mature deciduous forest, mid-successional communities, and no-tilled agricultural fields and stored intact at 4°C for one week, after which they were then separated into four different depth increments (0-5, 5-10, 10-20, and 20-30 cm). Each soil increment was transferred to a 473 mL Mason jar for 0-5 cm and 5-10 cm increments and to a 946 mL Mason jar for 10-20 cm and 20-30 increments; jars were left open and allowed to pre-incubate with atmospheric methane at room temperature for one week in order to reduce soil moisture levels to ca. 15% w/w. At the end of this period, methane oxidation was measured by sealing each jar with a cap and removing 0.5 mL of headspace at four 10-minute intervals. The headspace aliquot was immediately injected into the gas

chromatograph for methane analysis. After the CH_4 oxidation study, soil samples were sieved and separated for soil moisture, soil inorganic N content, and nitrification assays.

Nitrification Assay

A sieved soil sample of 10 g was placed in a 100 mL Erlenmeyer flask and combined with 50 mL of the phosphate buffer solution and ammonium sulfate (Hart et al. 1994), and sealed with Parafilm. The flasks were placed on a shaker table and shaken for 24 h. Soil slurry samples of 10 mL were taken before and another after 24 h incubation, centrifuged for 30 min and filtered through 0.2 μm filter paper. The aliquots were frozen until analysis of nitrate as above. Rates of nitrate production were calculated on the basis of the difference between the initial and final nitrate concentrations.

Statistic Analyses

I used Proc Mixed of SAS version 8.0 (SAS Institute 1999) for the analysis of variance (ANOVA) and analysis of covariance (ANCOVA) for field data, in which I treated site and treatment as fixed effects and day and site x treatment x day as random effects. I also used SPSS version 10.0.1 (SPSS Inc. 2001) for ANOVA and ANCOVA for the soil depth data, and also for correlation analyses for both field and laboratory samples. Methane and CO_2 data were natural log transformed before ANOVA and ANCOVA to homogenize variances. I used untransformed data for correlation analysis.

Results

Field study

Methane Oxidation

Soil CH_4 consumption was significantly different among sites (Table 4.2). Among control plots, CH_4 uptake was highest in deciduous forest at 44.1 ± 3.9 μg CH_4-C m^{-2} h^{-1} (±s.e, n=3 replicate sites x 9 sample dates) and lowest in mid-successional communities at 23.1 ±1.5 μg CH_4-C m^{-2} h^{-1}, while the coniferous forest was intermediate (Table 4.3).

Added nitrogen had a significant effect on soil CH_4 oxidation (Table 4.2); the site x treatment and the site x treatment x day interactions were also significant: nitrogen additions significantly reduced CH_4 oxidation rates only in the coniferous forest sites, where average rates declined from 28.7 ± 3.6 to 16.4 ± 2.3 μg CH_4-C m^{-2} h^{-1} N with low fertilizer addition and to 11.1 ± 2.6 μg CH_4-C m^{-2} h^{-1} with high N addition (Table 4.3 and Figure 4.1). Removing the forest floor of the high N coniferous forest plots further lowered oxidation rates to 6.2 ± 1.8 μg CH_4-C m^{-2} h^{-1}. In contrast, only forest floor removal significantly affected CH_4 oxidation rates in the deciduous forest sites, where rates declined from 44.1 ± 3.9 to 23.7 ± 5.8 μg CH_4-C m^{-2} h^{-1} following the removal. In the mid-successional communities oxidation rates were likewise unaffected by N-fertilizer addition.

Sampling date also had no effect on CH_4 oxidation rates (Table 4.2). Uptake rates varied very little among sample dates in the mid-successional communities; rates in control plots were between 20 and 35 μg CH_4-C m^{-2} h^{-1} for all 9 dates (Figure 4.2c). In deciduous forest control plots, CH_4 oxidation rates ranged from 17 to 59 μg CH_4-C m^{-2} h^{-1}, with the higher rates between June and September (Figure 4.2b). In the coniferous forest, CH_4 uptake was high only in August (to 52 μg CH_4-C m^{-2} h^{-1}) (Figure 4.2a); otherwise control plot rates were similar to those in the mid-successional sites.

Carbon Dioxide Fluxes

CO_2 production rates were also significantly different among sites and the site x treatment interaction was significant (Table 4.4). Among control plots, CO_2 flux was highest in mid-successional communities at 127 ±10 mg CO_2-C m^{-2} h^{-1}followed by coniferous forest at 109 ± 11 mg CO_2-C m^{-2} $h^{-1}$and then deciduous forest (70 ± 7 mg CO_2-C m^{-2} h^{-1} (Table 4.3).

Added nitrogen had a significant effect on CO_2 emissions (Table 4.4). The high N additions significantly decreased CO_2 fluxes from 109 ± 11 to 80 ± 8 mg CO_2-C m^{-2} h^{-1} in coniferous forest. In contrast, high N-additions significantly increased CO_2 emissions from 70 ± 7 to 101 ± 8 mg CO_2-C m^{-2} h^{-1} in deciduous forest (Table 4.3 and Figure 4.3). Forest floor removal significantly decreased CO_2 fluxes only in the coniferous forest, by 40% (Table 4.2).

Carbon dioxide fluxes also varied significantly by sample dates (Table 4.4). The emission rates in coniferous forests and mid-successional communities reached their highest levels (approximately 180 mg CO_2-C m^{-2} h^{-1}) in early August. The peak occurred two weeks earlier in deciduous forest – in late July emissions were around 188 mg CO_2-C m^{-2} h^{-1} (Figure 4.4).

Soil Properties

Soil moisture significantly differed only among sites (Table 4.5). For example, in control plots, average soil moisture was highest in mid-successional communities (18.6 ± 0.7 g H_2O·100 g $soil^{-1}$), followed by deciduous forest (9.4 ± 0.9 g H_2O·100 g $soil^{-1}$) and

coniferous forests (8.9 ± 0.7 g $H_2O \cdot 100$ g $soil^{-1}$) (Table 4.3). Treatments did not affect soil moisture, which varied (P=0.069) by sample dates (Table 4.3, 4.5 and Figure 4.5).

Soil temperature was slightly different among sites (P=0.044) (Table 4.5). Mid-successional control soils were on average about a degree warmer (18.6 °C) than the others (18.3 vs. 17.3 and 17.1 °C; Table 4.3) (Table 4.2). N addition did not significantly affect soil temperature, but soil temperature significantly varied with sample dates (Table 4.5). Temperatures were highest in mid June (24.0 ± 3.1 °C, n=3 replications) and started to decline at the end of August (Figure 4.6).

Soil nitrate significantly differed by site (Table 4.5). Among control plots, soil nitrate was highest in coniferous forest (5.4 ± 0.6 μg N g $soil^{-1}$) followed by deciduous forest (3.4 ± 0.6 μg N g $soil^{-1}$), and mid-successional communities (0.7 ± 0.2 μg N g $soil^{-1}$), respectively (Table 4.2). Although nitrate did not significantly differ by sampling dates (Table 4.5) it increased substantially after fertilization (Figure 4.7). The increases following fertilization were highest in deciduous forest soils, intermediate in mid successional communities, and lowest in coniferous forest. It was highest in mid-July and climbing up at the mid-September (Figure 4.7b).

Soil ammonium was also significantly different among sites (Table 4.5). Both deciduous (15.7 ± 2.8 μg N g $soil^{-1}$) and mid-successional communities (15.4 ± 2.8 μg N g $soil^{-1}$) had higher soil ammonium levels than those of coniferous forest (12.1 ± 1.6 μg N g $soil^{-1}$; Table 4.2 and Figure 4.9). N addition had no significant effect on soil ammonium (Table 4.5). However, ammonium levels changed significantly changed seasonally (Table 4.5). Soil ammonium in all treatments, including control, began to increase from the end of May until reaching the highest point at the end of June after the

second fertilization, and then declined steadily for the rest of the sampling period even after the third fertilization (Figure 4.8).

Soil Depth Profiles

As for *in situ* fluxes, overall soil CH_4 oxidation rates in laboratory-incubated cores were significantly different among sites (Table 4.8). Deciduous forest soils had the highest rates of oxidation at 0.49 ± 0.039 (s.e., n=6 soil cores x 4 depth x 2 treatments) μg CH_4-C kg dry soil^{-1} h^{-1} followed by mid-successional communities (0.20 ± 0.018 μg CH_4-C kg dry soil^{-1} h^{-1}), and coniferous forest (0.17 ± 0.024 μg CH_4-C kg dry soil^{-1} h^{-1}). Methane uptake did not much differ among soil depths except in deciduous forest soils, where consumption at the 5-10 cm depth was 28-58% higher than consumption at 0-5 cm, 10-20 cm, and 20-30 cm depth (Table 4.7 and Figure 4.9).

Fertilizer application (10 kg N ha^{-1}) in the forested sites also did not much affect soil CH_4 consumption within each site (Tables 4.7 and 4.8).

Soil moisture in cores was significantly different among sites, treatments, and soil depths (Table 4.9). Surface soils had the highest soil moisture when compared with lower level soil profiles (Table 4.7 and Figure 4.10). Moisture decreased up to 53% at 20-30 cm depth comparing to surface soils in deciduous fertilized soils.

Nitrate significantly differed by site and treatment but not by depth (Table 4.9). Although not significant, soil nitrate was generally highest in surface soils rather than deeper soils (Figure 4.11). For example, nitrate was 87% lower in surface soils than deeper layers in coniferous control plots (Table 4.7).

In contrast, soil ammonium significantly differed only among soil depths and not among sites or treatments (Table 4.9). Ammonium was highest in surface soils then significantly decreased with depths up to 88% in soils from deciduous control plots (Table 4.7 and Figure 4.12).

Soil nitrification also varied significantly among sites, depth and nearly differed among treatments (P=0.067; Table 4.9). Nitrification was lowest in mid-successional communities soils (Table 4.7). Nitrification also significantly decreased with soil depth, up to 95% in cores from coniferous control plots (Figure 4.13).

Controls on Methane and Carbon Dioxide Fluxes

From analysis of covariance of field data, none of the physical and chemical soil properties measured significantly affected CH_4 uptake in either *in situ* or in soil core incubation (Table 4.2 and 4.8). There were also no significant correlations between CH_4 flux and soil properties in the field (Tables 4.6). Methane uptake was weakly correlated with nitrate (r = -0.162) and ammonium (r = 0.152; Table 4.10). Methane and CO_2 fluxes were not significantly correlated (Table 4.6).

Soil moisture nearly affected (P=0.063) *in situ* CO_2 fluxes (Table 4.4) although moisture and CO_2 flux were not significantly correlated (Table 4.6). Only nitrate was significantly but weakly correlated with CO_2 (r = -0.144) (Table 4.6).

Discussion

Field study

Methane Oxidation

As in an earlier study (Chapter 2), soil CH_4 oxidation was significantly different among study sites. In control plots, CH_4 uptake was highest in mature deciduous forest followed by rates in coniferous forests and mid-successional communities, which were similar (Table 4.3 and Figure 4.1) Deciduous forest soils had lower soil bulk density than both coniferous and mid-successional communities (Table 4.1), which may have allowed faster CH_4 diffusion into the soil. Deciduous forest soils also had more total carbon, total nitrogen, and higher ammonium levels than coniferous forest soils. The higher soil nitrate in coniferous forests and higher soil ammonium in mid-successional communities might explain their lower CH_4 uptake. In contrast, deciduous forest soil had more acidity than coniferous forests and mid-successional communities. Acidity is thought to suppress soil CH_4 consumption but this did not appear to be true in this study.

N fertilizer inhibited soil CH_4 oxidation only in coniferous forest sites, where rates declined from 28.7 ± 3.6 to 16.4 ± 2.3 $\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$ with low fertilizer addition and to 11.1 ± 2.6 $\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$ with high N addition (Table 4.3). This suggests that soil CH_4 oxidation in mid-successional and mature native communities is unaffected by up to 3 times the current levels of N deposition in this area. CH_4 oxidation in planted coniferous stands, on the other hand, appears suppressible by small N additions.

Soil nitrate slightly increased following N-fertilization in all sites (Figure 4.7), but relatively low levels, especially in the coniferous forest and mid-successional communities during the growing season, suggests strong biological sinks for nitrogen. Levels added appeared not to create excess soil N, except in the deciduous forest where fertilized plots had more N than control plots for most of the year.

Forest floor removal significantly reduced CH_4 uptake in both coniferous and deciduous forests. This suggests that the litter layer is either a habitat for CH_4 oxidation or affects soil conditions in underlying layers. Perhaps the high C:N forest floor material immobilizes inorganic N and its removal allows N to increase in the mineral soil, thereby suppressing oxidation. Yavitt et al. (1993) also found a slight reduction of CH_4 consumption after removing roots and leaf litter from the soil surface in a northern hardwood forest in the Adirondack region of New York. These results also indicate that leaf litter in these sites does not contain a CH_4 oxidation inhibitor as found in some soil surface extracts from boreal and temperate forest (Amaral and Knowles 1997).

Carbon dioxide

As for CH_4 oxidation, CO_2 emissions were also significantly different among sites. Overall CO_2 production was highest in mid-successional communities, somewhat lower in coniferous forests, and much lower in deciduous forests. This might be caused by higher soil moisture in the mid-successional communities, which would have favored microbial activity. Although soil ammonium was a fairly good predictor of CO_2 emission, it was not different among sites.

Nitrogen fertilizer addition significantly affected CO_2 emissions in coniferous and deciduous forests. Fertilizer significantly increased soil microbial activity. Forest floor removal only significantly decreased CO_2 emission in the high N fertilization coniferous forest plots. This suggests that the forest floor was providing carbon and nutrients to soil microorganisms in lower horizons in these plots.

CH_4 oxidation with Soil Depth

In laboratory incubations, deciduous forest soil cores had the highest methane oxidation rates, followed by mid-successional communities, and coniferous forests. Although statistical analysis showed no detectable impact of soil physical and chemical properties on CH_4 uptake in soil cores, mid-successional communities had lower soil nitrate and nitrification rates and less CH_4 consumption than deciduous forest soil.

As in the field study, fertilizer treatment (10 kg N ha^{-1}) did not affect soil CH_4 consumption in soil cores. Fertilizer increased soil nitrate in coniferous and deciduous forests but not in the mid-successional communities. Plants and bacteria in mid-successional communities soil appear to have a high ability to deplete nitrate after the fertilization (see Figure 4.8). Soil ammonium increased slightly in fertilized plots of coniferous and deciduous forests but, again, not in mid-successional communities. The fertilized plots also had a higher nitrification rate than control plots, especially in deciduous forest.

Soil CH_4 consumption also did not significantly differ among soil depths, except in deciduous forest. There was a slight subsurface maximum at 5-10 cm depth in deciduous forest soil cores. CH_4 oxidation in soil below 5 cm depth was slightly higher than in 0-5 cm for mid-successional communities. Soil moisture also slightly decreased with soil depth, especially in deciduous forest soil cores. Soil nitrate was highest at 5-20 cm depth of coniferous forest, deciduous forest, and no-till cornfield but rarely found at depths lower than 5 cm in mid-successional communities. Only soil ammonium showed a significant decrease along soil profiles of all study sites. Soil nitrification was also higher near the surface.

Nitrification seems to be a good indicator for soil CH_4 uptake inhibition. Hütsch (2001) found a very strong negative correlation ($r = -0.92$) between CH_4 oxidation and nitrification from long-term fertilization treatments of the field experiment "Ewiger Roggenbau" at Halle, Germany. The use of nitrification inhibitors has also increased CH_4 oxidation by 28% (Weiske et al. 2001). Kravchenko (2002) recently showed that ammonium inhibition in peat soils was not a competitive mechanism but rather caused by toxic substances from nitrifiers. This suggests that nitrifiers are not responsible for methane oxidation as has been suggested in the past (see Hütsch 2001), but rather than inhibit methanotrophs by producing nitrite. This notion is consistent with our results, which showed no relationships between nitrification and CH_4 oxidation.

Conclusion

Low N deposition (10 kg N ha^{-1}) had no detectable effect on CH_4 fluxes in all sites while high N deposition (30 kg N ha^{-1}) significantly reduced CH_4 oxidation only in coniferous forest. N deposition also significantly increased CO_2 emission in deciduous forest while in contrast, only high N deposition significantly reduced CO_2 fluxes in coniferous forest. Forest floor removal also significantly decreased CH_4 oxidation in both coniferous and deciduous forests while CO_2 emission was significantly reduced only in coniferous forest by litter removal. Methane oxidation significantly differed among soil depths only in deciduous forest. The highest oxidation rate was in 5-10 cm soil depth. Soil factors had no significant effect on CH_4 uptake. Only soil moisture significantly associated with CO_2 fluxes.

References

Amaral, J. A., and R. Knowles. 1997. Inhibition of methane consumption in forest soils and pure cultures of methanotrophs by aqueous forest soil extracts. Soil Biology & Biochemistry **29**:1713-1720.

Austin, F. R. 1979. Soil Survey of Kalamazoo County, Michigan. United States Department of Agriculture, Soil Conservation Service, in cooperation with Michigan Agricultural Experiment Station. The Soil Conservation Service, Washington.

Borjesson, G., and H. O. Nohrstedt. 2000. Fast recovery of atmospheric methane consumption in a Swedish forest soil after single-shot N-fertilization. Forest Ecology and Management **134**:83-88.

Bradford, M. A., P. A. Wookey, P. Ineson, and H. M. Lappin-Scott. 2001. Controlling factors and effects of chronic nitrogen and sulphur deposition on methane oxidation in a temperate forest soil. Soil Biology & Biochemistry **33**:93-102.

Bundy, L. G., and J. J. Meisinger. 1994. Nitrogen availability indices. Pages 951-984 *in* R. W. Weaver, J. S. Angle, and B. S. Bottomley, editors. Methods of soil analysis. Part 2. Microbiological and biochemical properties. SSSA Book Series, no. 5., Madison, WI.

Butterbach-Bahl, K., L. Breuer, R. Gasche, G. Willibald, and H. Papen. 2002. Exchange of trace gases between soils and the atmosphere in Scots pine forest ecosystems of the northeastern German lowlands 1. Fluxes of N_2O, NO/NO_2 and CH_4 at forest sites with different N-deposition. Forest Ecology and Management **167**:123-134.

Butterbach-Bahl, K., R. Gasche, C. H. Huber, K. Kreutzer, and H. Papen. 1998. Impact of N-input by wet deposition on N-trace gas fluxes and CH_4-oxidation in spruce forest ecosystems of the temperate zone in Europe. Atmospheric Environment **32**:559-564.

Castro, M. S., P. A. Steudler, J. M. Melillo, J. D. Aber, and S. Millham. 1992. Exchange of N_2O and CH_4 between the atmosphere and soils in spruce-fir forests in the northeastern united-states. Biogeochemistry **18**:119-135.

Crum, J. R., and H. P. Collins. 2003. KBS soils *in* http://lter.kbs.msu.edu/Soil/characterization/.

Hart, S. C., J. M. Stark, E. A. Davidson, and M. K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. Pages 985-1018 *in* R. W. Weaver, J. S. Angle, and B. S. Bottomley, editors. Methods of soil analysis. Part

2. Microbiological and biochemical properties. SSSA Book Series, no. 5., Madison, WI.

Holland, E. A., B. H. Braswell, J. F. Lamarque, A. Townsend, J. Sulzman, J. F. Muller, F. Dentener, G. Brasseur, H. Levy, J. E. Penner, and G. J. Roelofs. 1997. Variations in the predicted spatial distribution of atmospheric nitrogen deposition and their impact on carbon uptake by terrestrial ecosystems. Journal of Geophysical Research-Atmospheres **102**:15849-15866.

Holland, E. A., F. J. Dentener, B. H. Braswell, and J. M. Sulzman. 1999. Contemporary and pre-industrial global reactive nitrogen budgets. Biogeochemistry **46**:7-43.

Hutchinson, G. L., and G. P. Livingston. 1993. Use of chamber systems to measure trace gas fluxes. Pages 63-78 *in* L. A. Harper, A. R. Mosier, J. M. Duxbury, and D. E. Rolston, editors. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. American Society of Agronomy, Madison, Wisconsin.

Hütsch, B. W. 2001. Methane oxidation, nitrification, and counts of methanotrophic bacteria in soils from a long-term fertilization experiment ("Ewiger Roggenbau" at Halle). Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde **164**:21-28.

King, G. M., and S. Schnell. 1998. Effects of ammonium and non-ammonium salt additions on methane oxidation by *Methylosinus trichosporium* OB3b and Maine forest soils. Applied and Environmental Microbiology **64**:253-257.

Klemedtsson, A. K., and L. Klemedtsson. 1997. Methane uptake in Swedish forest soil in relation to liming and extra N-deposition. Biology and Fertility of Soils **25**:296-301.

Kravchenko, I. K. 2002. Methane oxidation in boreal peat soils treated with various nitrogen compounds. Plant and Soil **242**:157-162.

MacDonald, J. A., U. Skiba, L. J. Sheppard, B. Ball, J. D. Roberts, K. A. Smith, and D. Fowler. 1997. The effect of nitrogen deposition and seasonal variability on methane oxidation and nitrous oxide emission rates in an upland spruce plantation and moorland. Atmospheric Environment **31**:3693-3706.

Saari, A., P. J. Martikainen, A. Ferm, J. Ruuskanen, W. De Boer, S. R. Troelstra, and H. J. Laanbroek. 1997. Methane oxidation in soil profiles of Dutch and Finnish coniferous forests with different soil texture and atmospheric nitrogen deposition. Soil Biology & Biochemistry **29**:1625-1632.

SAS Institute. 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc., Cary, NC.

Schnell, S., and G. M. King. 1994. Mechanistic analysis of ammonium inhibition of atmospheric methane consumption in forest soils. Applied and Environmental Microbiology **60**:3514-3521.

Sitaula, B. K., J. I. B. Sitaula, A. Aakraz, and L. R. Bakken. 2001. Nitrification and methane oxidation in forest soil: Acid deposition, nitrogen input, and plant effects. Water Air and Soil Pollution **130**:1061-1066.

SPSS Inc. 2001. SPSS Base 11.0 for Windows User's Guide. SPSS Inc.

Steinkamp, R., K. Butterbach-Bahl, and H. Papen. 2001. Methane oxidation by soils of an N limited and N fertilized spruce forest in the Black Forest, Germany. Soil Biology & Biochemistry **33**:145-153.

Wang, Z. P., and P. Ineson. 2003. Methane oxidation in a temperate coniferous forest soil: effects of inorganic N. Soil Biology & Biochemistry **35**:427-433.

Weiske, A., G. Benckiser, T. Herbert, and J. C. G. Ottow. 2001. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biology and Fertility of Soils **34**:109-117.

Yavitt, J. B., J. A. Simmons, and T. J. Fahey. 1993. Methane fluxes in a northern hardwood forest ecosystem in relation to acid precipitation. Chemosphere **26**:721-730.

Table 4.1 Soil properties for the mature deciduous forest, coniferous forest, mid-successional forest, and no-till agricultural fields at the KBS LTER site. Values are means (±s.e., n=varied). Data are from the KBS LTER website http://kbs.msu.edu/lter/.

Soil property	Deciduous forest	Coniferous forest	Mid-successional forest	No-till cornfield
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam
pH[1]	5.20 ± 0.12 a	5.58 ± 0.07 b	5.66 ± 0.04 b	6.45 ± 0.09 c
Bulk density (g cm^{-3})[2]	1.22 ± 0.08 a	1.42 ± 0.06 b	1.40 ± 0.04 b	1.57 ± 0.06 bc
Total carbon (mg C g $soil^{-1}$)[3]	1.52 ± 0.12 a	1.47 ± 0.27 a	0.97 ± 0.03 ab	0.73 ± 0.09 b
Total nitrogen (mg N g $soil^{-1}$)[3]	0.123 ± 0.09 a	0.107 ± 0.009 a	0.093 ± 0.003 ab	0.087 ± 0.009 b
Nitrate (μg N g $soil^{-1}$)[4]	1.93 ± 0.21 ab	2.67 ± 0.23 abd	0.38 ± 0.05 c	2.79 ± 0.30 bd
Ammonium (μg N g $soil^{-1}$)[4]	3.50 ± 0.37 ab	3.17 ± 0.23 ab	4.46 ± 0.58 a	2.08 ± 0.73 b

[1] sampled 8 March 2000 to a depth of 10 cm.

[2] sampled 10 April 1996 to a depth of 15 cm.

[3] sampled 7 April 1999 to a depth of 25 cm.

[4] average values from 2000 season to a depth of 25 cm.

Table 4.2 Analysis of covariance to determine effects of site, treatment, and soil properties on methane oxidation rates in mature deciduous forest, coniferous forest, and mid-successional communities at the KBS LTER site.

Variable	Num. df [a]	Den. df [a]	F	P
Site	2	276	32.5	<0.0001
Treatment	3	276	19.6	<0.0001
Site x Treatment	5	276	2.6	0.024
Day	8	276	0.7[b]	0.235
Site x Treatment x Day	39	276	-3.3[b]	0.001
Moisture	1	276	0.9	0.334
Temperature	1	276	0.1	0.762
Nitrate	1	222	0.6	0.424
Ammonium	1	222	1.6	0.205

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] Z value

Table 4.3 Effects of N fertilizer on methane oxidation, carbon dioxide emission, and soil properties in coniferous forest, deciduous forest, and mid-successional forest at the KBS LTER site. Values are means ± standard error of mean for 3 replicates x 9 sample dates. Values followed by different lower case letters are significantly different ($P<0.05$) between treatment and control within site. Values followed by different higher case letters are significantly different ($P<0.05$) of the same treatment among sites.

Treatment	CH_4 ($\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$)	CO_2 ($mg\ CO_2\text{-}C\ m^{-2}\ h^{-1}$)	Moisture ($g\ H_2O\ 100g\ soil^{-1}$)	Temperature (°C)	Nitrate ($\mu g\ N\ g\ soil^{-1}$)	Ammonium ($\mu g\ N\ g\ soil^{-1}$)
Coniferous Forest						
Control	28.7 ± 3.6 Aa	109 ± 11 Ba	8.9 ± 0.7 A	17.3 ± 0.6	5.4 ± 0.6	12.1 ± 1.6
Low N	16.4 ± 2.3 Ba	95.4 ± 9.9 Aa	10.1 ± 1.0 A	17.6 ± 0.8	6.8 ± 1.0 A	11.4 ± 1.5
High N	11.1 ± 2.6 Bb	80.1 ± 8.1 Bb	10.3 ±1.0 A	17.4 ±0.8	8.2 ± 0.7	11.8 ± 1.4
High N no forest Floor	6.2 ± 1.8 Ab	66.1 ± 7.0 Ab				
Deciduous Forest						
Control	44.1 ±3.9 Aa	70.5 ± 6.8 Ba	9.4 ± 0.9 A	17.1 ± 0.7	3.4 ± 0.6 a	15.4 ± 2.8
Low N	41.0 ± 7.1 Aa	91.1 ± 6.4 Ab	12.0 ±1.1 A	17.0 ± 0.6	11.0 ± 1.7 Ab	15.3 ± 1.5
High N	41.0 ± 8.0 Aa	101 ± 8 ABb	7.9 ± 0.7 A	16.9 ± 0.7	7.5 ± 1.9 a	18.8 ± 2.8
High N no forest Floor	23.7 ± 5.8 Ab	79.5 ± 6.9 Aa				
Mid-successional Forest						
Control	23.1 ± 1.5 Aa	127 ± 10 Aa	18.6 ± 1.7 B	18.3 ± 0.7	0.7 ± 0.2	15.7 ± 2.8
Low N	26.4 ± 1.7 Aa	113 ± 14 Aa	21.0 ± 2.3 B	17.9 ± 0.9	1.8 ± 0.3 B	15.1 ± 2.0
High N	25.1 ± 2.3 Aa	134 ± 10 Aa	19.6 ±2.2 B	17.7 ± 0.7	3.5 ± 1.2	18.0 ± 2.7

Table 4.4 Analysis of covariance to determine effects of site, treatment, and soil properties on carbon dioxide emissions in mature deciduous forests, coniferous forests, and mid-successional communities at the KBS LTER site.

Variable	Num. df [a]	Den. df [a]	F	P
Site	2	276	13.3	<0.0001
Treatment	3	276	3.4	0.018
Site x Treatment	5	276	4.5	0.001
Day	8	276	1.8[b]	0.036
Site x Treatment x Day	39	276	0.9[b]	0.386
Moisture	1	276	3.5	0.063
Temperature	1	276	1.4	0.233
Nitrate	1	222	0.1	0.698
Ammonium	1	222	3.0	0.083

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] Z value

Table 4.5 Analysis of variance to determine effects of site, treatment, and day on soil properties from coniferous forest, mature deciduous forest, and mid-successional forest at the KBS LTER site.

Variable	Num. df [a]	Den. df [a]	Moisture		Temperature		Nitrate		Ammonium	
			F	P	F	P	F	P	F	P
Site	2	278[b]	54.5	<0.0001	3.2	0.044	25.6	<0.0001	10.1	<0.0001
Treatment	3	278[b]	1.8	0.156	0.2	0.923	11.2	<0.0001	2.0	0.138
Site x Treatment	5	278[b]	0.7	0.626	0.1	0.983	3.8	0.005	0.5	0.744
Day	8	278[b]	1.5[c]	0.069	2.0[c]	0.025	1.4[c]	0.083	1.9[c]	0.026
Site x Treatment x Day	39	278[b]	0.8[c]	0.405	-2.1[c]	0.032	-1.1[c]	0.268	-0.5[c]	0.621

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] 226 for nitrate and ammonium

[c] Z value

Table 4.6 The relationships among methane oxidation, carbon dioxide and other soil properties in coniferous forest, mature deciduous forest, and mid-successional communities at the KBS LTER site. Values are Pearson correlation coefficients (r); n= 3 replicate plots x 9 sampling dates x 4 treatments.

	CO_2	Moisture	Temperature	Nitrate	Ammonium
CH_4	0.027	0.005	-0.089	-0.042	0.062
CO_2	1.000	0.095	0.113	-0.144*	0.002

* Correlation is significant at the 0.05 level (2-tailed)

Table 4.7 Fertilization effects on average methane oxidation, soil properties, and nitrification rates among crops in coniferous forest, deciduous forest, mid-successional forest, and no-till agricultural field at the KBS LTER site. Values are means ± standard error of mean for six replicates. Values followed by different lower case letters are significantly different ($P<0.05$) among sites. Values followed by different upper case letters are significantly different ($P<0.05$) among soil depths.

	Coniferous forest		Deciduous forest		Mid-successional forest		No-till agriculture
	Control	Fertilizer	Control	Fertilizer	Control	Fertilizer	Control
CH_4 ($\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$)							
0-5 cm	0.23 ±0.08a	0.20 ±0.06a	0.53±0.06ABb	0.51± 0.08b	0.12 ±0.04a	0.14 ±0.04a	0.04 ±0.01a
5-10 cm	0.12 ±0.06a	0.10± 0.03a	0.71±0.05Ab	0.71 ±0.15b	0.22 ±0.05a	0.26 ±0.04a	0.07 ±0.01a
10-20 cm	0.34 ±0.11	0.13± 0.08	0.34±0.09B	0.47 ±0.12	0.23 ±0.07	0.22 ±0.05	0.10 ±0.03
20-30 cm	0.14 ±0.04	0.11± 0.02	0.38±0.13Ba	0.30 ±0.09	0.18 ±0.07	0.24 ±0.02	0.07 ±0.02b
Moisture ($g\ H_2O.100g\ soil^{-1}$)							
0-5 cm	11.4 ±1.4	9.5 ±1.0a	12.9± 0.3A	14.0± 1.1Ab	15.2± 0.8*	11.0± 0.8Aab*	15.0± 0.6
5-10 cm	8.0 ±1.3a	8.0 ±1.0a	9.6± 0.3BCa	12.0± 0.7Ab	11.6± 1.0a	9.0± 0.4Aa	14.0± 0.6b
10-20 cm	7.4 ±1.3a	7.5 ±0.8	8.1± 0.6BCDa	8.7± 0.3B	11.1± 1.0b	8.4± 0.6A	14.0± 0.4b
20-30 cm	7.5 ±1.7a	6.6 ±1.3	6.1± 0.8CDa	6.6± 0.3B	11.7± 1.8a	7.6± 0.9B	14.1± 1.3b
Nitrate ($\mu g\ N\ g\ soil^{-1}$)							
0-5 cm	10.3± 5.3	3.7± 2.0	3.2± 1.1	1.8± 0.3	5.6± 1.1A	4.7± 0.6A	10.2± 1.8
5-10 cm	2.2± 0.8ab	9.0± 2.9	1.9± 0.4a	8.3± 2.7	0.6± 0.1Ba	1.5 ±0.3B	14.5± 6.2b
10-20 cm	1.4± 0.4a	10.9± 3.6	1.1± 0.3a	7.8± 2.4	0.5± 0.0Ba	0.9 ±0.2B	18.9± 7.4b
20-30 cm	1.4± 0.3a	4.6± 1.6	1.4± 0.7a	5.1± 1.6	0.4± 0.0Ba	0.7 ±0.1B	11.7± 4.5b
Ammonium ($\mu g\ N\ g\ soil^{-1}$)							
0-5 cm	6.2± 1.2A	8.3± 1.1A	11.5± 2.4A	9.6± 2.9A	7.8± 0.6AB	7.2± 0.5AB	8.1± 1.9A
5-10 cm	3.4± 0.3B	4.4±0.9AB	4.4± 0.5B	5.7± 0.9AB	6.0± 1.7ABC	6.0± 1.8ABC	4.3± 0.6AB
10-20 cm	2.4± 0.2B	4.0± 1.2B	2.0± 0.1B	3.4± 0.5AB	2.7± 0.1BCD	2.9± 0.3BCD	2.7± 0.3B
20-30 cm	1.6± 0.2B	3.2± 0.9B	1.4± 0.1B	2.8± 0.8B	1.5± 0.1CD	1.6± 0.2CD	2.1± 0.3B
Nitrification ($\mu g\ N\ g\ soil^{-1}\ h^{-1}$)							
0-5 cm	0.20± 0.03A	0.17± 0.02	0.11± 0.05	0.18± 0.05A	0.07± 0.02A	0.10± 0.03A	0.21± 0.06
5-10 cm	0.04± 0.01B	0.08± 0.03	0.03± 0.01	0.09± 0.02AB	0.02± 0.01B	0.05± 0.01AB	0.23± 0.13
10-20 cm	0.01± 0.01Ba	0.10± 0.05	0.03± 0.02a	0.05± 0.03B	0.01± 0.00Ba	0.01± 0.00B	0.17± 0.05b
20-30 cm	0.01± 0.00B	0.04± 0.02	0.05± 0.04	0.02± 0.01B	0.01± 0.00B	0.01± 0.00B	0.08± 0.03

* significant differences at 0.05 level between treatments within the same site.

Table 4.8 Analysis of covariance for methane oxidation as affected by site, fertilization, and soil depth on soil properties from coniferous forest, mature deciduous forest, mid-successional communities, and no-till agricultural fields at the KBS LTER site.

Source	SS	df	MS	F	P
Site	$3.2x10^{-2}$	3	$1.0x10^{-2}$	37.3	0.000
Treatment	$1.2x10^{-4}$	1	$1.2x10^{-4}$	0.43	0.513
Depth	$1.8x10^{-3}$	3	$6.2x10^{-4}$	2.18	0.092
Site x Treatment	$7.0x10^{-4}$	2	$3.5x10^{-4}$	1.25	0.291
Site x Depth	$7.5x10^{-3}$	9	$8.4x10^{-4}$	2.95	0.003
Treatment x Depth	$5.3x10^{-5}$	3	$1.8x10^{-5}$	0.06	0.979
Site x Treatment x Depth	$1.3x10^{-3}$	6	$2.2x10^{-4}$	0.77	0.593
Nitrate	$2.3x10^{-5}$	1	$2.3x10^{-5}$	0.08	0.777
Ammonium	$2.7x10^{-4}$	1	$2.7x10^{-4}$	0.96	0.329
Moisture	$5.5x10^{-6}$	1	$5.5x10^{-6}$	0.02	0.889
Nitrification	$2.1x10^{-4}$	1	$2.1x10^{-4}$	0.74	0.390
Error	$3.9x10^{-2}$	136	$2.8x10^{-4}$		
Total	910	168			
Corrected Total	$8.9x10^{-2}$	167			

Table 4.9 Analysis of variance to determine effects of site, treatment, and depth on soil properties from coniferous forest, mature deciduous forest, mid-successional forest, and no-till agricultural fields at the KBS LTER site.

Variable	df	Moisture		Nitrate		Ammonium		Nitrification	
		F	P	F	P	F	P	F	P
Site	3	27.9	0.000	21.1	0.000	1.0	0.384	13.5	0.000
Treatment	1	6.3	0.014	5.0	0.027	2.5	0.116	3.4	0.067
Depth	3	21.0	0.000	1.4	0.259	44.3	0.000	12.1	0.000
Site x Treatment	2	11.2	0.000	1.1	0.330	1.1	0.330	0.1	0.934
Site x Depth	9	2.2	0.023	1.8	0.077	1.5	0.152	1.1	0.400
Treatment x Depth	3	0.9	0.458	3.1	0.027	0.4	0.755	0.4	0.771
Site x Treatment x Depth	6	0.2	0.978	0.7	0.621	0.4	0.854	0.6	0.715

Table 4.10 The relationship among methane oxidation and other soil properties from the soil depth study in coniferous forest, mature deciduous forest, mid-successional communities, and no-till agricultural fields at the KBS LTER site. Values are Pearson correlation coefficients (r); n = 6 soil cores x 4 sites x 2 treatments.

	Moisture	Nitrate	Ammonium	Nitrification
CH_4	-0.044	-0.162*	0.152*	-0.032

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

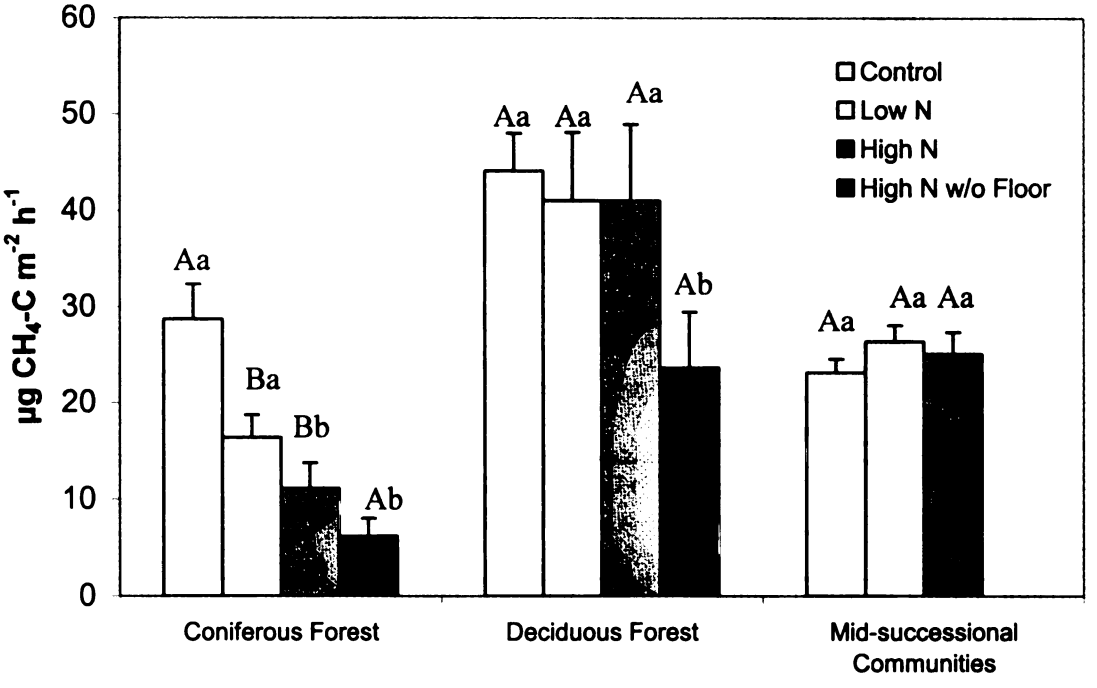


Figure 4.1 The effects of different N fertilizer levels on net methane oxidation in coniferous forest, deciduous forest, and mid-successional communities at the KBS LTER site. Bars are mean seasonal rates; vertical lines indicate standard errors (n= 3 replicate plots x 9 sampling dates). The different higher and lower case letters indicate significant differences (P>0.05) of the same treatment among sites, and between control and the treatment within the site, respectively.

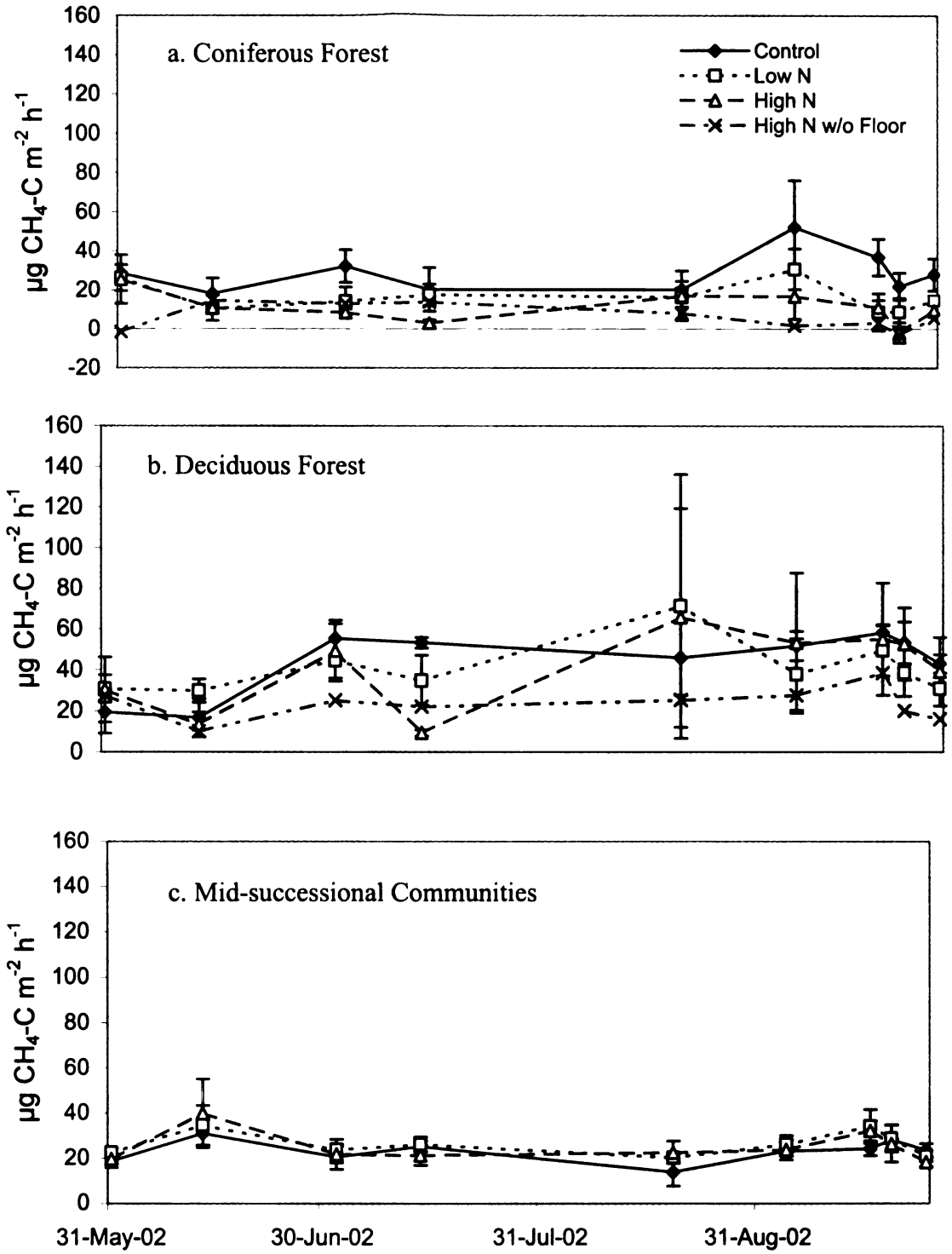


Figure 4.2 The effects of different N fertilizer levels on net daily methane oxidation in coniferous forest (top), deciduous forest (middle), and mid-successional communities (bottom) at the KBS LTER site. Vertical lines indicate standard errors (n= 3 replicate plots).

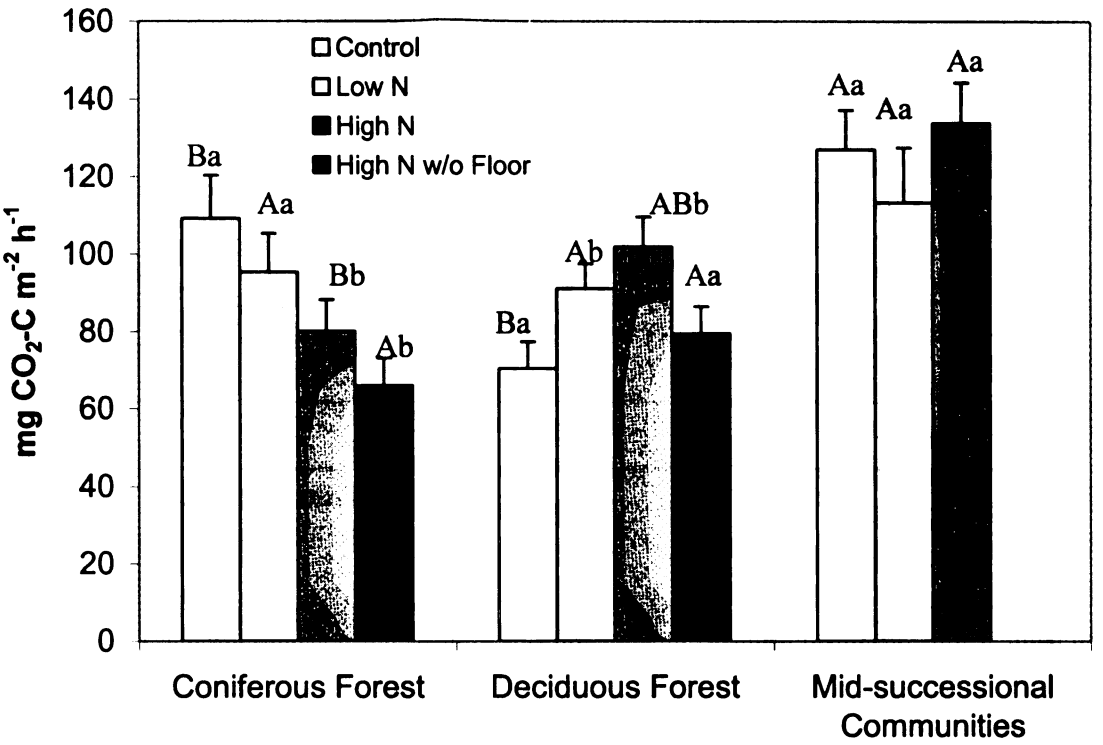


Figure 4.3 The effects of different N fertilizer levels on net carbon dioxide emission in coniferous forest, deciduous forest, and mid-successional communities at the KBS LTER site. Bars are mean seasonal rates; vertical lines indicate standard errors (n= 3 replicate plots x 9 sampling dates). The different higher and lower case letters indicate significant differences ($P<0.05$) of the same treatment among sites, and between control and the treatment within the site, respectively.

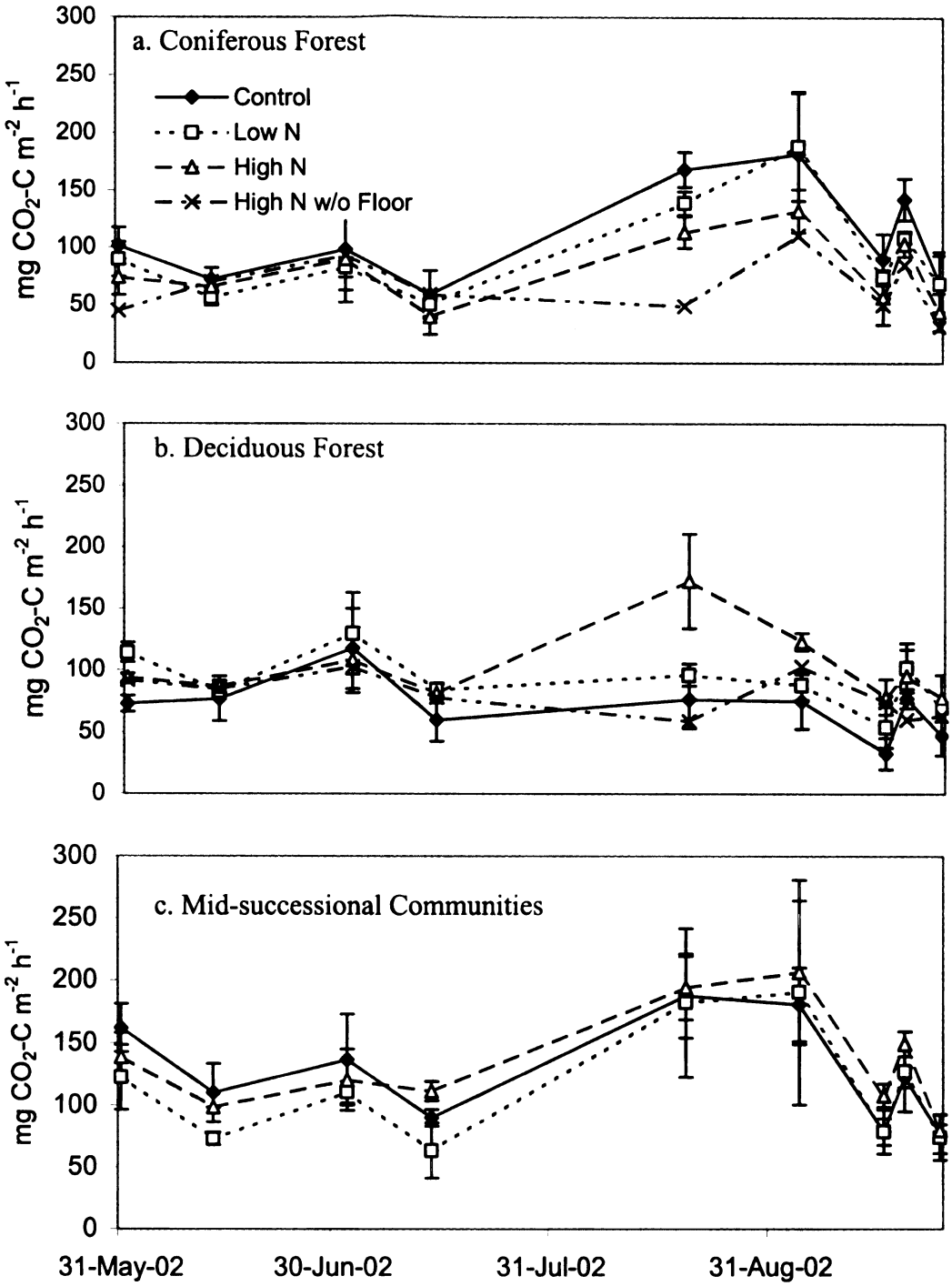


Figure 4.4 The effects of different N fertilizer levels on net daily carbon dioxide emission in coniferous forest (top), deciduous forest (middle), and mid-successional communities (bottom) at the KBS LTER site. Vertical lines indicate standard errors (n= 3 replicate plots).

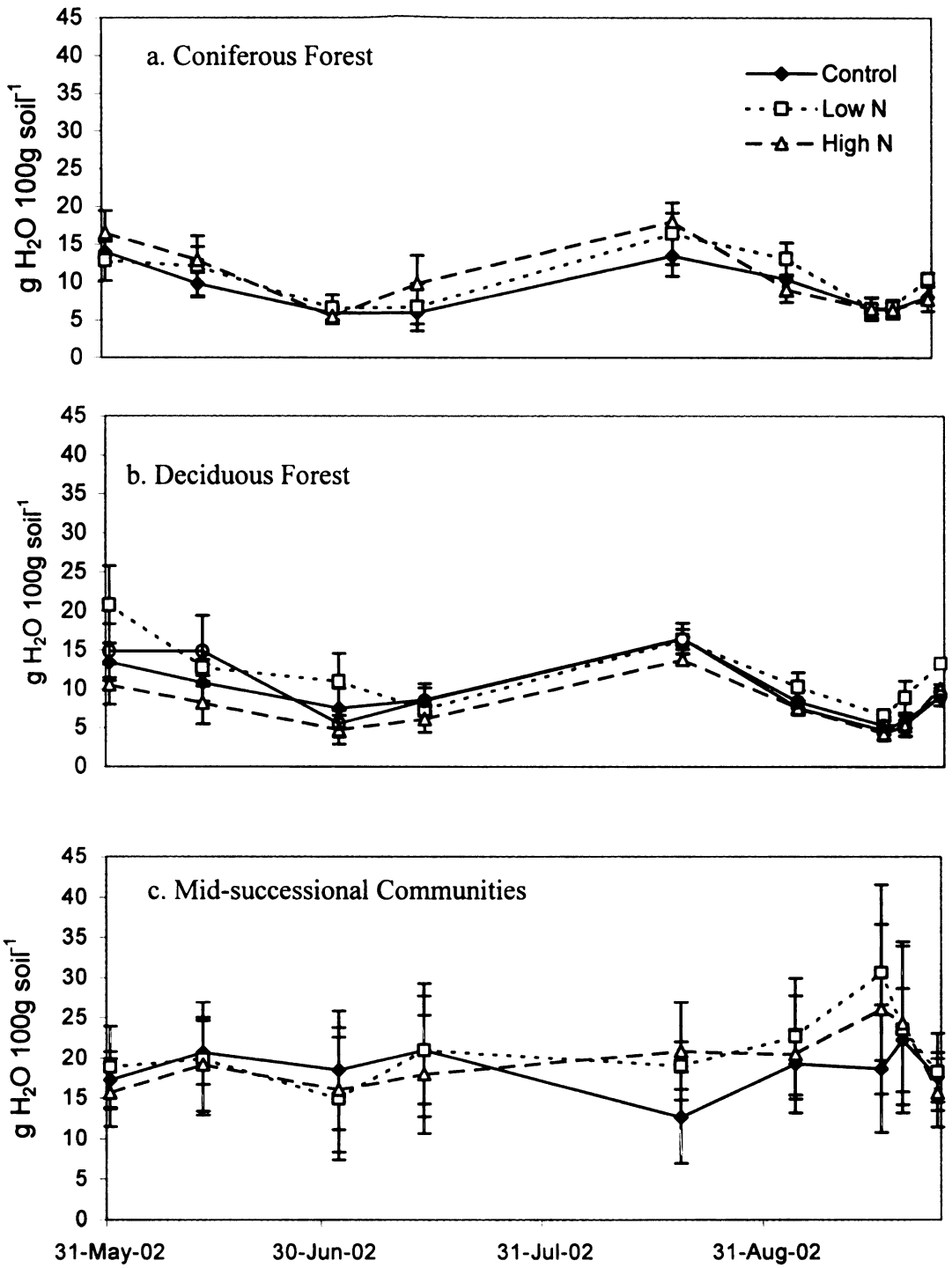


Figure 4.5 The effects of different N fertilizer levels on daily soil moisture in coniferous forest (top), deciduous forest (middle), and mid-successional communities (bottom) at the KBS LTER site. Vertical lines indicate standard errors (n= 3 replicate plots).

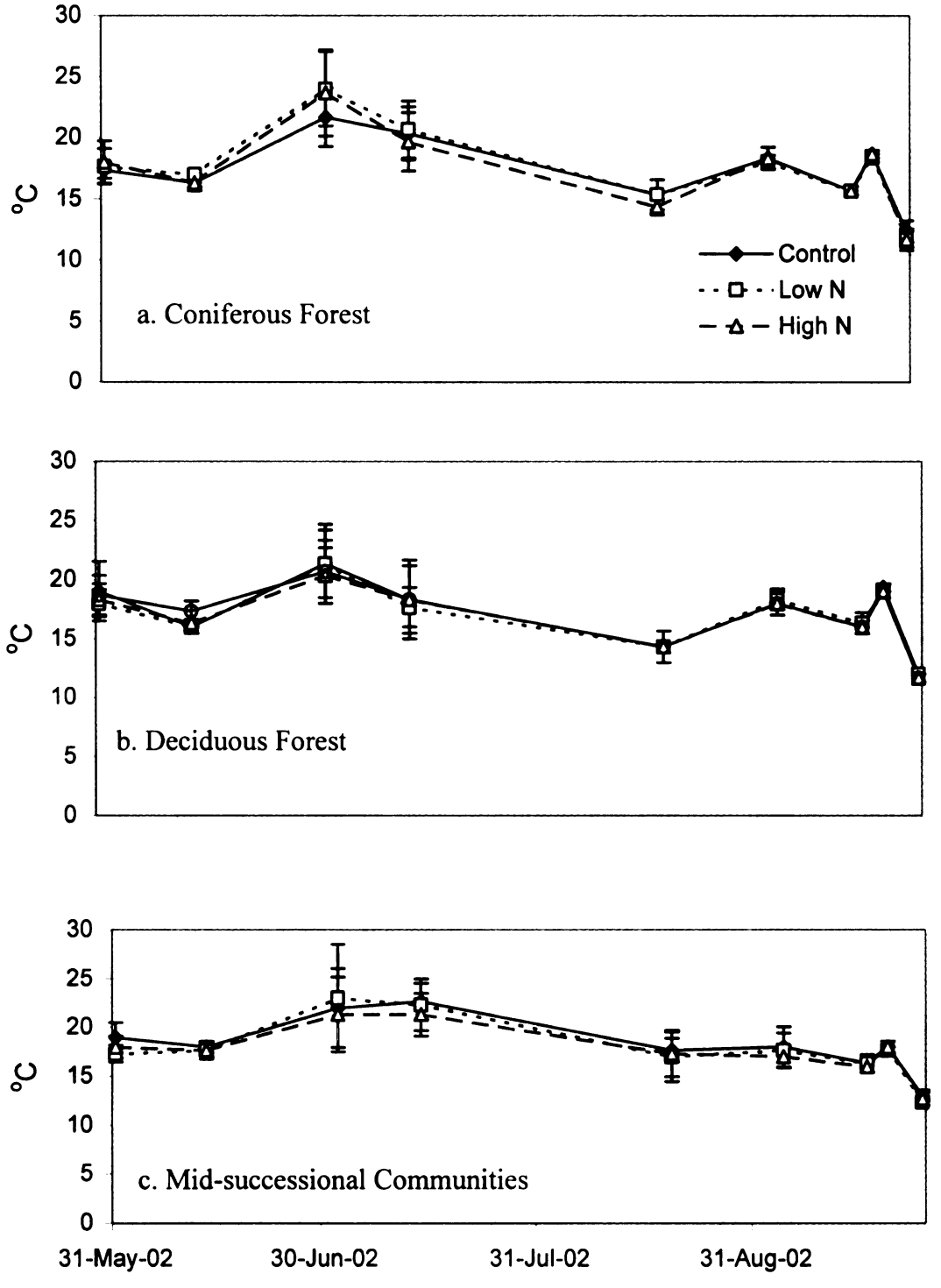


Figure 4.6 The effects of different N fertilizer levels on daily soil temperature in coniferous forest (top), deciduous forest (middle), and mid-successional communities (bottom) at the KBS LTER site. Vertical lines indicate standard errors (n= 3 replicate plots).

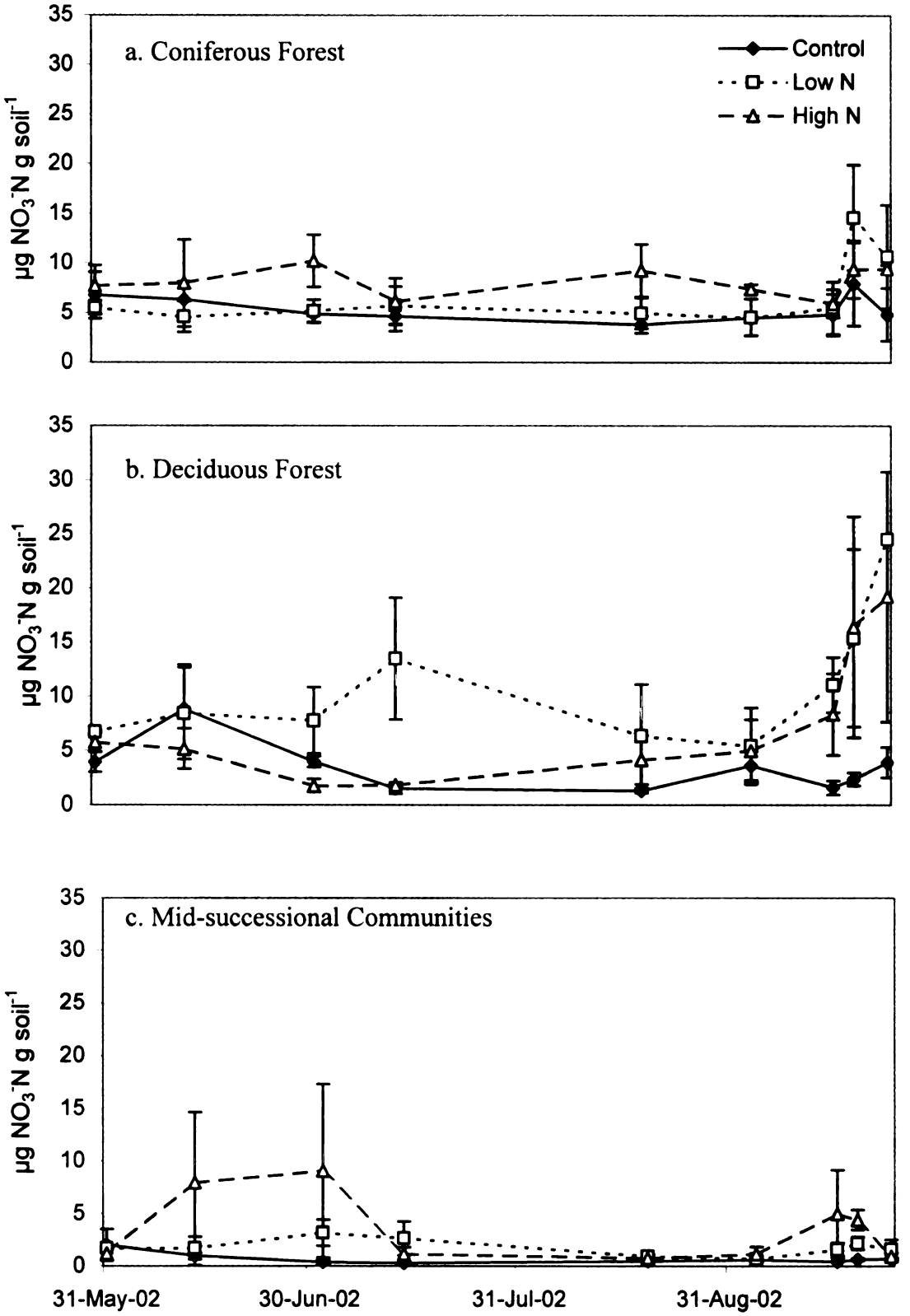


Figure 4.7 The effects of different N fertilizer levels on daily soil nitrate in coniferous forest (top), deciduous forest (middle), and mid-successional communities (bottom) at the KBS LTER site. Vertical lines indicate standard errors (n= 3 replicate plots).

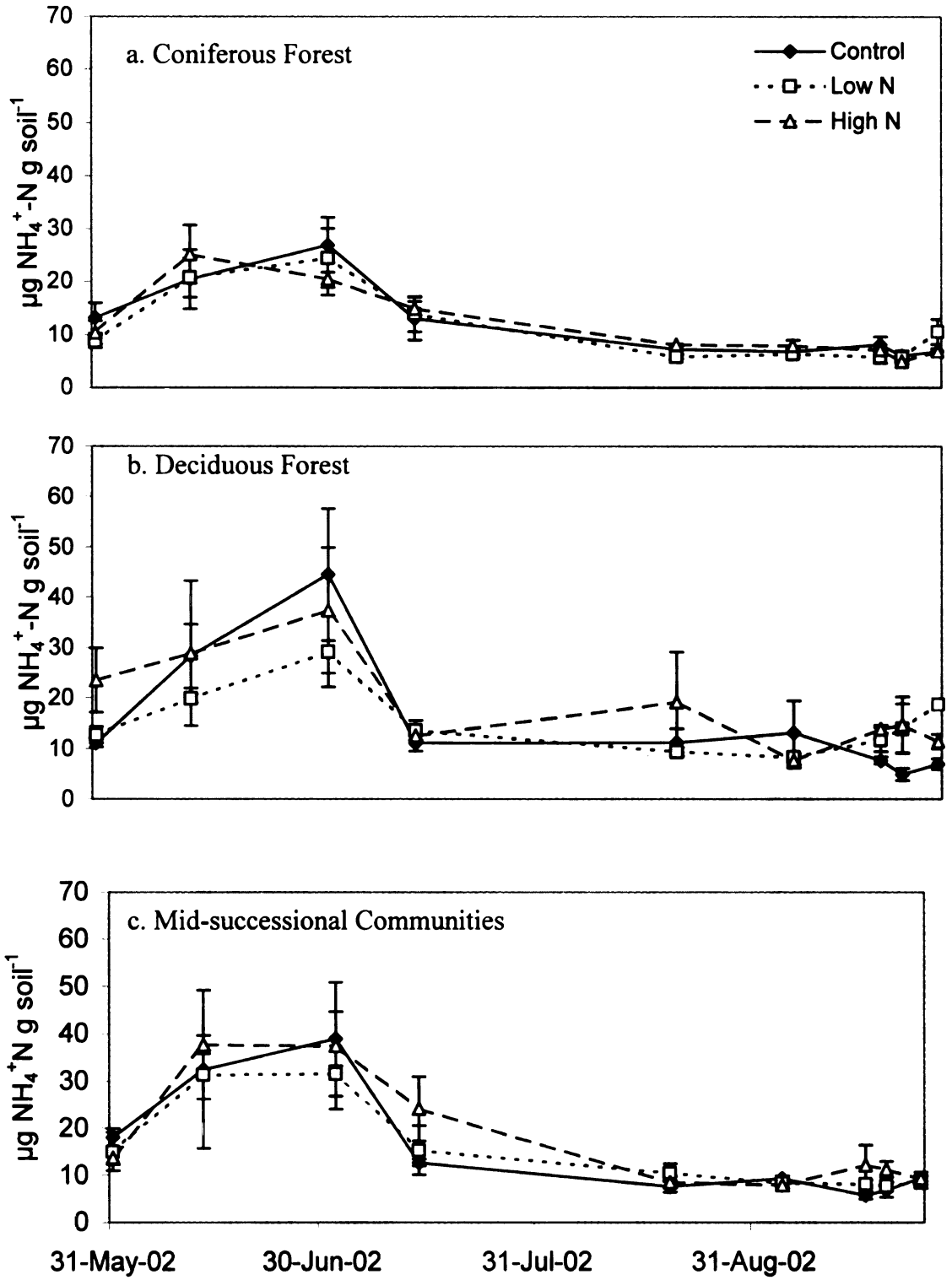


Figure 4.8 The effects of different N fertilizer levels on daily soil ammonium in coniferous forest (top), deciduous forest (middle), and mid-successional communities (bottom) at the KBS LTER site. Vertical lines indicate standard errors (n= 3 replicate plots).

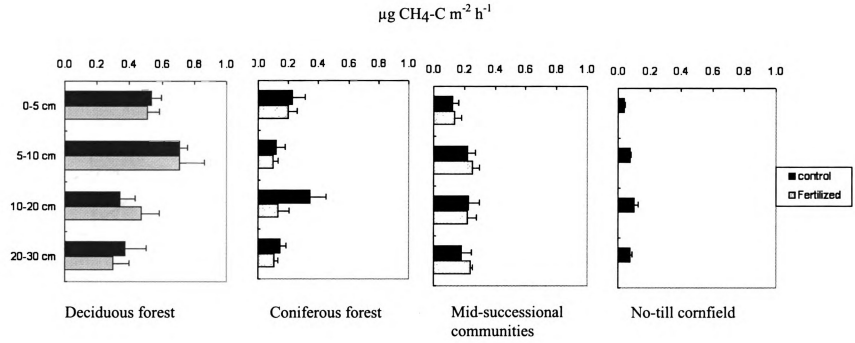


Figure 4.9 The effects of N fertilizer on methane oxidation along soil depth from soil cores taken from deciduous, coniferous forest, mid-successional communities, and no-till cornfields at the KBS LTER site, after incubation in laboratory. Error bars are standard errors, n= 6 replicates.

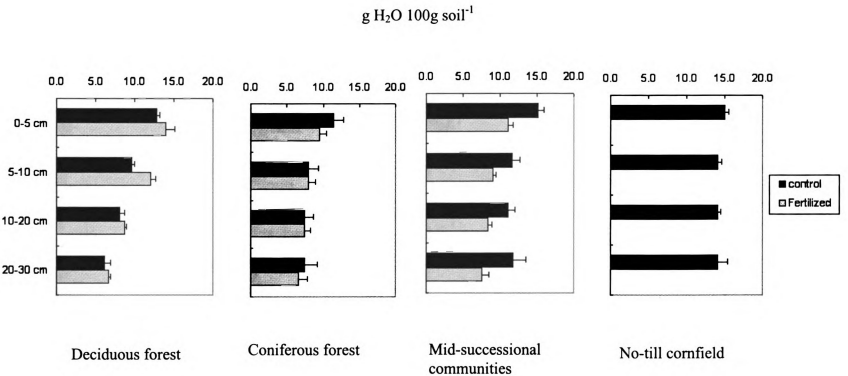


Figure 4.10 The effects of N fertilizer on soil moisture along soil depth from soil cores taken from deciduous, coniferous forest, mid-successional communities, and no-till cornfields at the KBS LTER site, after incubation in laboratory. Error bars are standard errors, n= 6 replicates.

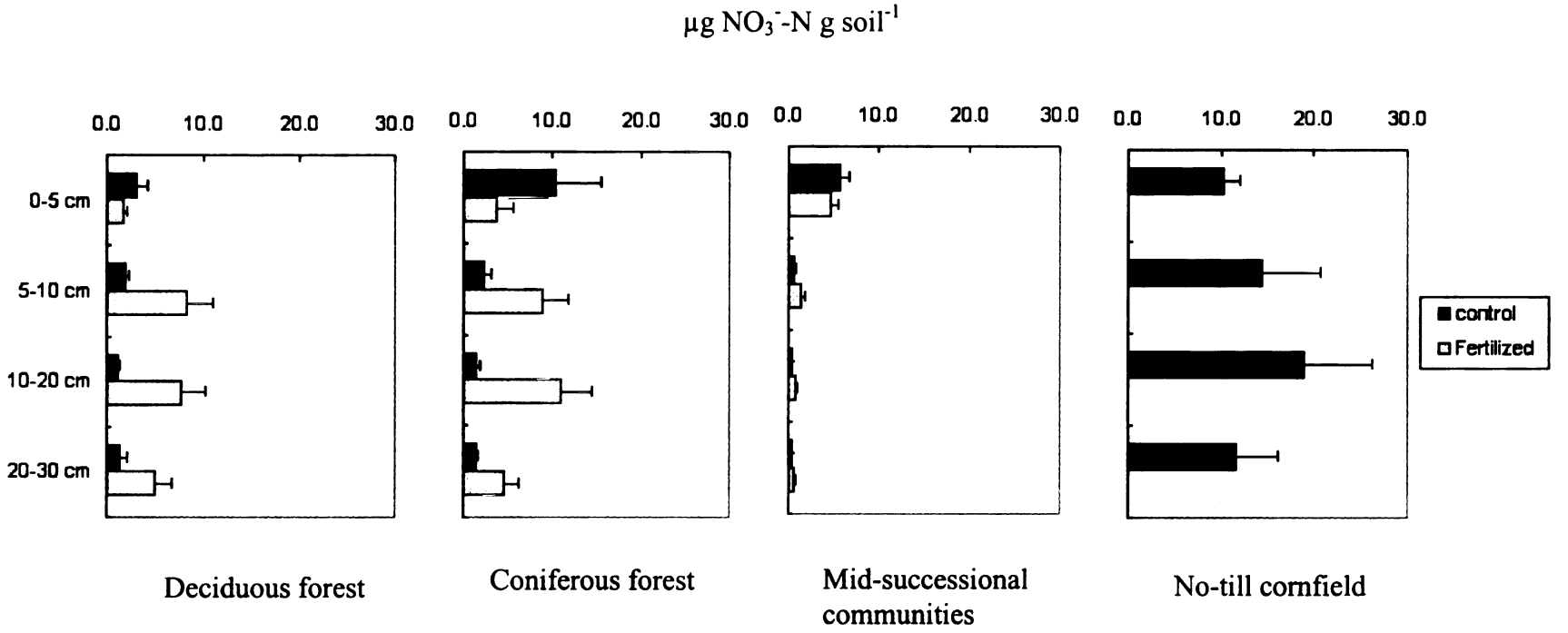


Figure 4.11 The effects of N fertilizer on soil nitrate along soil depth from soil cores taken from deciduous, coniferous forest, mid-successional communities, and no-till cornfields at the KBS LTER site, after incubation in laboratory. Error bars are standard errors, n= 6 replicates.

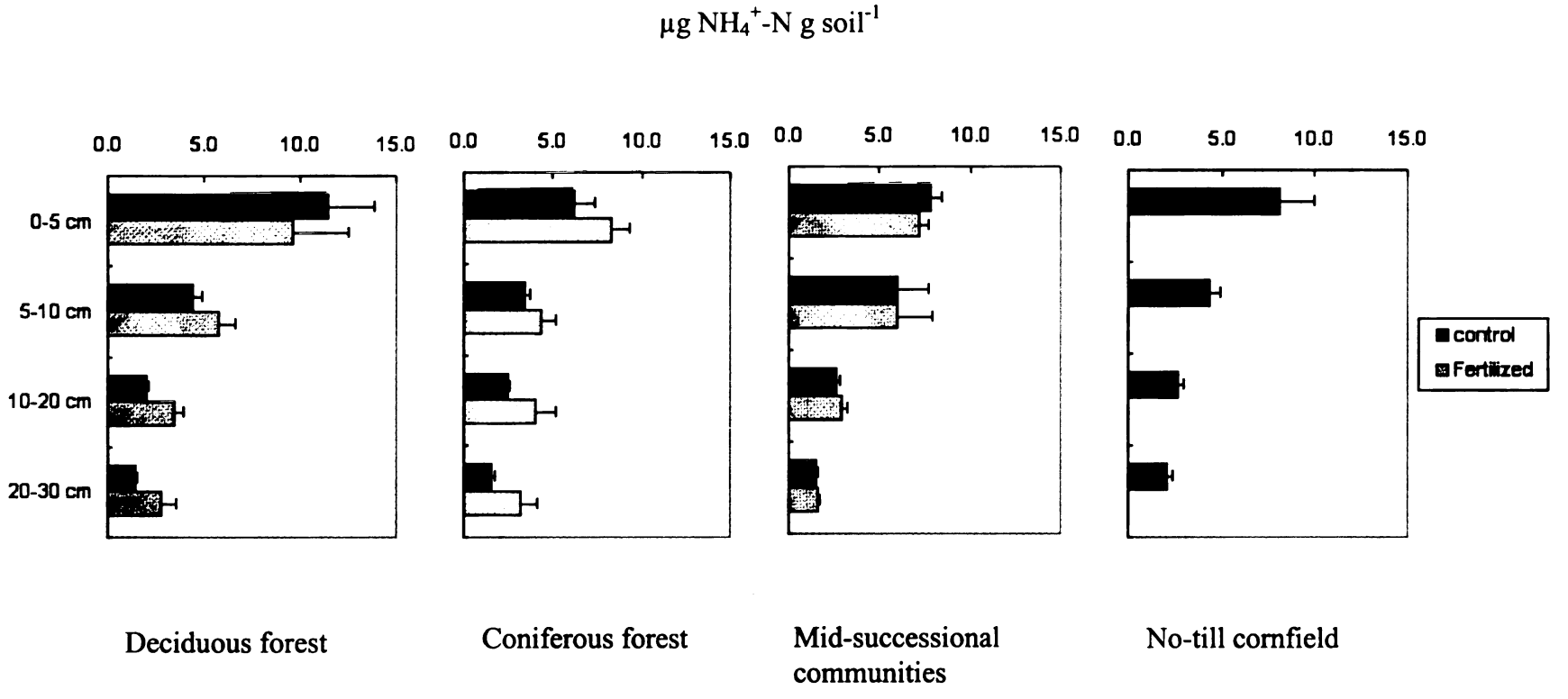


Figure 4.12 The effects of N fertilizer on soil ammonium along soil depth from soil cores taken from deciduous, coniferous forest, mid-successional communities, and no-till cornfields at the KBS LTER site, after incubation in laboratory. Error bars are standard errors, n= 6 replicates.

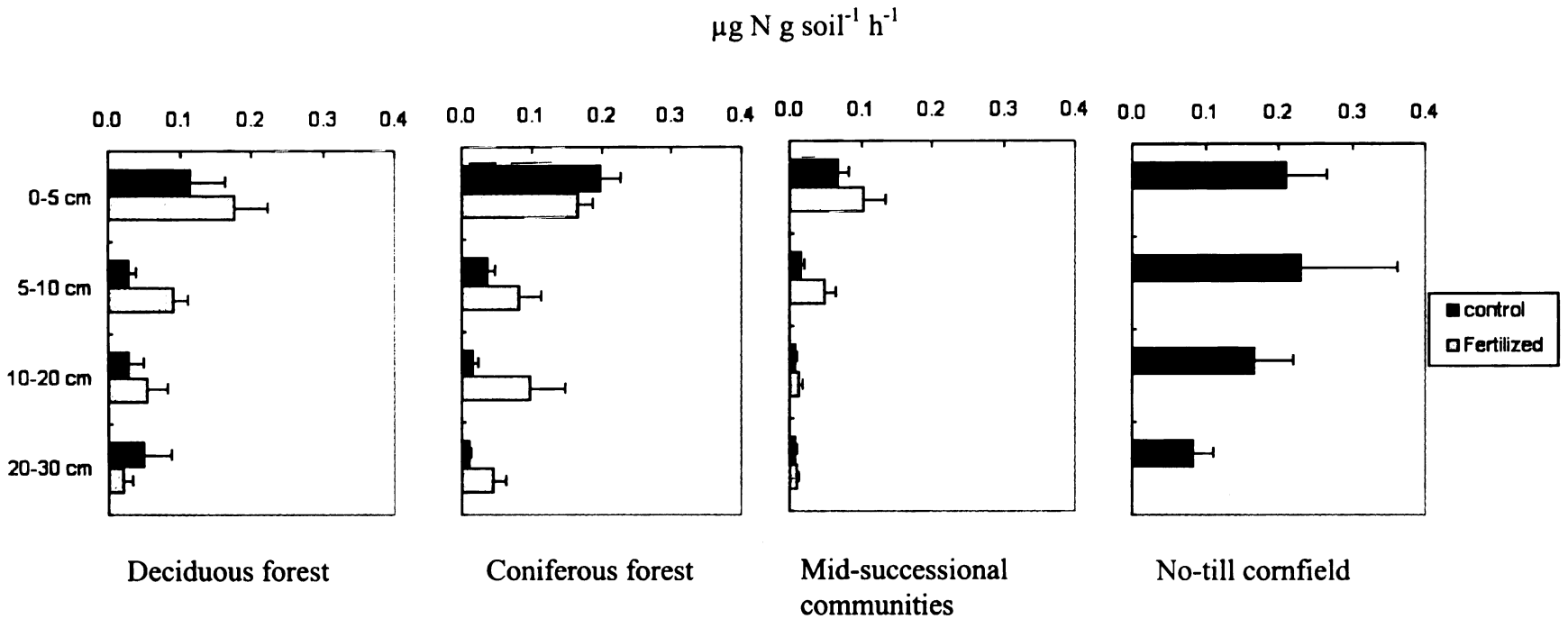


Figure 4.13 The effects of N fertilizer on soil nitrification along soil depth from soil cores taken from deciduous, coniferous forest, mid-successional communities, and no-till cornfields at the KBS LTER site, after incubation in laboratory. Error bars are standard errors, n= 6 replicates.

Chapter 5

Effects of Different Levels of Nitrogen Fertilization on Soil CH_4 Oxidation and CO_2 Flux in Continuous Corn

Introduction

Methane oxidation rates in agricultural soils tend to be substantially lower than rates in forest and grassland soils (Mosier et al. 1991, Goulding et al. 1995, Arif et al. 1996, Mosier et al. 1997, Powlson et al. 1997, Robertson et al. 2000, Hütsch 2001). Various studies have identified N fertilizer as a potentially important inhibitor of soil CH_4 uptake (Steudler et al. 1989, Topp 1993, Willison et al. 1995, Hütsch 1996, Prieme and Ekelund 2001).

Because the reduction of CH_4 oxidation may depend on the amount of N added to soil, reduced amounts of N fertilizer may allow increased CH_4 oxidation in agricultural soils. However, few investigators have studied the effects of different N fertilizer levels on CH_4 oxidation in agriculture. For example, N fertilizer had no effect on CH_4 oxidation in a regularly fertilized wheat field (Mosier and Schimel 1991), on irrigated crops in Colorado (Bronson and Mosier 1993), or on an acid oxisol in Puerto Rico (Mosier et al. 1998). Although Robertson et al. (2000) documented no increases in CH_4 oxidation in a reduced-fertilizer corn-soybean-wheat rotation, the sensitivity of CH_4 oxidation in agricultural soils to different levels of N fertilizer remains unknown.

The aim of this study is to evaluate whether different rates of N fertilizer will have correspondingly different effects on soil CH_4 consumption in continuous corn.

Methodology

Site Description

The study was conducted at the Long-term Ecological Research (LTER) sites at the W.K. Kellogg Biological Station (KBS), Hickory Corners, Michigan (42° 24'N, 85° 24'W, elevation 288 m). Annual rainfall at KBS averages 890 mm y^{-1} with about half falling as snow; potential evapotranspiration (PET) exceeds precipitation for about 4 months of the year. Mean annual temperature is 9.7 °C.

The study site is on the Kalamazoo/Oshtemo soil series (Austin 1979). The soil is Typic Hapludalfs (fine or coarse-loamy, mixed, mesic soils) derived from glacial till about 12,000 years ago (Crum and Collins 2003).

We measured methane fluxes at the N rate study plots established in 1999 to document the effect of different N-fertilizer levels on the growth and yield of corn (*Zea mays* L.). The experiment has 9 levels of N fertilizer from 0 to 291 kg N ha^{-1} applied to continuous corn rotation in a randomized complete block design with 4 replications (Figure 1). In 2002, the year of this study, N-fertilizer was applied as 28% urea ammonium nitrate (UAN). The first 34 kg ha^{-1} N fertilizer was injected between rows at planting and the remainder was injected at the 6-leaf stage of corn development on June 27th.

In Situ Gas Sampling

We measured in situ methane oxidation rates using the static chamber technique (Hutchinson and Livingston 1993). Static chambers were fashioned from 25 cm diameter PVC pipe: bases (25 cm diameter x 10 cm high) were installed in each plot and left in

place except during agronomic operations. Immediately prior to sampling, a 4.5 cm high cap was placed on each base and sealed with a latex skirt wrapped with an elastic band. At 30-40 minute intervals, four 10 mL headspace samples were removed through rubber septa in each cap using a syringe, and put into 3 mL glass sample vials pre-flushed with headspace air. Within 3 days vial contents were measured for CH_4 using a gas chromatograph (GC. 5890 Series II, Hewlett Packard) equipped with a flame ionization detector (FID), and measured for CO_2 using an infrared gas absorption (IRGA) analyzer (EGA-15996 CO_2 Analyzer, Analytical Development CO. LTD., Hoddesdon, England).

Chambers were sampled one day before fertilization and 1, 4, 12, 17, 33, 54, and 104 days after fertilization.

Soil Analyses

Soil temperature was measured at 0-5 cm depth using a soil temperature probe. Soil samples for other analysis were taken from the top 10 cm of soil using a 2.5 cm diameter soil probe. Fresh soils were passed through a 4 mm sieve and mixed by hand, and then sub samples were taken for moisture content and mineral N analysis. Prior to analysis, soils were stored for a week in a refrigerator at 4°C. Soil moisture content was measured gravimetrically by drying the soil samples at 65°C for 3 days or until dry. Further drying at 105°C removes an additional 0.8 g $H_2O \cdot 100g\ soil^{-1}$ moisture in these soils. Mineral N measurements were obtained by extracting 20 g of dry soil with 100 mL 1 M KCl for 24 h then filtering through 2-μm pore size fiberglass filters. The filtrates were frozen prior to analysis for NH_4^+ and NO_3^- using an Alpkem continuous flow

analyzer (Alpkem 3550, OI Analytical, College Station, TX) (Bundy and Meisinger 1994).

Statistical Analyses

The data were divided into two parts: before fertilization (day 0; n= 1 date) and after fertilization (days 1, 4, 12, 17, 33, 54, and 104; n = 7 dates). I used SPSS version 10.0.1 (SPSS Inc. 2001) for the analysis of covariance (ANCOVA) for the first part and also for the correlation analyses. I used Proc Mixed of SAS version 8.0 (SAS Institute 1999) for the analysis of covariance of the second data set. Methane and CO_2 data were natural log transformed before ANOVA and ANCOVA to homogenize variances. I used untransformed data for correlation analysis.

Results

Methane Oxidation

Methane oxidation averaged 3.34 ± 0.32 (s.e., n= 4 replicate blocks x 8 sampling dates x 9 N-fertilizer levels) μg CH_4-C m^{-2} h^{-1} across all sampling dates and fertilizer levels. There were no treatment effects and CH_4 consumption was not significantly affected by N addition (Tables 5.1, 5.2, 5.3 and Figure 5.2) except for the 34 kg N level (P=0.013), which had the average highest rate (5.53 ±1.51 μg CH_4-C m^{-2} h^{-1}) (Table 5.1). Oxidation rates ranged from –9 to 43 μg CH_4-C m^{-2} h^{-1} (negative number indicates net CH_4 production).

Methane consumption did not significantly change by sample dates (Table 5.3 and Figure 5.3); average daily rates were low, between 1 and 5 μg CH_4-C m^{-2} h^{-1} for all treatments except for one sample date. The exception was on July 30th following a July

29th rainfall, for which the average daily rate increased to 10 μg CH_4-C m^{-2} h^{-1}, with the 34 kg N ha^{-1} fertilization level as high as 19 μg CH_4-C m^{-2} h^{-1}.

Carbon Dioxide Fluxes

Fertilizer significantly affected CO_2 fluxes (Tables 5.5) but only at a fertilizer level of 291 kg N ha^{-1}, in which CO_2 fluxes (102.9 ± 11.4 mg CO_2-C m^{-2} h^{-1}) were slightly higher than control and other treatments (Table 5.1 and Figure 5.4). CO_2 production rates ranged from 4 to 249 mg CO_2-C m^{-2} h^{-1}. Carbon dioxide fluxes were strongly and significantly (p=0.043) different by sampling date (Table 5.5), and rates were also highest on July 30th (Figure 5.5).

Soil Properties

Soil moisture significantly differed among both fertilizer levels and sample dates (Table 5.6 and Figure 5.6). Moisture ranged from 5.20 g H_2O·100g $soil^{-1}$ (±0.18) on July 14th to 14.11 g H_2O·100g $soil^{-1}$ (±0.48) on July 30th (Figure 5.7).

Soil nitrate and ammonium also significantly varied by fertilizer levels and sample dates (Table 5.6). Nitrate and ammonium significantly increased with levels of N addition (Figure 5.8 and 5.10). Ammonium and nitrate levels also exponentially increased immediately after N application but declined rapidly until August 20th (Figure 5.9 and 5.11).

Soil temperature was not significant different among N levels but changed significantly with dates of sampling (Table 5.6 and Figure 5.12). Soil temperature was

highest in early July around 35°C then steadily declined to 10 °C in early October (Figure 5.13).

Controls of Methane Oxidation and Carbon Dioxide Fluxes

Analysis of covariance reveals a significant association of nitrate and ammonium levels with CH_4 oxidation (Table 5.3). In both cases correlations were negative, indicating that higher levels of soil N were associated with lower CH_4 oxidation ($r=-0.22$ for nitrate and -0.19 for ammonium; Table 5.7) although the correlations were not significant ($P>0.05$). On the other hand, CH_4 oxidation was positively correlated with soil moisture both before ($r=0.35$) and after fertilization ($r=0.18$). Carbon dioxide flux was the strongest predictor of CH_4 oxidation ($r=0.35$, $P<0.01$) for post fertilization fluxes.

In contrast, nitrate and ammonium were poor predictors of CO_2 flux (Tables 5.5 and 5.7), which was best predicted by soil temperature ($r=0.30$ and $P<0.01$).

Discussion

Methane oxidation rates in this study were low, on the order of 1-5 $\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$ for most sample dates. With one exception, CH_4 oxidation rates were not significantly affected by N-fertilizer levels, where oxidation was significantly greater than at other levels (Figure 5.2). There were also no significant differences in seasonal oxidation rates among the nine fertilizer levels. The exceptional response of the 34 kg N level appears due to a single post-rain sample date, where oxidation in this treatment was as high as 20 $\mu g\ CH_4\text{-}C\ m^{-2}\ h^{-1}$ (Figure 5.3).

Despite a lack of response to fertilizer level, CH_4 oxidation was related to soil inorganic N contents; higher nitrate and ammonium levels were weakly associated with lower CH_4 oxidation (Tables 5.3 and 5.7). This may indicate low population of CH_4 oxidizing bacteria or fewer activities due to the competitive effect of ammonium to methane monooxygenase enzyme.

The best prediction of CH_4 oxidation across all N levels was CO_2 flux and soil moisture. Higher oxidation rates were associated with higher soil respiration rates and soil moisture. Faster soil carbon cycling – as indicated by higher microbial activity and root respiration – appears to accelerate CH_4 oxidation as well.

Soil CO_2 fluxes were largely related to soil temperature differences (Tables 5.5 and 5.7). Rates of CO_2 emission varied over an order of magnitude over the course of this study (from < 20 to > 200 mg CO_2-C m^{-2} h^{-1}), and were highest following rainfall events in mid-summer (Figure 5.5). CO_2 fluxes were likewise unaffected by N-fertilizer levels (Figure 5.4).

The low rates of CH_4 oxidation in the highly and moderately fertilized plots are consistent with a number of other studies that have also found low oxidation rates in fertilized wheat (Mosier and Schimel 1991, Sitaula et al. 2000).

I was surprised, however, that oxidation rates were equally low or (even lower) in the low-fertilizer-N plots. The only other multiple-level N-fertilizer experiment in the literature (Hütsch et al. 1993) shows a progressive decline of soil CH_4 oxidation according to the amount of N-fertilization (0, 48, 96, and 144 kg N ha^{-1}) in the Rothamsted "Broadbalk Wheat Experiment." This, together with two available fertilizer experiments in wheat (Mosier and Schimel 1991), and timothy and clover (Sitaula et al.

2000), in which oxidation was also low at low fertilizer levels, suggests that some factors other than (but affected by) fertilizers may affect CH_4 oxidation.

It is also possible that a legacy of high fertilization at our site is still suppressing oxidation. That is, in the Broadbalk experiment plots have received low rates of fertilizer N for more than 140 years; the methanotroph community may take more than the 3 years of low fertilizer-N in our study to oxidize at higher rates. In another study, near our sites, soil in successional communities did not oxidize CH_4 at rates higher than agricultural sites for several years after the initiation of succession (Robertson et al. 2000), with rates 50% of nearby forest rates only after 50 years of succession. Thus, higher oxidation rates at lower fertilization rates may take additional years to develop.

Surprisingly, fertilizer at 34 kg N ha^{-1} stimulated soil CH_4 consumption in this study. The low level of N added might be adequate to stimulate the growth of CH_4 oxidizing bacteria as found in one nitrate fertilizer study (Hütsch et al. 1994) but not too high to suppress soil CH_4 oxidation.

Carbon dioxide production was also not affected by different amounts of N fertilizer except at the highest fertilizer level of 291 kg N ha^{-1}. Carbon dioxide fluxes were mainly related to soil temperature (Tables 5.5 and 5.7).

The substantial increase in both CH_4 oxidation and CO_2 production on July 30th was likely caused by the sharp increase in soil moisture from 5% on July 14th to 15% on July 30th due to heavy rain on July 29th. On the other hand, an early June rainfall resulted in a significant CO_2 response (Figure 5.5) but no CH_4 response (Figure 5.4). This may be due to the high soil N levels still present in early June (Figure 5.9 and 5.11).

Conclusion

Nitrogen fertilizer added to these continuous corn plots did not affect soil CH_4 oxidation nor CO_2 emission at any level of fertilizer addition but the lowest (CH_4) or highest (CO_2). Both CH_4 uptake rates and CO_2 fluxes varied seasonally. Soil nitrate and ammonium significantly affected soil CH_4 consumption but they did not influence CO_2 production. Only soil temperature was a significant predictor for CO_2 fluxes in this soil.

References

Arif, M. A. S., F. Houwen, and W. Verstraete. 1996. Agricultural factors affecting methane oxidation in arable soil. Biology and Fertility of Soils **21**:95-102.

Austin, F. R. 1979. Soil Survey of Kalamazoo County, Michigan. United States Department of Agriculture, Soil Conservation Service, in cooperation with Michigan Agricultural Experiment Station. The Soil Conservation Service, Washington.

Bronson, K., and A. Mosier. 1993. Nitrous oxide emissions and methane consumption in wheat and corn-cropped systems in northeastern Colorado. Pages 133-144 *in* L. A. Harper, A. Mosier, J. Duxbury, and D. E. Rolston, editors. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. American Society of Agronomy, Madison.

Bundy, L. G., and J. J. Meisinger. 1994. Nitrogen availability indices. Pages 951-984 *in* R. W. Weaver, J. S. Angle, and B. S. Bottomley, editors. Methods of soil analysis. Part 2. Microbiological and biochemical properties. SSSA Book Series, no. 5., Madison, WI.

Crum, J. R., and H. P. Collins. 2003. KBS soils *in* http://lter.kbs.msu.edu/Soil/characterization/.

Goulding, K. W. T., B. W. Hütsch, C. P. Webster, T. W. Willison, and D. S. Powlson. 1995. The effect of agriculture on methane oxidation in soil. Philosophical Transactions of the Royal Society of London Series a-Mathematical Physical and Engineering Sciences **351**:313-324.

Hutchinson, G. L., and G. P. Livingston. 1993. Use of chamber systems to measure trace gas fluxes. Pages 63-78 *in* L. A. Harper, A. R. Mosier, J. M. Duxbury, and D. E.

Rolston, editors. Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. American Society of Agronomy, Madison, Wisconsin.

Hütsch, B. W. 1996. Methane oxidation in soils of two long-term fertilization experiments in Germany. Soil Biology & Biochemistry **28**:773-782.

Hütsch, B. W. 2001. Methane oxidation in non-flooded soils as affected by crop production. European Journal of Agronomy **14**:237-260.

Hütsch, B. W., C. P. Webster, and D. S. Powlson. 1993. Long-term effects of nitrogen-fertilization on methane oxidation in soil of the Broadbalk Wheat Experiment. Soil Biology & Biochemistry **25**:1307-1315.

Hütsch, B. W., C. P. Webster, and D. S. Powlson. 1994. Methane oxidation in soil as affected by land-use, soil-pH and N-fertilization. Soil Biology & Biochemistry **26**:1613-1622.

Mosier, A., J. A. Delgado, and M. Keller. 1998. Methane and nitrous oxide fluxes in an acid oxisol in western Puerto Rico; effects of tillage, liming, and fertilization. Soil Biology & Biochemistry **30**:2087-2098.

Mosier, A., and D. Schimel. 1991. Influence of agricultural nitrogen on atmospheric methane and nitrous oxide. Chemical Industry **24**:334-336.

Mosier, A., D. Schimel, D. Valentine, K. Bronson, and W. Parton. 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. Nature **350**:330-332.

Mosier, A. R., J. A. Delgado, V. L. Cochran, D. W. Valentine, and W. J. Parton. 1997. Impact of agriculture on soil consumption of atmospheric CH_4 and a comparison of CH_4 and N_2O flux in subarctic, temperate, and tropical grasslands. Nutrient Cycling in Agroecosystems **49**:71-83.

Powlson, D. S., K. W. T. Goulding, T. W. Willison, C. P. Webster, and B. W. Hütsch. 1997. The effect of agriculture on methane oxidation in soil. Nutrient Cycling in Agroecosystems **49**:59-70.

Prieme, A., and F. Ekelund. 2001. Five pesticides decreased oxidation of atmospheric methane in a forest soil. Soil Biology & Biochemistry **33**:831-835.

Robertson, G. P., E. A. Paul, and R. R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science **289**:1922-1925.

SAS Institute. 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc., Cary, NC.

Sitaula, B. K., S. Hansen, J. I. B. Sitaula, and L. R. Bakken. 2000. Methane oxidation potentials and fluxes in agricultural soil: Effects of fertilisation and soil compaction. Biogeochemistry **48**:323-339.

SPSS Inc. 2001. SPSS Base 11.0 for Windows User's Guide. SPSS Inc.

Steudler, P. A., R. D. Bowden, J. M. Melillo, and J. D. Aber. 1989. Influence of nitrogen fertilization on methane uptake in temperate forest soil. Nature **341**:314-316.

Topp, E. 1993. Effects of selected agrochemicals on methane oxidation by an organic agricultural soil. Canadian Journal of Soil Science **73**:287-291.

Willison, T. W., C. P. Webster, K. W. T. Goulding, and D. S. Powlson. 1995. Methane oxidation in temperate soils - Effects of land-use and the chemical form of nitrogen fertilizer. Chemosphere **30**:539-546.

Table 5.1 Net methane oxidation, soil carbon dioxide emission, and soil properties at various N fertilizer levels in continuous corn plots after fertilization across all sample dates. Values are means (±s.e., n= 4 replicates[a] x 7 sampling dates or 2 replicates[b] x 7 sampling dates).

Variable	Fertilizer Level (kg N ha^{-1})								
	Control	34	67	101	134	168	202	246	291
CH_4 (μg CH_4-C m^{-2} h^{-1})[a]	2.50 ± 0.88	5.53 ± 1.51*	3.02 ± 0.80	2.81± 0.64	4.00 ± 1.07	3.84 ± 0.73	2.91 ± 1.02	4.20 ± 1.69	3.61 ± 0.95
CO_2 (mg CO_2-C m^{-2} h^{-1})[a]	84.7 ± 8.0	92.4 ± 8.9	80.5 ± 8.8	84.5 ± 8.7	83.0 ± 8.6	83.2 ± 8.5	86.9 ± 10.0	83.9 ±10.6	102.9±11.4
Moisture (g H_2O 100 g $soil^{-1}$)[b]	12.1 ± 1.0	11.5 ± 1.1	11.5 ± 1.0	11.3 ± 1.0	10.9 ± 1.0**	10.9 ± 0.9**	9.7 ± 0.9***	10.4 ± 0.9**	10.6 ± 1.0**
Temperature (°C)[a]	24.2 ± 1.7	24.3 ± 1.7	24.0 ± 1.6	24.4 ± 1.8	24.5 ± 1.7	23.9 ± 1.6	24.7 ± 1.8	25.2 ± 1.8	25.3 ± 1.9
Nitrate (μg N g $soil^{-1}$)[b]	7.5 ± 1.4	19.2± 3.6	26.1± 4.3*	32.3±1.0**	47.3±8.6***	37.9±8.0**	50.0±7.4***	67.0±11.2***	66.3±11.4***
Ammonium (μg N g $soil^{-1}$)[b]	2.6±0.3	26.1± 6.8	37.3±9.6	44.8±10.5	83.6±22.3*	70.0±26.1*	96.3±20.5**	139.6±33.4***	148.9±36.5***

*, **, *** values are significantly different from control values at 0.05, 0.01, and 0.0001 level, respectively.

Table 5.2 Analysis of covariance to determine effects of N fertilizer and soil properties on methane oxidation rates before fertilization.

Source	SS	df	MS	F	P
Fertilizer	0.27	8	0.03	0.42	0.867
Moisture	0.24	1	0.24	2.93	0.148
Temperature	1.5×10^{-4}	1	1.5×10^{-4}	0.002	0.968
Nitrate	0.12	1	0.12	1.52	0.273
Ammonium	0.12	1	0.12	1.49	0.277
Error	0.41	5	0.09		
Total	64.8	18			
Corrected Total	0.92	17			

Table 5.3 Analysis of covariance to determine effects of N fertilizer and soil properties on methane oxidation rates after fertilization.

Variable	Num. df [a]	Den. df [a]	F	P
Fertilizer	8	236	0.9	0.558
Date	6	236	1.3[b]	0.106
Fertilizer x Date	48	236	0.6[b]	0.543
Moisture	1	107	2.0	0.159
Temperature	1	236	0.3	0.619
Nitrate	1	107	4.7	0.033
Ammonium	1	107	5.5	0.021

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] Z value

Table 5.4 Analysis of variance to determine effects of N fertilizer and soil properties on carbon dioxide emission rates before fertilization.

Source	SS	df	MS	F	P
Fertilizer	1.76	8	0.22	2.4	0.175
Moisture	0.18	1	0.18	2.00	0.216
Temperature	0.01	1	0.01	0.15	0.718
Nitrate	0.05	1	0.05	0.61	0.470
Ammonium	0.07	1	0.07	0.80	0.413
Error	0.46	5	0.09		
Total	245	18			
Corrected Total	2.41	17			

Table 5.5 Analysis of covariance to determine effects of N fertilizer and soil properties on carbon dioxide emission rates after fertilization.

Variable	Num. df [a]	Den. df [a]	F	P
Fertilizer	8	236	2.67	0.008
Date	6	236	1.72[b]	0.043
Fertilizer x Date	48	236	-1.9[b]	0.059
Moisture	1	107	2.24	0.137
Temperature	1	236	15.5	0.0001
Nitrate	1	107	0.7	0.416
Ammonium	1	107	1.4	0.237

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively.

[b] Z value

Table 5.6 Analysis of covariance to determine effects of N fertilizer on soil properties after fertilization.

Variable	Num. df [a]	Den. df [a]	Moisture		Nitrate		Ammonium		Temperature	
			F[c]	P	F[c]	P	F[c]	P	F[c]	P
Treatment	8	111[b]	5.24	<0.0001	12.1	<0.0001	7.6	<0.0001	0.3	0.974
Day	6	111[b]	1.7	0.043	1.6	0.052	1.6	0.054	1.7	0.043
Treatment x Day	48	111[b]	-3.7	0.000	1.5	0.129	2.3	0.021	2.0	0.046

[a] Num. df and Den. df refer to numerator and denominator degrees of freedom, respectively

[b] 237 for temperature

[c] Z value for day and treatment x day

Table 5.7 The relationships among methane oxidation, carbon dioxide and other soil properties before and after fertilization across nine different N rates in continuous corn at the KBS LTER site. Values are Pearson correlation coefficients (r); [a] n = 9 fertilizer levels x 4 replicates x 1 sample date, [b] n = 9 fertilizer levels x 4 replicates x 7 sample dates.

	CO_2	Moisture	Temperature	Nitrate	Ammonium
Before Fertilization[a]					
CH_4	-0.140	0.346	-0.168	-0.223	-0.186
CO_2	1.000	-0.022	0.100	-0.003	-0.007
After Fertilization[b]					
CH_4	0.345**	0.176*	-0.067	-0.095	-0.174
CO_2	1.000	0.115	0.295**	0.059	0.038

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

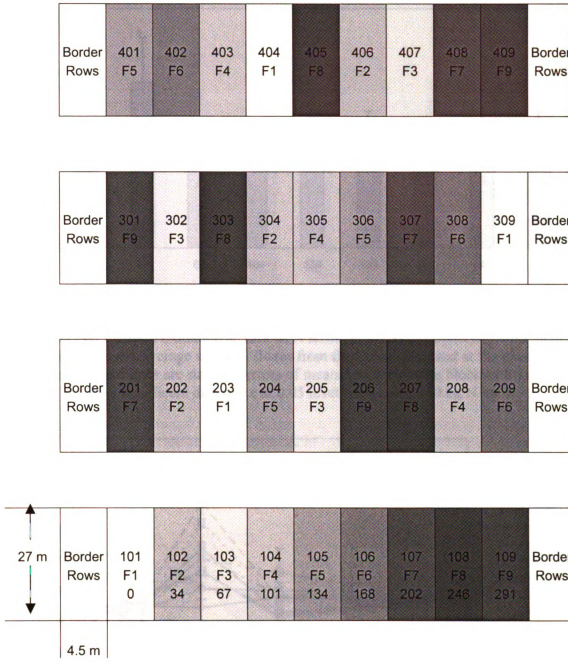


Figure 5.1 Layout of the N-fertilizer rate study at the KBS LTER site. Unit = kg N ha^{-1}.

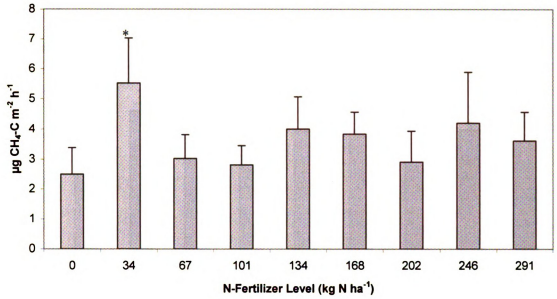


Figure 5.2 Net methane oxidation at various N fertilizer levels in continuous corn plots at KBS. Bars represent average seasonal fluxes from field plots fertilized at the rates indicated. Vertical lines are standard errors of means (n= 4 replicate blocks x 8 sampling dates). * means a significant difference at 0.05 level from control (0 kg N ha^{-1}).

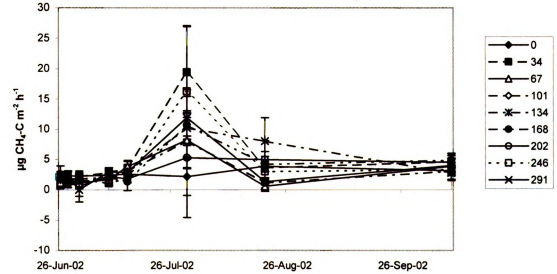


Figure 5.3 Net daily methane oxidation at various N fertilizer levels in continuous corn plots. Vertical lines are standard errors of means (n= 4 replicate plots). Fertilization occurred on June 27th.

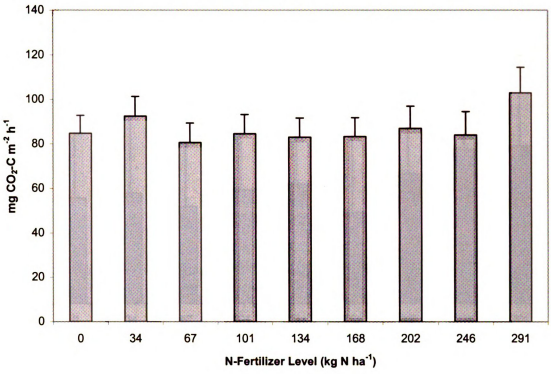


Figure 5.4 Net carbon dioxide emission at various N fertilizer levels in continuous corn plots. Bars represent average seasonal fluxes from field plots fertilized at the rates indicated. Vertical lines are standard errors of means (n= 4 replicate blocks x 8 sampling dates).

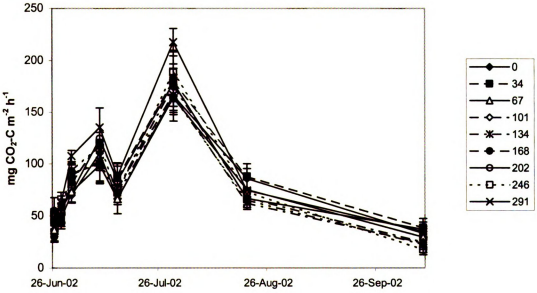


Figure 5.5 Net daily carbon dioxide flux at various N fertilizer levels in continuous corn plots. Vertical lines are standard errors of means (n= 4 replicate plots). Fertilization occurred on June 27th.

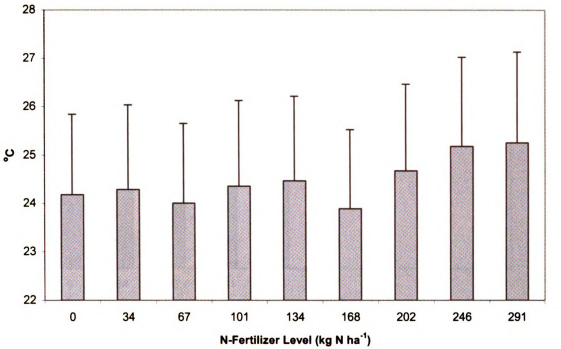


Figure 5.6 Net soil temperature at various N fertilizer levels in continuous corn plots at KBS. Bars represent average seasonal fluxes from field plots fertilized at the rates indicated. Vertical lines are standard errors of means (n= 4 replicate blocks x 8 sampling dates).

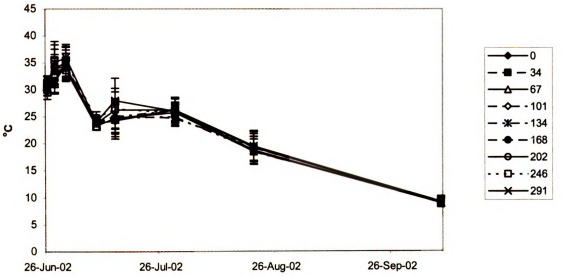


Figure 5.7 Net daily soil temperature at various N fertilizer levels in continuous corn plots at the KBS LTER site. Vertical lines are standard errors of means (n= 4 replicate plots).

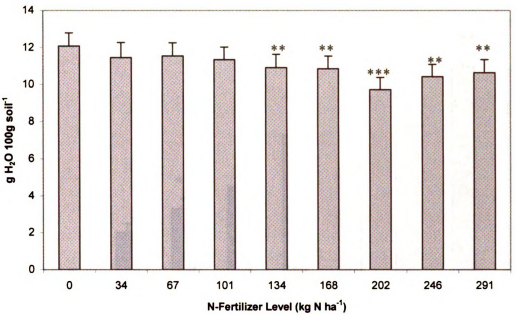


Figure 5.8 Net soil moisture at various N fertilizer levels in continuous corn plots at the KBS LTER site. Bars represent average seasonal fluxes from field plots fertilized at the rates indicated. Vertical lines are standard errors of means (n= 4 replicate blocks x 8 sampling dates). *,** and *** are significant differences at $P<0.05$, 0.01 and 0.0001 from control (0 kg N ha^{-1}).

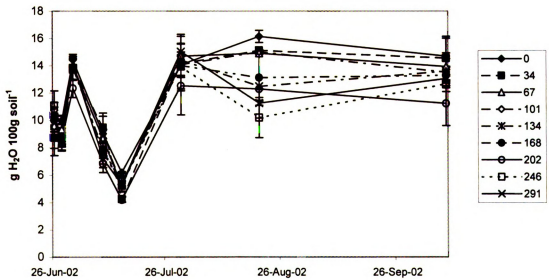


Figure 5.9 Net daily soil moisture at various N fertilizer levels in continuous corn plots at the KBS LTER site. Vertical lines are standard errors of means (n= 4 replicate plots).

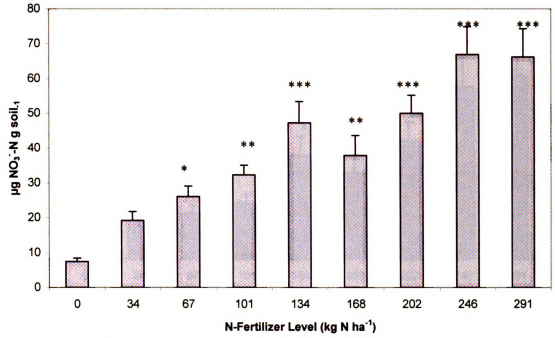


Figure 5.10 Net soil nitrate at various N fertilizer levels in continuous corn plots at KBS. Bars represent average seasonal fluxes from field plots fertilized at the rates indicated. Vertical lines are standard errors of means (n= 4 replicate blocks x 8 sampling dates). * ,** and *** are significant differences at P<0.05, 0.01 and 0.0001 from control (0 kg N ha^{-1}).

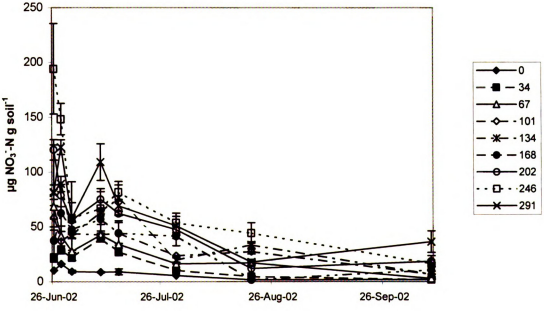


Figure 5.11 Net daily soil nitrate at various N fertilizer levels in continuous corn plots at the KBS LTER site. Vertical lines are standard errors of means (n= 4 replicate plots). Fertilization occurred on June 27th.

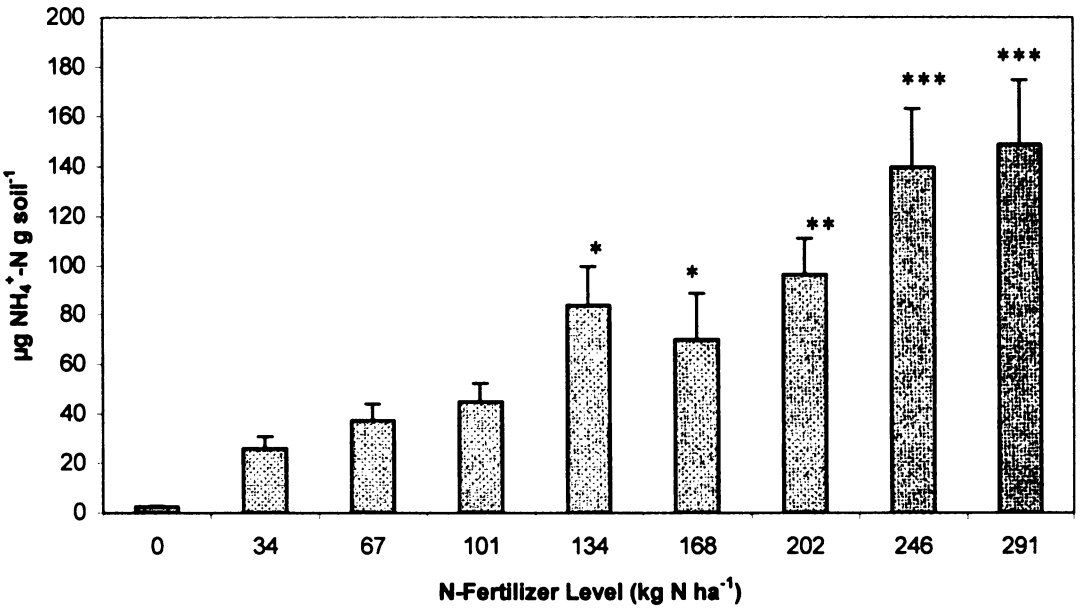


Figure 5.12 Net soil ammonium at various N fertilizer levels in continuous corn plots at KBS. Bars represent average seasonal fluxes from field plots fertilized at the rates indicated. Vertical lines are standard errors of means (n= 4 replicate blocks x 8 sampling dates). * ** and *** are significant differences at P<0.05, 0.01 and 0.0001 from control (0 kg N ha^{-1}).

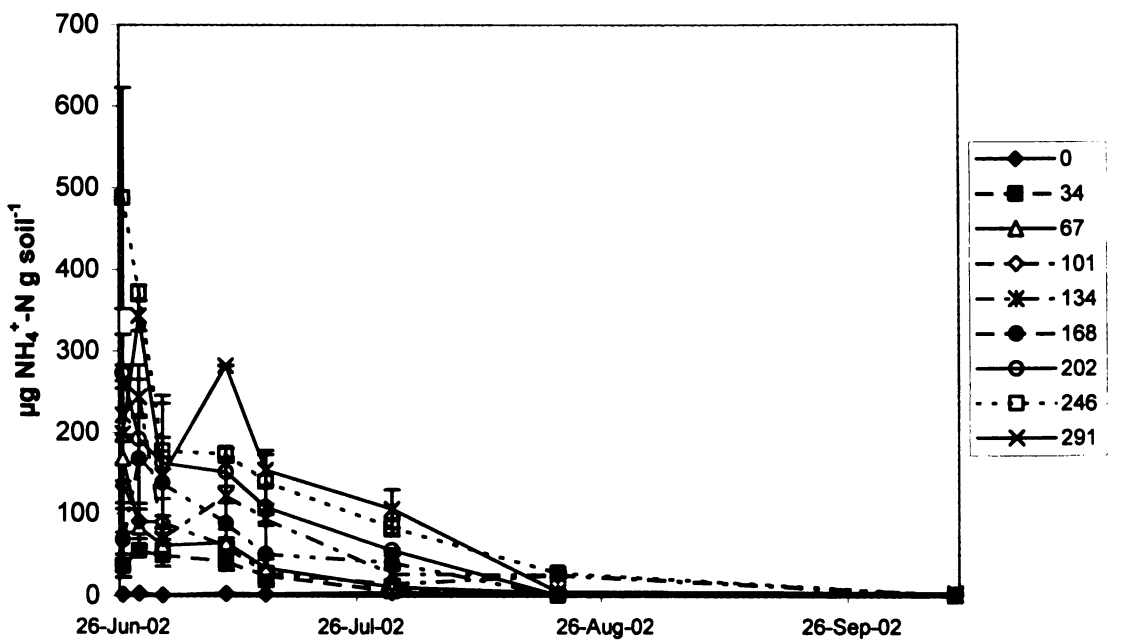


Figure 5.13 Net daily soil ammonium at various N fertilizer levels in continuous corn plots at the KBS LTER site. Vertical lines are standard errors of means (n= 4 replicate plots). Fertilization occurred on June 27th.