

Methane Oxidation and Production Activity in Soils from Natural and Agricultural Ecosystems

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ABSTRACT

Methane (CH₄) flux from soil to the atmosphere is the result of two microbial processes, methanogenesis and CH₄ oxidation. Land use may have a profound impact on the relative activities of these groups of organisms. In this study, the CH₄ production and consumption potentials of soils from agricultural and nonagricultural ecosystems were assessed in laboratory incubations. Methane production potentials of most soils were low and in the range of 0.02 to 0.35 nmol CH₄ g soil⁻¹ h⁻¹; however, soils from two of the agricultural sites that experience periodic water saturation had CH₄ production potentials from 100 to 300 nmol CH₄ g soil⁻¹ h⁻¹. The high methanogenic potential suggests that CH₄ consumers may not be wholly dependent on atmospheric CH₄ for their survival and maintenance. The prairie soils exhibited the highest CH₄ oxidation under ambient atmospheric CH₄ concentrations, and CH₄ oxidation activity was markedly enhanced in incubations with an atmosphere enriched in CH₄. This stimulated CH₄ oxidation activity was generally greater in the agricultural soils as compared with the forest and prairie soils. Methane oxidation appeared to be related to soil nitrogen status. Under ambient atmospheric CH₄ concentrations, CH₄ oxidation was negatively related to soil mineral N (NO₂⁻ + NO₃⁻ + NH₄⁺) concentration. However, a positive relationship between soil mineral N status and CH₄ oxidation activity was observed in incubations with atmospheres enriched in CH₄. This pattern suggests that the agricultural lands contain different populations of CH₄ oxidizers than the natural systems.

METHANE is a major atmospheric component contributing to global climate changes. It has a relative global warming potential 21 times that of carbon dioxide over a 100-yr time horizon, and contributes approximately 20% to the radiative force driving global climate change (Intergovernmental Panel on Climate Change, 1996). Methane's potency and increasing atmospheric concentration may have a paramount impact on future global warming. This potential impact has led to many terrestrial studies of methods and techniques to quantify CH₄ flux at the soil-atmosphere interface (Rolston, 1986; Mosier, 1990; Verma et al., 1992; Hutchinson and Livingston, 1993; Chan et al., 1998). There have also been several studies conducted to identify and assess the sources and sinks of CH₄ (Galchenko et al., 1989; Bouwman, 1990; Denmead, 1991; Duxbury et al., 1993; Topp and Pattey, 1997). These studies are generally in agreement, namely, that water-saturated systems like wetlands (swamps, marshes) and paddy soils (rice fields)

are net contributors of CH₄ to the atmosphere whereas upland soils (with the exception of landfills) are generally sinks for CH₄.

Methane flux is the difference between CH₄ oxidation and methanogenesis, which may occur simultaneously even in arable terrestrial ecosystems (Conrad, 1995), and the three groups of organisms that may be involved are the methanotrophic bacteria, ammonia oxidizing bacteria, and methanogenic bacteria (Schimel and Gullede, 1998). Upland soils are recognized as important sinks for atmospheric CH₄ (Conrad, 1996); however, atmospheric CH₄ concentrations typically are too low to support the growth of common CH₄ oxidizers in the laboratory (Bender and Conrad, 1993; King, 1993). Methane oxidation in soil can also be carried out by nitrifying bacteria, which have an affinity for CH₄ similar to that of the common methanotrophs (Bedard and Knowles, 1989). Methane production has the potential for influencing CH₄ oxidation, in that CH₄ oxidizers may not depend completely on atmospheric CH₄ for their survival in an ecosystem (Megraw and Knowles, 1987). Conrad (1995) hypothesized that in oxic soils, CH₄ production in anaerobic microsites could be an important source of CH₄ for CH₄ oxidizers.

Anthropogenic influences are generally considered to decrease the CH₄ consumption activity of soils (Ojima et al., 1993). Nitrogen fertility has been shown to dramatically decrease CH₄ consumption activity of forested and grassland systems (Stuedler et al., 1989; Mosier et al., 1991; Castro et al., 1994). Cultivation also appears to decrease net CH₄ consumption, as studies have shown that net CH₄ consumption in cultivated soils is less than in grasslands (Mosier et al., 1991, 1996; Kessavalou et al., 1998). However, changes in soil nitrogen status due either to N fertilizer additions or to cultivation may differentially affect the CH₄ oxidizing community. Schimel and Gullede (1998) discuss three levels of inhibition of CH₄ consumption as influenced by NH₄⁺. These levels were immediate inhibition, delayed inhibition, and no inhibition. These researchers concluded that the response of CH₄ oxidation to N inputs, and possibly other changes, will vary from system to system depending on the nature of the CH₄ oxidizer population. It is possible that the differential effect of NH₄⁺ fertility on CH₄ consumption in agricultural vs. forest systems may be due to differences in the populations of nitrifying bacteria. Regular application of NH₄⁺ to agricultural soils may result in increased populations of nitrifying bacteria, which will coincidentally metabolize CH₄, and may also stimulate methanotrophic bacterial populations, as both

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Abbreviations: DT50, time required for 50% of the initial headspace CH₄ to disappear.

Table 1. Characteristics of study sites.

Site	Soil map unit	Management
McFarland Forest	Lester sandy loam (Mollic Hapludalf)	Old grove woodland site (approximately 100 yr old), sparse understory. Good drainage.
Doolittle Prairie	Kossuth silty clay loam (Typic Endoaquoll)	Never-filled native prairie; hydric site vegetated by mixed grasses and forbes. Moderate to poor drainage.
Doolittle Farm	Kossuth silty clay loam (Typic Endoaquoll)	Corn-soybean rotation. Cultivation: fall chisel plow following corn, spring field cultivation following soybean. Nitrogen fertilization: approximately 200 kg N ha ⁻¹ (as anhydrous ammonia) applied in the fall after soybean. Poor drainage.
Roadside Prairie	Webster clay loam (Typic Endoaquoll)	Restored tallgrass prairie, approximately 80 yr old. Moderate to poor drainage.
Roadside Farm	Webster clay loam (Typic Endoaquoll)	Corn-soybean rotation. Cultivation: fall chisel plow following corn, spring field cultivation following soybean. Nitrogen fertilization: approximately 150 kg N ha ⁻¹ (as anhydrous ammonia) applied in the fall after soybean. Moderate to poor drainage.
Walnut Creek Hilltop	Clarion loam (Typic Hapludoll)	Corn-soybean rotation. Cultivation: fall chisel plow following corn, spring field cultivation following soybean. Nitrogen fertilization: approximately 138 kg N ha ⁻¹ (as anhydrous ammonia) applied in the fall after soybean. Good drainage.
Walnut Creek Sideslope	Clarion loam (Typic Hapludoll)	Crop, cultivation, and fertilization regime as above. Moderate drainage.
Walnut Creek Pothole	Okoboji mucky silt loam (Cumulic Vertic Endoaquoll)	Crop, cultivation, and fertilization regime as above. Poor drainage.
Cluever Control	Clarion loam (Typic Hapludoll)	Nitrogen fertilization: no added N.
Cluever Nitrogen	Clarion loam (Typic Hapludoll)	Nitrogen fertilization: spring-applied urea ammonium nitrate, 252 Kg ha ⁻¹ .
Cluever Broadcast	Clarion loam (Typic Hapludoll)	Nitrogen fertilization: swine manure slurry (60 Mg ha ⁻¹), spring-broadcast (approximately 224 kg N ha ⁻¹).
Cluever Injected	Clarion loam (Typic Hapludoll)	Nitrogen fertilization: swine manure slurry (51.2 Mg ha ⁻¹), spring-injected (approximately 132 Kg N ha ⁻¹).

methanotrophs and nitrifying bacteria can oxidize both CH₄ and NH₄⁺ (Hanson and Hanson, 1996).

In our recent study describing CH₄ flux from a variety of ecosystems (Chan and Parkin, 2001), agricultural systems tended to have reduced CH₄ fluxes; however, application of N fertilizer per se did not appear to have an inhibitory effect on CH₄ consumption. Clearly, more information is needed on the CH₄ production and consumption potential of soils in order to more clearly delineate the impact of land management on CH₄ flux. Thus, the objective of this study was to assess methanogenic and CH₄ oxidizing activity in a variety soils, and to relate these activities to soil N status.

MATERIALS AND METHODS

Sites

The study sites were located in central Iowa and represented a range of common agricultural systems under a variety of fertilization regimes, including inorganic N fertilization and fertilization with swine (*Sus scrofa*) manure, and natural systems including a hardwood forest site and native and restored prairies. Many of the sites were included in a recent evaluation of field CH₄ flux rates (Chan and Parkin, 2001). Site characteristics are given in Table 1.

Sample Collection and Processing

Two intact soil cores (4 cm diameter) were collected at each site in August 1995 by driving a metal probe containing a hollow plastic cylinder into the soil. The cylinders containing the cores were capped with butyl rubber stoppers and transported back to the laboratory. The top 5 cm of each core was removed, sieved (0.5-cm mesh), and used in the CH₄ activity assays described below. Soil cores were processed within 48 h.

Soil Properties

Nitrate (+nitrite) and ammonium were determined by colorimetric analyses of 2 M KCl soil extracts (4:1 KCl to soil) on

a Lachat (Mequon, WI) autoanalyzer following the procedure described by Keeney and Nelson (1982). The pH was determined on 1:1 (H₂O to soil) extracts with a standard glass electrode as described in McLean (1982). Soil water content was determined gravimetrically after overnight drying at 105°C (Gardner, 1982). General soil properties are given in Table 2.

Methane Activity Assays

Field-moist soils (equivalent of 1 g dry soil) were placed into 40-mL screw cap vials. The moisture content of each sample was adjusted to 50% gravimetric water content by additions of sterile distilled water. Different subsamples were used for each of the assays described below. Vials were capped with butyl rubber septa (Laboratory Supply Distributors, Mt. Laurel, NJ) and incubations were done at laboratory temperature (approximately 24°C) in the dark, except during sampling. Vials were sampled approximately every 2 d and monitored for about a 3-wk period. Methane production and consumption potentials were assessed by modifying the headspace gas conditions in the vials.

Methane consumption activity was determined in vials with an aerobic headspace, and was measured by monitoring de-

Table 2. General soil properties at the study sites.†

Site	Bulk density	Water content	pH	NO ₃ ⁻	NH ₄ ⁺
	g cm ⁻³	g g ⁻¹		- mg N kg ⁻¹ -	
McFarland Forest	1.04	0.14	6.1	3.6	1.9
Doolittle Prairie	0.95	0.40	5.6	4.1	<0.1
Doolittle Farm	1.11	0.23	6.4	5.0	0.9
Roadside Prairie	0.95	0.44	6.2	0.9	1.1
Roadside Farm	1.14	0.29	5.8	15.4	1.7
Cluever Control	1.40	0.21	5.0	3.8	1.2
Cluever Nitrogen	1.44	0.16	4.7	6.1	1.6
Cluever Broadcast	1.38	0.15	4.4	14.1	1.3
Cluever Injected	1.48	0.13	4.3	4.8	1.2
Walnut Creek Hilltop	1.33	0.08	4.7	7.5	0.7
Walnut Creek Sideslope	na	0.11	5.1	9.0	1.8
Walnut Creek Pothole	na	0.20	5.5	26.3	0.8

† Values for NO₃⁻, NH₄⁺, and soil water content were computed on a dry soil weight basis. na = values are not available.

creases in CH₄ concentration in the headspace of the vials over time. Two types of CH₄ oxidation incubations were conducted. In one regime the initial CH₄ concentration was near ambient levels (~1.7 to 2.0 μL L⁻¹) and in the other regime a headspace enriched in CH₄ (10 mL L⁻¹) was used. The elevated CH₄ treatment was prepared by adding CH₄ from a tank of ultra-high purity grade CH₄ (Air Products and Chemicals, Allentown, PA). Ambient O₂ concentrations (~19%) were present for both incubation regimes.

Methane production was also measured in two different incubation regimes. In one regime, methyl fluoride (CH₃F) (Matheson Gas Products, Montgomeryville, PA) was used as an inhibitor of CH₄ oxidation (Oremland and Culbertson, 1992; Chan and Parkin, 2000), and increases in headspace CH₄ with time were measured. These incubations were performed in an aerobic headspace and with a CH₃F concentration of 0.5%. In the second regime, CH₄ production was measured under anaerobic conditions with a headspace containing 75% H₂ and 25% CO₂. These conditions were established by alternately evacuating the vial headspace and flushing with helium to remove air. After five cycles of evacuation and flushing, a vacuum was drawn on each vial and H₂ and CO₂ (Air Products and Chemicals) were added to achieve the proportions stated above. Methane production potential was determined by monitoring increases in headspace CH₄ concentration with time.

Gas Sampling and Analysis

Methane, CH₃F, and O₂ were monitored using a dual detector Tracor (Austin, TX) 540 gas chromatograph. A 0.5-mL sample loop (valve injection) was used to draw a sample from the vials, split, and directed to two gas detectors in the gas chromatograph. Methane and methyl fluoride were measured with a flame ionization detector running at 200°C, oven temperature at 65°C, a Porapak Q 1.8-m glass column (Alltech, Deerfield, IL), and He carrier gas flowing at the rate of 30 mL min⁻¹. Oxygen was measured with a thermal conductivity detector running at 150°C, oven temperature at 65°C, a molecular sieve, 2.4-m stainless steel column, and He carrier gas at a flow rate of 75 mL min⁻¹. Standard curves were constructed for CH₄, CH₃F, and O₂ by injecting a range of concentrations corresponding to the specific gas. Certified standards of CH₄ and O₂ were obtained from Scott Speciality Gases (Troy, MI). The minimum detectable flux rate was calculated following the procedures described by Chan et al. (1998) using a 3% coefficient of variation for the calculations. The minimum CH₄ flux rate detectable in this study was 7.12 pmol CH₄ g⁻¹ h⁻¹ (for both consumption and production).

Rate Calculations

The maximum rates of consumption and production were used to compare ecosystem CH₄ cycling activity for each incubation regime. Estimation of the maximum rate (V_{opt} in Bender and Conrad, 1995) was accomplished by least-squares regression of the CH₄ concentration vs. time data and calculating the maximum (in cases of CH₄ production) or minimum (in cases of CH₄ consumption) first derivative of the function. This process was accomplished by employing the Table Curve software package (SPSS, 1996). When rates were less than our minimum detectable rate (7.12 pmol CH₄ h⁻¹ per microcosm), we followed the recommendations of Gilbert (1987) and included the values of these “nondetects” in our mean and comparison calculations. All rates are expressed on a dry-weight soil basis. Analysis of the kinetic patterns of CH₄ consumption was done by comparing the DT-50 values (time required for 50% of the initial headspace CH₄ to disappear) (Parkin et al., 1991; Bender and Conrad, 1995).

Statistical Analyses

The data presented in the bar plots represent means and ranges of duplicate samples. Site and group comparisons on CH₄ oxidation and production data were accomplished by using SAS one-way analysis of variance (ANOVA) (SAS Institute, 1999). Sites were grouped into three different categories based on land use similarities. The three categories are: (i) Natural; (ii) Agricultural—corn and soybean rotation, plow till, no manure; and (iii) Agricultural—corn and soybean rotation, plow till, manure fertility. To satisfy conditions of normality for the CH₄ production values, statistical analyses were performed on log-transformed data.

RESULTS AND DISCUSSION

Methane Production Potential

All of the soils possessed the capacity for methanogenesis under anaerobic conditions with added H₂ and CO₂ (Fig. 1). Methane production potential from the forest system was lower than the prairie sites (Fig. 1A), and average rates ranged from 0.05 to 0.36 nmol CH₄ g⁻¹ h⁻¹, with Roadside Prairie being the highest and McFarland Forest the lowest. Statistically significant differences were detected between the natural and no-manure plow till systems ($P < 0.01$), and also between the no-manure and manure group ($P < 0.01$). No differences were detected between the natural and plow till manure group even though manure was applied for three consecutive years to the manure systems. Methanogenic organisms operate best in neutral pH environments (Garcia, 1990; Zinder, 1993). The low pH (<4.5) observed in the manure systems may have had an effect on methanogenic activity.

Several agricultural sites exhibited very high CH₄ production activity (Fig 1, panel B2). The Walnut Creek Pothole site (WC Pothole) and the Doolittle Farm site (DL Farm) had average rates of 298 and 112 nmol CH₄ g⁻¹ h⁻¹, respectively (note scale change on Fig. 1, panel B2). The Walnut Creek Pothole site is a low area of an agricultural field that maintains standing water for extended periods during the year (Cambardella et al., 1994), and the Doolittle Farm site is also a hydric site, supporting a shallow water table. It is thought that these areas maintain anaerobic conditions conducive for the development and maintenance of methanogenic bacterial populations.

The detection of CH₄ production indicates that all these ecosystems possess populations of methanogenic bacteria. Given the right conditions these systems have a potential to produce and release CH₄ into the atmosphere. In situ methanogenic activity from Walnut Creek Pothole and Doolittle Farm may even fuel CH₄ oxidation activity. Yavitt et al. (1995) found methanogenesis occurring in a northern hardwood ecosystem when samples were incubated anaerobically as well as aerobically with methyl fluoride. In both laboratory and field studies, Wang and Bettany (1997) observed methanogenesis in their prairie and forest systems. Methane production began after periods of increased moisture (snowmelt, precipitation, flooding). In a laboratory experiment using a cultivated soil, Megraw and Knowles (1987) ob-

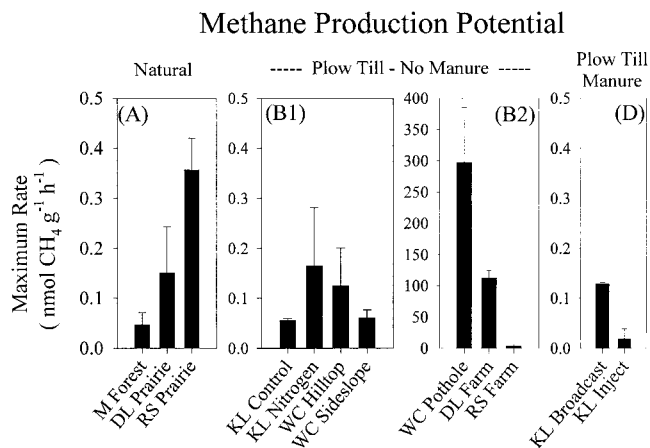


Fig. 1. Methane production potential of soils. Samples were incubated anaerobically with a gas mixture of 75% H₂ and 25% CO₂. Bars indicate the average rate and error bars indicate the range of the rates (*n* = 2). M Forest = McFarland Forest, DL Prairie = Doolittle Prairie, RS Prairie = Roadside Prairie, KL Control = Kluever Control, KL Nitrogen = Kluever Nitrogen, KL Broadcast = Kluever Broadcast Manure, KL Inject = Kluever Injected Manure, WC Hill Top = Walnut Creek Hilltop, WC Sideslope = Walnut Creek Sideslope, WC Pothole = Walnut Creek Pothole, DL Farm = Doolittle Farm, RS Farm = Roadside Farm.

served CH₄ production in anaerobic incubations as well as CH₄ oxidation under aerobic conditions. These researchers also suggested that the methanotrophs in their cultivated soil were not entirely dependent on atmospheric CH₄ for survival and growth. As is the case in many natural ecosystems, release of CH₄ has also been observed in cultivated systems after periods following irrigation or rainfall (Delgado and Mosier, 1996; Chan and Parkin, 2001).

Methane Oxidation Potential

Net CH₄ flux is the balance between production and consumption processes. In our assay, potential rates of CH₄ consumption were determined by changes in headspace CH₄ concentration. Thus, CH₄ oxidation would be underestimated if methanogenesis was also occurring. In order to assess CH₄ production in the absence of CH₄ consumption, we performed a separate set of incubations in which CH₃F was used. Methyl fluoride, an inhibitor of CH₄ consumption, allows for the measurement of production processes in the absence of consumption (Oremland and Culbertson, 1992; Chan and Parkin, 2000). When incubated in an aerobic headspace with CH₃F, only soils from three sites exhibited CH₄ production activity (Fig. 2). These CH₄ production rates were used to compute the CH₄ oxidation rates presented below.

When soils were incubated in an aerobic headspace containing ambient CH₄ concentrations (1.7 to 2.0 μL L⁻¹), the natural systems exhibited the highest CH₄ oxidation activity (Fig. 3A). The two prairie soils had CH₄ oxidation rates of 91.1 and 74.6 pmol CH₄ g⁻¹ h⁻¹, and these rates were significantly higher (*P* < 0.01) than the oxidation rate exhibited by the McFarland Forest soil (24.3 pmol CH₄ g⁻¹ h⁻¹). The agricultural systems showed much lower CH₄ oxidation activity. This was the case for the agricultural systems without liquid swine

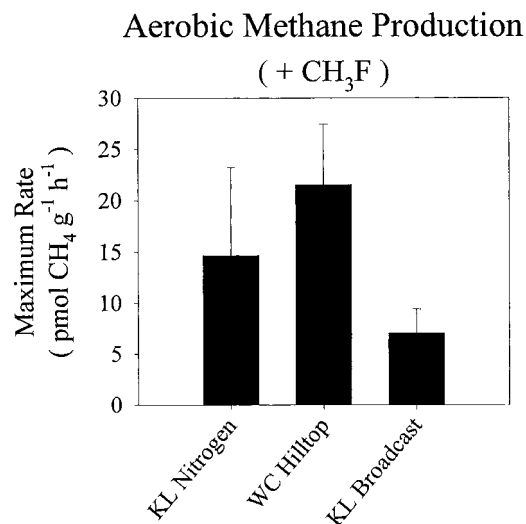


Fig. 2. Aerobic methane production. Samples were incubated aerobically with 0.5% CH₃F to inhibit CH₄ oxidation. Only soils exhibiting CH₄ production are shown. Bars indicate the average rate and error bars indicate the range of the rates (*n* = 2). Symbols are the same as in Fig. 1.

manure (Fig. 3B) as well as the two systems that received swine manure (Fig. 3C). One of the agricultural sites, the Doolittle Farm, actually exhibited CH₄ production in this aerobic incubation regime. It is not known why this production activity was not expressed in the presence of CH₃F.

Our observations of CH₄ oxidation activity in natural and agricultural ecosystems follow the general pattern of CH₄ oxidation activity reported in the literature; namely, natural ecosystems consume CH₄ at a higher rate than agricultural ecosystems (Bender and Conrad,

Methane Oxidation Assay (Ambient CH₄)

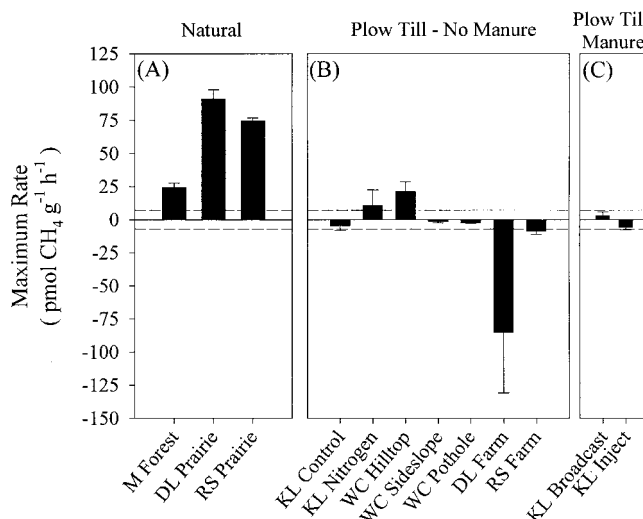


Fig. 3. Methane oxidation under conditions of ambient headspace CH₄ concentration. Positive rates indicate gross methane oxidation and negative rates indicate CH₄ release. Bars indicate the average rate and error bars indicate the range of the rates (*n* = 2). Horizontal dashed lines indicate minimum detection limit of ±7.12 pmol CH₄ h⁻¹ g⁻¹ for the assay. Symbols are the same as in Fig. 1.

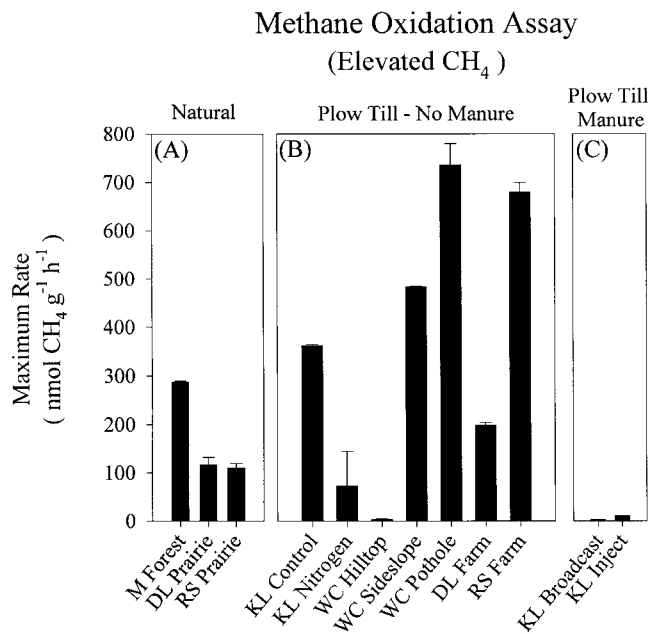


Fig. 4. Methane oxidation assay at elevated headspace methane concentration (~1% CH₄). Positive rates indicate gross methane oxidation. Bars indicate the average rate and error bars indicate the range of the rates (*n* = 2). Symbols are the same as in Fig. 1.

1993; Dobbie and Smith, 1996; Powlson et al., 1997). The CH₄ oxidation rates of our natural ecosystem group were significantly greater (*P* < 0.01) than both the non-manured and manured agricultural systems. Ambient CH₄ oxidation rates extrapolated from studies by Bender and Conrad (1992, 1993) yielded rates of approximately 100 to 200 pmol CH₄ g⁻¹ h⁻¹ for a German forest cambisol. These rates are about four to eight times larger than our McFarland Forest ecosystem. However, Heipieper and de Bont (1997) reported average rates of 120 pmol CH₄ g⁻¹ h⁻¹ for a Dutch grassland soil incu-

bated with 1 μL L⁻¹ CH₄. This value is similar to those of our prairie soils.

Because past reports indicate that CH₄ oxidizing bacteria may differ with regard to their affinity for CH₄ (Bender and Conrad, 1992; Conrad, 1995), we also measured CH₄ oxidation potential under elevated (~1%) headspace CH₄ concentrations (Fig. 4). In all cases, a higher headspace CH₄ concentration stimulated CH₄ oxidation rates (note scale change from Fig. 3 to Fig. 4). Under this elevated CH₄ incubation regime, oxidation activities of the agricultural soils were stimulated to a greater extent than the forest and prairie soils. The Roadside Farm and Walnut Creek Pothole soils had the highest rates of CH₄ oxidation. At the Walnut Creek site there appeared to be a landscape effect. Lowest rates were observed in soil collected from the Hilltop location (3.0 nmol CH₄ g⁻¹ h⁻¹), and the highest rate was observed in soil from the depressional Pothole location (736 nmol CH₄ g⁻¹ h⁻¹). The Sideslope location had an intermediate rate of 483 nmol CH₄ g⁻¹ h⁻¹. The two sites that receive liquid swine manure application (Kluever Broadcast and Kluever Injected) had very low CH₄ oxidation activity compared with most of the other sites. Manure applications, or some other factor at this site, seemed to have an adverse effect on the population of CH₄ consumers, which have affinity to high concentrations of CH₄ (Fig. 4). We suspect that the low soil pH (pH < 5.0) may have caused a reduction in the population sizes and/or activity of the CH₄-consuming community, although antibiotics (Hanson and Hanson, 1996; Schnell and King, 1995) or other compounds in the swine manure may have played a role.

The kinetics of CH₄ oxidation are illustrated in Fig. 5. After a lag time of 2 d, CH₄ oxidation was rapid in the Roadside Farm, the Doolittle Farm, and the Walnut Creek Pothole soils. The time for 50% disappearance of the initial CH₄ (DT-50) in these incubations ranged

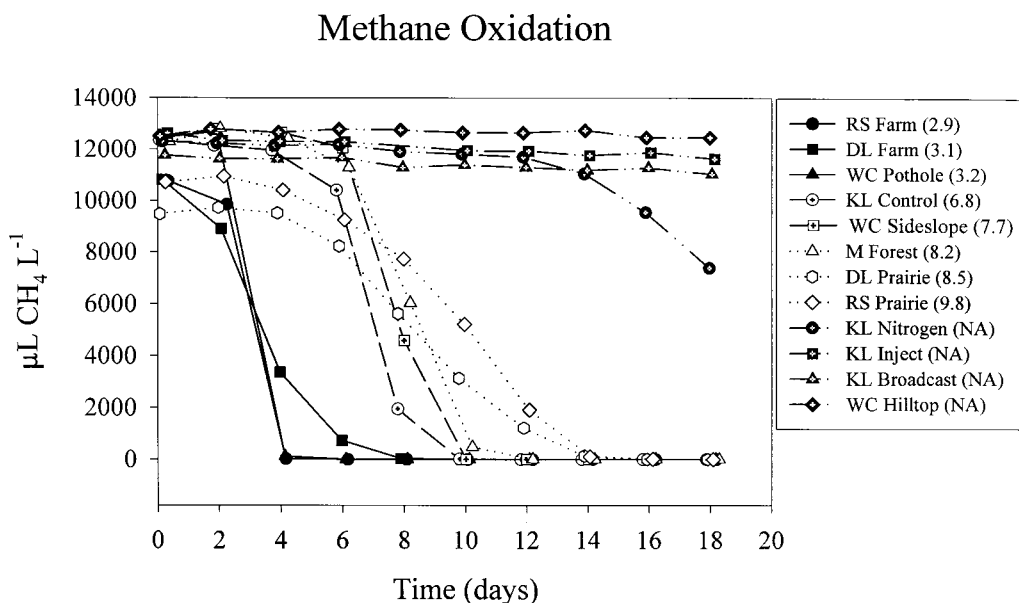


Fig. 5. Kinetics of methane oxidation. Curves are the average of two samples. Values in brackets behind the site identification indicate the time (days) for reduction of the initial headspace CH₄ concentration by 50% (DT-50). NA indicates no CH₄ oxidation activity. Symbols are the same as in Fig. 1.

from 2.9 to 3.2 d (Fig. 5). Methane oxidation also occurred rapidly in the Kluever Control soil and the soil from the Walnut Creek Sideslope location. However, with these soils the lag time was greater (approximately 6 d), and the resulting DT-50 values were longer (6.8 and 7.7 d). The forest soil and the two prairie soils showed a slightly different pattern of CH₄ oxidation. Methane oxidation commenced after a lag period of 4 to 5 d, but oxidation rates did not increase as rapidly as observed in some of the agricultural soils. The other sites showed little CH₄ oxidation activity over the 20-d incubation period, although CH₄ oxidation in the Kluever Nitrogen soil appeared to be increasing after a lag time of 14 d. The different kinetic patterns may be a reflection of differences in CH₄-oxidizing bacterial populations.

A short lag time followed by a rapid increase in CH₄ oxidation rate could indicate the presence of an active population of CH₄ oxidizers relative to soils that exhibited longer lags.

Methane oxidation activity appeared to be related to nitrogen status of the systems (Fig. 6). Methane oxidation activity under conditions of ambient atmospheric CH₄ was negatively related to soil mineral N (NO₂⁻ + NO₃⁻ + NH₄⁺) concentrations (Fig. 6A). The two prairie sites, which had the lowest mineral N concentrations, also showed the highest CH₄ oxidation activity. The forest site had a mineral N concentration of 5.5 mg N kg⁻¹ and showed a CH₄ oxidation rate intermediate to those of the prairie and agricultural sites. The agricultural sites had the highest mineral N concentrations,

Effect of Soil Inorganic N on Methane Oxidation

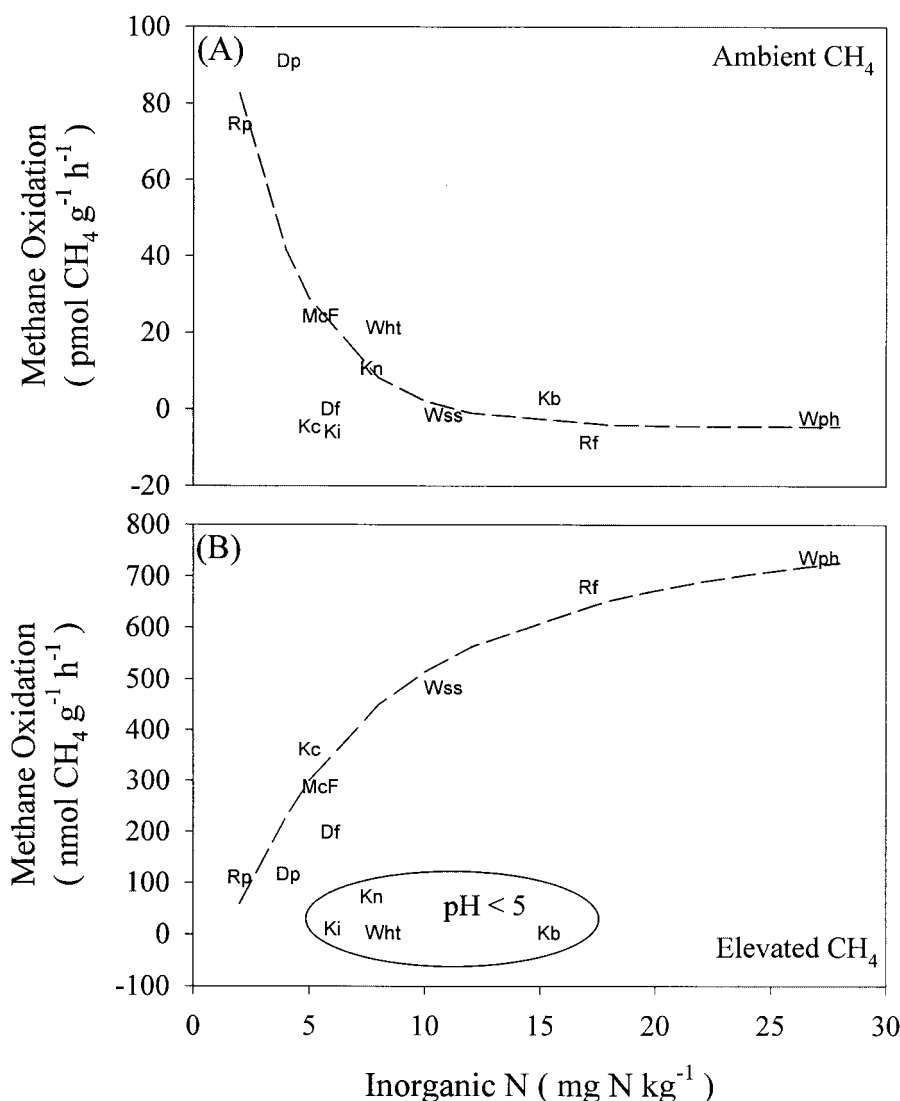


Fig. 6. Relationship between soil inorganic N concentrations and methane oxidation rates as determined in incubations with ambient headspace CH₄ concentrations (A) and elevated headspace CH₄ concentrations (~1% CH₄) (B). McF = McFarland Forest; Dp = Doolittle Prairie; Rp = Roadside Prairie; Kc = Kluever Control; Kn = Kluever Nitrogen; Kb = Kluever Broadcast Manure; Ki = Kluever Injected Manure; Wht = Walnut Creek Hilltop; Wss = Walnut Creek Sideslope; Wph = Walnut Creek Pothole; Df = Doolittle Farm; Rf = Roadside Farm.

and showed the lowest ambient CH₄ oxidation activity. Under conditions of elevated CH₄ concentrations, a different pattern was observed (Fig. 6B). Methane oxidation activity measured in an atmosphere of 1% CH₄ was positively related to soil mineral N levels. The forest and prairie sites had the lowest CH₄ oxidation activity, which corresponded to low soil mineral N concentrations. Conversely, soils from the agricultural sites had higher soil mineral N levels, and higher corresponding CH₄ oxidation rates. However, a group of the agricultural sites did not fit this pattern. It is thought that the CH₄ oxidation activity of these soils was inhibited by low soil pH (<5).

Several studies have shown that CH₄ oxidation is lower in agricultural systems than in natural systems (Bender and Conrad, 1993; Dobbie and Smith, 1996; Powlson et al., 1997). This effect, in part, is thought to be due to fertilizer N inhibition of CH₄ consumption activity in arable soils (Stuedler et al., 1989; Mosier et al., 1991; Bronson and Mosier, 1994). Ammonium (NH₄⁺) has been reported to be a competitive inhibitor of CH₄ oxidation (Whittenbury et al., 1970; Hyman and Wood, 1983; Jones and Morita, 1983). Our observations of lower CH₄ oxidation in agricultural soils incubated at ambient CH₄ concentrations are consistent with these past studies. However, under conditions of elevated atmospheric CH₄, our agricultural sites with pH > 5.0 had significantly higher activity ($P < 0.01$) than the natural systems.

Recent literature indicates there are two populations of CH₄ oxidizers present in the environment (Conrad, 1995). One population, having a low affinity to CH₄, typically has a K_m in the range of 1000 nM CH₄, and the other population, having a high affinity for CH₄, has a K_m in the range of 30 to 60 nM CH₄. In contrast, Dunfield et al. (1999) suggest that low- and high-affinity oxidations are carried out by the same methanotrophs, in their case a Type II methanotroph, but that affinity for CH₄ changes as a function of growth conditions. It is well documented, however, that ammonia-oxidizing bacteria can also oxidize CH₄ as an alternative substrate for ammonia monooxygenase (Suzuki et al., 1976; Hyman and Wood, 1983; Jones and Morita, 1983; Ward, 1987), perhaps making this group of organisms important in the cycling of CH₄ in agricultural systems. In both field and spiked (100 μL CH₄ L⁻¹) laboratory studies, Goldman et al. (1995) found a positive correlation between CH₄ consumption and soil NH₄⁺. These findings are similar to those of our study, which shows that under an elevated CH₄ atmosphere, N may actually stimulate CH₄ oxidation activity. We suspect that the CH₄ oxidation activity carried out under elevated atmospheric CH₄ levels may be predominantly due to nitrifying bacteria, especially in the agricultural systems, where regular ammoniacal N fertilizer applications may serve to create and sustain high populations of nitrifying bacteria.

Methane oxidation by nitrifying bacteria may be important in situations where elevated CH₄ concentrations are present. Although the maximum rate of CH₄ oxidation by nitrifying bacteria has been reported to be several orders of magnitude less than for common CH₄-

oxidizing bacteria, the contribution of the nitrifying bacteria to total CH₄ oxidation could be significant if high numbers of these organisms are present (Conrad, 1995). As shown by our laboratory experiments, soils from all of the sites sampled were capable of producing CH₄; however, some sites were much more active than others. Hydric systems, such as our Walnut Creek Pothole site, or the Doolittle Farm site, which showed high CH₄ production potential and have been observed to produce CH₄ in the field (Chan and Parkin, 2001), may serve to support an active CH₄-oxidizing bacterial population.

CONCLUSIONS

Methane flux dynamics in soil are complex and may be reflected by the activities of three distinct microbial populations: the methanotrophs, the ammonia oxidizing nitrifiers, and the methanogens. This study shows that the prairie and forest soils had the greatest potential to oxidize atmospheric concentrations of CH₄; however, soil from many of the agricultural sites exhibited greater CH₄ oxidation activity when exposed to elevated CH₄ concentrations. All of the soils exhibited CH₄ production potential. The high methanogenic potential at two hydric sites (Walnut Creek Pothole and Doolittle Farm) may indicate that CH₄ oxidizers may not be wholly dependent on atmospheric CH₄ for their survival and maintenance. In soils with pH less than 5.0, CH₄ oxidation activity was nearly eliminated. The nitrogen and water status as well as pH of a given system may selectively affect these groups and subsequently influence the net flux of CH₄ from a given site.

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