Methane cycling in soils

Alvarus Sang Keong Chan
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Agriculture Commons, Environmental Sciences Commons, Microbiology Commons, and the Soil Science Commons

Recommended Citation
Chan, Alvarus Sang Keong, "Methane cycling in soils " (2000). Retrospective Theses and Dissertations. 13889.
https://lib.dr.iastate.edu/rtd/13889
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600
Methane cycling in soils

by

Alvarus Sang Keong Chan

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Microbiology

Major Professors: Timothy B. Parkin and Robert E. Andrews

Iowa State University

Ames, Iowa

2000

Copyright © Alvarus Sang Keong Chan, 2000. All rights reserved.
This is to certify that the Doctoral dissertation of

Alvarus Sang Keong Chan

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Co-major Professor

Signature was redacted for privacy.

Co-major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College
# TABLE OF CONTENTS

## ACKNOWLEDGMENTS

*vi*

## ABSTRACT

*vii*

## GENERAL INTRODUCTION

1

- Dissertation Organization
- Literature Review
- Objectives
- References

30

## COMPARISON OF CLOSED-CHAMBER AND BOWEN-RATIO METHODS FOR DETERMINING METHANE FLUX FROM PEATLAND SURFACE

30

- Abstract
- Introduction
- Materials and Methods
- Results and Discussion
- Acknowledgments
- References
- Disclaimer

53

## EVALUATION OF POTENTIAL INHIBITORS OF METHANOGENESIS AND METHANE OXIDATION IN A LANDFILL COVER SOIL

59

- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- References
- Disclaimer

78

## A COMPARISON OF METHANE FLUXES FROM NATURAL AND AGRICULTURAL ECOSYSTEMS

84

- Abstract
- Introduction
- Materials and Methods
- Results and Discussion
- Conclusions
- Acknowledgments
- References
- Disclaimer

114
METHANE OXIDATION AND PRODUCTION ACTIVITY IN SOILS FROM
NATURAL AND AGRICULTURAL ECOSYSTEMS

Abstract 136
Introduction 137
Materials and Methods 139
Results and Discussion 143
Conclusions 152
References 153
Disclaimer 159

GENERAL CONCLUSIONS 171
Summary 171
Suggestions for Additional Research 174
References 177
ACKNOWLEDGMENTS

I thank my committee members, Drs. Andrews, Dispirito, Karlen, Moorman, Loynachan and Parkin for their time, support, recommendations and patience throughout my studies and research at Iowa State University.

Dr. Moorman: thanks for all the interesting and refreshing discussions on microbial ecology, politics and bureaucracy during late afternoons and weekends. You have no idea how much I enjoyed these moments in time.

Dr. Parkin: thank you for providing support, encouragement, research freedom and an ideal environment for unlimited discussions, critical thinking and problem solving. Special thanks go to the entire Parkin family, including Rudy, Isabel, Bailey and Sparky for opening your home to me on various holidays and social occasions.

Thank you Mr. Otis B. Smith (O man), Mrs. Eva Bryne-Pursey and Mr. Darrin Hansen for your assistance in sampling, analysis and for being great lab partners and friends. Otis, I will miss the Godfather's, King's Buffet and KFC visits.

Thank you to all the people in the National Soil Tilth Laboratory for support and help during my research. I am most honored to have worked with such an excellent team.

I also would like to take this opportunity to thank fellow graduate students, the Ames Dutch group, Mr. Dennis Wendell, Mr. and Mrs. Andy Tang, Dr. Neal “Baldman” Eash, Dr. Krish (Jay) Jayachandran, Dr. Anne Kimber (and family), Dr. Jeff “Ortho-Boy” Novak, Dr. Robert “Doc” Sjogren, Dr. Peter Stahl and friends world wide for their assistance, encouragement and friendship.

During my course of study I lost many family members that were very close,
leaving me with many serious obligations and responsibilities. A support network was quickly formed which allowed me to continue my studies. A very special thank you goes to my mom Carmen and my brothers Emil (Axe), Sven, and Ewart (Ewi) and their families for their continued support, encouragement and belief in me.

Last, but most certainly not least, I thank my wife Stephanie for her tremendous support and tolerance of my presence.
ABSTRACT

Methane (CH₄) is an important greenhouse gas which captures heat in the atmosphere by absorbing infrared radiation and thereby contributing to global warming. Atmospheric CH₄ concentration has been increasing since the industrial age and recent data suggest that the growth rate may be on the rise. This increase of CH₄ in the atmosphere may have a significant impact on future global warming. It has been estimated that 50% of annual CH₄ comes from terrestrial environments. The role of natural and agricultural ecosystems in terms of their contribution to the atmosphere is unclear. This study was conducted to: i) evaluate methodology for studying CH₄ fluxes from terrestrial systems, ii) quantify and compare CH₄ flux as well as CH₄ activity from a variety of ecosystems, and iii) determine climatic, soil, landscape, and anthropogenic controls on CH₄ production and consumption activity. Two methods to measure CH₄ flux were evaluated and compared in terms of their sensitivity. It was found that the bowen-ratio method, a micrometeorological technique, was much less sensitive than soil chamber techniques for measuring CH₄ flux. Inhibitor techniques for determination CH₄ production and oxidation activity were evaluated. Acetylene (C₂H₂) or ethylene (C₂H₄) is recommended for the inhibition of CH₄ oxidation and methyl chloride (CH₃Cl) for the inhibition of methanogenesis. Methane flux from agricultural systems under a variety of tillage and fertility practices, a hardwood forest, native and restored prairies, and a municipal landfill were quantified. Generally, the natural systems were net consumers of CH₄ and the landfill was a net producer of CH₄. Agricultural systems were more variable, and CH₄ flux was highly influenced by precipitation, landscape position and nitrogen status. Laboratory evaluations of CH₄
production and consumption indicated that two populations of CH₄ oxidizers may be operating, each with a different affinity for CH₄. Also, all of the oxic soils demonstrated the capacity for CH₄ production and CH₄ oxidation, indicating that CH₄ flux from a given ecosystem may be due to a complex interaction between CH₄ production and CH₄ consumption activities.
GENERAL INTRODUCTION

Dissertation Organization

This dissertation consists of this general introduction including a literature review, four chapters which are manuscripts that have been submitted for publication or already published, and a general conclusion. References in the general introduction and general conclusions sections are formatted per the Journal of Environmental Quality specifications. The manuscripts were written in the format of the targeted journal for publication. The first manuscript entitled "Comparison of Closed-Chamber and Bowen-Ratio Methods for Determining methane Flux from Peatland Surfaces", was published in the Journal of Environmental Quality (1998, Vol. 27, no. 1, p. 232-239.). This paper evaluates the use of Closed-chambers and the Bowen-ratio method for measuring methane (CH₄) fluxes and also addresses protocols to evaluate collected data. The second manuscript entitled "Evaluation of Potential Inhibitors of Methanogenesis and Methane Oxidation in a Landfill Cover Soil", was prepared for Soil Biology and Biochemistry. This paper assesses potential inhibitors of CH₄ oxidation and methanogenesis and also deals with some of the implications of using inhibitors to estimate gross production or consumption. The third and fourth manuscripts entitled "A Comparison of Methane Flux from Natural and Agricultural Ecosystems" and "Methane Oxidation and Production Activity in Soils from Natural and Agricultural Ecosystems", were prepared for the Journal of Environmental Quality. In the third paper, CH₄ fluxes for a wide range of Iowa ecosystems were measured over a two year period. The impact of agriculture on CH₄ flux is assessed by comparing the natural and agricultural systems. Also, a CH₄ flux estimate for the state of Iowa was
calculated based on our flux estimates and reported land use patterns. To better understand the differences and dynamics seen in the field CH$_4$ fluxes (paper 3), laboratory experiments were performed to determine CH$_4$ oxidation and production potential (paper 4). The studies leading to these manuscripts were supervised by Dr. Timothy B. Parkin. The first manuscript was also co-authored by Dr. John H. Prueger who was very generous to collaborate and help with the meteorological methods and techniques required for that study.

**Literature Review**

**Methane and Global Climate Change**

Methane is a colorless and odorless gas which captures heat by absorbing infrared radiation thereby contributing to global warming (Topp and Pattey, 1997). Methane's lifetime in the atmosphere is approximately 14 years and it has a relative global warming potential of approximately 21 times that of carbon dioxide (CO$_2$) over a time horizon of 100 years (Table 1) (IPCC, 1996). This increased warming potential relative to carbon dioxide is partly due to methane's larger absorptive capacity and partly due to methane's participation in other chemical atmospheric processes where other radiatively active gas compounds are formed (Duxbury et al., 1993). Estimates from the Intergovernmental Panel on Climate Change indicate that CH$_4$ contributes about 20% to the radiative force driving global climate change (IPCC, 1996). Ice core data indicate that the CH$_4$ concentration has been increasing since the industrial age (Topp and Pattey, 1997). The atmospheric CH$_4$ concentration stands currently at 1.7 parts per million (vol/vol) or µL L$^{-1}$. 
The concentration of CH₄ has increased by 7% between 1983 and 1993 (IPCC, 1995). The growth rate declined in the 1980s and dramatically dropped in 1991 and 1992 (IPCC, 1995). However, recent data suggest that the growth rate may be on the rise again (NOAA, The Climate Monitoring and Diagnostics Laboratory, Carbon Cycle Greenhouse Gas Group, Boulder, CO.). The increase of CH₄ in the atmosphere coupled with the potency of CH₄ may have a significant impact on future global warming.

**Methanogenesis**

Methanogenesis is the process of biological CH₄ production by a group of bacteria generally labeled methanogens. The methanogens are a large and diverse group of microorganisms. Their taxonomic realm spans over three orders, seven families and twenty genera and include approximately 68 species (Garcia, 1990). They belong to the domain Archaea (Woese et al., 1978, 1990) and are different from the true bacteria in many ways (Boone et al., 1993). As reviewed by Boone et al. (1993) distinguishing characteristics of the Archaea include the possession of membrane lipids composed of isoprenoids either-linked to glycerol or other carbohydrates, lack of peptidoglycan containing muramic acid and a distinctive ribosomal RNA sequences. Methanogens are dependent on other groups of microbial associates (cellulolytic, hydrolytic, fermentative microorganisms) for the breakdown of biological polymers (proteins, lipids, carbohydrates) to render them metabolic substrate (carbon dioxide, methanol, acetate, methylamines).
Methanogens are the terminal microorganisms in a microbial food chain (Large, 1983) and obtain energy by converting their metabolic substrates (carbon dioxide, methanol, acetate, methylamines) to CH₄ or CH₄ and CO₂. These organisms contain various unique cofactors which allow them to produce methane. These cofactors are: Methanofuran (MRF) (a), tetrahydromethanopterin (H₄MPT) (b), coenzyme F₄₂₀ (c), coenzyme F₄₃₀ (d), 2-mercaptoethanesulfonic acid (coenzyme M or CoM) (e), 7-mercaptoheptanoyl threonine phosphate (HS-HTP) and (Fig. 2). MFR, H₄MPT and CoM carry the C₁ unit when CO₂ is reduced to CH₄ (Fig. 3). Coenzyme F₄₂₀ is a carrier for electrons and hydrogen, coenzyme F₄₃₀ serves as a cofactor for methyl-CoM methylreductase, and 7-mercaptoheptanoyl threonine phosphate serves as an electron donor to methyl reductase (Fig. 3). As reviewed by Boone (1991) the biological formation of CH₄ culminates in the reductive cleavage of a methyl group from the one carbon carrier coenzyme M (CoM, 2-mercaptoethanesulfonate) and that the reactions leading to this step depend on the metabolic substrate used (Fig. 3). Catabolic pathways leading to the final step can be categorized into three groups: CO₂ reducing, methylotrophic and aceticlastic (Boone et al. 1993). The most common way of CH₄ production is the Hydrogen (H₂) mediated reduction of CO₂ (Jones, 1991; Zinder, 1993; Schimel and Gulledge, 1998). Methanogens are obligately anaerobic and thus very sensitive to traces of oxygen (Jones, 1991). They also require a low redox potential (threshold -150mV; Masscheleyn et al., 1993) and thus may be inhibited by the presence of alternative electron acceptors such as NO₃⁻, NO₂⁻, or Fe³⁺ (Boone, 1991). Most methanogens are mesophiles (Topp and Pattey, 1997) but they can be found in a wide
temperature range (Zinder, 1993). Methanogens prefer a pH neutral environment; however, there are examples of some that favor more acidic environments (Garcia, 1990; Zinder, 1993).

Gaseous inhibitors have been widely utilized to study the impact of methanogenesis on the dynamics of CH₄ cycling (Oremland and Capone, 1988; Chan and Parkin, 2000c). A common inhibitor used to block biological methane production is acetylene (C₂H₂) (Chan and Parkin 2000c). Sprott et al. (1982) concluded that the blocking of methanogenesis with C₂H₂ was due to a disruption in the transmembrane pH gradient and not an effect on the methanogenic enzymes. Acetylene did not seem toxic to the methanogens, as cells were still viable after 16 hours of exposure (Sprott et al., 1982). Ethylene (C₂H₄) also inhibits methanogenesis and appears to be a reversible inhibitor (Oremland and Capone, 1988). Many other gaseous compounds inhibit methanogenesis (Oremland and Capone, 1988) however for a large portion of them the true nature of their inhibition kinetics remains a mystery.

Despite their sensitivity to oxygen, methanogens can be readily isolated from non flooded, oxic soils (Peters and Conrad, 1995). Assessment of the contribution and importance of these organisms to CH₄ dynamics in non flooded, oxic ecosystems has been largely overlooked.

**Methane Oxidation**

Methanotrophs (obligate methylotrophs) are thought to play a key role in the oxidation of CH₄ in the environment. They are obligate aerobes that are ubiquitous
throughout the environment and that utilize CH₄ as their sole carbon and energy source by employing methane monooxygenase, the vital enzyme system in methanotrophs. They commonly oxidize CH₄ to CO₂ by way of methanol (CH₃OH), formaldehyde (HCOH) and formate (HCOOH) (Fig. 4) (Hanson, 1996; Zahn, 1996). The first step in this series of two electron oxidations is the oxidation of CH₄ to CH₃OH by methane monooxygenase (Fig. 4). Physiologically these organisms are divided into three groups: Type I, Type II and Type X, based on their carbon (formaldehyde) assimilation pathway (ribulose monophosphate or serine cycle) and ultrastructural arrangement of their cell membrane (Zahn, 1996). Type I has a vesicular disc-shaped cellular membrane and the ribulose monophosphate pathway, Type II has a paired cellular membrane and the serine pathway while Type X has a vesicular disc-shaped cellular membrane and both the serine and ribulose monophosphate pathways (Zahn, 1996). Taxonomically the methanotrophs span over 6 genera: *Methylobacter, Methylomonas, Methylosinus, Methylocystis, Methylococcus*, and *Methylomicrobium* (Hanson and Hanson, 1996). Methanotrophs are generally mesophilic and prefer a pH neutral environment (Topp and Pattey, 1997).

A wide selection of gaseous inhibitors have been employed in the past to study CH₄ oxidation and the dynamics CH₄ cycling in the environment (Oremland and Capone, 1988; Bedard and Knowles, 1989; Chan and Parkin, 2000c). Most of these inhibitors target the methane monooxygenase enzyme. Acetylene (C₂H₂), a commonly used inhibitor for CH₄ oxidation, targets methane monooxygenase and seem to behave as an irreversible inhibitor (Prior and Dalton, 1985; Matheson et al., 1997). Acetylene apparently has the same effect on ammonia monooxygenase (Hyman and Wood, 1985). Methyl fluoride (CH₃F) and
difluoromethane (CH$_2$F$_2$) have also been used as inhibitors for CH$_4$ oxidation, however their effect on methane monooxygenase differ from that of C$_2$H$_2$ in that they behave like reversible inhibitors (Matheson et al., 1997, Miller et al., 1998).

Discrepancies exist between CH$_4$ oxidation potentials of cultured methanotrophs and upland (non flooded) soil oxidation potentials (Conrad, 1995; Conrad, 1996; Topp and Pattey, 1997). Cultured methanotrophs (sometimes termed known or common methanotrophs) exhibit a low CH$_4$ affinity ($K_m = 800$-$66,000$ nM, Threshold $\sim 1.0$ nM) while the observed affinity in soils is much higher ($K_m = 20$-$200$ nM, Threshold $= 0.03$-$0.7$ nM) (Conrad, 1995; Conrad, 1996). Based on these observations, it is suggested that the methanotrophs isolated to date may not be the same organisms consuming atmospheric CH$_4$ in upland non flooded soils (Conrad, 1995; Conrad, 1996; Topp and Pattey, 1997). However, recent experiments by Dunfield et al. (1999) suggest that low and high affinity oxidations may be carried out by the same methanotrophs, in their case a type II methanotroph, but that affinities varied depending on the growth conditions. As reviewed by Hanson and Hanson (1996) methanotrophs are inhibited by ammonium (NH$_4$$^+$), nitrate (NO$_3$$^-$) and nitrite (NO$_2$$^-$). Antibiotics have also been reported to decrease the CH$_4$ oxidation rates in soil (Hanson and Hanson, 1996).

Sensitive to many of the same inhibitors as the methanotrophs and also considered to have a role in the oxidation of CH$_4$ are the NH$_4^+$ oxidizing (nitrifying) bacteria (Conrad, 1995; Topp and Pattey, 1997). A key enzyme component of the NH$_4^+$ oxidizing bacteria is ammonia monooxygenase which oxidizes NH$_3$ to NH$_2$OH and H$_2$O. Methane is also an alternative substrate for ammonia monooxygenase and NH$_4^+$ oxidizing bacteria can also
consume CH₄ (Suzuki et al., 1976; Hyman and Wood, 1983; Ward, 1987; Jones and Morita, 1983). Methane $K_m$ values for the NH₄⁺ oxidizers (nitrifiers) range 6,600-2,000,000 nM which are much higher than for the common methanotrops. Also, $K_m$'s of nitrifiers and the common methanotrophs are much higher than the apparent $K_m$'s of upland soils (Conrad, 1996), indicating that the high affinity organisms operating in upland soils may be distinctly different. Still NH₄⁺ oxidizers (nitrifiers) could contribute to CH₄ oxidation if present in adequate numbers (Conrad, 1995). In both field and laboratory studies, Goldman et al. (1995) found a positive correlation between CH₄ consumption and soil NH₄⁺ content. Chan and Parkin (2000a) also observed similar trends in N fertilized systems versus controls for their CH₄ field fluxes and also a similar correlation as Goldman et al. (1995) in their laboratory incubations (Chan and Parkin, 2000b).

Upland Ecosystem Influence on Atmospheric Methane

Methane flow between terrestrial systems and the atmosphere is determined by measurement of the CH₄ flux at the soil / atmosphere interface. Methane flux measured at the soil / atmosphere interface is the net effect of CH₄ production (methanogenesis) and consumption (Knowles, 1993). Upland soil systems often support a mixture of anaerobic and aerobic sites where both processes can potentially occur simultaneously. A positive CH₄ flux indicates net CH₄ production and is observed when the magnitude of the methanogenic process is larger than CH₄ uptake. This is the case in rice paddies and wetlands (flooded or water saturated areas) where the local ecosystem is predominately anaerobic (Schutz et al., 1990; Lauren and Duxbury, 1993). It is generally recognized that
flooded systems such as rice paddies, bog lands, and other natural wetlands are large contributors to the atmospheric CH$_4$ pool (Stewart et al., 1989; Bouwman, 1990; Amstel and Swart, 1994; Topp and Pattey, 1997). A negative CH$_4$ flux indicates consumption of CH$_4$ by soil and is observed when the magnitude of CH$_4$ consumption is larger than methanogenesis. This is generally observed in arable soils, where conditions are predominately aerobic (Schutz et al. 1990; Bronson and Mosier, 1993; Mosier et al., 1991; Hansen et al., 1993). However, non flooded upland terrestrial systems including arable soils are more complex and, depending upon the land use coupled with local conditions, may be either net producers or consumers of CH$_4$ (Bronson and Mosier, 1993; Goulding et al., 1996; MacDonald et al., 1996). Natural forest and grassland systems have been widely studied and are also generally considered to be net consumers of CH$_4$ (Steudler et al., 1989; Mosier et al., 1991; Castro et al., 1993; Castro et al., 1994a; Lessard et al., 1994; Ambus and Christensen, 1995; Castro et al., 1995; Mosier et al., 1996; Mosier et al., 1997; Prieme and Christensen, 1997).

Decreases in CH$_4$ consumption capacities of soils are generally linked to anthropogenic influences including agriculture (Ojima et al., 1993). Sharp decreases of CH$_4$ consumption in agricultural ecosystems in comparison to natural ecosystems are potentially due to the use of fertilizer nitrogen (Steudler et al., 1989; Mosier et al., 1991; Adamsen and King, 1993; Hansen et al., 1993; Castro et al., 1994b; Castro et al., 1995; Goulding et al., 1995; Mosier et al., 1996; Goulding et al., 1996; Syamsul Arif et al., 1996), tillage practices (Mosier et al., 1997), soil compaction (Hansen et al, 1993; Keller and Reiners, 1994), and agrochemical applications (Topp, 1993; Syamsul Arif et al., 1996).
The negative effect of antibiotics on CH$_4$ oxidation may also play a role in decreasing CH$_4$ consumption rates in cases where fertilization is accomplished using animal manure (Schnell and King, 1995).

Past CH$_4$ flux work have not addressed how each process (production and consumption), functioning simultaneously, affect CH$_4$ flux at the soil surface. Also largely unknown are the effects that soil and climatic factors have on each process (production and consumption) in relation to soil management.

**Measuring Field Methane Fluxes**

Techniques to measure CH$_4$ fluxes can be divided into two categories: (i) small scale techniques and (ii) large scale techniques. Small scale techniques include laboratory and field incubations of intact soil cores, as well as open-chamber and closed-chamber methods placed on the soil surface (Robertson, 1993; Topp and Pattey, 1997). The scale at which these techniques function may range from a few cm$^2$ to 1 m$^2$. Determination of CH$_4$ flux with these methods usually involves monitoring changes in headspace CH$_4$ concentration with time. A gas chromatograph equipped with a flame ionization detector is typically used to determine CH$_4$ concentration; however, other detectors such as photo acoustic or ultrasonic have also been used.

Potential disadvantages of soil core techniques may include initial soil disruption when the sample is collected as well as potential changes in the soil microenvironment, such as oxygen depletion, during incubation. Field chambers can also induce disturbances that may impact the CH$_4$ flux rate. In addition to perturbing the microclimate at the soil
surface, diffusion of gas from the soil can be affected by gas concentration in the chamber headspace (Hutchinson and Livingston, 1993). The high spatial variability frequently associated with soil gas flux measurements often requires that many samples be collected.

A major advantage of small scale techniques, is that the measured flux can be more closely associated with measurements of ancillary factors that may exert controls on production and consumption reactions. Thus, these methods are more amenable to determination of the mechanistic relationships between controlling factors and microbial processes (Parkin, 1993).

Large scale techniques measure areas in the range of 100 m² to 200 km² (Robertson, 1993; Topp and Pattey, 1997) and include tower or aircraft based meteorological techniques. A few major advantages of these techniques are the measurement of spatially integrated fluxes over a large areas and these methods are generally non-disruptive to the local environment. It is generally thought that flux estimates obtained with large scale methods have lower associated variability; however, variability usually cannot be assessed. A major disadvantage of these methods is that they may be less sensitive than small chamber methods by a factor of 10 depending upon method used for CH₄ quantification (Topp and Pattey, 1997; Chan et al., 1998).

Accurate quantitative assessment of CH₄ fluxes is critical for the accurate estimation of CH₄ budgets. Publications reporting field fluxes using large scale techniques are numerous, however very few studies compare the magnitudes of fluxes determined by large and small scale methods. Our recent publication (Chan et al., 1998) is the only one which compares the precision and accuracy of CH₄ flux measurements as determined by
chamber and micrometerological techniques.

Gaseous Inhibitors of Methanogenesis and Methane Oxidation

Soils may support a mixture of anaerobic and aerobic sites where both methanogenesis and CH$_4$ oxidation can function at the same time. Specific inhibitors of either CH$_4$ oxidation or methanogenesis potentially provide the opportunity to study the effects of various factors on these two processes independently. Gaseous compounds that are capable of completely shutting down either CH$_4$ oxidation or methanogenesis present powerful tools for investigating the factors controlling these processes. In past years several gaseous compounds have been used in the study of methanogenesis and CH$_4$ oxidation, though the compound dosage and efficacy appear to vary. An example of this is the compound acetylene (C$_2$H$_2$) which historically has been a popular inhibitor of both methanogenesis and CH$_4$ oxidation. Its application dosage and efficacy varies immensely as reported from different research groups. Full inhibition of methanogenesis has been reported at levels of 0.5% (King, 1996) and at 1.25% (Oremland and Taylor, 1975), while partial inhibition (60%) has been observed with a 0.01% headspace C$_2$H$_2$ concentration (King, 1996). Methane oxidation however, is much more sensitive to C$_2$H$_2$. Watanabe et al. (1995) reported a 90% inhibition of CH$_4$ oxidation at C$_2$H$_2$ concentrations as low as 0.003 %. King (1996) found 89% inhibition of CH$_4$ uptake associated with washed, excised $S$. eurycarpum roots at C$_2$H$_2$ concentrations of 0.01% and greater than 95% inhibition at C$_2$H$_2$ concentrations exceeding 0.1%. Miller et al. (1998) observed total oxidation inhibition with soils exposed to 1.0 kPa (0.99%) C$_2$H$_2$, and soils remained
inhibited even after removal of C$_2$H$_2$. Similar discrepancies in application dosage and efficacy were also found in the literature regarding methyl fluoride (CH$_3$F) and dimethyl ether (CH$_3$OCH$_3$).

Past approaches to study the differential effects of inhibitors on methanogenesis and CH$_4$ oxidation tested only a few compounds at only a few concentrations. Discrepancies in reported compound dosages for the inhibition of CH$_4$ oxidation and the varied efficacy of these dosages needed further evaluation. Our work provides a systematic evaluation of five gaseous compounds over a wide concentration range for potential use in CH$_4$ cycling studies.

**Objectives**

Over the past 200 years anthropogenic factors have led to a significant increase in atmospheric trace gas concentrations and fueled recent concerns about global climate change. With regard to CH$_4$, anthropogenic activities such as fossil fuel consumption, rice cultivation, animal husbandry, biomass burning, and landfills have been reported to be responsible for 60-80% of the current CH$_4$ emissions (IPCC, 1995). Whereas past emphasis has been CH$_4$ emissions, the effects of anthropogenic practices, and especially agricultural activities, on the ability of soil to serve as a sink (or source) of atmospheric CH$_4$ has been largely neglected. In an era where international treaties on climate change may facilitate the brokering of trace gas credits, it is critical to have a firm understanding of the relationship between land use and CH$_4$ flux and to have a mechanistic understanding of the soil/environmental factors controlling CH$_4$ production and consumption. This research
was initiated to fill the deficits in our knowledge of the role of terrestrial ecosystems in atmospheric CH$_4$ cycling by addressing the following objectives:

1. To determine the extent to which land management influences the capacity of soils to serve as sources or sinks for atmospheric CH$_4$. Specific objectives here were to: i) assess whether agricultural soils are net sources or sinks for CH$_4$ and ii) compare the CH$_4$ fluxes from natural ecosystems, agricultural lands, and lands impacted by anthropogenic influences (i.e. landfill).

2. To determine the major factors controlling CH$_4$ flux in soil. This included relating climatic, soil and landscape controls as well as anthropogenic activities such as fertilizer addition on observed CH$_4$ production and consumption activities.

3. To develop and evaluate methods for studying CH$_4$ cycling in soils, including determination of the errors associated with field flux measurements and an assessment of the efficacy of different inhibitors of CH$_4$ production and consumption.

References


Table 1. Global Warming Potentials relative to CO$_2$.

<table>
<thead>
<tr>
<th>Gas Species</th>
<th>Formula</th>
<th>Lifetime (years)</th>
<th>Global Warming Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 years</td>
<td>100 years</td>
</tr>
<tr>
<td>Methane$^\dagger$</td>
<td>CH$_4$</td>
<td>$14.5\pm2.5^\ddagger$</td>
<td>62</td>
</tr>
<tr>
<td>Nitrous Oxide</td>
<td>N$_2$O</td>
<td>120</td>
<td>290</td>
</tr>
</tbody>
</table>

This table was adapted from IPCC, 1995.

$^\dagger$ The methane Global Warming Potential includes the direct and indirect effects from the production of tropospheric ozone and stratospheric water vapour. Indirect effects due to CO$_2$ production is not included.

$^\ddagger$ The adjustment time is given here rather than lifetime.
Figure 1. Breakdown of biological polymers to render substrate to the methanogens. (Adapted from Brock et al., 1994.)
Figure 2. Unique methanogen cofactors. Methanofuran (MRF) (a), tetrahydromethanopterin (H₄MPT) (b), coenzyme F₄₂₀ (c), coenzyme F₄₃₀ (d), 2-mercaptoethanesulfonic acid (coenzyme M or CoM) (e) and 7-mercaptoheptanoyl threonine phosphate (HS-HTP) (f). (Adapted from Brock et al., 1994 and Prescott et al., 1996.)
Figure 3. General pathway for the production of CH₄ from CO₂, acetate, methanol and formate. Symbol Key: MFR, methanofuran; H₄MPT, tetrahydromethanopterin; F₄₃₀ red, coenzyme F₄₃₀ reduced; F₄₃₀ ox, coenzyme F₄₃₀ oxidized; CoM, 2-mercaptoethanesulfonic acid (coenzyme M or CoM); HS-HTP, 7-mercaptoheptanoyl threonine phosphate. (Adapted from Jones, 1991.)
Figure 4. Common pathway for methane oxidation for methanotrophs. Symbol key: MMO, methane monooxygenase; MeDH, methanol dehydrogenase; FalDH, formaldehyde dehydrogenase; FDH, formate dehydrogenase; RuMP cycle, ribulose monophosphate cycle.
COMPARISON OF CLOSED-CHAMBER AND BOWEN-RATIO METHODS
FOR DETERMINING METHANE FLUX FROM PEATLAND SURFACES

A manuscript published in the Journal of Environmental Quality

A.S.K. Chan, J.H. Prueger and T.B. Parkin

ABSTRACT

Methane is an important greenhouse gas, and it has been estimated that 50% of annual CH₄ comes from terrestrial systems. Better and more accurate methods are needed to quantify CH₄ flux from terrestrial environments. Two general methods commonly applied to measure trace gas fluxes are soil cover (chamber) techniques, and micrometeorology methods. Both of these methods have advantages and disadvantages, yet little information is available concerning the relative performance of the techniques. This study was conducted to compare CH₄ flux measurements obtained by using a closed-chamber soil cover technique and a micrometeorological method (Bowen-ratio Energy Balance (BREB)). Methane flux rates obtained by both methods were compared using 9 time points over a 3 day period at a peatland site in north central Minnesota. Mean CH₄ fluxes obtained by both methods were of the same magnitude (2.43 to 5.88 mg CH₄ m⁻² h⁻¹); however, differences were observed in the magnitudes of temporal variability as well as the detection sensitivities (minimum detectable flux). Spatial variability associated with the closed-chamber flux determinations was high and coefficients of variation of 87.3 to 134% were observed (10 replicate chambers). Spatial variability of the BREB method could not be assessed because only one Bowen-ratio station was used. Temporal variations
for CH₄ flux were found to be large using the BREB method (CVs 90.3 to 285%). Temporal variability associated with the chamber method was low (CVs 7.76 to 20.5%). The minimum detectable flux for the closed-chamber method was 9.32 x 10⁻² mg CH₄ m⁻² h⁻¹ (for the detection of both consumption & production), while the minimum detectable flux for the BREB method ranged from 2.16 to 25.5 mg CH₄ m⁻² h⁻¹. Due to analytical uncertainties associated with gas chromatographic determination of CH₄ gradients, the BREB is not recommended.

**INTRODUCTION**

Methane concentration in the atmosphere has been increasing by about 1% per year (Khalil et al., 1990). Methane's relative global warming potential is 63 times that of carbon dioxide (CO₂) (Duxbury et al., 1993). This increased warming potential is partly due to methane's larger absorptive capacity and partly due to methane's participation in other chemical atmospheric processes where other radiatively active gas compounds are formed (Duxbury et al., 1993). Pearce (1989) predicted that CH₄ could become the primary greenhouse gas within 50 years. Because of the increase of CH₄ in the atmosphere and the potential impact on global warming, assessment of the sources and sinks of CH₄ is crucial. Accurate quantitative assessment of CH₄ flux rates and of the factors that influence CH₄ flux from terrestrial systems is critical.

Chamber methods have been widely used for soil cover techniques to measure trace gas fluxes, and numerous chamber configurations have been developed (Rolston, 1986). Closed-chambers generally employ an open bottom enclosure which is inserted into the
soil surface. Flux measurements are determined by estimating the rate of change of gas concentrations within the enclosure. Because of their low cost, simplicity of design and operation, closed chambers are commonly used to determine fluxes of many gaseous compounds from soil (Mosier et al., 1991a; Freijer et al., 1991; Livingston et al., 1995).

Despite their design simplicity, closed-chamber methods have limitations. Disruption of the local microenvironment due to modification of atmospheric pressure, wind, and gas concentration gradients can influence measured fluxes (Denmead, 1991; Hutchinson et al., 1993; Rolston, 1986; Wesely et al., 1989; Livingston et al., 1995). Although modifications have been developed to minimize the effect of some of these factors (Mosier, 1990; Hutchinson et al., 1993; Matthias et al., 1980; Rolston, 1986; Livingston et al., 1995) they still remain a source of concern. Another limitation of closed chamber methods is large spatial variability in flux estimates from site to site. Previous studies reported high spatial variability for CH₄ flux, with coefficients of variation ranging from 31 to 168% (Mosier, 1990).

In recent years micrometeorological methods have been applied to measure surface/atmosphere trace gas exchanges (Verma et al., 1992; Wofsy et al., 1993; Wesely et al., 1989). Micrometeorological techniques are non-disruptive to the local environment and enable determination of fluxes without perturbations induced by covering the soil (Denmead, 1991; Wesely et al., 1989). These methods can also allow for continuous measurements (Baldocchi et al., 1988; Wofsy et al., 1993). Flux measurements obtained by these methods are time averaged point measurements which, if sufficient fetch is available, can represent temporal and spatially integrated estimates (Denmead, 1991; Wesely et al., 1989).
Eddy correlation in conjunction with a tunable diode laser to measure methane concentrations have been used with a high degree of success (Thurtell et al. 1991, Verma et al. 1992, Edwards et al. 1994). Using the eddy correlation technique with a tunable diode laser can result in very accurate estimates of methane flux, however it should be noted that this type of system is expensive ($120,000) and requires considerable ancillary equipment as well expertise in operating the tunable diode laser.

The Bowen Ratio Energy Balance (BREB) method is a micrometeorological technique that relies on flux gradient theory to estimate gas flux (Baldocchi et al., 1988). The BREB method has been shown to be reliable for latent heat flux estimation typically yielding errors in flux estimates on the order of 10-20% (Verma and Rosenberg, 1975; Sinclair et al., 1975). This method has also been applied to estimate methane fluxes (Prueger et al., 1995, Denmead, 1991).

The primary reason for using the BREB technique was to evaluate a more simple and less costly micrometeorological method in conjunction with gas chromatography (total cost: approx. $25,000) to estimate methane flux from a natural ecosystem and determine the suitability of this type of method in the field.

Accurate determination of concentration gradients is critical for applying the BREB method for estimating trace gas flux; however, few guidelines exists concerning the precise degree of accuracy required. Wesely et al, (1989) state that estimation of trace gas flux using the BREB method requires that the concentration gradient of the component of interest be measured with a "high degree of relative accuracy". Subsequently, it was
reported that for a rice paddy site CH₄ gradients between 10⁻³ and 10⁻² ppmv were difficult to distinguish from system noise (Denmead, 1991). Other limitations of micrometeor techniques are the unknown spatial variability (unless more than one tower is used) and the scale of measurement which is not conducive, in many cases, to process oriented studies.

In this study the two methods were used to estimate CH₄ flux from a peatland system known to display a predominately methanogenic characteristic. The objective of this study was to compare a static closed-chamber method and the BREB method for estimating CH₄ fluxes, and to evaluate the utility of using gas chromatographic analysis for determining CH₄ fluxes with these methods. The temporal and spatial variability of the estimates is also assessed.

**MATERIALS AND METHODS**

**Sampling Site**

This study was conducted between the 21st and 23rd of September 1993 on a northern peatland within the U.S. Forest Service Marcell Experimental Forest (47° 32'N, 93° 28'W) in Itasca County, Minnesota. The region is a complex of small upland watersheds and kettle-hole bogs. Average annual temperature is 3 °C, and average annual precipitation is 77 cm, of which 30 % falls as snow. This area is known to be highly methanogenic (Dise, 1992, 1993; Crill et al., 1988; Verma et al., 1992).

The study area (referred to as the Bog Lake Peatland) is an open bog, and was the location of a recent study by Verma et al. (1992). The soils of this site are classified as greenwood peat (USDA-SCS, 1987), and at the time of sampling standing water covered
the site. During the 3 day sampling period the average day time air temperatures ranged from 8.5 °C to 12.3 °C.

The Bowen-ratio station was erected on a wooden platform which extended approximately 50 meters into the peatland, and chambers were installed around the platform area. Methane flux measurements were performed three times each day (morning, noon, afternoon) for the closed-chamber method and continuously (morning through the afternoon) for the Bowen-ratio method on three successive days. At each sampling time flux estimates were determined by the BREB method and with soil chambers.

Bowen-ratio Energy Balance Method - Theory

Flux-gradient theory is based on the assumption that turbulent transfer of scalers is analogous to molecular diffusion and can be determined as the product of a mean vertical mixing ratio gradient for any atmospheric constituent and an eddy diffusivity coefficient (Baldocchi et al., 1988). The flux-gradient profile for an atmospheric constituent can thus be expressed in general form as

\[ F = k_z \frac{\partial \chi}{\partial z} \]  

(1)

where \( F \) is the flux of an entity in question (ug m\(^{-2}\) d\(^{-1}\)), \( k_z \) is the eddy diffusivity coefficient (m\(^2\) s\(^{-1}\)), and \( \partial \chi / \partial z \) the change in mixing ratio of an entity with height \( z \) (m) above a surface. More specifically Eq. 1 can be recast to express methane flux as:

\[ F_{CH_4} = k_{me} \frac{\partial C}{\partial z} \]  

(2)

where \( F_{(CH_4)} \) is methane flux, \( k_{me} \) the eddy diffusivity coefficient for methane, and \( \partial C / \partial z \) the
methane concentration gradient with height $z$.

Atmospheric stability near the surface can generally be described as neutral, stable or unstable. Neutral conditions are generally found for very short periods of time surrounding sunrise and sunset hours and involve very small or no thermal stratification of the atmosphere near the surface. Buoyancy forces under these conditions are usually very small or nonexistent. For the stable condition, temperatures near the surface are cooler than the air above the surface thus suppressing vertical turbulent transfer of gas constituents to the atmosphere. Unstable conditions are associated with warm to hot surfaces with cooler air above thus enhancing buoyancy forces which increase turbulence transfer in the vertical. The effects of thermal stability on the shape of the wind speed profile and on turbulent exchange rates can be expressed by a non-dimensional parameter that relates buoyancy to mechanical shear forces. This parameter is called the Richardson number ($R_i$) and is given by

$$R_i = \frac{g(\partial \theta / \partial z)}{T(\partial u / \partial z)^2}$$

where $g$ is the acceleration due to gravity, $\partial \theta / \partial z$ and $\partial u / \partial z$ are mean gradients of potential temperature and horizontal wind speed, and $T$ is the mean absolute temperature ($^\circ$K).

Under neutral atmospheric stability conditions an assumption of similarity is made for the transport of methane, sensible heat and water vapor to the atmosphere. The eddy diffusivity coefficient usually denoted as $k$ with a subscript specifically assigning it to an atmospheric constituent, represents any atmospheric constituent scalar relating the turbulent flux to the gradient of the mean associated variable (Stull, 1988). The eddy
diffusivity for water vapor $k_v$ can be derived from the aerodynamic profile for latent heat flux (evaporation) expressed as

$$E = \frac{(M_w/M_a)}{P} \rho_a k_v \frac{\partial e_a}{\partial z}$$

(4)

where $E$ is evaporation, $M_w/M_a$ is the ratio of the molecular weights of water and air, ($a$ constant, 0.622), $P$ is atmospheric pressure (kPa), $\rho_a$ is density of air ($\text{kg m}^{-3}$), $k_v$ ($\text{m}^2 \text{s}^{-1}$) is the eddy diffusivity coefficient for water vapor, and $\partial e_a/\partial z$ is the change in water vapor pressure with height $z$. The BR£B method is used to measure $\partial e_a/\partial z$ and estimate $E$ in Eq. [4]. The Reynolds analogy assumes equality of eddy diffusivities for water vapor, heat, and momentum (Dyer, 1974). In this study the assumption is extended to include methane. Boundary layer studies conducted by Dyer and Hicks (1970), Businger et al. (1971) and Pruitt et al. (1973) reported that the assumption of equality of k's for different atmospheric entities is generally valid for neutral atmospheric stability conditions but not necessarily so for stable or unstable conditions. Corrections for stable and unstable atmospheric conditions must be applied to the eddy diffusivity.

**Bowen-ratio Energy Balance Method Instrumentation**

Micrometeorological instrumentation was deployed to measure the surface energy balance components of net radiation, soil, sensible, and latent heat flux densities. The Bowen-ratio station consisted of four aspirated psychrometers vertically placed at 1, 30, 80 and 230 cm above the peatland surface. The maximum tower height of 230 cm was determined by the upwind fetch area of the tower and adhering to the conservative ratio of
100:1 of upwind fetch area to the height of the tower. Thus there was an upwind area in excess of 250 m of peatland bog. Wet and dry bulb thermocouples were placed in a 4 cm diameter by 20 cm long white polyvinyl chloride (PVC) pipe. This pipe was insulated from solar radiation with a layer of foam padding which was wrapped with chrome reflective tape. A fan attached to one end of the PVC pipe pulled ambient air through the pipe and past the wet and dry bulb thermocouples at a constant rate. Wind speed and aspirated psychrometer masts were constructed near the Bowen-ratio station to evaluate wind speed and vapor pressure profiles over the peatland surface. A net radiometer (Q*6 Radiation and Energy Balance Systems (REBS), Seattle, Washington) was used to measure net radiation at a height of 1.25 m, while a soil heat flux plate (REBS, Seattle, Washington) was buried at a depth of 8 cm below the sphagnum moss to measure heat flux below the moss surface.

Methane concentration gradients were measured by sampling air at two heights above the bog surface. An air sampling mast was constructed with intake ports at 1 and 230 cm, with each sampling port was connected to a low volume pump (15 ml min⁻¹) and a pressure actuated multiport sampling valve with 3.2 mm diameter teflon tubing. Sixteen mylar air sample bags (1235 cm³) were connected by teflon tubing to each multiport valve. The mylar bags were connected to multiposition valves (with 3.2 mm diameter teflon tubing) and bags were contained inside a large plastic container for protection from wind, precipitation and solar radiation.

Sampling was accomplished by drawing air into mylar bags at a constant rate of 15 ml min⁻¹ over the course of 1 hour. After 1 hour when approximately 900 ml of air had
been collected from each height, the multiposition valves were switched, and a new set of air sampling bags were brought on line with the sampling pumps. This design enabled uninterrupted air sampling throughout the day. Gas samples were collected from 0800 h to 2000 h CST for day 1 and day 2, and from 0800 h to 1600 h CST on day 3. Three 8 ml samples from each mylar bag were collected by syringe and transferred to three 7 ml evacuated glass vials and stored for subsequent CH$_4$ concentration analysis in the laboratory (see closed-chamber method).

**Bowen-ratio Energy Balance Flux Calculations**

Evaporation estimated from the BREB technique was calculated as

$$\lambda E = \frac{(R_n - G)}{1 + B}$$

where $\lambda E$ is latent heat flux, $R_n$ is net radiation, $G$ is soil heat flux (all in W m$^{-2}$). Heat storage in the vegetative layer is assumed to be negligible relative to $R_n$, $Le$, $H$ and $G$, particularly so when most of the vegetative layer is submerged in water. $B$ is the Bowen-ratio (dimensionless) calculated as

$$B = \frac{T_1 - T_2}{e_1 - e_2}$$

where $\gamma$ is the psychrometric constant (Pa K$^{-1}$), $T$ is dry bulb air temperature ($^\circ$ C) at two heights and $e$ is the vapor pressure (kPa) at two heights above the surface. Using Reynolds analogy (Reynolds, 1894) and Eq. [5], the eddy diffusivity for methane $k_e$ (m$^2$ s$^{-1}$)
is calculated and used in Eq. [2] with the CH₄ gradient data to compute CH₄ flux rates.

**Closed-Chamber Method**

Twenty-four hours before the start of the experiment, ten 0.15 m diameter and 0.45 m in length open aluminum cylinders were installed near the micrometeorological equipment. The cylinders were pressed 0.15 m below the soil/water interface (surface water), leaving 0.30 m of sampling head space.

Methane flux estimates were obtained 3 times per day over a 3 day period. These measurements were performed by covering each cylinder with a steel cover equipped with a butyl stopper, and collecting headspace samples with a syringe at 0, 30, 60 and 120 minutes. Chamber covers were removed after 120 minutes. Gas sampling was performed by collecting 8 ml of chamber headspace gas using a syringe and transferring the gas sample to 7 ml evacuated vials. All chamber and sampling manipulations were performed from the platform to minimize soil disturbances; however, for four of the chamber incubations CH₄ production kinetics indicated that bubbles had been dislodged during sampling resulting in a large and immediate increase in chamber CH₄ concentration. These data were considered outliers and not used. Gas samples were transported to the lab and analyzed for CH₄. Storage, transport, and analysis protocols for the samples are the same for both the BREB and the chamber methods.

Methane was measured using a Tracor 540 gas chromatograph (Tracor Instruments Austin, Inc., Austin, TX) equipped with a flame ionization detector running at 200 °C, oven temperature at 45 °C, a Porapak Q column and helium carrier gas flowing at the rate of 30 ml min⁻¹. A 0.5 ml sample loop (valve injection) was used to draw a sample from the vials.
Standard curves were constructed using zero umol l\(^{-1}\) (Helium Blank), 0.204 umol l\(^{-1}\) (made from 0.408 umol l\(^{-1}\)), 0.408 umol l\(^{-1}\), 4.08 umol l\(^{-1}\), 40.8 umol l\(^{-1}\), 408 umol l\(^{-1}\) and 4.08 x 10\(^3\) umol l\(^{-1}\) standards (Methane in Helium, Scott Specialty Gases, Troy, MI).

Methane fluxes were obtained by linear regression of the CH\(_4\) concentration vs. time data. In cases where a linear model was used (94% of the chamber fluxes) flux rates were judged significant if the 95% confidence limits of the slope did not include zero. For the remaining 6% of the CH\(_4\) fluxes measurements that did not conform to a linear model, rates were computed using the zero, 30 and 60 min gas samples according to the mathematical procedures described by Hutchinson et al. (1981).

The minimum detectable flux rate was calculated using the following procedure. The variability associated with sampling and analysis was determined by analyzing 25 air samples collected using the same equipment and techniques applied for the collection of the Minnesota samples. Methane concentrations of the 25 samples were averaged and the percent coefficient of variation (% CV) calculated. Percent CV was then used along with the minimum detectable flux curves to estimate the detection limit range of each method.

**RESULTS AND DISCUSSION**

**Closed Chamber Flux Estimates**

One criterion that must be considered in a comparison of Bowen-ratio and closed-chamber methods is the detection sensitivity (the minimum detectable flux, MDF). Detection sensitivity of the closed-chamber method is dependant on the ability to detect changes in CH\(_4\) concentration in the chamber headspace over time. The measured CH\(_4\)
concentration difference between two samples, $t_1$ and $t_2$, must be greater than the sampling and analytical error associated with the CH$_4$ determination technique for a flux to be considered significantly different than 0. A theoretical detection limit range based on this principle was developed (Fig. 1). In construction of this curve we used the criteria that the difference between CH$_4$ concentrations at successive time points (30 min) had to exceed a value of two standard deviations. By computing these minimum detectable concentration differences over a range of possible analytical/sampling errors, and applying the known chamber headspace volume we could compute a minimum detectable CH$_4$ flux rate for the closed-chamber method as a function of analytical/sampling error. From analysis of 25 air samples we determined that the actual variability associated with gas sampling and CH$_4$ analysis was 3.44%. Analysis of precision, evaluated using the 0.204 umol l$^{-1}$ (5 ppmv) and 0.408 umol l$^{-1}$ (10 ppmv) standards, yielded coefficients of variations of 2.53% and 1.17%, respectively. It should be noted that these estimates refer to the precision associated with sampling and analysis, not simply analysis alone. Steele et al. reports a precision of CH$_4$ analysis in the range of 0.2%. Our precision of analysis is 1.7% for standard air, 0.6% for 0.408 umol l$^{-1}$ (10 ppmv) CH$_4$ standard and 0.3% for 4.08 umol l$^{-1}$ (100 ppmv) CH$_4$ standard. As a conservative estimate to calculate the minimal detectable flux, we used the value of 3.44%, thus from Fig. 1 we estimate that our minimum detectable CH$_4$ flux is $9.32 \times 10^{-2}$ mg CH$_4$ m$^{-2}$ h$^{-1}$ (for the detection of both CH$_4$ consumption & production).

Our detection limit is of the same magnitude observed by others for closed chamber methods. Moore and Roulet (1991) reported a detection limit of 0.042 mg CH$_4$ m$^{-2}$ h$^{-1}$ for
static closed chambers. Similarly, Dise (1992) reported a minimum detectable flux of 0.013 mg CH$_4$ m$^{-2}$ d$^{-1}$ for closed chambers.

A linear model adequately fit ($r^2 > 0.96$) the chamber data in 81 of the 86 chamber measurements. For these data, in all cases, the fluxes were significantly different from 0 as indicated by the 95% confidence intervals of the slopes. In the measurements where a linear model was not applicable, the data were observed to be curvilinear (concave). This effect was presumably due to diffusional constraints on CH$_4$ flux into the headspace of the chambers, and flux estimates were determined by the method of Hutchinson and Mosier (1981). Assessment of the significance of the fluxes computed by this latter method was not possible using standard regression analysis so fluxes were determined non zero if they exceed our estimated minimum detectable flux.

Most of the fluxes (98.8%) for individual chambers exceeded the minimum detectable CH$_4$ flux rate during all sample dates and times (Fig. 2). It was observed that all chambers exhibited positive CH$_4$ flux, indicating that CH$_4$ production was the dominant process. Methane fluxes of individual chambers ranged from 0.08 to 27.3 mg CH$_4$ m$^{-2}$ h$^{-1}$ over the 3 day period. Mean CH$_4$ fluxes ranged from 2.29 to 7.18 mg CH$_4$ m$^{-2}$ h$^{-1}$. These values are slightly lower than the range of fluxes (6.29 to 17.21 mg CH$_4$ m$^{-2}$ h$^{-1}$) measured by Crill et al. (1988) at a Marcel Forest bog site in the spring. However, they are similar to the September fluxes of 0.83 to 4.6 mg CH$_4$ m$^{-2}$ h$^{-1}$ observed by Dise (1993).

The pronounced spatial variability associated with chamber measurements is evident. Coefficients of variation associated with mean fluxes at each sample time ranged from 87.3 to 134%. Whalen et al. (1988) reported coefficients of variations of 50 to 100%
in chamber measurements of CH₄ flux within a tundra environment. However, the % CVs observed by Dise (1993) were lower and in the range of 10-82%. The lower spatial variability associated with Dise's chamber-derived CH₄ flux estimates were likely due to a larger chamber design than the ones used in our study.

There was no discernable temporal trend in CH₄ fluxes from the chambers. Dise (1993) reported that periodicity in daily temperatures did not induce diurnal variability in observed CH₄ fluxes. This effect was attributed to the fact that diurnal temperature fluctuations are dampened with depth.

While oftentimes an individual chamber yielded a high CH₄ flux at one sampling time, and a low flux at another time, there appeared to be some consistency in the magnitude of CH₄ fluxes exhibited by some chambers. Ranking of chambers by flux value revealed that chambers 1, 4, and 6 always yielded fluxes in the lower 50th percentile, while chambers 3, 8, and 9 always yielded fluxes in the upper 50th percentile. The magnitude of the temporal variation will be discussed later in comparison of the chamber with the BREB flux estimates.

Bowen-ratio Flux Estimates

Detection sensitivity of the BREB method is dependent upon the precision of the measured CH₄ gradient in the atmosphere above the surface (∂c/∂z). The measured methane concentration difference (∂c) between heights z₁ and z₂ must be greater than the precision of the gas analysis technique for the concentration difference to be considered significantly different from zero. In a manner similar to the closed-chamber, minimum detectable CH₄ concentration differences were computed over a range of theoretical
analytical errors (Fig. 1). These minimum detectable concentration differences, were used in Eq. [2] to calculate the minimum CH₄ fluxes that could be determined by the BREB method. In these calculations the turbulent transfer coefficient \( k_v \) was set at both the measured minimum \((8.87 \times 10^{-3} \text{ m}^2 \text{ s}^{-1})\) and maximum \((0.105 \text{ m}^2 \text{ s}^{-1})\) value observed over the three day period to illustrate the wide range of minimum detectable fluxes using the BREB system. For an analytical precision of 3.44% associated with CH₄ concentration determinations, we calculate that the minimum detectable CH₄ fluxes associated with the BREB to be 2.16 to 25.5 mg CH₄ m⁻² h⁻¹ for the range of \( k_v \) used (for the detection of both consumption & production). These minimum detectable flux values are 23.2 to 274 times higher than the closed-chamber method. The sampling error (3.44%) may have been reduced by installation of the gas chromatograph in the field and coupling it to the BREB method sampling valves. This may have lead to a better BREB method minimum detectable fluxes and hence to the resolution of the BREB method obtained methane fluxes.

It was observed from the field studies that \( k_v \) was not constant, but fluctuated throughout the measurement period during the field study (Fig. 3). Thus, a single minimum detectable flux cannot be established for the BREB method, rather detection limits must be established based on \( k_v \) at each hourly interval when the BREB CH₄ fluxes were estimated.

Hourly CH₄ flux rates over the three day sampling period along with the minimum detectable fluxes are shown in Fig. 4. The average detection limit ranged from a minimum 2.16 mg CH₄ m⁻² h⁻¹ to a maximum of 25.5 mg CH₄ m⁻² h⁻¹ (for the detection of both
consumption & production) over the sampling period. Unlike the chamber method where most of the field fluxes (98.8%) obtained exceeded the minimum detectable flux, with the BREB method only 43.8% of the fluxes observed exceeded the minimum detectable flux.

Detection limits associated with the BREB method sensitivities obtained in this study ranged from 2.16 to 25.5 mg CH$_4$ m$^{-2}$ h$^{-1}$. Hansen et al. (1993) reported net fluxes between -0.004 and -0.02 mg CH$_4$ m$^{-2}$ h$^{-1}$ for their studies on non-flooded agricultural soils. Schutz et al. (1989) reported flux values between -3.6 and 0.1 mg CH$_4$ m$^{-2}$ h$^{-1}$ for dry soils. Other CH$_4$ flux studies on arable land reported fluxes varying between 0 and -0.054 mg CH$_4$ m$^{-2}$ h$^{-1}$ (Goulding et al., 1995; Mosier et al., 1991b; Dobbie et al., 1994; Prieme, 1994).

Despite the poor detection sensitivity of the BREB method (coupled with gas chromatographic analysis of concentration gradients), the CH$_4$ fluxes obtained were of the same magnitude as those obtained with the closed chambers. Mean fluxes of 2.43, 5.09, and 2.92 mg CH$_4$ m$^{-2}$ h$^{-1}$ were observed over the 3 day sampling period. The temporal variability exhibited by the BREB fluxes was high with coefficients of variation ranging from 90.3 to 285%. This high variability is a result of the variability associated with the CH$_4$ gradients, as well as variability associated with the turbulent transfer coefficient ($k_*$). The large variations in $k_*$, coupled with the small negative CH$_4$ gradients observed on day 3 resulted in negative CH$_4$ fluxes during the morning. It is unlikely that these negative fluxes represent actual methane consumption, as the numerous past studies conducted in this study area have all reported positive methane fluxes. These negative fluxes, although large, are at or near the detection sensitivity of the method.
Comparison of fluxes estimated by both methods is presented in Table 1. Mean fluxes obtained by the chamber method were not significantly different (P>0.05) than fluxes obtained from the BREB method on each of the sampling days. The three day averages obtained from the two methods were also very similar.

Whereas mean fluxes compared well, the temporal variability associated with the estimates provided by the two methods differed substantially. Coefficients of variation associated with the chamber methods were low and ranged from 7.76 to 20.5%. In contrast, the BREB method exhibited high variability, and coefficients of variation ranged from 90.3 to 285% over the three day period.

Both techniques have advantages and disadvantages. An advantage of the closed-chamber method is the ability to take a soil sample at the same location the flux measurement was taken, allowing for a more direct correlation between soil characteristic and the observed flux measurement. However, the high degree of spatial variability associated with chamber methods indicates that a large number of chambers must be used in order to obtain an accurate spatial estimate of CH$_4$ flux. The BREB method provides a spatially integrated CH$_4$ flux estimate (Baldocchi et al., 1988). In our study we estimate, based on wind speed, that the BREB flux values represent an upwind source ellipsoid area with a length of approximately 200 meters and a width of approximately 100 meters. Whereas the BREB technique may have advantages in providing spatially integrated estimates, the temporal variability associated with this technique was substantially higher than for the closed chambers.

Based on the above reported flux estimates and our results, we conclude that the
BREB method coupled with gas chromatographic analysis (GC/FID or GC/ECD) may not perform well in determining CH₄ flux from non-flooded agricultural lands, where the microbial CH₄ production/consumption activity is expected to be below the minimum detection sensitivity of the method.

ACKNOWLEDGMENTS

We would like to thank E.S. Verry and his staff from the U.S.D.A., Forest Service in Grand Rapids, Minnesota for their help and allowing us access to the study site.

REFERENCES


Hutchinson G.L., and G.P. Livingston. 1993. Use of chamber system to measure trace gas fluxes. p. 63-78. In D.M. Kral (ed.) Agricultural ecosystem effects on trace gases and global climate change. ASA, Madison, WI.


DISCLAIMER

Trade names are used in this publication to provide specific information, and do not constitute a guarantee or warranty of the product or equipment by the United States Department of Agriculture, nor an endorsement over other similar products.
Table 1. Comparison of CH₄ fluxes determined using closed-chambers and the Bowen-ratio Energy Balance method.

<table>
<thead>
<tr>
<th>Time</th>
<th>Closed Chamber</th>
<th>Bowen Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg CH₄ m⁻² h⁻¹</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0900 h</td>
<td>4.21 (87.3)</td>
<td>1.47</td>
</tr>
<tr>
<td>1250 h</td>
<td>3.08 (102)</td>
<td>0.88</td>
</tr>
<tr>
<td>1700 h</td>
<td>2.92 (101)</td>
<td>4.94</td>
</tr>
<tr>
<td>Mean</td>
<td>3.41 (20.5)</td>
<td>2.43 (90.3)</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800 h</td>
<td>5.46 (109)</td>
<td>2.02</td>
</tr>
<tr>
<td>1200 h</td>
<td>7.18 (134)</td>
<td>1.25</td>
</tr>
<tr>
<td>1600 h</td>
<td>5.01 (97.0)</td>
<td>12.0</td>
</tr>
<tr>
<td>Mean</td>
<td>5.88 (19.4)</td>
<td>5.09 (118)</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0900 h</td>
<td>4.94 (101)</td>
<td>-3.92</td>
</tr>
<tr>
<td>1130 h</td>
<td>4.86 (110)</td>
<td>12.2</td>
</tr>
<tr>
<td>1430 h</td>
<td>5.59 (102)</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean</td>
<td>5.13 (7.76)</td>
<td>2.92 (285)</td>
</tr>
<tr>
<td>3 Day Mean</td>
<td>4.81 (26.4)</td>
<td>3.48 (40.7)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are % Coefficient of variation.
Figure 1. Closed-chamber & Bowen-ratio minimum detectable flux (MDF) vs Analytical variability, Sampling time interval (for closed-chamber) = 30 minutes. Minimum \((8.87 \times 10^{-2} \text{ m}^2 \text{s}^{-1})\) and maximum \((1.05 \times 10^{-1} \text{ m}^2 \text{s}^{-1})\) hourly \(k_v\), of the 3 sampling days was computed and used to estimate MDF for the Bowen-ratio system. The \(\delta z\) was kept constant at 2.29 meters.
Figure 2. Closed-chamber CH₄ field fluxes. The minimum detectable flux (MDF) for the closed-chamber system was 9.32 x 10⁻² mg m⁻² h⁻¹, based on 3.44 % analytical variability. The fluxes of most chambers (98.8%) exceeded the MDF.
Figure 3. Hourly variations in $k_v$, over the study period.
Figure 4. Bowen-ratio CH₄ field fluxes. Hourly CH₄ fluxes were computed using the corresponding kᵥ (Fig. 3). The minimum detectable flux lines were constructed based on 3.44% analytical variability, a δz of 2.29 meters and the corresponding hourly kᵥ values.
EVALUATION OF POTENTIAL INHIBITORS OF
METHANOGENESIS AND METHANE OXIDATION
IN A LANDFILL COVER SOIL

A manuscript submitted to Soil Biology and Biochemistry

A.S.K. Chan and T. B. Parkin

ABSTRACT

Biological methane (CH₄) production is an anaerobic process, while CH₄ consumption occurs predominantly under aerobic conditions; however, both processes can occur simultaneously in soil. Thus, field measurements of CH₄ flux reflect the net result of both consumption and production reactions. Specific inhibitors of either CH₄ consumption or production processes offer the opportunity for independent assessment of the rates for these two processes. Objectives of this work were to identify potential gaseous inhibitors of either CH₄ oxidation or methanogenesis and to evaluate the effect of inhibitor concentration on these two processes. For these studies sieved soil from a municipal landfill cover was treated with a variety of compounds including acetylene (C₂H₂), ethylene (C₂H₄), ethane (C₂H₆), methyl chloride (CH₃Cl) and methyl fluoride (CH₃F). Each experiment consisted of 6 different treatments which included sterile soil, untreated soil and soil with test compound concentrations of 0.00%, 0.01%, 0.1% and 1%. Incubations were conducted under both aerobic and anaerobic conditions. Several compounds completely inhibited CH₄ oxidation while not significantly influencing CH₄ production; including C₂H₂ at 0.001%, C₂H₄ at 0.1%, and CH₃F at 0.1%. One compound (CH₃Cl) was
unique, in that a concentration of 0.1% inhibited methanogenesis by 88.9% but CH₄ oxidation was not significantly affected. We recommend the use of C₂H₂ or C₂H₄ for inhibition of CH₄ oxidation, and CH₃Cl for inhibition of methanogenesis.

INTRODUCTION

Recent concern about global warming has intensified interest in the role of terrestrial systems in controlling atmospheric CH₄ levels. Terrestrial systems can function as net sources or sinks for atmospheric CH₄. Methane flux measured at the soil / atmosphere interface is the net effect of two processes; CH₄ oxidation and methanogenesis (Knowles, 1993). A negative CH₄ flux, i.e. consumption of CH₄ by soil, occurs when the magnitude of the CH₄ uptake process is larger than methanogenesis. This is commonly observed in arable soils, when conditions are predominately aerobic (Schutz et al. 1990; Mosier et al., 1991; Bronson and Mosier, 1993; Hansen et al., 1993). On the other hand a positive CH₄ flux indicates net CH₄ production and is observed when the magnitude of the methanogenic process is larger than CH₄ uptake. This is the case in rice paddies and wetlands (flooded or water saturated areas) which are predominately anaerobic (Schutz et al., 1990; Lauren and Duxbury, 1993). Both soil and wetland systems often support a mixture of anaerobic and aerobic sites, and it is under these conditions that the use of inhibitors are of value in distinguishing CH₄ consumption and production activities.

Inhibitors have been applied to a variety of ecosystems to quantify the role of CH₄ oxidizers in mitigating atmospheric CH₄ increases. Boeckx and Van Cleemput (1997) using CH₃F in field chambers determined that CH₄ oxidation was responsible for
consumption of 34% to 67% of the CH₄ produced in a wetland system. Using CH₃F in investigations of CH₄ cycling in the rhizosphere of Pontederia cordata, Sagittaria lancifolia and Typha latifolia, Lombardi et al. (1997) determined that CH₄ oxidation consumed 22.9% of the CH₄ produced in field experiments and 64.9% of the CH₄ produced in greenhouse experiments. King (1996) used C₂H₂ as an inhibitor of CH₄ oxidation and determined that from 1-58% of the potential CH₄ produced was consumed by CH₄ oxidation associated with burweed (Sparganium eurycarpum) roots. Data from Oremland and Culbertson (1992a) show that CH₄ oxidation in a seasonally exposed sandbar accounted for 75% to 96% of the total CH₄ production. By utilizing the CH₃F technique on plants (Sagittaria lancifolia), Schipper and Reddy (1996) determined that 65% of the total CH₄ produced was oxidized.

Several gaseous compounds have been examined as potential inhibitors of CH₄ oxidation and methanogenesis. Ethylene was observed to inhibit methanogenesis in marine sediments at headspace concentrations of 20% and 5%, but not at 1.25%; however C₂H₆ had no effect on CH₄ production (Oremland and Taylor, 1975). The effects of C₂H₄ or C₂H₆ on CH₄ oxidation have not been evaluated. Historically, C₂H₂ has been used as an inhibitor of both CH₄ oxidation and methanogenesis, depending upon the concentration. Inhibition of methanogenesis has been reported at C₂H₂ concentrations of 0.5% (King, 1996) and at 1.25% (Oremland and Taylor, 1975), while CH₄ oxidation is much more sensitive to C₂H₂ and inhibition has been reported to occur at an C₂H₂ concentration as low as 0.003% (Watanabe et al.,1995).

Methyl fluoride has a similar differential effect on CH₄ oxidation and production.
Oremland and Culbertson (1992b) recommended that in soil, a CH₃F concentration of 0.4% would selectively block CH₄ oxidation without affecting methanogenesis. Similar results have been reported by Schipper and Reddy (1996). They found no significant effect of 1% CH₃F on methanogenesis but CH₄ oxidation was completely inhibited in soil slurries. Also, Epp and Chanton (1993) observed that while CH₄ oxidation in the rhizosphere of aquatic macrophytes was inhibited by 1.5% of CH₃F, methanogenesis was not significantly affected. However, there have been reports of methanogenesis inhibition at lower CH₃F concentrations. Frenzel and Bosse (1996) observed that in CH₄ production in soil slurries was reduced by 75% in the presence of 0.1% CH₃F and 90% inhibition of methanogenesis occurred at 1% CH₃F. Also, >99% inhibition of methanogenesis has been observed at CH₃F concentrations as low as 0.01% in anaerobic sediments (King, 1996).

Whereas inhibitors have been evaluated in materials collected from a wide variety of habitats, none have been performed with land-fill cover soil. Landfills represent major contributors to atmospheric CH₄ (Topp and Pattey, 1977) and the cover soils of landfills have been reported to have high CH₄ oxidation activity (Jones and Nedwell, 1993; Bogner, 1997; Borjesson and Svensson, 1997). Given importance of landfills to the global CH₄ budget, along with the lack of information about inhibitor efficacy for landfill cover soils, our investigations were designed to evaluate 5 gaseous compounds, over a wide concentration range for their inhibitory effects on methanogenesis and CH₄ oxidation associated with a land-fill cover soil. This study includes compounds which have been previously investigated (C₂H₂, C₂H₄, C₂H₆ and CH₃F), as well as an evaluation of the inhibitory effects of CH₂Cl and low concentrations of C₂H₄ and C₂H₆, which have not been
previously been determined.

MATERIALS AND METHODS

2.1. Soil Properties

The soil used in our experiments was topsoil (0-10 cm) collected from a capped city landfill located on Dayton Road, Ames, Iowa. Initial testing indicated that this soil had high CH$_4$ oxidizing activity under aerobic conditions and high methanogenic activity under anaerobic conditions.

Nitrate (+ nitrite) (NO$_3^-$, NO$_2^-$) and ammonium (NH$_4^+$) were determined by colorimetric analyses of 2 M KCl soil extracts (4:1 KCl:soil) on a Lachat autoanalyzer (Lachat Instruments, Mequon, WI.) following the procedure described by Keeney and Nelson (1982). Soil pH was determined on 1:1 (dH$_2$O: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). Microbial biomass carbon (MBC) was measured by fumigation-extraction (Rice et al., 1996). Fifty grams of 5 mm sieved soil was extracted with 100 ml of 0.5 M potassium sulfate K$_2$SO$_4$. Soluble organic carbon (SOC) was measured on a Dohrmann DC-180 carbon analyzer (Rosemount Analytical Services, Santa Clara, CA). Biomass Carbon was calculated using the correction factor ($K = 0.33$) of Sparling and West (1988). Total N and C were determined by combustion on a Carlo Erba Carbon/Nitrogen Analyzer (Fisons Instruments, Rodano, Milano, Italy). Soil texture analyses (sand, silt and clay) were performed by Midwest Laboratories, Inc. (Omaha, NE). General properties of this soil are listed in Table 1.
2.2. Inhibition Experiments

The effects of the five compounds (C₂H₂, C₂H₄, C₆H₆, CH₃Cl and CH₃F) on CH₄ consumption and CH₄ production were evaluated in two different incubation regimes. Aerobic conditions (headspace of approximately 20% O₂) with added CH₄ (initial headspace concentration of 1 to 3%) were used to evaluate compound influences on CH₄ oxidation, while anaerobic conditions (helium (He) atmosphere) with a 10% Hydrogen (H₂), 10% carbon dioxide (CO₂) headspace were established to evaluate compound effects on methanogenesis. For these tests 20 gram portions of sieved (0.5 cm mesh) soil were incubated in 250 mL erlenmeyer flasks. Flasks were sealed with a rubber stopper in which a glass tube accommodating a butyl rubber septum (20mm) (Bellco Glass, Vineland, NJ, USA) was mounted. Compounds were tested at concentrations of 0%, 0.001%, 0.01%, 0.1%, 1%, and in some cases 10% (v/v). Autoclaved soil was included as a sterile control. Each treatment was replicated 3 times. Incubations were performed in the dark at room temperature (21° C) and spanned up to 30 hours for the evaluation of CH₄ consumption and up to 7 days for the evaluation of CH₄ production.

2.3. Gas Chromatography

Methane and the initial test compound concentrations were measured by injecting 0.2 ml of gas sample from the headspace of each flask into a Tracor 540 gas chromatograph (Tracor Instruments Austin, Inc., Austin, TX) equipped with a flame ionization detector (FID) running at 200 °C, a Porapak Q column (Alltech Associates, Inc., Deerfield, IL, USA) and He carrier gas flowing at the rate of 30 mL min⁻¹. Certified
standards of CH₄, C₂H₂, C₂H₄, and C₃H₆ were obtained from Scott Speciality Gases, Troy, MI, USA. Methyl chloride and CH₃F were obtained from Matheson Gas Products, Montgomeryville, PA.

2.4. Rate Determinations

An example of the response of these processes to increasing concentrations of CH₃F is shown in Figure 1. Methane consumption appeared to be a first order process (Fig. 1A), but increased concentrations of CH₃F reduced CH₄ consumption. Rate coefficients for CH₄ consumption were estimated by applying linear regression to the natural log of the concentration vs time data (slope = -k, Paul and Clark, 1996). Methane production rate increased with time but was not well described by first order kinetics (Fig. 1B). The impact of inhibitors on this process was evaluated by computing the maximum rate of CH₄ production. This analysis was done by fitting the CH₄ concentration vs. time data with a curve and evaluating the first derivative of the fit equation using the Table Curve software package (SPSS Inc., Chicago, IL).

2.5. Statistical Analysis

Results for each inhibitor tested are presented in terms of a mean and a confidence limit. Statistical differences between treatments and the control were determined by Tukey’s test (Sigma Stat software package, SPSS Inc., Chicago, IL). Effects within test concentrations can be determined by observation of the overlap of 97.5% confidence limits (Birnbaum 1961; Natrela, 1963; Parkin, 1993).
RESULTS

Methane oxidation was inhibited by all of the test compounds, but inhibitor effectiveness differed (Fig. 2). Acetylene had a strong inhibitory effect on CH$_4$ oxidation over the entire concentration range (Fig. 2A). At a C$_2$H$_2$ concentration of 0.001% the first order rate constant dropped 4.2 x 10$^{-2}$ min$^{-1}$, corresponding to an average of 93.5% inhibition and at higher C$_2$H$_2$ concentrations CH$_4$ oxidation was 100% inhibited. Methane oxidation was less sensitive to C$_2$H$_4$ (Fig. 2B). At 0.001% C$_2$H$_4$, CH$_4$ oxidation was unaffected, but partial inhibition (33%) was observed at 0.01% C$_2$H$_2$, with near complete inhibition (96%) occurring at concentrations of 1%. Neither C$_2$H$_6$ nor CH$_3$Cl (Fig. 2C, 2D) were effective inhibitors of CH$_4$ oxidation at concentrations less than 0.1%; however at a concentration of 1%, a 53% and 78% inhibition was observed for C$_2$H$_6$ and CH$_3$Cl respectively. Methyl chloride was completely inhibitory at a concentration of 10%.

Increased concentrations of CH$_3$F progressively inhibited CH$_4$ oxidation (Fig. 2E), and concentrations at and exceeding 0.1% complete inhibition was observed.

The effects of the test compounds on methanogenesis are presented in Fig. 3. Acetylene only slightly inhibited CH$_4$ production at 0.001%, but with increasing concentration greater inhibition was observed (Fig. 3A). Ethylene was ineffective at inhibiting CH$_4$ production at low concentrations, but at 1% significant inhibition was observed. In contrast, C$_2$H$_6$ showed essentially no effect on CH$_4$ production activity (Fig. 3C). Methyl chloride, on the other hand was completely inhibitory at concentrations of 1% and 10%, and partial inhibition (89%) was observed at 0.1% (Fig. 3D). Methyl fluoride exhibited slight inhibition (39%) at 1% and inhibition was nearly complete (93%) at 10%.
A comparison of the effects of the inhibitors on both processes is presented in Fig. 4., where CH₄ consumption and production activity are expressed as a percentage of the respective rates with no inhibitor added. Acetylene, at a concentration of 0.001% did not significantly reduce CH₄ production activity, but did inhibit CH₄ oxidation (Fig. 4A). Methane oxidation at this 0.001% C₂H₂ concentration was reduced to 6.6% of the control. At higher C₂H₂ concentrations both CH₄ oxidation and methanogenesis were significantly lower than the control (no inhibitor). There was no significant effect of C₂H₄ on CH₄ production at concentrations less than 1%, but 89% inhibition of methanogenesis was observed at 1% C₂H₄ (Fig. 4B). Methane oxidation was more sensitive to C₂H₄ than methanogenesis and a C₂H₄ concentration of 0.1% and greater resulted in 90% inhibition. Methanogenesis was unaffected by C₂H₄ over the range of concentrations tested (Fig. 4C). Methane oxidation was only partially inhibited (35% and greater) by C₂H₄ at higher concentrations (> 0.1%).

The pattern of inhibition produced by CH₃Cl was unlike those of the other inhibitors tested, in that methanogenesis was more sensitive than CH₄ oxidation (Fig 1D). Methanogenesis was impacted by CH₃Cl and at concentrations exceeding 0.01%, and at a CH₃Cl concentration of 0.1% methanogenesis was inhibited by 89%. Methane oxidation was not significantly affected by less than 0.1% CH₃Cl, but concentrations of CH₃Cl greater than1% did inhibit CH₄ oxidation. Methanogenesis was unaffected at CH₃F concentrations at and below 0.1% (Fig. 4E). Partial inhibition of methanogenesis (38.8%) was observed at 1% CH₃F and 92.6% inhibition at 10% CH₃F. Methane oxidation was
partially inhibited (39.5%) by CH$_3$F at 0.01% and completely inhibited by CH$_3$F concentrations > 0.1%.

**DISCUSSION**

Inhibitors present potentially powerful tools in the study of microbial processes; however, they must be used judiciously due to potential nonspecific effects or unintended effects. As pointed out by Oremland and Capone (1988), "...employing inhibitors in ecological / biogeochemical studies must be tempered by a knowledge of their limitations."

This caution is underscored by the variability in the literature concerning inhibitory effects of C$_2$H$_2$ and CH$_3$F on CH$_4$ oxidation and production in the wide range of systems they have been applied.

Generally, there is consistency in reports regarding the inhibitory concentrations of CH$_3$F required to inhibit CH$_4$ oxidation. Data from Oremland and Culbertson (1992b) indicate complete inhibition of CH$_4$ oxidation by soil at CH$_3$F concentrations of 0.3% and 1.7% over a 150 h period. Decreasing CH$_3$F concentrations resulted in a decrease in the time inhibition was sustained, with CH$_3$F concentrations of 0.16% and 0.06% resulting in nearly complete inhibition of CH$_4$ oxidation for 100 and 30 hr, respectively. In short term incubations (approximately 30 hr) of *S. eurycarpum* roots, King (1996) observed nearly complete inhibition of CH$_4$ oxidation (86% to 96%) over a CH$_3$F concentration range of 0.01% to 1.0%. Our aerobic incubations of landfill topsoil yielded results similar to these past studies. Over a 30 h incubation we observed a 39% inhibition of CH$_4$ oxidation at a CH$_3$F concentration of 0.01%, and complete inhibition at 0.1% CH$_3$F.
The effects of CH$_3$F of methanogenesis are less consistent. Oremland and Culbertson (1992a) reported a 36% inhibition of methogenesis in anoxic salt marsh sediments incubated with 1.25% CH$_3$F, but observed no inhibition of methanogenesis at 0.4% CH$_3$F in anaerobic incubations of composted paper waste. Epp and Chanton (1993) found that in the rhizosphere of aquatic macrophytes a CH$_3$F concentration of 1.5% was sufficient to inhibit CH$_4$ oxidation, but did not affect methanogenesis. However, King (1996) observed that methogenesis was affected by much lower CH$_3$F concentrations. He reported that methane production in anaerobic incubations of peat material was inhibited by 89% and 96% at CH$_3$F concentrations of 0.01% and 0.1%, respectively and suggested that a possible reason for the higher sensitivity of methanogenesis to CH$_3$F may be due to differences in sediment sources (marine vs freshwater). Recently, Frenzel and Bosse (1996) observed that methanogenesis in different systems exhibited a differential sensitivity to CH$_3$F. In the rhizosphere of cottontail (Typha latifolia) these workers found that methanogenesis was not affected by CH$_3$F up to concentrations of 1.0%. Methane production in a hypersaline microbial mat was also found to be insensitive to CH$_3$F concentrations up to 4%, but in anoxic rice field soil low CH$_3$F concentrations (0.1%) inhibited methanogenesis by 75%. The differential effects of CH$_3$F on methanogenesis were thought to be related to the characteristics of the methanogenic populations, with acetoclastic methanogens showing a greater sensitivity to CH$_3$F than organisms using methylamine or formate (Frenzel and Bosse, 1996). Evidence supporting this effect is provided by Janssen and Frenzel (1997) who observed that the growth of acetoclastic methanogens was inhibited by lower CH$_3$F concentrations than growth of methanogens
using H$_2$ or formate. Our results are consistent with past observations in that for anaerobic incubations of soil with added H$_2$ and CO$_2$, methanogenesis was not impacted by CH$_3$F concentrations of $<0.1\%$, but at high CH$_3$F levels ($>1.0\%$) CH$_4$ production was inhibited.

Unlike CH$_3$F, there is more agreement in the literature concerning the inhibitory effects of C$_2$H$_2$ on CH$_4$ oxidation and production. Early work indicated that C$_2$H$_2$ at high concentrations ($>1.25\%$) strongly inhibit methogenesis (Oremland and Taylor, 1975; Sprott et al., 1982). Recently, CH$_4$ production has also been reported to be sensitive to much lower C$_2$H$_2$ concentrations. King (1996) observed that 0.01% C$_2$H$_2$ inhibited methanogenesis associated with peat by $>60\%$. Also, data of Watanabe et al. (1997) indicate that in 7 d incubations of soil amended with glucose, C$_2$H$_2$ at a concentration of 0.001% only had a slight effect on methanogenesis, but that 0.01% C$_2$H$_2$ resulted in 60% inhibition. Our observations of 80% inhibition of methanogenesis at a 0.01% C$_2$H$_2$, but no significant effect at 0.001% C$_2$H$_2$ are consistent with these recent studies.

Acetylene also inhibits CH$_4$ oxidation, but most past studies only have examined C$_2$H$_2$ concentrations exceeding 0.1% (Yavitt et al., 1990; Bender and Conrad, 1995; McDonald et al., 1996; Miller et al., 1998). For C$_2$H$_2$ to be useful in distinguishing between gross and net rates of methane production, it must inhibit CH$_4$ oxidation at a concentration of $<0.01\%$ because methanogenesis is also sensitive to 0.01% C$_2$H$_2$. King (1996) reported a 89% inhibition of CH$_4$ oxidation associated with excised *S. eurycarpum* roots at 0.01% C$_2$H$_2$. An evaluation of lower C$_2$H$_2$ concentrations on CH$_4$ oxidation have only been presented by Wantanabe et al. (1995). In laboratory incubations of rice field soils, these investigators observed 90% inhibition of CH$_4$ oxidation at a C$_2$H$_2$ concentration
The effects of C₂H₄ on CH₄ cycling reactions are largely unknown. Oremland and Taylor (1975) found nearly complete inhibition of methanogenesis by 5% C₂H₄, but there have been no reports of C₂H₄ effects on CH₄ oxidation. Our results not only confirm that high C₂H₄ concentrations inhibit methanogenesis, but also that CH₄ oxidation is nearly completely inhibited (90%) at C₂H₄ concentrations exceeding 0.1%. Despite the fact that C₂H₄ is produced in soils by microorganisms (Arshad and Frankenberger, 1989; Arshad and Frankenberger, 1990) it is doubtful that this process significantly impacts CH₄ oxidation in nature. In anaerobic incubations of forest soils, Sexstone and Mains (1990) measured C₂H₄ production rates of 205 to 71.8 nM C₂H₄-C kg soil⁻¹ h⁻¹. Assuming a bulk density of 1.0 g cm⁻³ and an air filled porosity of 50%, we calculate that these rates of production would result in soil atmosphere C₂H₄ concentrations of only 0.0005% and 0.00017% over a 1 hour period (assuming no loss through diffusion). Based on our results these concentrations are too low to impact either CH₄ consumption or production.

There is little information on the other compounds we examined. Our observations that methanogenesis is not significantly affected by C₂H₄ at concentrations up to 1% are similar to results of Oremland and Taylor (1975) who reported no inhibitory effect of C₂H₄ on CH₄ production at concentrations of 1.25% and 20% using marine sediments. Methyl chloride, as far as we know, has never been used to study its effects on CH₄ oxidation or methanogenesis; however our results indicate that at CH₃Cl is distinctly different than the other compounds of our study, in that methanogenesis was more sensitive than CH₄ oxidation. Methanogenesis was significantly inhibited (89%) by 0.1% CH₃Cl, but CH₄
oxidation was not affected by concentrations < 0.1%.

Recommendations of inhibitor concentrations which may be useful for distinguishing between CH₄ oxidation and CH₄ production are summarized in Table 2. Acetylene at a concentration of 0.001% inhibited CH₄ oxidation by 93.5% but did not significantly impact CH₄ production. Ethylene at a higher concentration (0.1%) had a similar effect. Methyl chloride is unique, in that at a concentration of 0.1% methanogenesis was inhibited by 89%, but CH₄ oxidation was not significantly affected. Finally, CH₃F at a concentration of 0.1% showed complete inhibition of CH₄ oxidation but did not significantly influence CH₄ production. Of the compounds we investigated, C₂H₂, C₂H₄ and CH₃Cl show the greatest application for use in CH₄ cycling studies. Despite its widespread use, CH₃F should be used with caution, because of the reported differential response of methanogenic bacteria to CH₃F (Frenzel and Bosse, 1996; Janssen and Frenzel, 1997).

Finally, it is realized that extrapolation of our results to other soils may be tenuous; however, for many of the compounds tested, similar efficacious concentrations have been reported over a wide range of habitats, including mineral and organic soils, sediments, and plant systems. Also, the landfill soil used in our study had the highest CH₄ oxidation and production activity of any soil we have examined, and we speculate that the inhibitor concentrations we report (with the exception of CH₃F) may be at least as effective in other soils. However, a prudent course of action would be to evaluate the precise inhibitor concentration required to achieve the desired degree of inhibition in each specific system of interest.
REFERENCES


DISCLAIMER

Trade names are used in this publication to provide specific information, and do not constitute a guarantee or warranty of the product or equipment by the United States Department of Agriculture, nor an endorsement over other similar products.
Table 1. Properties of Landfill Soil used in inhibitor evaluation studies.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>CoV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.71</td>
<td>0.79</td>
</tr>
<tr>
<td>NO$_3^-$ (ug N g$^{-1}$ dry soil)</td>
<td>3.9</td>
<td>1.34</td>
</tr>
<tr>
<td>NH$_4^+$ (ug N g$^{-1}$ dry soil)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>% Moisture</td>
<td>54</td>
<td>0.82</td>
</tr>
<tr>
<td>Microbial Biomass (ug C g$^{-1}$ dry soil)</td>
<td>596</td>
<td>12.63</td>
</tr>
<tr>
<td>Soluble C (ug C g$^{-1}$ dry soil)</td>
<td>31</td>
<td>7.09</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.15</td>
<td>3.94</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>1.92</td>
<td>1.04</td>
</tr>
<tr>
<td>Soil Texture</td>
<td>57% Sand</td>
<td>25% Silt</td>
</tr>
</tbody>
</table>

Values in brackets are % Coefficients of Variation of 3 measurements

Table 2. Recommended compound concentrations to selectively inhibit either methanogenesis or methane oxidation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>CH$_4$ production</th>
<th>CH$_4$ oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylene</td>
<td>0.001</td>
<td>8.8</td>
<td>93.5</td>
</tr>
<tr>
<td>Ethylene</td>
<td>0.1</td>
<td>17.7</td>
<td>90</td>
</tr>
<tr>
<td>Methyl chloride</td>
<td>0.1</td>
<td>88.9</td>
<td>11.2</td>
</tr>
<tr>
<td>Methyl fluoride</td>
<td>0.1</td>
<td>7.7</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 1. Effect of headspace methyl fluoride concentration on methane consumption (A) and methane production (B). Each line and symbol type represent different methyl fluoride concentrations from 0 to 10%.
Figure 2. Effects of different concentrations of inhibitors on methane oxidation first order rate constant. Error bars are the 97.5% confidence limits of the mean of 3 replicates. In cases where only 2 replications are available the distance between the error bars are actual ranges of the individual rate constants. Asterisks indicate that the treatment is statistically significant ($P < 0.05$) from the control (no inhibitor). Ten percent (10%) concentration was not tested for acetylene, ethylene and ethane.
Figure 3. Effects of different concentrations of inhibitors on methane production maximum rate. Error bars are the 97.5% confidence limits of the mean of three replicates. In cases where only 2 replications are available the distance between the error bars are actual ranges of the individual rates. Asterisks indicate that the treatment is statistically significant (P<0.05) from the control (no inhibitor). Ten percent (10%) concentration was not tested for acetylene, ethylene and ethane.
Figure 4. Effects of different concentrations of inhibitors on methane oxidation and methane production activity. Black bars indicate the mean methane oxidation activity. Grey bars indicate the mean methanogenic activity. Error bars are the 97.5% confidence limits (n=3). In cases where only 2 reps were available error bars indicate the range. Asterisks indicate that the treatment is statistically significant (P<0.05) from the control (no inhibitor). Ten percent (10%) concentration was not tested for acetylene, ethylene and ethane.
A COMPARISON OF METHANE FLUXES FROM NATURAL AND AGRICULTURAL ECOSYSTEMS

A manuscript to be submitted to the Journal of Environmental Quality

A.S.K. Chan and T.B. Parkin

ABSTRACT

The role of natural and agricultural ecosystems in terms of their methane (CH₄) contributions to the atmosphere is uncertain. This study was conducted to quantify and compare CH₄ fluxes from a variety of ecosystems in central Iowa. We investigated agricultural systems under different agricultural management practices, a hardwood forest site, native and restored prairies, and a municipal landfill. Flux measurements were obtained using a closed-chamber method, and measurements were compiled by sampling over the 1993 and 1994 growing seasons. In 1993 most of the agricultural sites were net CH₄ producers with mean CH₄ fluxes ranging from -0.02 g m⁻² to 3.19 g m⁻² over the 258 day sampling season, while the natural ecosystems were net CH₄ consumers with mean seasonal flux rates ranging from -0.27 to -0.07 g m⁻² 258 d⁻¹. In 1994 only the landfill and the agricultural site treated with broadcast swine manure were net CH₄ producers while the remainder of the natural and agricultural ecosystems were net CH₄ consumers with mean seasonal flux rates ranging from -0.43 to -0.008 g m⁻² 271 d⁻¹. We hypothesize that the differences in CH₄ fluxes between the two years are due to differences in rainfall. Also, at the agricultural sites we observed no reduction of CH₄ consumption activity as a result of ammonium (NH₄⁺) application. Rather, there appeared to be a stimulation of CH₄
consumption in response to fertilization. It is suspected that out-gassing of CH₄ dissolved in the manure slurry was the underlying mechanism for the net positive fluxes observed at the manure treated sites. Area weighted CH₄ fluxes were calculated for each ecosystem type and cumulative seasonal net fluxes for the state of Iowa were estimated. For the state of Iowa our calculations yielded net positive fluxes of 36748 Mg CH₄ and 240278 Mg CH₄ for 1993 and 1994. These estimates were dominated by a hydric agricultural site in 1993 and by the Landfill site in 1994.

**INTRODUCTION**

Methane (CH₄) is a potent greenhouse gas with a relative global warming potential 21 times (over a time horizon of 100 years) that of carbon dioxide (IPCC, 1996). Estimates from the Intergovernmental Panel on Climate Change indicate that CH₄ contributes about 20% to the radiative force driving global climate change (IPCC, 1996). The concentration of CH₄ has increased by 7% between 1983 and 1993 (IPCC, 1995). The growth rate declined in the 1980s and dramatically dropped in 1991 and 1992 (IPCC, 1995). However, recent data (NOAA, The Climate Monitoring and Diagnostics Laboratory, Carbon Cycle Greenhouse Gas Group, Boulder, CO.) suggest that the growth rate may be on the rise again. This increase in the atmospheric concentration coupled with the potency of CH₄ may have a major potential impact on future global warming.

Critical to the development of strategies to offset this trend in climate changes, is the assessment of the role of terrestrial systems in the production and consumption of atmospheric CH₄. It is generally recognized that flooded systems such as rice paddies, bog
lands, and other natural wetlands are large contributors to the atmospheric CH₄ pool (Stewart et al., 1989; Bouwman, 1990; Amstel and Swart, 1994; Topp and Pattey, 1997). Non-flooded terrestrial systems on the other hand are more complex and, depending upon the land use coupled with local conditions, may be net producers or consumers of CH₄ (Bronson and Mosier, 1993; Goulding et al., 1996; McDonald et al., 1996). Natural forest and grassland systems have been widely studied and are generally considered to be net consumers of CH₄ (Steudler et al., 1989; Mosier et al., 1991; Castro et al., 1993; Castro et al., 1994a; Lessard et al., 1994; Ambus and Christensen, 1995; Castro et al., 1995; Mosier et al., 1996; Mosier et al., 1997; Prieme and Christensen, 1997a).

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 (Steudler et al., 1989; Mosier et al., 1991; ). Cultivation also appears to decrease net CH₄ consumption, as studies have shown that net CH₄ consumption in cultivated grasslands is less than in native grasslands (Mosier et al, 1991; Mosier et al.1996). Kessavalou et al. (1998a) examined CH₄ fluxes in grass sod and winter wheat-fallow management systems over a 2 ½ year period. Measurement of CH₄ flux from sod, no-till wheat, a subtill treatment, and a plow treatment indicated that only consumption was observed. Average annual CH₄ uptake was higher under sod and generally consumption decreased with increasing disturbance. Methane flux determinations in wheat and corn cropping systems of Eastern Colorado indicated that net CH₄ consumption was greatest in dryland wheat, and that consumption activity decreases under irrigated wheat, and irrigated corn (Bronson
Current information is enabling the development of generalizations concerning the role of land use on the magnitude of CH₄ flux, but clearly more information is needed on specific systems and especially agricultural systems to refine our estimates of CH₄ flux from terrestrial systems. The objective of our field study was to measure CH₄ flux from a variety of agricultural and non-agricultural systems and relate these measurements to land management and climate.

**MATERIALS AND METHODS**

**Sites**

The sites we investigated represent a range of ecosystems found in the upper Midwestern United States. This study concentrated on agricultural sites under a variety of tillage and fertility regimes, including chisel plow and no-till, inorganic N fertilization and fertilization with swine manure. The natural systems investigated included a hardwood forest site, and native and restored prairie sites. For comparative purposes, the CH₄ flux from a municipal landfill was also measured. All sites were located in central Iowa. The site locations and other general site characteristics are given in Table 1.

**Weather Data**

Weather data from central Iowa weather stations were obtained from The University of Nebraska’s High Plains Climate Center (Lincoln, NE). Stations include: Ames 5 SE, Story County, 42° 0' N latitude and 93° 36' W longitude, Precipitation; Ames 8 WSW, Boone County, 42° 1' N latitude and 93° 46' W longitude, Precipitation and
Temperature; Colo, Story County, 42° 1' N latitude and 93° 19' W longitude. Precipitation and Temperature; Zearing, Story County, 42° 10' N latitude and 93° 18' W longitude. Precipitation. Data from all weather stations expressed similar trends, thus only data from the Ames 8 WSW station is reported.

Soil Properties

Soil texture analyses were performed by Midwest Laboratories, Inc. (Omaha, NE). Nitrate (+ nitrite) (NO$_3^-$, NO$_2^-$) and ammonium (NH$_4^+$) were determined by colorometric analyses of 2 M KCl soil extracts (4:1 KCl:soil) on a Lachat autoanalyzer (Lachat Instruments, Mequon, WI.) following the procedure described by Keeney and Nelson (1982). Soil pH was determined on 1:1 (dH$_2$O: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). Microbial biomass carbon (MBC) measurements were determined by fumigation-extraction (Rice et al., 1996). Fifty grams of 5 mm sieved soil was extracted with 100 ml of 0.5 M potassium sulfate K$_2$SO$_4$. Soluble organic carbon (SOC) was measured on a Dohrmann DC-180 carbon analyzer (Rosemount Analytical Services, Santa Clara, CA). Biomass carbon was calculated using the correction factor ($K = 0.33$) of Sparling and West (1988). Soil aggregation, defined as the percent of water stable aggregates > 0.25 mm, was determined by the wet sieving method of Yoder (1936), as modified by Eash et al., (1994). Total N and C were determined by combustion on a Carlo Erba Carbon/Nitrogen Analyzer (Fisons Instruments, Rodano, Milano, Italy). The general soil properties of each site are shown in Table 2 and 3.
Methane Flux Measurements

Methane fluxes were determined using a closed-chamber method. Two types of chamber configurations were used. At some sites (Kluver in 1993 and Nashua) temporary chambers (15 cm diameter x 8 cm long) inserted to a depth of 3.5 cm, were installed each time flux measurements were performed. These chambers were installed 30 min before the measurements were started, and were left open to the atmosphere up until the time sampling was started. At all of the other sites and times permanent chambers were used. These chambers consisted of aluminum cylinders (15 cm diameter x 15 cm long) inserted into the ground to a depth of 7.5 cm. Permanent chambers were installed at least 24 hours before the first sampling of the season and remained in position during the remainder of season. Four to 21 chambers were installed at each site. Flux measurements were made during the 1993 and 1994 growing seasons generally on a bi-monthly interval but in some instances only 1 measurement per month was possible. Methane fluxes were determined by closing the chambers off from the atmosphere at time 0. For the permanent chambers this was done by installing a steel cover, equipped with a 20 mm butyl rubber stopper, on top of the cylinder. For the temporary chambers, a 20 mm butyl rubber stopper septum was inserted into the vent hole. For both types of chambers, gas samples were taken generally at 0, 10, and 20 minutes (some sites, 0, 20 & 40 min. or 0, 30 & 60 min.). Samples were collected by withdrawing 8 ml of air from the chamber headspace with a 25 gauge needle and a 20 cc syringe at each time point. Gas samples were transferred to evacuated 7 ml vials fit with 20 mm butyl rubber stoppers and were transported back to the lab for CH₄ analysis.
Methane Analysis

Methane was measured using a Tracor 540 gas chromatograph (Tracor Instruments Austin, Inc., Austin, TX) equipped with a flame ionization detector as described by Chan et al. (1998). The variability associated with our sample collection and CH₄ analysis at CH₄ concentrations at or near ambient levels ranged from 2.5 to 3.5% (coefficient of variation) (Chan et al., 1998)

Methane Flux Calculations

Hourly CH₄ fluxes (positive and negative) were obtained by applying linear regression to the CH₄ concentration versus time data. Fluxes were taken as the slope of the line if the linear regression was significant at the 10% level. In some cases the data exhibited curvilinear trends with time. For these data the mathematical procedures described by Hutchinson and Mosier (1981) were used. The minimum detectable flux rate was calculated following the procedures described by Chan et al. (1998), and the minimum CH₄ flux rates detectable in this study ranged from 12.2 to 61.0 µg CH₄ m⁻² h⁻¹ depending on the chamber size and the deployment time. Some of the fluxes measured from the individual chambers were smaller than our detection limit. In these situations, we followed the recommendation of Gilbert (1987) and included the measured values of these “non-detects” in computing mean fluxes. Seasonal fluxes were estimated for the period of time when the mean daily temperature exceeded 0 °C. We have no winter measurements and were reluctant to extrapolate our rates over the entire year due to recent reports of significant CH₄ fluxes at low temperatures (Kessavalou et al. 1998a; Mast et al. 1998; Wickland et al. 1999). Seasonal fluxes for each chamber were determined by integrating
the hourly flux measurements using the trapezoid rule. These values were divided by the number of days within the sampling period and then multiplied by the number of days when the mean daily temperature exceeded 0 °C. A mean seasonal flux for each site, and an associated variance was computed from the seasonal estimates of each chamber. These seasonal estimates extend over a period of 258 days in 1993 and 271 days in 1994.

The regional net CH₄ flux inventory for Iowa was estimated by the general procedure of Livingston and Hutchinson (1995). To accomplish this, we grouped our sites into six different strata based on land use similarities and available land use statistics. The six strata are: 1) Forested Land, 2) Prairie, Grassland, Pasture and Range, 3) Agricultural - corn/soybean rotation, plow till, chemical fertilizer, 4) Agricultural - corn/soybean rotation, plow till, manure fertility, 5) Agricultural - corn/soybean rotation, no-till, and 6) Landfill. The mean seasonal CH₄ flux rate for each stratum was calculated by averaging the seasonal flux rates of the sites included in that stratum. The within stratum variance was also calculated. In cases where a stratum is represented by only a single site, the average and variance associated with the seasonal estimate of that stratum is taken to be the site mean and variance. The seasonal net CH₄ flux inventory for the region of interest, in this case, the state of Iowa was calculated by multiplying the seasonal fluxes for each strata by the area of land represented by each strata in the state.
RESULTS AND DISCUSSION

Agricultural Ecosystems

Methane flux from agricultural systems under a variety of cropping, tillage, and fertility management were measured. Cultivated sites under a corn/soybean rotation showed little consistency (Fig 1). At the Walnut Creek hilltop site most measurements were below the detection limit of our analysis, with a few occasional high CH$_4$ flux measurements. Similarly, at the Doolittle farm site, at most sampling times, rates were low; however on July 28, 1993 all the chambers exhibited positive CH$_4$ fluxes, with a mean hourly CH$_4$ flux of 1990 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$. The mean hourly fluxes observed at Doolittle farm on sampling dates immediately before and after the July 28 date were significantly lower (P≤0.05). It is thought that these high rates resulted from the intense rainfall which occurred during July, 1993 (Fig. 2). Rates at the Roadside farm (Fig. 1) showed a slightly different pattern. Throughout the season, many negative fluxes were observed. Mean hourly fluxes ranged from -45.4 to 16.4 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$ for 1993. The large positive fluxes in response to the July 1993 precipitation were not as evident at the Roadside farm site, although there appears to be a trend of increased CH$_4$ flux during the late summer and fall of 1993. We cannot precisely determine why Roadside farm site responded differently from the other two cultivated sites, but in 1993 the Walnut Creek Hilltop site and the Doolittle farm site were cropped to soybean, while the Roadside farm site was cropped to corn. We speculate that the cause of the lower the CH$_4$ fluxes at the Roadside farm is due to the N fertilizer treatment (anhydrous ammonia). It is thought that this fertilizer addition may have had a positive effect on the nitrifying bacteria population, specifically the
ammonia oxidizing bacteria. A key enzyme component of the ammonia oxidizing bacteria is the ammonia monooxygenase which oxidizes NH₃ to NH₂OH and H₂O. Methane is an alternative substrate for ammonia monooxygenase and ammonia oxidizing bacteria can also consume CH₄ (Suzuki et al., 1976; Hyman and Wood, 1983; Jones and Morita, 1983; Ward, 1987). In both field and laboratory studies, Goldman et al. (1995) found a positive correlation between CH₄ consumption and soil NH₄⁺.

Methane flux from the no-till site showed a mixed pattern (Fig. 3). On each of the sampling dates some chambers indicated a positive CH₄ flux, while others exhibited a negative CH₄ flux. Unlike the sites shown in Fig. 1 where the chambers were placed in relatively close proximity to one another (approximately 1 meter), at the no-till site the 21 chambers were placed along an east-west transect at 10 meter spacings. This transect traversed low and high areas of the field. Chambers at the east end of the field (high area) tended to exhibit negative fluxes, while those at the west end (low area) of the field exhibited positive fluxes (Fig 4A). This pattern reflected the spatial distribution of soil water content across the field (Fig. 4B). Past work on the influence of tillage on CH₄ flux indicates that no tillage systems may be more active in consuming atmospheric CH₄ than are plowed systems (Kessavalou et al., 1998a; Hutsch, 1998). Our observations, that the wet locations within the no-till field were net CH₄ producers while the drier locations were weak net CH₄ consumers, indicate that this generalization may not always be true.

At the Kluver site several fertility management practices were evaluated (Fig. 5). Chambers from the control plots (no N fertilizer) exhibited both positive and negative fluxes, with mean hourly rates ranging from -3.4 to 9.6 μg CH₄ m⁻² h⁻¹ in 1993 and -18.3 to
15.0 µg CH₄ m⁻² h⁻¹ in 1994 (Fig 5A). Fewer positive CH₄ fluxes were observed in the urea ammonium nitrate fertilizer treatment (Fig 5B) and mean hourly fluxes ranged from -23.2 to 5.7 µg CH₄ m⁻² h⁻¹ in 1993 and -46.1 to 9.7 µg CH₄ m⁻² h⁻¹ in 1994. It is also interesting to note that a decrease in the mean hourly CH₄ flux was observed immediately following UAN application. The general decrease of observed positive fluxes and mean CH₄ fluxes in the urea ammonium nitrate fertilized treatment within the Kluver N fertilized system for both years may be attributed to our previous speculations concerning the stimulatory effect of NH₄⁺ on nitrifying bacteria populations and the subsequent increased metabolism.

Application of liquid swine manure to agricultural land resulted in increased fluxes of CH₄ (Fig. 5C and 5D). Large fluxes were detected immediately following the 1993 manure application in both the broadcast and injected treatments; however, a greater number of larger fluxes were noted in the injected treatment. Mean CH₄ flux rate after manure application within the injected treatment was significantly different (P ≤ 0.001) than the mean CH₄ flux rate of the previous sampling before the manure application. It is thought that the large initial flush of CH₄ was due to out-gassing of dissolved CH₄ in the manure slurry rather than an immediate increase in in situ CH₄ production.

To determine if it is even possible that out-gassing of CH₄ can account for the CH₄ flux rates observed over the injection band we estimated the mass of CH₄ dissolved in the manure slurry. We assumed that the manure was saturated with CH₄ and used a CH₄ absorption coefficient of 0.033 L CH₄ L⁻¹ H₂O. The swine manure at the site was knifed injected at 76.5 cm row spacing at a rate of 37400 L manure ha⁻¹. The flux chamber placed directly over the injection band covered the equivalent of 0.43 L of manure. If all the CH₄
dissolved in this volume of manure out-gassed during the 60 min cover period, the resulting maximum flux rate would be $5.2 \times 10^3 \mu g \text{ CH}_4 \text{ m}^{-2} \text{ h}^{-1}$. This maximum potential rate far exceeds our measured values, indicating that the out-gassing may be a feasible mechanism. We realize that this is a maximum potential since salt effects may reduce the amount of dissolved CH$_4$ and it is unlikely that all dissolved CH$_4$ out-gassed during the 60 minutes. A similar out-gassing effect was observed in laboratory studies of soils by Chadwick and Pain (1997). These researchers determined that the primary source of CH$_4$ from soils treated with animal manure was from the manures themselves and not from the soil.

Fluxes in the broadcast manure treatment remained low throughout the 1993 season and mean hourly fluxes ranged from -6.3 to 11.6 $\mu g \text{ CH}_4 \text{ m}^{-2} \text{ h}^{-1}$. However, in the injected treatment elevated fluxes were also observed in August 1993. At the end of June 1994 a small increase in CH$_4$ flux was observed in the manure treatments. The elevated CH$_4$ fluxes observed long after manure application may have been the result of an interaction between rainfall and manure resulting in a stimulation of CH$_4$ production activity.

Additional investigations of the effects of swine manure, were performed in the fall of 1994 when CH$_4$ fluxes were determined immediately before and after manure application (Fig. 6). Fluxes of from 4 chambers in each of three replicate plots before manure application were negative (Fig. 6A). Within 24 h after manure injection, fluxes were measured at locations between and directly over injection bands. Fluxes between bands were elevated (Fig. 6B) as compared to the pre-manure hourly rate averages of -28.8 to -14.7 $\mu g \text{ CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ (significant at $P = 0.004$). The fluxes of chambers located directly
over the manure injection bands showed high positive fluxes, which were significantly different (P<0.001) than between band fluxes (Fig. 6C). Out-gassing is also suspected here to be a significant contributor to the large CH$_4$ fluxes from the manure injected bands.

Natural Ecosystems

The McFarland forest site was a net consumer of atmospheric methane in both 1993 and 1994 (Fig. 7A). Mean hourly flux rates for 1993 ranged from -74.2 to -2.00 µg CH$_4$ m$^{-2}$ h$^{-1}$. The trend of increasing mean fluxes observed in 1993 could be due to the high precipitation observed in the summer of this year. In 1994 mean hourly CH$_4$ flux rates ranged from -115.4 to -15.9 µg CH$_4$ m$^{-2}$ h$^{-1}$. Our estimates are within the range of -293.3 to -1.6 µg CH$_4$ m$^{-2}$ h$^{-1}$ reported for other forest systems (Lessard et al. 1994; Born et al., 1990; Prieme and Christensen, 1997a; Castro et al., 1994a).

Mean CH$_4$ fluxes from the Roadside prairie also were negative (Fig 7B). The only positive mean hourly flux was observed in July, 1993. Elevated rainfall during this period may have caused smaller net CH$_4$ consumption rates resulting from either an inhibition of CH$_4$ oxidation or a stimulation of CH$_4$ production. Methane activity at the Doolittle prairie site was more dynamic (Fig 7C). Unlike other reports of grassland systems of the arid west always functioning as net CH$_4$ consumers (Kessavalou et al., 1998a; Mosier et al., 1997), the Doolittle prairie site was both a consumer and a producer of CH$_4$ with mean hourly fluxes ranging from -90.8 to 32.6 µg CH$_4$ m$^{-2}$ h$^{-1}$. Tate and Striegl (1993) also reported a range of CH$_4$ flux values for a tallgrass prairie that included both positive and negative fluxes, although the site was predominantly a net CH$_4$ consumer. Our Doolittle prairie site appeared to be similar to a Canadian grassland site studied by Wang and Bettany (1997).
Those researchers observed positive CH$_4$ flux rates (0.8 to 26.7 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$) following precipitation events at sites located at low slope positions.

**Landfill**

The landfill site was an active contributor of atmospheric CH$_4$ (Fig. 8). Mean hourly fluxes ranged from 173 to 50,468 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$ for 1993 and 1,422 to 2,600,000 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$ for 1994. Boeckx et al. (1996) reported rates in the range of -246 to 38,100 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$ for a Belgium landfill in Antwerp. Jones and Nedwell (1993) reported rates in the range of 1,760,000 to and 9,360,000 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$ for 2 sites in a landfill in Essex, England. In a study spanning from 1988 to 1994 Bogner et al. (1995) observed fluxes ranging from -64 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$ to 46,625,000 $\mu$g CH$_4$ m$^{-2}$ h$^{-1}$. Large variations in measured CH$_4$ fluxes ranging six orders of magnitudes are not unusual (Bogner et al., 1995). Negative chamber flux rates were also observed for both years.

**Ecosystems Comparisons**

In an effort to compare and classify the systems independently from the magnitude of the CH$_4$ flux rate estimate, a ranking system was designed. A score for each ecosystem was determined by calculating the difference between number of positive and negative fluxes measured over the entire season, and expressing this difference as a percentage of the total number of measurements performed. The resulting range for this net polarity ranking system is -100% to 100%; with negative values indicating a tendency for the system to be a net consumer of CH$_4$ while a positive value indicating the system is a net CH$_4$ producer. In 1993 the majority of the sites were ranked as positive flux systems, with the Doolittle farm site being the strongest with over 80% of the flux measurements.
showing net CH₄ production (Fig 9). The sites showing the strongest tendency for CH₄ consumption were the Roadside prairie and Mcfarland forest. In 1994 all the sites except for the landfill were classified as either net CH₄ consumers or neutral (consumption = production). This ranking system indicates that 60% of the sites surveyed in 1993 had an overall producing tendency while in 1994 only 10% had a producing tendency. The overall shift towards predominately CH₄ consuming ecosystems in 1994 may be due to decreased rainfall for this year. Whereas this ranking allows a semi-quantitative ordering of the systems we studied, for computation of regional inventories, mean seasonal or annual estimates are needed.

Despite the fact that 1993 had the highest rainfall on record (1893 to 1998), the mean seasonal flux rates of all the natural systems were negative, indicating that these systems were net sinks for CH₄ (Fig. 10). The Roadside prairie site exhibited the highest CH₄ consumption activity, followed by the McFarland forest site. While the seasonal CH₄ flux indicates net consumption at the Doolittle prairie site, the 90% confidence interval includes 0. Kessavalou et al. (1998b) observed different CH₄ uptake rates for systems under different tillage regimes and concluded that these differences were due to differences in water-filled pore space (WFPS) resulting from the change in tillage management. These researchers reported an exponential decline of CH₄ uptake as WFPS increased. Other researchers also found similar decreases in CH₄ uptake rates as WFPS or soil water content increased (Adamsen and King, 1993; Castro et al., 1994a; Castro et al., 1995; Kessavalou et al., 1998a). We observed lower seasonal CH₄ uptake rates within the natural ecosystem group (forest and prairie’s) as the average seasonal WFPS increased. This was evident for
both years under study. However this trend was not apparent in our agricultural ecosystem group.

Mean seasonal CH$_4$ fluxes from the non-manured agricultural systems were low (-0.025 to 0.079 g m$^{-2}$), and generally, confidence intervals included 0. However there was a tendency of higher seasonal CH$_4$ flux from the hydric Doolittle farm site (3.19 g m$^{-2}$) and the sites receiving swine manure (0.14 to 0.31 g m$^{-2}$). The no-till site also tended to have a higher CH$_4$ flux (0.079 g m$^{-2}$ per season) than the non manured plow till sites (-0.025 to 0.011 g m$^{-2}$ per season). This observation is in contrast to others who have reported that a shift to no tillage results in enhanced CH$_4$ uptake by soil (Kessavalou et al., 1998; data of G.P. Robertson as presented by Paustian et al., 1995). The Landfill site was a strong source of CH$_4$ with a mean seasonal rate of 52.4 g CH$_4$ m$^{-2}$.

In a pattern similar to 1993, the natural systems were net consumers of CH$_4$ in 1994 (Fig. 11). The McFarland forest site exhibited the highest CH$_4$ consumption activity followed by the Roadside prairie site. Averaged over the season, the Doolittle prairie site tended to be a net consumer of CH$_4$; however, the 90% confidence interval included 0.

The mean seasonal CH$_4$ flux rate from the Roadside farm site was lower (-0.13 g m$^{-2}$) than all the other agricultural sites (-0.082 to 0.12 g m$^{-2}$) in 1994. For 1994 mean seasonal CH$_4$ flux rate for the Kluver broadcast manure site was higher (0.12 g m$^{-2}$) than all the other agricultural sites (-0.13 to -0.0075 g m$^{-2}$) but was not significantly different. Manure applications tended to increase the mean seasonal CH$_4$ flux rate with in the Kluver agricultural systems. This trend was also apparent in 1993 at the Kluver agricultural site.

Similar to 1993 the Landfill site, with a mean seasonal flux rate of 7445 g CH$_4$ m$^{-2}$, was a
large source for CH$_4$.

For both years under study, CH$_4$ fluxes from the agricultural systems tended to be higher than the natural systems. Mosier et al. (1991) also observed higher daily consumption rates in native and uncultivated grasslands versus cultivated grasslands. Macdonald et al. (1996) found the largest oxidation rates in mineral forest soils. Prieme et al. (1997b) observed a CH$_4$ flux rate decrease (consumption rate increase) when land use was changed from arable agricultural to woodland. Decreased methane consumption in agricultural ecosystems in comparison to natural ecosystems are potentially due to the use of fertilizer nitrogen (Steudler et al., 1989; Mosier et al., 1991; Adamsen and King, 1993; Hansen et al., 1993; Castro et al., 1994b; Castro et al., 1995; Goulding et al., 1995; Mosier et al., 1996; Goulding et al., 1996; Syamsul Arif et al., 1996), tillage practices (Mosier et al., 1997), soil compaction (Hansen et al, 1993; Keller and Reiners, 1994), and agrochemical applications (Topp, 1993; Syamsul Arif et al., 1996). The negative effect of antibiotics on CH$_4$ oxidation may also play a role in decreasing CH$_4$ consumption rates in cases where fertilization is accomplished using animal manure (Schnell and King, 1995).

In our study, fertilizer N did not appear to have a inhibitory effect on CH$_4$ consumption as has been reported for forest systems (Steudler et al., 1989). No significant differences were detected between the mean seasonal flux rates of Kluver control and Kluver N-fertilized in either 1993 or 1994. Delgado and Mosier (1996) measuring CH$_4$ field flux rates in a barley system, also found no effect of N fertilizer on CH$_4$ flux. Similar findings were also reported by Bronson and Mosier (1993) in wheat and corn cropped systems. Schimel and Guldje (1998) discuss three levels of inhibition of CH$_4$
consumption as influenced by NH$_4^+$. These levels were immediate inhibition, delayed inhibition and no inhibition. These researchers concluded that the response of CH$_4$ oxidation to N inputs, and possibly other changes, will vary from system to system depending on the nature of the methane oxidizer population. It is possible that the differential effect of NH$_4^+$ fertility on CH$_4$ consumption in agricultural vs. forest systems may be due to differences in the populations of nitrifying bacteria. Regular application of NH$_4^+$ to agricultural soils may result in increased populations of nitrifying bacteria which will coincidently metabolize CH$_4$, and may also stimulate methanotrophic bacterial populations, as both methanotrophs and nitrifying bacteria can oxidize both CH$_4$ and NH$_4^+$ (Hanson and Hanson, 1996).

Manure application may impact CH$_4$ consumption and production activity in soil differently than chemical fertilizer N. We observed higher CH$_4$ fluxes in the manure treated sites than in non-manure treatments. Two potential mechanisms underlying this observation are: (i) the out-gassing of the CH$_4$ that was dissolved in the liquid phase of the manure (Chadwick and Pain, 1997); (ii) and stimulation of methanogenesis as a result of the creation of anaerobic zones due to manure application. We suspect that out-gassing of methane immediately after application to be the primary source of the emissions we observed at the Kluver and Nashua site.

A higher positive mean seasonal CH$_4$ flux rate was detected between the landfill versus all the other ecosystems for both 1993 and 1994. The mean seasonal CH$_4$ flux rate of 1994 is 142 times higher than the seasonal mean in 1993. Jones and Nedwell (1993) reported yearly fluxes of 7920 and 14500 g m$^{-2}$ y$^{-1}$ at two landfill sites in Essex, United
Kingdom. These reported yearly fluxes are 1.1 to 277 times higher than our mean seasonal estimates for 1993 and 1994 for Iowa.

**Iowa CH₄ Flux Inventory**

Spatial extrapolation of our seasonal CH₄ flux estimates was performed in order to evaluate the relative contribution of each of the systems we studied to the atmospheric CH₄ budget. For this evaluation we used the approach of Livingston and Hutchinson (1995), wherein the systems we studied were allocated to one of six strata. The strata were created based on available land use information for the state of Iowa.

In 1993 areal CH₄ flux estimates represent approximately 67% of the total Iowa land area (Table 4). For this area we calculated a positive flux of 36748 Mg CH₄ into the atmosphere. A major source of CH₄ (33936 Mg) was from the plow till, non-manured system. However, this large input from crop land was the result of a single large CH₄ flux event from the Doolittle farm site (see Fig. 1). Without this single event, the CH₄ flux from the plow till, non-manured stratum is reduced to 0.13 g CH₄ m⁻² for the season, and the resulting areal contribution to the Iowa CH₄ budget becomes 6928 Mg. We suspect that this single high event may be an anomaly brought about by a rise in the shallow water table and subsequent degassing of CH₄ from the groundwater which is saturated with respect to CH₄ (Simpkins and Parkin, 1993). Also, a high water table could stimulate increased methanogenic activity in soil. We have observed in laboratory incubations a high rate of soil methanogenic activity at this site (Chan and Parkin, 2000). The plow-till manure and the no-till systems were net positive contributors to the atmospheric CH₄ pool with rates of 2421, and 1147 Mg CH₄ per 1993 season, respectively. Despite the large specific CH₄ flux
rates from the landfill, we estimate that, because of the relatively small total land area comprised by municipal landfills in the state (0.02%), their contribution to the atmospheric CH₄ pool was relatively low in 1993.

In 1994 a somewhat different pattern is observed (Table 5). Consumption of CH₄ by the forests is greater, but CH₄ consumption by the grassland systems is slightly less. However, the greatest differences are those exhibited by the agricultural systems. The plow till, non-manured systems switched from net contributors of atmospheric CH₄ to a CH₄ consuming systems. And although specific areal rates of consumption are less than the grassland system, by taking into account the land area in the type of agricultural management, this stratum is an important sink for CH₄. As in 1993, the plow till systems with swine manure fertility are a net source of CH₄. Unlike 1993, when the contribution of CH₄ by landfills was of the same magnitude as the cropped systems, in 1994 a large increase in the specific CH₄ production rate was observed at the Landfill site resulting in an increase of the total CH₄ flux by 142 fold. We can offer no explanation why Landfill rates exhibited such differences in the two years.

**CONCLUSIONS**

Our results support a major generalization arising from past work, namely that natural forest and grassland systems are net consumers of atmospheric methane. This was true even in 1993, a year in the top 100th percentile for precipitation (calculated from 1893 through 1998, 105 years). No simple characterizations of CH₄ flux from agricultural systems can be made. Many of the agricultural systems we studied exhibited low rates of
both CH₄ consumption and production; and thus, can be considered as neutral with regard to their impact on atmospheric CH₄ concentrations. Most of the time it was impossible to discern the precise reasons for the variability observed; however, in some instances it appears that soil type and/or soil water content may play a primary role in determining whether a particular location in a field is functioning as a net producer or consumer of CH₄. Such was the case with our no-till site. The lower elevations within the field tended to be wetter and supported net CH₄ production, while the higher elevations were drier and exhibited net CH₄ consumption. Also, the poorly drained agricultural site having a shallow water table (Doolittle farm) was a strong producer of CH₄ during 1993; however in 1994 when precipitation was 86% of the 30 year average this site was primarily a net consumer of CH₄. The inhibitory effect of NH₄⁺ on methanotrophic activity in soils is well documented; however, we did not observe this effect. In fact our data indicate a possible stimulatory effect of NH₄⁺ on CH₄ consumption. Evidence for this include: i) an increase in CH₄ consumption activity following fertilizer N addition, and ii) in a controlled experiment, a trend of greater mean seasonal CH₄ flux in the unfertilized site as compared to the fertilized sites. We postulate a possible reason for these observations is that addition of NH₄⁺ fertilizer to agricultural fields having a history of fertilizer application, may result in an increase in the populations of nitrifying bacteria, which in turn, can metabolize CH₄. Of course this is only presumptive evidence, as the population dynamics of nitrifying bacteria were not determined in this study. Application of liquid swine manure to agricultural land resulted in increased fluxes of CH₄ as evident at the Kluver manure injected, broadcasted and Nashua sites. Initial increased fluxes may be due to out-gassing
of dissolved CH$_4$ in the manure slurry. Subsequent elevated CH$_4$ flux later in the season, such as was observed with the Kluver broadcast manure site in 1994 and the Kluver injected manure site in 1993 site, may be due to stimulation of the methanogenic bacteria enriched from the manure application. In 1993, a year of high rainfall, the agricultural systems were net producers CH$_4$, while the natural forest and grassland systems were net consumers of CH$_4$. However, in 1994, a year of more normal rainfall, only the agriculture lands with manure fertility were net producers of CH$_4$. Finally, it appears that municipal landfills, despite the relatively small proportion of land they represent, have a large effect on total CH$_4$ emissions.

ACKNOWLEDGMENTS

We thank Dr. David W. Meek for his assistance with programming of the SAS package and Dr. Randy J. Killorn for allowing us access to the Kluver plots. Otis Smith, Darrin Hansen, and Eva Bryne for assistance with sampling and analyses.

REFERENCES


Jones, R.D., and R.Y. Morita. 1983. Methane oxidation by *Nitrooccus oce anus* and

1998a. Fluxes of carbon dioxide, nitrous oxide, and methane in grass sod and
27, p. 1094-1104.

fluxes following tillage and wetting in a wheat-fallow cropping. Journal of
Environmental Quality, Vol. 27, p. 1105-1116.

Part 2. 2nd ed. ASA and SSSA, Madison, WI.

oxide and methane under secondary succession of pasture to forest in the Atlantic

Methane and carbon dioxide fluxes from poorly drained adjacent cultivated and

exchange: application and sources of error. P. 14-51. *In* P.A. Matson and R.C.
Harriss (ed.) Methods in Ecology - Biogenic Trace Gases: Measuring Emissions
1996. Soil environmental variables affecting the flux of methane from a range of
forest, moorland and agricultural soils. Biogeochemistry 34: p. 113-132.

and CH₄ from subalpine soils in Rocky Mountain National Park, Colorado. Global

R.H. Miller, and D.R. Keeney (ed.) Methods of soil analysis. Part 2. 2nd ed. ASA
and SSSA, Madison, WI.

350, no 6316, p. 330-332.

1996. CH₄ and N₂O fluxes in the Colorado shortgrass steppe 1. Impact of
387-399.

1997. CH₄ and N₂O fluxes in the Colorado shortgrass steppe 2. Long-term impact
of land use change. Global Biogeochemical Cycles, Vol. 11, no 1, p. 29-42.

of land use change on methane oxidation in temperate forest and grassland soils.
Chemosphere 26, p. 675-685.


DISCLAIMER

Trade names are used in this publication to provide specific information, and do not constitute a guarantee or warranty of the product or equipment by the United States Department of Agriculture, nor an endorsement over other similar products.
### Table 1. Characteristics of Study Sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil map unit</th>
<th>Location / Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mcfarland Forest</td>
<td>Lester sandy loam</td>
<td>Story County, IA T84n R23W Sec 7. Old grove woodland site (approximately 100 years old), sparse understory. Drainage: good.</td>
</tr>
<tr>
<td></td>
<td>(Mollic Hapludalf)</td>
<td></td>
</tr>
<tr>
<td>Doolittle Prairie</td>
<td>Kossuth silty clay loam</td>
<td>Story County, IA T85N R23W NE1/4 Sec 25 (north edge fence). Never tilled native prairie; mesic site vegetated by mixed grasses and forbes. Drainage: moderate to poor.</td>
</tr>
<tr>
<td></td>
<td>(Typic Haplaquoll)</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>Soil Type/Mercury</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Roadside Prairie</td>
<td>Webster clay loam</td>
<td>Story County, IA T85N R22W NW1/4 Sec 20, 300 m from east edge of Sec 20. Restored tallgrass prairie, approximately 80 years old. Drainage: moderate to poor.</td>
</tr>
<tr>
<td></td>
<td>(Typic Hapludoll)</td>
<td></td>
</tr>
<tr>
<td>Roadside Farm</td>
<td>Webster clay loam</td>
<td>Approximately 10-20 m across the fence south of the Roadside Prairie site.</td>
</tr>
<tr>
<td></td>
<td>(Typic Hapludoll)</td>
<td>In corn/soybean rotation. Tillage: 2 times field cultivation in 1993 and 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>times disk chisel in fall of 1993. Fertilization: 198-33-100 Kg, N-P-K per</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corn was planted on this site in 1993 and soybeans in 1994.</td>
</tr>
<tr>
<td>Landfill</td>
<td>Sandy loam</td>
<td>Story County, IA T83N R24W Sec 1, located on Dayton road, Ames, Iowa. Capped municipal city landfill. Drainage: moderate to poor.</td>
</tr>
<tr>
<td></td>
<td>(Textural analysis)</td>
<td></td>
</tr>
</tbody>
</table>
| Kluver Sites          | Clarion loam        | Boone County, IA T84N R25W Sec 35, approximately 3-4 miles west, Ames, Iowa. Plot sizes were 4.6 x 12.2 meters. Tillage: conservation and Nicollet loam (Aquic Hapludoll) drainage: moderate. Soybeans were planted on the plots for both years.
| Control              | see above           | Control plots: No treatment. 
| N-fertilized        | see above           | Urea Ammonium Nitrate, 252 Kg per hectare, applied between May 28 and June 3 in 1993, and on May 17 in 1994. 
| Spring Broadcasted  | see above           | Spring Broadcasted Manure plots: 60.6 Mg per hectare liquid swine manure (224-132-149 Kg, N-P-K per hectare), applied on May 17, 1993 and April 7, 1994. 
| Spring Injected     | see above           | Spring Injected Manure plots: 51.2 Mg per hectare liquid swine manure (188-120-132 Kg, N-P-K per hectare), application time is the same as the spring broadcast treatment. |
Table 1. (continued)

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nashua</td>
<td>Floyd loam and Readlyn loam</td>
<td>Floyd County, IA T94N R15W Sec 22, Northeast Research Center near Nashua, 1 mile south of Nashua. Drainage: moderate to good.</td>
</tr>
<tr>
<td></td>
<td>(Aquic Hapludoll)</td>
<td>Fertilization: Background check, all plots: No treatment. Control plots (area within treated plot): No treatment. Manure Injected plots: 37.4 x 10^3 liters per hectare hog manure, approximately 134.5 Kg per hectare N, computed based on NH₄-N + 25% organic N.</td>
</tr>
<tr>
<td>Walnut Creek</td>
<td>Clarion loam, Webster clay loam</td>
<td>Boone County, IA T83N R25W SE1/4, Sec. 24, approximately 2 miles south of Ames, Iowa. Soil type: Loam. Tillage: no till. Drainage: good to poor. Soybeans were planted on this site.</td>
</tr>
<tr>
<td></td>
<td>(Typic Hapludoll)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clarion loam</td>
<td>Boone County, IA T83N R25W E1/2, SE1/4, Sec 25, approximately 2 miles south of Ames, Iowa. Tillage: field cultivation. Fertilization: No Treatments. Drainage: good to moderate. Soybeans were planted.</td>
</tr>
<tr>
<td></td>
<td>(Typic Hapludoll)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nicollet loam</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Aquic Hapludoll)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. General soil properties at the study sites. Values are seasonal averages from 1993.

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>H$_2$O</th>
<th>WFPS</th>
<th>N</th>
<th>C</th>
<th>MBC</th>
<th>SOC</th>
<th>B.D.</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Agg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N kg$^{-1}$</td>
<td>%</td>
<td>- mg C kg$^{-1}$ -</td>
<td>g cm$^{-3}$</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Doolittle**

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>H$_2$O</th>
<th>WFPS</th>
<th>N</th>
<th>C</th>
<th>MBC</th>
<th>SOC</th>
<th>B.D.</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Agg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL Prairie</td>
<td>5.65</td>
<td>25.4</td>
<td>2.09</td>
<td>56.0</td>
<td>82.9</td>
<td>0.50</td>
<td>5.83</td>
<td>1992</td>
<td>30.3</td>
<td>0.95</td>
<td>46</td>
<td>35</td>
<td>20</td>
<td>67.7</td>
</tr>
<tr>
<td>DL Farm</td>
<td>6.78</td>
<td>12.5</td>
<td>0.62</td>
<td>35.9</td>
<td>68.5</td>
<td>0.32</td>
<td>3.68</td>
<td>804</td>
<td>23.7</td>
<td>1.11</td>
<td>30</td>
<td>33</td>
<td>37</td>
<td>62.2</td>
</tr>
<tr>
<td>Landfill</td>
<td>7.05</td>
<td>34.7</td>
<td>6.11</td>
<td>20.7</td>
<td>na</td>
<td>0.15</td>
<td>2.56</td>
<td>673</td>
<td>35.0</td>
<td>na</td>
<td>57</td>
<td>25</td>
<td>18</td>
<td>49.9</td>
</tr>
</tbody>
</table>

**Kluver**

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>H$_2$O</th>
<th>WFPS</th>
<th>N</th>
<th>C</th>
<th>MBC</th>
<th>SOC</th>
<th>B.D.</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Agg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KL Control</td>
<td>4.75</td>
<td>40.4</td>
<td>1.61</td>
<td>22.6</td>
<td>66.9</td>
<td>0.17</td>
<td>2.00</td>
<td>324</td>
<td>30.6</td>
<td>1.40</td>
<td>51</td>
<td>29</td>
<td>20</td>
<td>37.6</td>
</tr>
<tr>
<td>KL Nitrogen</td>
<td>4.68</td>
<td>83.9</td>
<td>10.8</td>
<td>22.9</td>
<td>72.1</td>
<td>0.18</td>
<td>2.07</td>
<td>320</td>
<td>30.7</td>
<td>1.44</td>
<td>53</td>
<td>27</td>
<td>21</td>
<td>34.7</td>
</tr>
<tr>
<td>KL Broadcast</td>
<td>4.72</td>
<td>49.5</td>
<td>2.88</td>
<td>21.2</td>
<td>60.9</td>
<td>0.16</td>
<td>1.83</td>
<td>318</td>
<td>32.8</td>
<td>1.38</td>
<td>54</td>
<td>27</td>
<td>19</td>
<td>36.2</td>
</tr>
<tr>
<td>KL Injected</td>
<td>4.78</td>
<td>112</td>
<td>13.2</td>
<td>22.4</td>
<td>75.0</td>
<td>0.16</td>
<td>1.70</td>
<td>328</td>
<td>42.0</td>
<td>1.48</td>
<td>56</td>
<td>25</td>
<td>20</td>
<td>38.8</td>
</tr>
</tbody>
</table>

**M Forest**

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>H$_2$O</th>
<th>WFPS</th>
<th>N</th>
<th>C</th>
<th>MBC</th>
<th>SOC</th>
<th>B.D.</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Agg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.42</td>
<td>38.2</td>
<td>4.33</td>
<td>41.9</td>
<td>71.8</td>
<td>0.35</td>
<td>4.80</td>
<td>1050</td>
<td>54.9</td>
<td>1.04</td>
<td>56</td>
<td>33</td>
<td>11</td>
<td>62.0</td>
</tr>
</tbody>
</table>
Table 2. (continued)

|              | RS Prairie | | RS Farm | | Walnut Creek | | | | | |
|--------------|------------|---|---------|---|-------------|---|---|---|---|
|              |            |   |         |   |             |   |   |   |   |
| Roadside     |            |   |         |   |             |   |   |   |   |
|              |            |   |         |   |             |   |   |   |   |
| RS Prairie   | 6.13       | 15.1 | 0.74   | 46.5 | 68.9 | 0.46 | 5.62 | 1573 | 34.5 | 0.95 | 60 | 6 | 34 | 71.3 |
| RS Farm      | 6.00       | 30.7 | 0.62   | 34.0 | 68.1 | 0.36 | 3.92 | 618  | 20.2 | 1.14 | 43 | 32 | 27 | 48.3 |
| Walnut Creek |            |   |         |   |             |   |   |   |   |
| W no-till    | 6.46       | 22.9 | 0.80   | 32.7 | 69.5 | 0.29 | 3.63 | 728  | 44.1 | 1.18 | 43 | 36 | 21 | 42.3 |
| W Hilltop    | 5.60       | 23.6 | 0.60   | 20.5 | 54.7 | 0.15 | 1.69 | 327  | 29.9 | 1.33 | 51 | 30 | 19 | 41.7 |

Values for NO$_3^-$, NH$_4^+$, H$_2$O, WFPS, N, C, MBC, and SOC are computed on a dry soil weight basis.

Water Filled Pore Space (WFPS)

Microbial Biomass Carbon (MBC)

Soluble Organic Carbon (SOC)

Bulk Density (BD)

Aggregation (Agg.)
Table 3. General soil properties at the study sites. Values are seasonal averages from 1994.

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>H$_2$O</th>
<th>WFPS</th>
<th>N</th>
<th>C</th>
<th>MBC</th>
<th>SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N kg$^{-1}$</td>
<td>%</td>
<td>- mg C kg$^{-1}$ -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doolittle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL Prairie</td>
<td>5.82</td>
<td>4.10</td>
<td>0</td>
<td>47.40</td>
<td>70.2</td>
<td>0.52</td>
<td>7.03</td>
<td>2753</td>
<td>28.27</td>
</tr>
<tr>
<td>DL Farm</td>
<td>6.48</td>
<td>6.28</td>
<td>0</td>
<td>32.17</td>
<td>61.5</td>
<td>0.28</td>
<td>3.45</td>
<td>744</td>
<td>24.47</td>
</tr>
<tr>
<td>Landfill</td>
<td>6.82</td>
<td>2.72</td>
<td>1.72</td>
<td>15.17</td>
<td>na</td>
<td>0.16</td>
<td>2.95</td>
<td>1458</td>
<td>53.05</td>
</tr>
<tr>
<td>KL Control</td>
<td>4.93</td>
<td>17.45</td>
<td>0.36</td>
<td>13.13</td>
<td>39.0</td>
<td>0.14</td>
<td>1.62</td>
<td>364</td>
<td>28.98</td>
</tr>
<tr>
<td>KL Nitrogen</td>
<td>4.79</td>
<td>41.63</td>
<td>7.20</td>
<td>12.96</td>
<td>40.9</td>
<td>0.13</td>
<td>1.50</td>
<td>320</td>
<td>31.07</td>
</tr>
<tr>
<td>KL Broadcast</td>
<td>4.80</td>
<td>80.74</td>
<td>3.70</td>
<td>14.32</td>
<td>41.2</td>
<td>0.15</td>
<td>1.64</td>
<td>329</td>
<td>35.04</td>
</tr>
<tr>
<td>KL Injected</td>
<td>4.86</td>
<td>26.86</td>
<td>0.48</td>
<td>14.84</td>
<td>49.8</td>
<td>0.13</td>
<td>1.60</td>
<td>305</td>
<td>32.28</td>
</tr>
</tbody>
</table>
Table 3. (continued)

<table>
<thead>
<tr>
<th>Location</th>
<th>NO\textsubscript{3}\textsuperscript{−}</th>
<th>NH\textsubscript{4}\textsuperscript{+}</th>
<th>H\textsubscript{2}O</th>
<th>WFPS</th>
<th>N</th>
<th>C</th>
<th>MBC</th>
<th>SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Forest</td>
<td>6.44</td>
<td>3.98</td>
<td>4.01</td>
<td>28.63</td>
<td>49.0</td>
<td>0.28</td>
<td>3.64</td>
<td>1681</td>
</tr>
<tr>
<td>Roadside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS Prairie</td>
<td>6.58</td>
<td>0.23</td>
<td>1.23</td>
<td>39.50</td>
<td>58.5</td>
<td>0.53</td>
<td>6.79</td>
<td>2368</td>
</tr>
<tr>
<td>RS Farm</td>
<td>6.07</td>
<td>6.52</td>
<td>0.42</td>
<td>27.61</td>
<td>55.2</td>
<td>0.33</td>
<td>4.00</td>
<td>951</td>
</tr>
</tbody>
</table>

Values for NO\textsubscript{3}\textsuperscript{−}, NH\textsubscript{4}\textsuperscript{+}, H\textsubscript{2}O, WFPS, N, C, MBC, and SOC are computed on a dry soil weight basis.

Water Filled Pore Space (WFPS)

Microbial Biomass Carbon (MBC)

Soluble Organic Carbon (SOC)
Table 4. Iowa methane flux inventory for 1993. Values for CH₄ fluxes are totals for 258 day sampling period.

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Land Area</th>
<th>CH₄ Flux Rate</th>
<th>Total CH₄ Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>7.96 x 10⁹</td>
<td>-0.18 (0.08)</td>
<td>-1394 (673)</td>
</tr>
<tr>
<td>Prairie, grassland range and pasture</td>
<td>6.14 x 10⁹</td>
<td>-0.17 (0.14)</td>
<td>-1051 (837)</td>
</tr>
<tr>
<td>Plow till, corn/soybean swine manure</td>
<td>1.50 x 10¹⁰</td>
<td>0.16 (0.18)</td>
<td>2421 (2679)</td>
</tr>
<tr>
<td>No-till</td>
<td>1.45 x 10¹⁰</td>
<td>0.08 (0.30)</td>
<td>1147 (4265)</td>
</tr>
<tr>
<td>Landfill</td>
<td>3.30 x 10⁷</td>
<td>52.4 (67.0)</td>
<td>1732 (2215)</td>
</tr>
<tr>
<td>Iowa Total</td>
<td>1.45 x 10¹¹</td>
<td>67.1</td>
<td>36748 (76748)</td>
</tr>
</tbody>
</table>

Land use information was compiled from a variety of sources (Daugherty, 1995, Taylor, 1995, Bull et al., 1996, U.S. Department of Agriculture, 1994 and the Iowa Department of Natural Resources).

Values in parentheses are standard deviations of the means.

Values in brackets are computed excluding the single CH₄ flux event which occurred at the Doolittle farm site on July 28, 1993.

Only municipal landfills included.
Table 5. Iowa methane flux inventory for 1994. Values for CH₄ fluxes are totals for 271 day sampling period.

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Land Area^</th>
<th>CH₄ Flux Rate±</th>
<th>Total CH₄ Flux±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>7.96 x 10⁹</td>
<td>-0.433 (0.08)</td>
<td>-3442 (642)</td>
</tr>
<tr>
<td>Prairie, grassland range and pasture</td>
<td>6.14 x 10⁹</td>
<td>-0.104 (0.12)</td>
<td>-637 (748)</td>
</tr>
<tr>
<td>Plow till, corn/soybean</td>
<td>4.08 x 10¹⁰</td>
<td>-0.063 (0.06)</td>
<td>-2558 (2247)</td>
</tr>
<tr>
<td>swine manure</td>
<td>1.60 x 10¹⁰</td>
<td>0.055 (0.09)</td>
<td>844 (1430)</td>
</tr>
<tr>
<td>No-till</td>
<td>1.54 x 10¹⁰</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Landfill§</td>
<td>3.30 x 10⁷</td>
<td>7445 (16250)</td>
<td>246030 (536970)</td>
</tr>
<tr>
<td>Iowa Total†</td>
<td>1.45 x 10¹¹</td>
<td>240278 (536977)</td>
<td></td>
</tr>
</tbody>
</table>

^ Land use information was compiled from a variety of sources (Daugherty, 1995, Taylor, 1995, Bull et al., 1996, U.S. Department of Agriculture, 1994 and the Iowa Department of Natural Resources).

± Values in parentheses are standard deviations of the means.

§ Only municipal landfills included.

† Excluding no-till.
Figure 1. Methane fluxes from agricultural ecosystems. The small circles indicate the hourly CH₄ flux rate of the individual chambers. The large circles are the mean hourly flux rate of the chambers. The horizontal lines indicate the detection sensitivities of our measurements based on a 3% system analytical variability.
Figure 2. Weather data from station Ames 8wsw. Bi-weekly precipitation summary and mean daily temperature. Annual precipitation for 1993 was 1435.6 mm (172% of 30 year normal, 100th percentile within 105 years, 1893-1998) and for 1994, 719 mm (86% of 30 year normal, 37.1th percentile within 105 years, 1893-1998). The 30 year normal is 836 mm.
Figure 3. Methane fluxes from the Walnut creek no-till agricultural ecosystem. The small circles indicate the hourly CH$_4$ flux rate of the individual chambers. The large circles are the mean hourly flux rate of the chambers. The horizontal lines indicate the detection sensitivities of our measurements based on a 3% system analytical variability.
Figure 4. Comparison of CH₄ flux vs. mean water content at the Walnut creek, no-till site. Panel A shows the seasonal mean CH₄ flux per chamber (1993) and panel B shows mean water content through the sampling period.
Figure 5. Methane fluxes from Kluver agricultural ecosystems. (A) Control (no fertilizer), (B) UAN, (C) Broadcast Manure and (D) Injected Manure. The small circles indicate the hourly CH₄ flux rate of the individual chambers. The large circles are the mean hourly flux rate of the chambers. The horizontal lines indicate the detection sensitivities of our measurements based on a 3% system analytical variability.
Figure 6. Methane fluxes from the Nashua manure experiment site. (A) Flux before manure application (15 days before manure application), (B) Flux between injection bands 1 day after manure application and (C) Flux over injection bands 1 day after manure application. The small circles indicate the hourly CH₄ flux rate of the individual chambers. The large circles are the mean hourly flux rate of the chambers. The horizontal lines indicate the detection sensitivities of our measurements based on a 3% system analytical variability.
Figure 7. Methane fluxes from natural ecosystems, (A) McFarland Forest, (B) Roadside Prairie and (C) Doolittle Prairie. The small circles indicate the hourly CH$_4$ flux rate of the individual chambers. The large circles are the mean hourly flux rate of the chambers. The horizontal lines indicate the detection sensitivities of our measurements based on a 3% system analytical variability.
Figure 8. Methane fluxes from landfill. The small circles indicate the hourly CH$_4$ flux rate of the individual chambers. The large circles are the mean hourly flux rate of the chambers. The horizontal lines indicate the detection sensitivities of our measurements based on a 3% system analytical variability.
Figure 9. Methane flux ecosystem ranking. Bars indicate the difference between number of positive and negative fluxes measured over the entire season expressed as a percentage of the total number of measurements performed.
Figure 10. Cumulative yearly methane flux rates of the sites in 1993. Symbols indicate the mean annual methane flux rate. Error bars indicate the 90% confidence interval.
1994 Cumulative Seasonal CH$_4$ Flux Rate

Figure 11. Cumulative yearly methane flux rates of the sites in 1994. Symbols indicate the mean annual methane flux rate. Error bars indicate the 90% confidence interval.
METHANE OXIDATION AND PRODUCTION ACTIVITY IN SOILS
FROM NATURAL AND AGRICULTURAL ECOSYSTEMS

A manuscript to be submitted to the Journal of Environmental Quality

A.S.K. Chan and T. B. Parkin

ABSTRACT

Biological methane (CH₄) flux from soil is the result of two processes, methanogenesis and methane oxidation. The interaction of soil and climatic variables with these processes complicates the interpretation of management effects on field CH₄ fluxes. In this study, laboratory incubations were performed with soils collected from a variety of ecosystems including forest, prairie and agricultural systems. Methane production as well as consumption potentials were measured. The lowest and highest mean CH₄ production potential was measured from a manure injected and N fertilized agricultural system with production rates of 0.02 and 298 nMol CH₄ g⁻¹ h⁻¹, respectively. High methanogenic potential in selected systems may indicate that CH₄ oxidizers may not be wholly dependent on atmospheric CH₄ for their survival and maintenance. Mean CH₄ oxidation rates under ambient CH₄ incubation conditions ranged from 2.8 to 91.1 pMol CH₄ g⁻¹ h⁻¹ with the prairie ecosystems performing the highest and selected agricultural systems the lowest. Methane oxidation potentials in the presence of elevated CH₄ yielded mean rates ranging from 2.8 nMol CH₄ g⁻¹ h⁻¹ to 736 nMol CH₄ g⁻¹ h⁻¹. Mean oxidation rates under this incubation regime were generally higher in agricultural systems than other systems. Methane oxidation activity appeared to be related to nitrogen status (NO₃⁻ and NH₄⁺) of the
ecosystems. Methane oxidation activity under ambient atmospheric CH₄ appeared to be negatively related to soil mineral N concentration. However, a positive relationship between soil mineral N status and CH₄ oxidation activity was observed in incubations with 1% CH₄. We suspect this pattern to be indicative of different populations of CH₄ oxidizers. Results of this study are consistent with our past observations of field CH₄ fluxes.

INTRODUCTION

Methane (CH₄) is a major component in atmospheric processes contributing to global climate changes. It has a relative global warming potential 62 times that of carbon dioxide over a time horizon of 20 years, and contributes approximately 20% to the radiative force driving global climate change (IPCC, 1996). Methane’s potency and increasing atmospheric concentration may have a paramount impact on future global warming. This potential impact has lead to many terrestrial studies in methods and techniques to quantify methane flux rates 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 many terrestrial studies involving the identification and assessment of the many sources and sinks of CH₄ (Galchenko et al., 1989; Bouwman, 1990; Denmead, 1991; Duxbury et al., 1993; Topp and Pattey, 1997). These studies generally agree that water saturated systems like wetlands (swamps, marshes) and paddy soils (rice fields) are heavy contributors of CH₄ whereas upland dry soils (except landfills) are generally sinks for CH₄.
Equally important are investigations that elucidate the possible controlling factors of CH₄ consumption or release from specific non-flooded terrestrial environments. For example, a study by Ojima et al. (1993) found that anthropogenic influences generally decrease CH₄ consumption. Other examples are studies where nitrogen fertility has been shown to decrease CH₄ consumption by forest and grassland systems (Mosier et al. 1991; Steudler et al., 1989).

Methane cycling in an environment is dependent on both methane oxidation and methanogenesis which exist simultaneously even in arable terrestrial ecosystems (Conrad, 1995). Methane flux measured at the soil / atmosphere interface is the net effect of these two processes (Knowles, 1993). These two processes involve three microbial populations. Methane oxidation is carried out by methanotrophic bacteria as well as the ammonia oxidizing (nitrifying) bacteria (Willison et al., 1995; Topp and Pattey, 1997; Willison et al., 1997; Schimel and Gulledge, 1998), while methanogenic bacteria serve as the source of CH₄ (Topp and Pattey, 1997; Schimel and Gulledge, 1998).

Whereas upland soils are recognized as important sinks for atmospheric CH₄ (Reeburgh et al., 1993), 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 be completely dependent on atmospheric CH₄ for their survival in an ecosystems (Megraw and Knowles, 1987). Conrad (1995) hypothesizes that in oxic soils
CH$_4$ production in anaerobic micro sites could be an important source of CH$_4$ for CH$_4$ oxidizers. Clearly, more information is needed on the CH$_4$ production and consumption potential of soils in order to more clearly delineate the impact of land management on CH$_4$ flux.

The objective of this study was to assess land management effects on CH$_4$ consumption and production potential and to relate these results to our earlier information on field CH$_4$ fluxes from natural and agricultural ecosystems (Chan and Parkin, 2000a).

**MATERIALS AND METHODS**

**Sites**

The sites investigated are located in central Iowa and represent a range of ecosystems found in the upper Midwestern United States. This study concentrated on agricultural sites under a variety of tillage and fertility regimes, including chisel plow, inorganic N fertilization and fertilization with swine manure. The natural systems investigated included a hardwood forest site, and native and restored prairie sites. The site locations and other general site characteristics are given in Table 1.

**Sample Collection and Processing**

Intact soil cores were collected by driving a metal probe containing a hollow plastic cylinder into the soil. Twenty four soil cores (4 cm diameter x 20 cm depth) were collected in August of 1995, two cores from each of the sites described in Table 1. The cylinders containing the cores were capped with butyl rubber stoppers and transported back to the laboratory. The top 5 cm (0-5 cm) of the core was sectioned off and sieved (0.5 cm mesh).
This sieved soil was used in the CH$_4$ activity assays (production and consumption) described below. Soil cores were processed within 48 hours.

**Soil Properties**

Nitrate (+ nitrite) (NO$_3^-$, NO$_2^-$) and ammonium (NH$_4^+$) were determined by colorometric analyses of 2 M KCl soil extracts (4:1 KCl:soil) on a Lachat autoanalyzer (Lachat Instruments, Mequon, WI.) following the procedure described by Keeney and Nelson (1982). Soil pH was determined on 1:1 (dH$_2$O: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**

The CH$_4$ production and consumption potential of the top 0-5 cm section of each soil core was measured. Soil cores were sieved and for all incubations regimes, an equivalent of 1 gram dry soil was weighed into 40 ml screw cap vials. The moisture content of each sample was adjusted to 50% gravimetric water content by additions of distilled water. Vials were capped with butyl rubber septa (Laboratory Supply Distributors, Mt. Laurel, NJ, USA) and incubations were done at laboratory temperature (approx. 24°C), in the dark except during sampling. Vials were sampled approximately every two days and monitored for about a three week period. Methane production and consumption potential was assessed by modifying the headspace gas conditions in the vials.

Methane consumption activity was performed in vials with an aerobic headspace, and was measured by monitoring CH$_4$ concentrations in the headspace of the vials over
time. Two types of incubations were conducted. In one regime the starting CH₄ concentration was near ambient levels (~1.7 to 2.0 μL L⁻¹) and in the other regime elevated CH₄ (~1% v/v) was placed in the headspace. The elevated CH₄ treatment was prepared by adding CH₄ from a tank of ultra high purity grade CH₄ (Air Products and Chemicals, Inc. Allentown, PA, USA). Ambient Oxygen (O₂) concentrations (~19%) were present for both types of incubations.

Methane production was also measured in two different incubation regimes. In one regime, methyl fluoride (CH₃F) (Matheson Gas Products, Montgomeryville, PA, USA), was used as an inhibitor of CH₄ oxidation (Oremland and Culbertson, 1992; Chan and Parkin, 2000b), and increases in headspace CH₄ with time were measured. These incubations were performed in an aerobic headspace and a CH₃F concentration of 0.5%. Methane production was also measured under anaerobic conditions with a headspace containing 75% hydrogen (H₂) and 25% carbon dioxide (CO₂). These conditions were established by alternately evacuating the vial headspace and flushing with helium (He) to remove air. After 5 cycles of flushing and evacuation, a vacuum was drawn on each vial and H₂ and CO₂ (Air Products and Chemicals, Inc. Allentown, PA, USA) 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 540 gas chromatograph (Tracor Instruments Austin, Inc., Austin, TX, USA). 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 \( \text{CH}_3\text{F} \) were measured with a flame ionization detector (FID) running at 200 °C, oven temperature at 65 °C, a Porapak Q, 6 foot glass column and He carrier gas flowing at the rate of 30 ml min\(^{-1}\). Oxygen was measured with a thermal conductivity detector (TCD) running at 150 °C, oven temperature at 65 °C, a Molecular Sieve 13x, 8 foot stainless steel column and He carrier at the rate of 75 ml min\(^{-1}\). Standard curves were constructed for \( \text{CH}_4 \), \( \text{CH}_3\text{F} \) and \( \text{O}_2 \) by injecting a range of concentrations corresponding to the specific gas. Certified standards of \( \text{CH}_4 \) and \( \text{O}_2 \) were obtained from Scott Speciality Gases, Troy, MI, USA. Methyl fluoride was obtained from Matheson Gas Products, Montgomeryville, PA, USA. The minimum detectable flux rate was calculated following the procedures described by Chan et al. (1998) and using a 3% coefficient of variation for the calculations. The minimum \( \text{CH}_4 \) flux rate detectable in this study is 7.12 pMol h\(^{-1}\) per vial (for either consumption or production).

**Rate Calculations**

The maximum rates of consumption and production were used to compare ecosystem \( \text{CH}_4 \) activity for each incubation regime. Estimation of the maximum rate \( (V_{\text{opt}} \text{ in } \text{Bender and Conrad, 1995}) \) was accomplished by least-squares regression of the \( \text{CH}_4 \) concentration vs. time data and calculating the maximum (in cases of \( \text{CH}_4 \) production) or minimum (in cases of \( \text{CH}_4 \) consumption) first derivative of the function. This process was accomplished by employing the Table Curve software package (SPSS Inc., Chicago, IL). In situations when rates were less than our minimum detectable rate (7.12 pMol h\(^{-1}\) per microcosm) we followed the recommendations of Gilbert (1987) and included the values of these “non-detects” in our mean and comparison calculations. All rates are expressed on a
dry weight soil basis. Possible adaptation of the methane oxidizing community at elevated (~1%) headspace incubation condition was evaluated by comparing the DT-50% values (time required for 50% of headspace CH₄ to disappear) (Parkin et al. 1991; Bender and Conrad, 1995) of each system.

Statistical Analyses

The data presented in the bar plots represent means and ranges of duplicate microcosms. Site and group comparisons on CH₄ oxidation data was accomplished by using SAS Anova (one way). Sites were grouped into three different categories based on land use similarities. The three categories are: 1) Natural, 2) Agricultural - corn/soybean rotation, plow till, no manure, 3) Agricultural - corn/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

While most arable soils are not typically net producers of CH₄, periodic conditions of anaerobiosis could support transient episodes of CH₄ production, if the methanogenic potential exists. Methane production activity under anaerobic conditions with added H₂ and CO₂, indicated that all of the systems investigated (Table 1) possessed the capacity for methanogenesis (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. Statistical
differences were detected between the natural and no manure plow till systems ($P<0.01$). Differences were also detected between the no manure and manure group ($P<0.01$). However, results of these comparisons are strongly influenced by the high CH$_4$ production activity exhibited by soil from the Walnut Creek Pothole (WC Pothole), Doolittle farm (DL Farm), and Roadside farm (RS Farm) sites (Fig. 1C). Curiously 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 pH neutral environments (Garcia, 1990; Zinder, 1993). Low pH ($<4.5$) observed in the manure systems may have had an effect on methanogenic activity. All of the agricultural systems exhibited some degree of methanogenic activity (Figs 1B, 1C, 1D); however, several agricultural sites exhibited very high CH$_4$ production activity (Fig 1C). The Walnut Creek Pothole site (WC Pothole) and the Doolittle farm site (DL Farm) had average rates of 298 and 112 nMol CH$_4$ g$^{-1}$ h$^{-1}$, respectively (note scale change on Fig. 1C). 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 where high rates of CH$_4$ flux were observed in the field (Chan and Parkin, 2000a). It is thought that these areas maintain anaerobic conditions conducive for the development and maintenance of high methanogenic bacterial populations. The detection of CH$_4$ production indicates that all these ecosystems possess populations of methanogenic bacteria. Given the right conditions these systems have a potential to produce and release CH$_4$ into the atmosphere. Methanogenic activity from Walnut Creek Pothole and Doolittle farm may even fuel CH$_4$ oxidation activity. Yavitt et al. (1995) found methanogenesis
occurring in a northern hardwood ecosystem under anaerobic and aerobic conditions with methyl fluoride. They concluded that the CH₄ release could support the growth of methanotrophic bacteria. A study by Wang and Bettany (1997) also found methanogenesis in prairie and forest systems in both laboratory incubations and field observations. Methane production was initiated after periods of increased moisture (snowmelt, precipitation, flooding). In a laboratory experiment using a cultivated soil Megraw and Knowles (1987) observed CH₄ production in anaerobic incubations as well as CH₄ oxidation in their aerobic incubations. These researchers also suggested that the methanotrophs in that cultivated soil are not entirely dependent on atmospheric CH₄ for survival and growth. As is the case in the natural ecosystems release of CH₄ has also been observed in arable systems after periods following irrigation or rainfall (increase moisture) (Delgado and Mosier, 1996; Chan and Parkin, 2000a).

**Methane Oxidation Potential**

Net CH₄ flux from a system is the result of the balance between production and consumption processes. In our assay of CH₄ oxidation, potential rates of CH₄ consumption were determined by changes in headspace CH₄ concentration, thus, CH₄ oxidation will be underestimated if methanogenesis is also occurring. In order to assess the CH₄ production in the absence of CH₄ consumption under the conditions employed in this CH₄ oxidation assay (soils at 50% gravimetric water content incubated in an aerobic headspace), 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, 2000b).
When incubated in an aerobic headspace with methyl flouride, only soils from three sites exhibited CH₄ production activity (Fig. 2). Average rates of production for Kluver nitrogen (KL Nitrogen), Kluver broadcast manure (KL Broadcast) and Walnut Creek Hill Top (WC Hill Top) were 14.7, 7.1 and 21.5 pMol CH₄ g⁻¹ h⁻¹, respectively. These rates of CH₄ production under conditions in which CH₄ oxidation were inhibited were used to compute the gross CH₄ oxidation results described 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 systems had the highest CH₄ oxidation activity with mean rates of 91.1 and 74.6 pMol CH₄ g⁻¹ h⁻¹, and these rates were significantly higher (P<0.01) than the 24.3 pMol CH₄ g⁻¹ h⁻¹ average rate exhibited by the McFarland forest soil. The agricultural systems showed much lower CH₄ oxidation activity. This was the case for the agricultural systems without manure (Fig. 3B) as well as the two systems which 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 the Doolittle soil did not also show CH₄ production when incubated with CH₃F. Similar results were recently reported by Boeckx et al. (1997). In aerobic incubation when heavy clayey and loamy soil cores from Belgium grasslands were assayed for CH₄ uptake rates, high rates of methane production were observed.

Our observations of CH₄ oxidation activity in natural and agricultural ecosystems follow the general pattern of CH₄ oxidation activity present in the literature; namely, natural ecosystems consume CH₄ at a higher rate than agricultural ecosystems (Bender and
Conrad, 1993; Dobbie and Smith, 1996; Powlson et al., 1997). The CH₄ oxidation rates of the 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 4 to 8 times larger than our McFarland forest ecosystem. In another study, Heipieper and de Bont (1997) reported average rates of 120 pMol CH₄ g⁻¹ h⁻¹ in a Dutch grassland soil incubated with 1μL L⁻¹ CH₄. This value is similar (1.4 times larger on the average) to our prairie results.

The pattern of CH₄ oxidation activity under conditions of ambient CH₄ concentrations are also consistent with our observations of field CH₄ fluxes at many of these same sites (Chan and Parkin, 2000a). Conversion of these laboratory rates (pMol CH₄ g⁻¹ h⁻¹) to field rates (g m⁻² d⁻¹) assuming a 5 cm activity depth resulted in rates that were approximately in the same range and magnitude as the observed field flux rates in the Chan and Parkin (2000a) field study. Generally, the field CH₄ fluxes were low or below detection limits for the agricultural ecosystems; however the prairie and forest systems exhibited high CH₄ consumption rates (Chan and Parkin, 2000a). The agricultural sites receiving manure (Kluver Broadcast and Kluver Injected) did not exhibit significant CH₄ consumption activity; rather these sites were net contributors of CH₄ to the atmosphere. The high field fluxes were mainly attributed to initial out-gassing of the dissolved CH₄ from the applied manure. The samples for this study were taken in August which is several months after manure application and samples were also sieved and homogenized, any dissolved CH₄ may have been removed thus out-gassing was not observed. The fact that
the Doolittle farm soil exhibited net CH$_4$ production is consistent with the high CH$_4$ fluxes from this site (Chan and Parkin, 2000a).

Because of past reports that CH$_4$ oxidizing bacteria may differ with regard to their affinity for CH$_4$ (Bender and Conrad, 1992; Conrad, 1995), we also measured CH$_4$ oxidation potential under elevated (~1%) headspace CH$_4$ concentrations (Fig. 4). In all cases, a higher headspace CH$_4$ concentration stimulated CH$_4$ oxidation rates (note scale change from Fig. 3 to Fig. 4). Under this elevated CH$_4$ incubation regime, oxidation activity of the forest soil was stimulated to a greater extent than the prairie soils (Fig. 4A). While CH$_4$ oxidation activity of the prairie and forest sites were stimulated, many of the agricultural sites exhibited a greater stimulation of activity. The Roadside farm site and the Walnut Creek Pothole site had the highest rates of CH$_4$ oxidation (680 and 736 nMol CH$_4$ g$^{-1}$ h$^{-1}$, respectively). 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 CH$_4$ g$^{-1}$ h$^{-1}$), and the highest rate was observed in soil from the depressional Pothole location (736 nMol CH$_4$ g$^{-1}$ h$^{-1}$). The side slope location had an intermediate rate of 483 nMol CH$_4$ g$^{-1}$ h$^{-1}$. The two sites which receive swine manure application (KL Broadcast and KL Injected) had very low CH$_4$ oxidation activity compared to most of the other sites. Manure applications, or some other factor at this site, seemed to have an adverse effect on the population of CH$_4$ consumers that have affinity to high concentrations of CH$_4$ (Fig. 4). We suspect antibiotics (Hanson and Hanson, 1996; Schnell and King, 1995) and other toxic substances accompanying swine manure or perhaps the low soil pH (pH < 5.0) may have caused a reduction in the population sizes and activity of the CH$_4$ consuming community.
Methane oxidation activity appeared to be related to nitrogen status (NO₃⁻ and NH₄⁺) of the systems (Fig. 5). Methane oxidation activity as expressed under conditions of ambient atmospheric CH₄ appeared to be negatively related to soil mineral N concentration (Fig 5A). The two prairie sites, which had the lowest mineral N concentrations also showed the highest CH₄ oxidation activity. The forest site had mineral N levels 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 levels, and showed the lowest levels of ambient CH₄ oxidation activity. Under conditions of elevated CH₄ concentrations, a different pattern of CH₄ oxidation activity was observed (Fig. 5B). 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. Note, however, that 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) in these systems.

Generally, it has been observed that CH₄ oxidation activity in agricultural systems is lower 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 methane consumption activity in arable systems (Steudler et al., 1989; Mosier et al., 1991; Bronson and Mosier, 1994). Ammonium (NH₄⁺) has been reported to be a competitive inhibitor for CH₄ in CH₄ oxidizing bacteria (Whittenbury et al., 1970; Hyman and Wood, 1983; Jones and Morita, 1983). Our observations of lower CH₄ oxidation at ambient CH₄
concentrations are consistent with these past studies. However, under conditions of elevated atmospheric CH$_4$, our non-manured agricultural sites had significantly higher activity (P<0.01) than the natural systems.

Recent literature indicate there are two populations of CH$_4$ oxidizers present in the environment (Conrad, 1995). One population, having a low affinity to CH$_4$, typically has a $K_m$ in the range of 1000 nM CH$_4$, and the other population, having a high affinity for CH$_4$ with a $K_m$ in the range of 30-60 nM CH$_4$. 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 CH$_4$ changes as a function of growth conditions. It is well documented, however, that ammonia oxidizing bacteria can also oxidize CH$_4$ 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 CH$_4$ in agricultural systems. In both field and spiked (100 $\mu$L CH$_4$ L$^{-1}$) laboratory studies, Goldman et al. (1995) found a positive correlation between CH$_4$ consumption and soil NH$_4^+$. Results of the Goldman et al. (1995) are similar to those of our study which show that under an elevated CH$_4$ atmosphere, N may actually stimulate CH$_4$ oxidation activity. We suspect that the CH$_4$ oxidation activity carried under elevated atmospheric CH$_4$ levels may be predominantly due to nitrifying bacteria, especially in the agricultural systems where regular N fertilizer applications may serve to create and sustain high population levels of nitrifying bacteria. Concentration time course data (Fig. 6) and DT-50% values (Fig. 6) indicates lower adaptation periods in incubations with 1% CH$_4$ headspace for systems with higher levels of mineral N (agricultural systems).
This data suggest that the low affinity CH$_4$ oxidizers were already present in the high mineral N soils and not enriched by the introduction of the 1% CH$_4$ in the headspace. In contrast the natural systems had longer DT-50% times indicating that the CH$_4$ oxidizing populations were different.

Methane oxidation by nitrifying bacteria may only be important in situations where elevated CH$_4$ concentrations are present. Although the maximum rate of CH$_4$ oxidation by nitrifying bacteria has been reported to be several orders of magnitude less than for common CH$_4$ oxidizing bacteria, the contribution of the nitrifying bacteria to total CH$_4$ oxidation could be significant if high numbers of these organisms are present (Conrad 1995). As shown by our laboratory experiments, soil from all of the sites sampled were capable of producing CH$_4$; however some sites were much more active than others. In hydric systems, such as our Walnut Creek Pothole site, or at the Doolittle farm site, which has a shallow water table, CH$_4$ production potential was high and may be of importance. It is unlikely that methanogenic conditions exist on a long term basis at these sites, but rather methanogenesis may occur in response to periodic wetting events. Methane concentrations in the soil pores was not measured, however, in our previous study we noted that at times these sites were net producers of CH$_4$ (Chan and Parkin , 2000a).

These temporary CH$_4$ bursts may affect the soil CH$_4$ flux measurements directly and indirectly. The direct effect would be the excess CH$_4$ diffusing up the soil profile and escaping to the atmosphere unable to be used by the CH$_4$ consuming community. Indirectly, the activity of the microbial populations which oxidize CH$_4$ may be stimulated when exposed to periodic elevated CH$_4$ levels, thus the subsequent oxidation of
atmospheric CH$_4$ may continue at an elevated rate.

CONCLUSIONS

The CH$_4$ flux dynamics in soil is complex and may be reflected by the activities of three distinct populations, the methanotrophs, the NH$_4^+$ oxidizers (nitrifiers), and the methanogens. The nitrogen and water status as well as pH of a given system will selectively impact these groups and subsequently impact the net flux of CH$_4$ from a given site. Natural sites receiving low N inputs had high potential to oxidize atmospheric concentrations of CH$_4$. Agricultural sites showed enhanced activity to oxidize CH$_4$ at elevated concentrations. The difference in the CH$_4$ oxidation pattern between the natural and agricultural ecosystems may reflect the activities of distinct CH$_4$ oxidizing communities in these systems. The agricultural site were dominated by low affinity CH$_4$ oxidizers (nitrifiers) whereas the natural sites were dominated by high affinity CH$_4$ oxidizers. Methane oxidation activity may be enhanced if conditions periodically favor methanogenesis. However, if soil pH was less than 5.0 CH$_4$ oxidation activity was nearly eliminated. It was impossible to establish the direct effects of swine manure application on CH$_4$ oxidation activity in this study, since the manured sites were also the sites with low pH.
REFERENCES


Hutchinson, G.L., and G.P. Livingston. 1993. Use of chamber system to measure trace gas fluxes. p. 63-78. In D.M. Kral (ed.) Agricultural ecosystem effects on trace gases and global climate change. ASA, Madison, WI.


**DISCLAIMER**

Trade names are used in this publication to provide specific information, and do not constitute a guarantee or warranty of the product or equipment by the United States Department of Agriculture, nor an endorsement over other similar products.
<table>
<thead>
<tr>
<th>Site</th>
<th>Soil map unit</th>
<th>Location / Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mcfarland Forest</td>
<td>Lester sandy loam (Mollic Hapludalf)</td>
<td>Story County, IA T84n R23W Sec 7. Old grove woodland site (approximately 100 years old), sparse understory. Drainage: good.</td>
</tr>
<tr>
<td>Doolittle Prairie</td>
<td>Kossuth silty clay loam (Typic Haplaquoll)</td>
<td>Story County, IA T85N R23W NE1/4 Sec 25 (north edge fence). Never tilled native prairie; mesic site vegetated by mixed grasses and forbes. Drainage: moderate to poor.</td>
</tr>
</tbody>
</table>
Table 1. (continued)

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roadside Prairie</td>
<td>Webster clay loam</td>
<td>Story County, IA T85N R22W NW1/4 Sec 20, 300 m from east edge of Sec 20. Restored tallgrass prairie, approximately 80 years old. Drainage: moderate to poor.</td>
</tr>
<tr>
<td></td>
<td>(Typic Hapludoll)</td>
<td></td>
</tr>
<tr>
<td>Roadside Farm</td>
<td>Webster clay loam</td>
<td>Approximately 10-20 m across the fence south of the Roadside Prairie site.</td>
</tr>
</tbody>
</table>
Table 1. (continued)

<table>
<thead>
<tr>
<th>Location</th>
<th>Soil Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walnut Creek</td>
<td>Canisteo silty clay loam</td>
<td>Boone County, IA T83N R25W E1/2, SE1/4, Sec 25, approximately 2 miles south of Ames, Iowa. Tillage: field cultivation. Fertilization: 138 Kg N per hectare (anhydrous ammonium) in fall of 1993. Drainage: good to moderate, poor in the pothole area. Soybeans were planted on this site for both 1993 and 1995. In 1994 corn.</td>
</tr>
<tr>
<td>Hilltop Site</td>
<td>(Typic Haplaquoll) and Clarion loam (Typic Hapludoll)</td>
<td></td>
</tr>
<tr>
<td>Clarion loam</td>
<td>(Typic Hapludoll)</td>
<td></td>
</tr>
<tr>
<td>Nicollet loam</td>
<td>(Aquic Hapludoll)</td>
<td></td>
</tr>
<tr>
<td>Kluver Sites</td>
<td>Clarion loam (Typic Hapludoll) and Nicollet loam (Aquic Hapludoll)</td>
<td>Boone County, IA T84N R25W Sec 35, approximately 3-4 miles west, Ames, Iowa. Plot sizes were 4.6 x 12.2 meters. Tillage: conservation tillage. Drainage: moderate. Soybeans were planted on the plots for all three years.</td>
</tr>
<tr>
<td>Control</td>
<td>----- see above -----</td>
<td>Control plots: Planted but no nitrogen, no manure (no treatment).</td>
</tr>
<tr>
<td>N-fertilized</td>
<td>----- see above -----</td>
<td>Urea Ammonium Nitrate, 252 Kg per hectare, applied between May 28 and June 3 in 1993, and on May 17 in 1994. Dates unavailable for 1995</td>
</tr>
<tr>
<td>Spring Broadcasted Manure plots: 60.6 Mg per hectare liquid swine manure</td>
<td>(224-132-149 Kg, N-P-K per hectare), applied on May 17, 1993 and April 7, 1994. Dates unavailable for 1995.</td>
<td></td>
</tr>
<tr>
<td>Spring Injected</td>
<td>Spring Injected Manure plots: 51.2 Mg per hectare liquid swine manure (188-120-132 Kg, N-P-K per hectare), application time is the same as the spring broadcast treatment. Dates unavailable for 1995.</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>----- see above -----</td>
<td></td>
</tr>
<tr>
<td>Injected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. General soil properties at the study sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>B.D</th>
<th>H₂O</th>
<th>pH</th>
<th>NO₃⁻</th>
<th>NH₄⁺</th>
<th>pH</th>
<th>NO₃⁻</th>
<th>NH₄⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Forest</td>
<td>1.04</td>
<td>14.1</td>
<td>6.1</td>
<td>3.6</td>
<td>1.9</td>
<td>4.6</td>
<td>0.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Doolittle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL Prairie</td>
<td>0.95</td>
<td>40.2</td>
<td>5.6</td>
<td>4.1</td>
<td>0</td>
<td>5.6</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>DL Farm</td>
<td>1.11</td>
<td>22.6</td>
<td>6.4</td>
<td>5.0</td>
<td>0.9</td>
<td>6.5</td>
<td>5.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Roadside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS Prairie</td>
<td>0.95</td>
<td>44.2</td>
<td>6.2</td>
<td>0.9</td>
<td>1.1</td>
<td>6.1</td>
<td>8.0</td>
<td>1.3</td>
</tr>
<tr>
<td>RS Farm</td>
<td>1.14</td>
<td>29.0</td>
<td>5.8</td>
<td>15.4</td>
<td>1.7</td>
<td>5.8</td>
<td>5.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Kluver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KL Control</td>
<td>1.40</td>
<td>21.2</td>
<td>5.0</td>
<td>3.8</td>
<td>1.2</td>
<td>4.9</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>KL Nitrogen</td>
<td>1.44</td>
<td>16.4</td>
<td>4.7</td>
<td>6.1</td>
<td>1.6</td>
<td>4.7</td>
<td>4.2</td>
<td>1.6</td>
</tr>
<tr>
<td>KL Broadcast</td>
<td>1.38</td>
<td>15.0</td>
<td>4.4</td>
<td>14.1</td>
<td>1.3</td>
<td>4.7</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>KL Injected</td>
<td>1.48</td>
<td>13.2</td>
<td>4.3</td>
<td>4.8</td>
<td>1.2</td>
<td>4.7</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Walnut Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W Hill Top</td>
<td>1.33</td>
<td>8.0</td>
<td>4.7</td>
<td>7.5</td>
<td>0.7</td>
<td>5.0</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>W Side Slope</td>
<td>na</td>
<td>11.4</td>
<td>5.1</td>
<td>9.0</td>
<td>1.8</td>
<td>5.1</td>
<td>3.0</td>
<td>1.4</td>
</tr>
<tr>
<td>W Pothole</td>
<td>1.48</td>
<td>19.9</td>
<td>3.5</td>
<td>26.3</td>
<td>1.0</td>
<td>5.8</td>
<td>4.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Values for NO₃⁻, NH₄⁺, H₂O, are computed on a dry soil weight basis. Bulk Density (BD).
Methane Production Assay
H₂ + CO₂ amended

Figure 1. Methane production assay. Microcosms 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. Horizontal dashed lines indicate minimum detection limit of +/− 7.12 pMol h⁻¹ vial⁻¹ for the assay. All rates are reported on a dry soil weight basis. Symbol Key: M Forest = McFarland forest; DL Prairie = Doolittle prairie; RS Prairie = Roadside prairie; KL Control = Kluver control; KL Nitrogen = Kluver nitrogen; KL Broadcast = Kluver broadcast manure; KL Inject = Kluver injected manure; WC Hill Top = Walnut Creek Hill Top; WC Side Slope = Walnut Creek Side Slope; WC Pothole = Walnut Creek Pothole; DL Farm = Doolittle farm; RS Farm = Roadside farm.
Aerobic Methane Production

( + CH₃F )

Figure 2. Aerobic methane production. Microcosms were incubated aerobically with 0.5% CH₃F to inhibit CH₄ oxidation. Only ecosystems exhibiting CH₄ production are shown. Bars indicate the average rate and error bars indicate the range of the rates. Horizontal dashed lines indicate minimum detection limit of +/− 7.12 pMol h⁻¹ vial⁻¹ for the assay. All rates are reported on a dry soil weight basis. Symbol Key: KL Nitrogen = Kluver nitrogen; KL Broadcast = Kluver broadcast manure; WC Hill Top = Walnut Creek Hill Top.
Figure 3. Methane oxidation assay at ambient headspace CH₄ concentration (~1.7 - 2.0 µL L⁻¹) incubation. 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. Horizontal dashed lines indicate minimum detection limit of +/- 7.12 pMol h⁻¹ vial⁻¹ for the assay. All rates are reported on a dry soil weight basis.

Symbol Key: M Forest = McFarland forest; DL Prairie = Doolittle prairie; RS Prairie = Roadside prairie; KL Control = Kluver control; KL Nitrogen = Kluver nitrogen; KL Broadcast = Kluver broadcast manure; KL Inject = Kluver injected manure; WC Hill Top = Walnut Creek Hill Top; WC Side Slope = Walnut Creek Side Slope; WC Pothole = Walnut Creek Pothole; DL Farm = Doolittle farm; RS Farm = Roadside farm.
Figure 4. Methane oxidation assay at elevated headspace methane concentration (~1%) incubation. Positive rates indicates gross methane oxidation. Bars indicate the average rate and error bars indicate the range of the rates. Horizontal dashed lines indicate minimum detection limit of +/− 7.12 pMol h⁻¹ vial⁻¹ for the assay. All rates are reported on a dry soil weight basis. Symbol Key: M Forest = McFarland forest; DL Prairie = Doolittle prairie; RS Prairie = Roadside prairie; KL Control = Kluver control; KL Nitrogen = Kluver nitrogen; KL Broadcast = Kluver broadcast manure; KL Inject = Kluver injected manure; WC Hill Top = Walnut Creek Hill Top; WC Side Slope = Walnut Creek Side Slope; WC Pothole = Walnut Creek Pothole; DL Farm = Doolittle farm; RS Farm = Roadside farm.
Effect of Soil Inorganic N on Methane Oxidation

Figure 5. Effect of soil inorganic N (combined NO$_3^-$ and NH$_4^+$) on methane oxidation rate as determined in incubations with ambient headspace CH$_4$ concentrations (panel A) and elevated headspace CH$_4$ concentrations (panel B). All rates are reported on a dry soil weight basis. Symbol Key: McF = McFarland forest; Dp = Doolittle prairie; Rp = Roadside prairie; Kc = Kluver control; Kn = Kluver nitrogen; Kb = Kluver broadcast manure; Ki = Kluver injected manure; Wht = Walnut Creek Hill Top; Wss = Walnut Creek Side Slope; Wph = Walnut Creek Pothole; Df = Doolittle farm; Rf = Roadside farm.
Figure 6. Time course data on methane oxidation at elevated headspace (~1%) methane incubation. Curves are the average of two samples. Values in brackets behind the site identification are DT-50% in hours. Symbol Key: M Forest = McFarland forest; DL Prairie = Doolittle prairie; RS Prairie = Roadside prairie; KL Control = Kluver control; KL Nitrogen = Kluver nitrogen; KL Broadcast = Kluver broadcast manure; KL Inject = Kluver injected manure; WC Hill Top = Walnut Creek Hill Top; WC Side Slope = Walnut Creek Side Slope; WC Pothole = Walnut Creek Pothole; DL Farm = Doolittle farm; RS Farm = Roadside farm.
GENERAL CONCLUSIONS

Summary

Accurate quantitative assessment of methane (CH$_4$) flux rates from terrestrial ecosystems is crucial for the determination of factors influencing the flux rate and for CH$_4$ budget estimations locally, regionally and globally. The minimum detectable flux for the Bowen-Ratio Energy Balance (BREB) system ranged from 2.16 to 25.5 mg CH$_4$ m$^{-2}$ h$^{-1}$ while it was $9.32 \times 10^{-2}$ mg CH$_4$ m$^{-2}$ h$^{-1}$ for the closed-chamber method. Based on reported flux estimates and our BREB instrument results, we conclude that the BREB method coupled with gas chromatographic analysis (GC/FID or GC/ECD) may not perform well in determining CH$_4$ flux from non-flooded non-manured agricultural lands, where the microbial CH$_4$ production and consumption activity is expected to be below the minimum detection sensitivity of the method.

Our inhibitor study has confirmed that several gaseous compounds may be useful for distinguishing between CH$_4$ oxidation and CH$_4$ production. Acetylene at a concentration of 0.001% inhibited CH$_4$ oxidation by 93.5% but did not significantly impact CH$_4$ production. Ethylene at a higher concentration (0.1%) had a similar effect. Methyl chloride is unique, in that at a concentration of 0.1% methanogenesis was inhibited by 88.9 %, but CH$_4$ oxidation was not significantly affected. Finally, methyl fluoride (CH$_3$F) at a concentration of 0.1% showed complete inhibition of CH$_4$ oxidation but did not significantly influence CH$_4$ production. It is our opinion that acetylene (C$_2$H$_2$), ethylene (C$_2$H$_4$) and methyl chloride (CH$_3$Cl) show the most promise for use in CH$_4$ cycling studies while CH$_3$F appears to be an effective inhibitor it should be used with care. All inhibitors
should be used with caution; however, as there is evidence that acetigenic methanogens
and methanogens that produce CH₄ from hydrogen (H₂) and carbon dioxide (CO₂) may
have differential sensitivities to these inhibitors.

Land management has a major influence on the capability of soils to serve as
sources or sinks for atmospheric CH₄. Our field experiment results support a major
generalization arising from past work namely that natural forest and grassland systems are
net consumers of atmospheric CH₄. This was true even in a year (1993) ranked in the top
100th percentile for precipitation (calculated from 1893 through 1998, 105 years).
However, no simple characterizations of CH₄ flux from agricultural systems can be made.
Many of the agricultural systems we studied exhibited low rates of both CH₄ consumption
and production; and thus, can be considered as neutral with regard to their impact on
atmospheric CH₄ concentrations. It was impossible to discern, precisely, the reasons for
the variability observed; however, it appears that soil type and/or soil water content may
play a primary role in determining whether a particular location in a field is functioning as
a net producer or consumer of CH₄. Such was the case with our no-till site where a
correlation existed between soil water content and CH₄ flux. The lower elevations within
the field tended to be wetter and supported net CH₄ production, while the higher elevations
were drier and exhibited net CH₄ consumption. In this case landscape position controlled
the moisture distribution and, thus was indirectly controlling CH₄ flux. The poorly drained
agricultural site having a shallow water table (Doolittle farm) was a strong producer of CH₄
during 1993; however, in 1994 when precipitation was 86% of the 30 year average this site
was primarily a net consumer of CH₄. At this site the water was a major controller of the
CH₄ flux at the soil surface.

The inhibitory effect of NH₄⁺ from fertilizer on methanotrophic activity in soils is well documented; however, we did not observe this effect. In fact our data indicate a possible stimulatory effect of NH₄⁺ on CH₄ consumption. Evidence for this include: i) an increase in CH₄ consumption activity following fertilizer N addition at our agricultural field sites, and ii) a trend of greater mean annual CH₄ flux in the unfertilized site as compared to the fertilized sites. We postulate a possible reason for these observations is that addition of NH₄⁺ fertilizer to agricultural fields having a history of fertilizer application, may result in an increase in the populations of nitrifying bacteria, which in turn, can metabolize CH₄. Of course this is only presumptive evidence, as the population dynamics of nitrifying bacteria were not determined in this field study. Application of liquid swine manure to agricultural land resulted in increased fluxes of CH₄ as was evident at the Kluver manure injected, broadcasted sites and the Nashua site. Initial increased fluxes following swine manure application may be due to out-gassing of dissolved CH₄ in the manure slurry. Subsequent elevated CH₄ flux later in the season, such as was observed with the Kluver broadcast manure site in 1994 and the Kluver injected manure site in 1993 site, may be due to stimulation of the methanogenic bacteria enriched from the manure application. In 1993, a year of high rainfall, the agricultural systems were net producers CH₄, while the natural forest and grassland systems were net consumers of CH₄. However, in 1994, a year of more normal rainfall, only the agriculture lands with manure fertility were net producers of CH₄. Finally, it appears that municipal landfills, despite the relatively small proportion of land they represent, have a large effect on total CH₄
emissions.

The CH₄ flux dynamics in soil is complex and may be reflected by the activities of three distinct populations, the methanotrophs, the NH₄⁺ oxidizers (nitrifiers), and the methanogens. The nitrogen and water status as well as pH of a given system will selectively impact these groups and subsequently impact the net flux of CH₄ from a given site. Natural sites receiving low N inputs had high potential to oxidize atmospheric concentrations of CH₄. Agricultural sites showed enhanced activity to oxidize CH₄ at elevated concentrations. The difference in the CH₄ oxidation pattern between the natural and agricultural ecosystems may reflect the activities of distinct CH₄ oxidizing communities in these systems. The agricultural site were dominated by low affinity CH₄ oxidizers (nitrifiers) whereas the natural sites were dominated by high affinity CH₄ oxidizers. Methane oxidation activity may be enhanced if conditions periodically favor methanogenesis. However, if soil pH was less than 5.0 CH₄ oxidation activity was nearly eliminated. It was impossible to establish the direct effects of swine manure application on CH₄ oxidation activity in this study, since the manured sites were also the sites with low pH.

Suggestions for Additional Research

There are a few reports describing CH₄ activity in the winter season (Mosier et al., 1996; Mast et al., 1998; Kessavalou et al., 1998). However reported CH₄ flux rates from these studies appeared to fall below the minimum detectable flux when the procedures of Chan et al. (1998) are applied to determine a minimum detectable flux. More research is
needed to precisely determine CH$_4$ activity in the winter months in non flooded ecosystems and it's contributions to the CH$_4$ budget.

Extreme temporal and spatial variability is observed when measuring CH$_4$ fluxes at the soil-atmosphere interface. In our field study we observed both net positive and negative CH$_4$ fluxes simultaneously from one ecosystem. There is a need to develop a mechanistic model to describe the factors that drive the underlying processes leading to a net CH$_4$ flux at the soil-atmosphere interface of non flooded ecosystems. Physical factors (i.e. tillage practices, soil compaction, soil water content and aeration), chemical factors (i.e. pH, fertilizers, and other agrochemicals) and biological factors (interaction of CH$_4$ consumers and CH$_4$ producers as well O$_2$ consumption) all affect the CH$_4$ flux and therefore must be factored into the model. The resultant model may explain the observed field variability.

Although many efforts have been made to minimize the uncertainty of field scale CH$_4$ flux measurements resulting from temporal and spatial variability (Mosier, 1998), it remains a serious problem when extrapolating field scale CH$_4$ fluxes across space and time to local, regional and global scales. Uncertainty increases by factors of magnitudes when extrapolation procedures and models are applied. Better methods to summarize field scale data is needed and better extrapolation methods, procedures and models are needed to summarize data on the local, regional and global scales. Except for efforts by Livingston and Hutchinson (1995) very little has been done concerning extrapolations of field scale fluxes to larger scales.

The role of NH$_4^+$ oxidizers (nitrifiers) in terms of their contributions to CH$_4$
consumption in non flooded ecosystems is uncertain. In a review by Schimel and Gulledge (1998) three effects on CH₄ consumption were discussed as a result of NH₄⁺ additions to systems; (i) immediate inhibition, (ii) delayed inhibition and (iii) no inhibition. However a study by Goldman et al. (1995) and our results (Chan and Parkin, 2000b) indicate a positive correlation between CH₄ consumption and NH₄⁺ which adds a possible fourth effect to the list, stimulation of CH₄ consumption. These effects are possibly dependent on the microbial composition (type and quantity) of the CH₄ consuming community.

Current molecular tools (gene probes, 16s RNA probes) can be specifically designed to target NH₄⁺ oxidizers (nitrifiers) and methanotrophs (even methanogens). These powerful molecular tools can be employed in conjunction with CH₄ consumption experiments to enhance our understanding of the role of the NH₄⁺ oxidizers in CH₄ consuming communities. Molecular techniques are powerful tools however likewise to other tools for measuring microbial presence and abundance (culture methods, fluorescent antibodies, phospholipid fatty acid methyl esters, fatty acid methyl esters) they also have drawbacks which researchers must be aware of (Hanson and Hanson, 1996).

Inhibitor methods may also be useful in separating between CH₄ oxidation activity by nitrifiers and activity by methanotrophs. A method by Bodelier and Frenzel (1999) involves CH₂F and C₂H₂ which are used to discriminate between methanotrophic and nitrifying bacteria activity on either CH₄ and NH₄⁺ oxidation. Similar methods are currently also being developed and tested by Dr. Alan Hooper (University of Minnesota) and Dr. Alan A. Dispirito (Iowa State University) (personal communication, Dr. Alan A. Dispirito).
Utilizing molecular tools to measure the microbial structure (and abundance) of both the CH₄ consuming and producing communities in conjunction with field CH₄ flux measurements and laboratory studies using inhibitors (Chan and Parkin, 2000a) to elucidate factors effecting the two primary processes may in totality ultimately provide crucial data and insight for the construction of a much needed mechanistic CH₄ flux model for non-flooded ecosystems.

References


