

Evidence for Anaerobic CH₄ Oxidation in Freshwater Peatlands

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This study involved *in vitro* assays of peat soil to investigate the occurrence, importance and potential mechanism(s) of anaerobic methane oxidation (AOM) in several northern peatlands ranging from ombrotrophic bog to minerotrophic fen. Although strong evidence suggests that AOM is linked to sulfate reduction in marine sediments, very little is known about AOM in freshwater systems such as northern peatlands, which have large methane (CH₄) production and are a significant source of atmospheric CH₄. Our results showed a mean net AOM rate of $17 \pm 2.6 \text{ nmol kg}^{-1} \text{ s}^{-1}$ with a maximum rate of $176 \text{ nmol kg}^{-1} \text{ s}^{-1}$ for a minerotrophic fen in central New York. AOM was demonstrated with three independent methods to verify our results: (a) additions of methanogenic inhibitors, (b) stable isotope enrichment (¹³C-CH₄), and (c) natural abundance stable isotope analysis (¹³C-CH₄). These experiments confirmed that AOM occurs simultaneously with methanogenesis, consumes a significant portion of gross CH₄ production, and significantly fractionates C isotopes ($\sim -12\%$). Experiments using a variety of potential electron acceptors demonstrated that Fe(III) and SO₄²⁻ are not quantitatively important, while the role of NO₃⁻ is uncertain and deserves more attention. The exact mechanism(s) for AOM in peat soils remains unclear; however the AOM rates reported in this study are similar to those reported for CH₄ production and aerobic CH₄ oxidation in northern peatlands, suggesting that AOM may be an important control on CH₄ fluxes in northern peatland ecosystems.

Keywords Anaerobic CH₄ oxidation, peat soil, wetland, archaea, electron acceptor, methanogenesis, stable isotopes, diffusion

INTRODUCTION

Northern peatlands are a large terrestrial carbon (C) sink, store approximately one third of global soil carbon (Gorham 1991), and represent a significant source of atmospheric methane (CH₄) (Fung et al. 1991; Prather et al. 1995). How C and CH₄

in these systems will respond to global environmental change is therefore a question of scientific and social concern (Moore et al. 1998; Wuebbles and Hayhoe 2002). Despite the importance of northern peatlands to the global CH₄ cycle, CH₄ fluxes between peatland soils and the atmosphere display a very broad range of values that can be explained, only in part, by environmental variables, such as temperature (Crill et al. 1993; Granberg et al. 2001), water table level (Moore and Knowles 1989; Moore and Roulet 1993), vegetation (Whiting and Chanton 1993; Bubier et al. 1995), and peat quality (Svensson and Sundh 1992; Yavitt et al. 2000). Although flux studies have provided important insight into spatial and temporal patterns, processes and mechanisms controlling these fluxes have received less attention and several questions regarding CH₄ cycling in northern peatlands remain (Segers 1998).

Conceptual models of CH₄ dynamics in peatlands start with the premise that atmospheric emissions are the balance between production by CH₄-producing archaea (methanogens) in anoxic portions of soils and sediments and oxidation by CH₄-consuming bacteria (methanotrophs) in the oxic portion (Whalen and Reeburgh 2000). Consequently, many process-based studies of CH₄ dynamics (e.g., Sundh et al. 1994; Yavitt et al. 1988) have used potential production and potential consumption assays to estimate net rates of anoxic production and oxic consumption. However, these approaches do not adequately explain variations in and constraints on peatland CH₄ emissions, and potential production rates often do not correlate well with fluxes (Yavitt et al. 1988; Smemo and Yavitt 2006). One reason for this discrepancy is the difficulty in measuring gross rates of CH₄ production. Indeed, production assays truly represent net production, suggesting that a more complicated dynamic might exist. Another process that could influence CH₄ cycling in freshwater wetlands is anaerobic CH₄ oxidation, yet the process is poorly understood and remains controversial (Kiene 1991).

Anaerobic oxidation of CH₄ (AOM) has long been viewed as the primary sink for CH₄ in marine systems, and most marine sediments exhibit net CH₄ oxidation (Hoehler et al. 1994; Valentine 2002). Although the microorganism(s) responsible

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have not yet been isolated and the exact mechanisms are not certain (Michaelis et al. 2002), recent studies (e.g., Boetius et al. 2000; Thomsen et al. 2001) suggest that the process is carried out by an organism(s) phylogenetically related to methanogens (Hallam et al. 2004) in a consortium with sulfate-reducing bacteria. Evidence for AOM in freshwater systems, however, is less convincing. Raghoebarsing et al. (2006) demonstrated that a related organism can carry out AOM in freshwater sediments in a consortium with denitrifying bacteria. Further evidence is limited to a few SO_4^{2-} -rich lakes (Panganiban et al. 1979; Iversen et al. 1987; Smith et al. 1993), one contaminated anoxic aquifer (Smith et al. 1991), experimental waste slurries (Malek and Weismann 1988; Islas-Lima et al. 2004), and a landfill leachate plume (Grossman et al. 2002), yet the reported data is limited and circumstantial. The only evidence of AOM in wetlands, that we are aware of, is a series of papers by Murase and Kimura (1994a, 1994b, 1994c) describing AOM in rice-paddy soils; however, reported rates were low and AOM was a small part of the total CH_4 budget. Thus, most authors have considered AOM quantitatively unimportant in freshwater wetlands even if it does occur (Topp and Pattey 1997).

One reason AOM in freshwater wetlands is controversial is that the terminal electron acceptor associated with this oxidation is unclear. With the exception of areas prone to acid rain or in watersheds containing acid SO_4^{2-} rocks, SO_4^{2-} concentrations in most freshwater systems are probably too little for the process to be thermodynamically favorable (Alperin and Reeburgh 1984). AOM via denitrification is a more favorable reaction (Raghoebarsing et al. 2006), but the process requires a well-developed population of denitrifying bacteria and abundant NO_3^- , which are lacking in most anoxic peat soils. Zehnder and Brock (1980) alternatively hypothesized an energetically favorable reaction where an unknown metal oxide served as the electron acceptor for AOM in Lake Mendota. Scott et al. (1998) demonstrated that humic substances in a variety of environments can act as electron acceptors during the oxidation of organic carbon by mediating the reduction of Fe (III), and others have proposed that Fe(III) could serve as the electron acceptor for AOM in freshwater wetlands (Murase and Kimura 1994a; Daniel et al. 1999).

However, electron acceptor identification is also complicated by inter-system electron acceptor availability related to trophic status. Although atmospheric deposition can strongly influence the inputs of N and S to wetland ecosystems, inputs of mineral elements are more related to groundwater. Thus, minerotrophic wetland systems connected to local groundwater tend to have greater concentrations of metals such as Fe and Mn, as well as NO_3^- and SO_4^{2-} . If AOM is related to groundwater inputs, then we expect the process to be more important in minerotrophic systems than in ombrotrophic systems that are not connected to local groundwater and depend on atmospheric inputs of nutrients and electron acceptors.

Here, we examine AOM in a variety of northern peatlands and test hypotheses pertaining to mechanisms, potential electron ac-

ceptors and the spatial extent of the process. We employed three independent methods to elucidate the process, evaluate the potential of Fe(III), NO_3^- and SO_4^{2-} as terminal electron acceptors and test the hypothesis that AOM rates decrease across a trophic gradient of peatland ecosystems. In particular, we hypothesized that (1) AOM is functioning simultaneously with CH_4 production resulting in underestimates of gross production, (2) AOM is linked to the oxidation and reduction of Fe, which serves as the electron acceptor for the process, and (3) AOM occurs in a variety of peatland types, but is quantitatively more important in minerotrophic systems that are linked to local groundwater and thus sources of potential electron acceptors.

METHODS

Study Sites

“Peatland” is a generic term used to describe wetlands with peat soil. Peatland ecosystems are common in northern latitudes and encompass a wide range of chemistry, hydrology and plant species. These variations, which range from truly ombrotrophic bogs to minerotrophic nutrient rich sedge-fens, influence biogeochemical cycles via differences in pH, redox, primary productivity, decomposition, and nutrient cycling (Bridgham et al. 1998). Most fens, for example, are minerotrophic (hydrologically open) and receive nutrients and other ions from groundwater. Bogs, on the other hand, are ombrotrophic (hydrologically closed) and rely on atmospheric deposition and internal cycling for nutrients (Wheeler and Proctor 2000). Since our initial hypotheses concerning AOM relate to the availability of potential electron acceptors, we focused most of our study on a nutrient rich riverine sedge-fen in New York State that has high rates of CH_4 production and emission in most years (Table 1), and for which we had previous anecdotal evidence for the process (Smemo and Yavitt 2006). In addition, we studied peatlands in Minnesota and Sweden. The American sites represent a gradient from minerotrophic fen to ombrotrophic bog and a variety of climates, ages and vegetation types. The Swedish site provided a similar trophic gradient within a single wetland complex, allowing us to control for differences in climate and substrate age. Each site has been studied previously, and Table 1 provides an overview of several site characteristics.

Michigan Hollow is a 15-ha minerotrophic riverine sedge-fen located in central New York State, USA, and is owned by the New York State Department of Environmental Conservation (Bernard and Macdonald 1974). The fen lies on the divide between the Susquehanna and St. Lawrence River watersheds. This site receives surface flow from the surrounding forested uplands and groundwater discharge. The site is characterized by distinct vegetation zones dominated by *Carex lacustris* L., *Typha latifolia* L., or *Juncus effusus* L.

Bog Lake and Junction Fen are poor fens located in North-central Minnesota, USA. Both wetlands occur on a glacial moraine landscape (Bridgham et al. 1998) in the United States Forest Service’s Marcell Experimental Forest. Another site,

TABLE 1
Study site characteristics. Unavailable data denoted as NA. Ion data given as porewater concentration.

Site	Location	Size (ha)	Functional classification	Mean annual precip (mm)	Mean annual temp (C)	Dominant plant species	pH	SO ₄ ²⁻ (mg L ⁻¹)	Fe(II + III) (mg L ⁻¹)	Mean summer CH ₄ flux (mmol m ⁻² s ⁻¹)
Michigan Hollow ¹	New York, USA (42°21'N, 76°28'W)	15	Minerotrophic fen	935	7.7	<i>Carex lacustris</i> , <i>Typha latifolia</i> , <i>Juncus effusus</i>	6	NA	160	284.32
Junction Fen ²	Minnesota, USA (47° 32'N, 93° 28'W)	NA	Poor fen	770	3	<i>Sphagnum spp.</i> , <i>Carex sp.</i> , <i>Vaccinium sp.</i>	4.5	NA	10	130
Bog lake ³	Minnesota, USA (47° 32'N, 93° 28'W)	10	Poor fen	766	3	<i>Sphagnum sp.</i> , <i>Carex sp.</i>	4.4	1-2	23.3	162.76
Alborn Fen ^{4,5}	Minnesota, USA (47° 00'N, 92° 34'W)	NA	Intermediate fen	691	3	<i>Sphagnum spp.</i> , <i>Carex sp.</i> , <i>Larix laricina</i>	5	NA	12.7	>500 ⁸
Stordalen ^{6,7}	Abisko, Sweden (68°22'N, 19°03'E)	25	Mixed-Mire	299	-0.7	<i>Sphagnum spp.</i> , <i>Carex sp.</i> , <i>Eriophorum angustifolium</i> , <i>Polytrichum commune</i> , <i>Vaccinium sp.</i> , <i>Cladonia sp.</i>	4	NA	22.7 -9.4	-0.35 -147.22

¹Smemo and Yavitt (2006). ²Dise (1991). ³Dise and Verry (2000). ⁴Santelmann (1991). ⁵Bridgham et al. (1998). ⁶Rosswall et al. (1975). ⁷Svensson et al. (1999). ⁸S. Bridgham (personal communication).

Alborn Fen, is also located in North-Central Minnesota in the highlands along the North Shore of Lake Superior (Bridgman et al. 1998). Ground cover at these sites is dominated by *Carex* sp. and *Sphagnum* sp. These wetlands have been previously described (Table 1).

Stordalen is a mixed-mire located in northern Sweden. The peatland is characterized by a mosaic of wetland types that differ in nutrient status, moisture, and vegetation (see Wheeler and Proctor (2000)). The peatland also has a discontinuous permafrost layer, which may be degrading (Malmer and Wallén 1996). This uneven permafrost distribution contributes to the mosaic of peatland types mentioned before (Svensson et al. 1999). We studied three sites representing a hydrologic gradient from wet to dry. The wet minerotrophic site did not have permafrost and the intermediate ombrotrophic site was, until recently, underlain by permafrost (Svensson et al. 1999). Both sites were dominated by *Sphagnum* mosses and *Carex* sp. The dry ombrotrophic site had permafrost present and was dominated by drier vegetation such as *Vaccinium* shrubs, *Cladonia* sp., *Polytrichum commune*, and lichens. Detailed studies of site characteristics and trace-gas dynamics can be found in Rosswall et al. (1975), Svensson and Rosswall (1984), and Svensson et al. (1999).

Sampling Methods

Peat was sampled by extracting cores using either a Russian style peat bore (8 cm barrel) or a PVC pipe (15 cm dia.). When possible, peat samples were extracted, placed directly into incubation jars and the headspace flushed with ultra-high purity N₂ in the field. When this method was not practical, peat samples were stored in sealed jars that were capped tightly and overfilled with porewater to remove excess air bubbles, and then transported immediately to the lab where they were sectioned, transferred to sterile jars, and flushed with N₂. The Minnesota peat samples were extracted with PVC cores (15 cm diameter, 60 cm length), saturated with porewater, sealed with air-tight rubber end caps and shipped overnight on ice to the lab at Cornell University where they were sectioned and transferred as above. Michigan Hollow peat was sampled monthly from April through October in 1998, 1999, 2000 and 2001, and was collected from three different sites that were characterized by different dominant vegetation. Minnesota samples were taken in July of 2001. All samples from Sweden were collected in September of 2001. Peat used in incubations was taken from depths of either 5–15 cm (surface) or 20–30 cm (deep). Surface peat data are reported unless denoted in table or figure.

Peat porewater was collected from the Michigan Hollow *Carex* site in the summer of 2000 and 2001 using a stainless steel porewater sampler that was connected to a 60 ml syringe fitted with a 3-way stopcock. Fifteen syringes were filled with O₂-free N₂ and the steel tube connected and flushed just prior to insertion into the peat. Approximately 60 ml of porewater was drawn into the syringe, and the stopcock closed before remov-

ing the collection tube. Syringes were then transported to the lab in a cooler with ice, transferred to sterile incubation jars and bubbled with N₂ for 10 minutes prior to being sealed.

Laboratory Assay Techniques

Our *in vitro* incubations were similar to those used by Yavitt et al. (1988). Intact peat (~150 g wet) or peat porewater (~150 ml) samples were placed in sterile ~1000 or ~250 ml Mason jars, flushed with N₂ and sealed with lids that were fitted with siliconed Swage-lok bulkhead fittings. A minimum of 4 replicates was used in each treatment. Gases and liquids were transferred through replaceable thermo-green butyl rubber septa (Supelco, Inc.) using a 20 ml syringe. Syringes were sealed before removal from the septa to maintain gas partial pressure. Prior to incubation experiments, jars were evacuated to -67 kPa and then over-pressurized with N₂ to 135 kPa. This process was repeated at least 10 times for each jar, with light shaking between cycles. Each jar was then overfilled with N₂ and slowly allowed to equilibrate to atmospheric pressure through a syringe filled with O₂-free DI-H₂O. In order to ensure anoxic conditions, jars were allowed to equilibrate for 24 hours to consume any remaining O₂ and the evacuation process was repeated prior to measurements.

CH₄ was then added to the headspace at varying concentrations, and the samples were incubated at room temperature (~25°C) in the dark to minimize potential photosynthetic production of O₂. Jars were shaken lightly for 30 seconds immediately following sample collection. We are very confident that this method maintains anoxic conditions, and we conducted several experiments using certified CH₄ standards, sulfurhexafluoride as a tracer, and stable isotopes that showed no statistically significant leakage over the incubation periods used in this study (*data not shown*).

Headspace gas samples were taken at 12–48 hour intervals and analyzed on a Varian 3400cx gas chromatograph (GC) equipped with a flame ionization detector (FID), a thermal conductivity detector (TCD) and dual Chromosorb 102 columns. Injection, detector and column temperatures were 130°C, 180°C, and 50°C, respectively. The FID was calibrated at each sampling period by analyzing triplicate external samples of certified 10, 100, 1000, and 10,000 μl l⁻¹ CH₄ standards. The equation of a linear line fit was used to estimate CH₄ concentrations and partial pressures. Reproducibility was ±25 μl l⁻¹ for standards of 10,000 μl l⁻¹ CH₄. The TCD was calibrated internally using certified standards (1,000 and 10,000 μl l⁻¹ CO₂).

Net vs. Gross Processes

We used 3 independent methods to quantify the difference between gross and net rates of AOM and methanogenesis. First, we used Bromoethanesulfonate (BES) and NO₃⁻ to inhibit methanogenesis. BES is known to inhibit the reductive demethylation of methyl-Coenzyme M (Müller et al. 1993), and has been commonly used in studies of microbial CH₄ cycling. BES was added as a 40 mM solution (Hoehler et al. 1994),

which was bubbled with O₂-free N₂ for 10 minutes and autoclaved prior to addition (20 mM final concentration). Although NO₃⁻ is not a specific methanogenic inhibitor, it is known to suppress methanogenic activity via the production of toxic intermediates (e.g., nitrite) during denitrification (Kluber and Conrad 1998; Roy and Conrad 1999). NO₃⁻ also is thought to serve as an alternate electron acceptor in AOM (Islas-Lima et al. 2004; Raghoebarsing et al. 2006), yet NO₃⁻ availability is low in anoxic peat soils (discussed later) and is biologically utilized rather quickly. NO₃⁻ was added as a sterile, anoxic, 20 mM solution (10 mM final concentration). Anoxic and autoclaved de-ionized water (DI-H₂O) was used as a control in each experiment. Inhibitors were added to incubations of *Carex*-, *Typha*- and *Juncus*-derived Michigan Hollow peat samples in July 1999, April 2000 and June 2001, and to all Minnesota peat samples in August 2001.

Second, we used a slightly modified version of an isotope dilution technique (von Fischer and Hedin 2002) to estimate gross rates of CH₄ production and AOM. Headspace CH₄ was enriched with pure ¹³C-CH₄ to bring the δ¹³C-CH₄ to approximately 1000‰ PDB (Pee Dee Belemite). The resulting headspace CH₄ samples were then divided for analysis using the GC methods already described and for isotopic analysis on a modified Europa Scientific ANCA TG and a Europa Scientific Geo 20-20 stable isotope ratio mass spectrometer (PDZ Europa). The sample volume was flushed into the system and carried by ultra-high purity helium under a pressure of 124 kPa. Due to the anoxic nature of our samples, standards and blanks were run with room air in order to maintain the oxidation state of the nickel and platinum catalyst utilized by the ANCA TG. Calibrations were made using 2 μl l⁻¹CH₄ with a δ¹³C of -40.95‰ PDB. Standard reproducibility was ±1‰. For a more detailed description of the technique and instrumentation refer to von Fischer and Hedin (2002).

By measuring the change in the mass of headspace CH₄ and the δ¹³C-CH₄ over time, and by assuming an accepted value of δ¹³C-CH₄ of “new” CH₄ from methanogenesis in peatlands of approximately -60‰ (Quay et al. 1988; von Fischer and Hedin 2002), we were able to use pool-dilution mixing model to estimate gross rates of CH₄ production. Gross consumption was then calculated as the difference between gross production and net flux. Because gross production is based on isotope dilution and assumes no consumption, some of our calculated rates of gross production were negative. This is important for our calculations, and we therefore left those numbers as negative in our tables.

A point of uncertainty in this method is the isotopic fractionation of C from AOM. We made our calculations based on the assumption that there was no isotopic fractionation of C during AOM. If this assumption is false then calculated rates of consumption were conservative and this error would not detract from our conclusions. Figure 1 provides a conceptual diagram of the expected relationship between headspace CH₄ mass and the isotopic composition of CH₄ as a function of time assum-

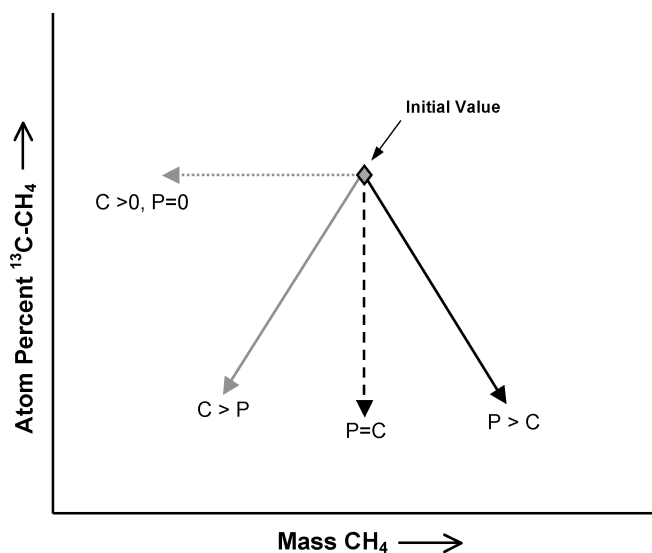


FIG. 1. Qualitative illustration of the expected relationship between headspace CH₄ mass and CH₄ isotopic composition as a function of time as a result of different gross rates of CH₄ production (P) and CH₄ consumption (C). Model assumes instantaneous isotopic fractionation during CH₄ production but no fractionation during CH₄ consumption. Adapted from von Fischer and Hedin (von Fischer and Hedin 2002).

ing strong isotopic discrimination during CH₄ production. This diagram provided the framework for interpreting our data and calculating gross process rates, as well as a qualitative estimate of the importance of production and consumption processes.

The technique was used in four different experiments. We applied the methods to Michigan Hollow *Juncus* derived peat ($n = 4$, blanks; $n = 8$, DI-H₂O treatment; $n = 8$, NO₃⁻ treatments) in October of 1999, and Michigan Hollow *Carex*-derived peat ($n = 4$, blanks; $n = 12$, all treatments) in October of 2000. In September of 2001, the technique was used on samples of Stordalen peat from three different sites and three depths ($n = 5$, control; and $n = 5$, each site at each depth). Controls in this case were autoclaved peat from the minerotrophic site. Headspace gas samples were stored in 10 ml glass serum vials with aluminum seals and rubber septa (Geomicrobial Technologies, Oechelata, OK) for 2 weeks and transported back to the lab at Cornell University. Our final isotope experiment, performed in October of 2001, used Michigan Hollow *Carex*-derived peat, Alborn Fen peat, and Stordalen minerotrophic peat ($n = 4$, site × control; $n = 4$, site × treatment). Controls in this experiment were acid-killed peat samples. Given the difficulty of a complete biological control (Brock 1978), particularly in intact saturated peat samples, we used several methods in this study with similar results.

Third, we measured the change in natural abundance stable isotopes during separate *in vitro* incubations of peat soils. Our experiment used *Juncus*-derived Michigan Hollow peat ($n = 6$, each treatment) with tank CH₄ (41.1‰ PDB) added (~3 kPa) to the incubation jars. CH₄ concentrations were measured as a function of time using a Varian 3400cx with an FID (see previous GC description). Subsamples were stored in 10 ml

glass serum vials and analyzed at the University of California, Irvine for carbon isotope ratios. Measurements of $\delta^{13}\text{C}\text{-CH}_4$ were made using a Finnigan MAT Model Delta Plus XP isotope ratio mass spectrometer. Sample measurement accuracy was $\pm 0.25\%$ (Chidthaisong et al. 2002).

Electron Acceptor Experiments

Anoxic peat samples were amended with NO_3^- , Fe(III), and SO_4^{2-} in order to evaluate the effect of electron-acceptors potentially involved in AOM. NO_3^- was added as 10 mM KNO_3 , SO_4^{2-} as 1 mM NaSO_4 , and Fe(III) as 10 mM poorly crystalline amorphous Fe(III)-oxide (Lovley and Phillips 1986). All solutions were bubbled with O_2 -free N_2 and autoclaved before being introduced to peat incubations via syringe and rubber septa. NO_3^- and Fe(III) were added to *Carex*-derived Michigan Hollow peat in April of 2001 ($n = 12$, each treatment), and to Minnesota peats in August of 2001 ($n = 4$, site \times depth \times treatment). SO_4^{2-} and Fe(III) were used in our final isotope experiment in October of 2001, which is described later. Measurements of Fe(II), Fe(III), and Fe^{total} in peat soil and porewater were made using the ferrozine assay described by Lovley and Phillips (1988).

Site vs. Electron Acceptor

In order to assess the combined effect of site differences and potential terminal electron acceptors on rates of AOM, as well as test our hypotheses related to peatland trophic status, we conducted an experiment using *Carex*-derived surface peat from Michigan Hollow, *Sphagnum/Carex*-derived surface peat from Alborn Fen, and minerotrophic *Sphagnum*-derived surface peat from Stordalen. Replicates were all amended with CH_4 (including $^{13}\text{C}\text{-CH}_4$ enrichment), and consisted of biological controls (acid-killed), $\text{DI-H}_2\text{O}$ controls, Fe(III) additions, and SO_4^{2-} additions ($n = 4$, site \times treatment). This experiment was conducted in October 2001, and the methods used were described before.

Data Analysis

We analyzed our data in two ways to test for treatment effects and to assess differences in production and/or consumption rates. Treatment effects were compared using the general linear model and analysis of variance procedures (ProcGLM) in SAS version 8.2. As we expected, measurements of microbial CH_4 cycling were highly variable. To deal with this inherent variability, we tested for rate differences between treatments and replicates by using the comparison of two or more regression functions procedure outlined by Neter et al. (1996), and a goodness of fit test in Minitab version 12. This method allowed us to compare the slopes of lines fitted to rate data and determine if slopes were significantly different from each other or zero. All process rates are given in terms of net flux, with oxidation or consumption given as negative numbers.

RESULTS

Net CH_4 Production vs. $[\text{CH}_4]$

Michigan Hollow peat collected from 1998 to 2001 exhibited significant ($p < 0.01$) net CH_4 oxidation when incubated under anoxic conditions with ~ 1 kPa headspace $[\text{CH}_4]$. Several experiments with fresh peat samples conducted over the four-year period resulted in a mean ($n = 350$) headspace CH_4 oxidation rate of -17 ± 2.6 nmol kg^{-1} (dry peat) s^{-1} with a maximum net oxidation rate of -176 nmol kg^{-1} s^{-1} . Much of the variation in observed rates of net oxidation was related to differences in vegetation zone sampled and time of year (Smemo and Yavitt 2006). Incubations using peat from the 3 different vegetation zones in late spring 1999 illustrated this spatial pattern and demonstrated that rates of AOM varied as a function of CH_4 concentrations (Figure 2). CH_4 production rates were consistently higher in incubations without CH_4 additions than in those with CH_4 added, and net consumption was common when CH_4 additions were ~ 1 kPa or greater. This pattern was robust; an experiment conducted over a 72-hour period with 28 jars of homogenized *Typha*-derived peat and random concentrations of CH_4 (ranging from 0.17 to 1.35 kPa) added to the headspace of each jar showed a strong negative linear relationship ($r^2 = 0.81$) between the net rate of CH_4 production and the amount of CH_4 available for oxidation. Greater $[\text{CH}_4]$ resulted in lower production rates, suggesting that net CH_4 production (production–consumption) was less due to the increasing importance of AOM.

We created an enrichment time-series using fresh *Carex*-derived peat collected in the autumn of 1998. Repeated additions of methane every 2 days for 10 days (the headspace was purged entirely before each new CH_4 addition) demonstrated that the net flux rate was more negative (from ~ -25 to -115 nmol kg^{-1} s^{-1} ; $p < 0.001$) over time with repeated additions of CH_4 (1 kPa

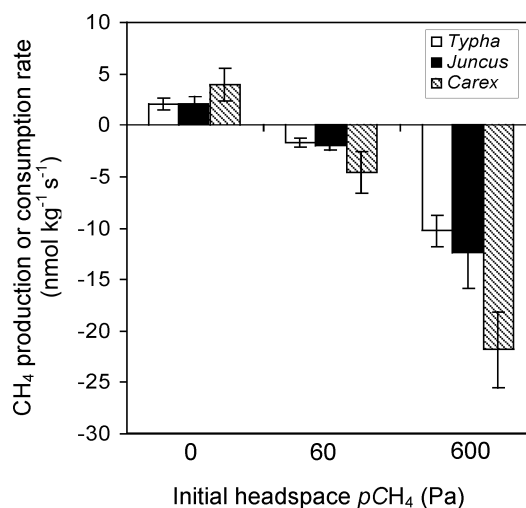


FIG. 2. Response of fresh Michigan Hollow peats to varying headspace $p\text{CH}_4$. Bars represent the mean rate ($n = 4$) of net CH_4 production or consumption from *Juncus*, *Typha*, and *Carex*-derived peat incubated at three initial headspace CH_4 concentrations. Error bars are \pm SE.

TABLE 2
Results and calculations from stable isotope pool-dilution experiment using Michigan Hollow peat samples without CH₄ additions.

Sample ¹	Net headspace flux (ng hr ⁻¹)	Gross consumption (ng hr ⁻¹)	Gross production (ng hr ⁻¹)	Calculated mass produced (mg) ^{2,3}	Measured mass produced or consumed (mg) ³	% of production consumed
Control ⁴	0.00	0.00	0.00	-0.01	0.04	—
Peat + DI A	-3.95	3.30	-0.65	-0.14	-0.75	425.3%
Peat + DI B	2.45	4.09	6.54	1.30	0.48	63.3%
Peat + DI C	1.67	3.03	4.70	0.91	0.31	65.4%
Peat + NO ₃ ⁻	-0.45	0.66	0.22	0.04	-0.06	268.47%

Net headspace flux and measured mass produced or consumed are measured numbers, while others are calculated estimates. Bolded columns emphasize the discrepancy between measured versus calculated values: the difference was then used to calculate % of gross production consumed. Gross consumption rate is simply the difference between gross production and net flux. Controls are empty sterilized jars.

¹Samples that had a $r^2 > 0.6$ for headspace CH₄ flux and ¹³C flux. ²Based on a $\delta^{13}\text{C-CH}_4$ of -60‰ for CH₄ production. ³Calculated for the entire incubation time of 180 hours. ⁴Non-zero values due to instrument drift.

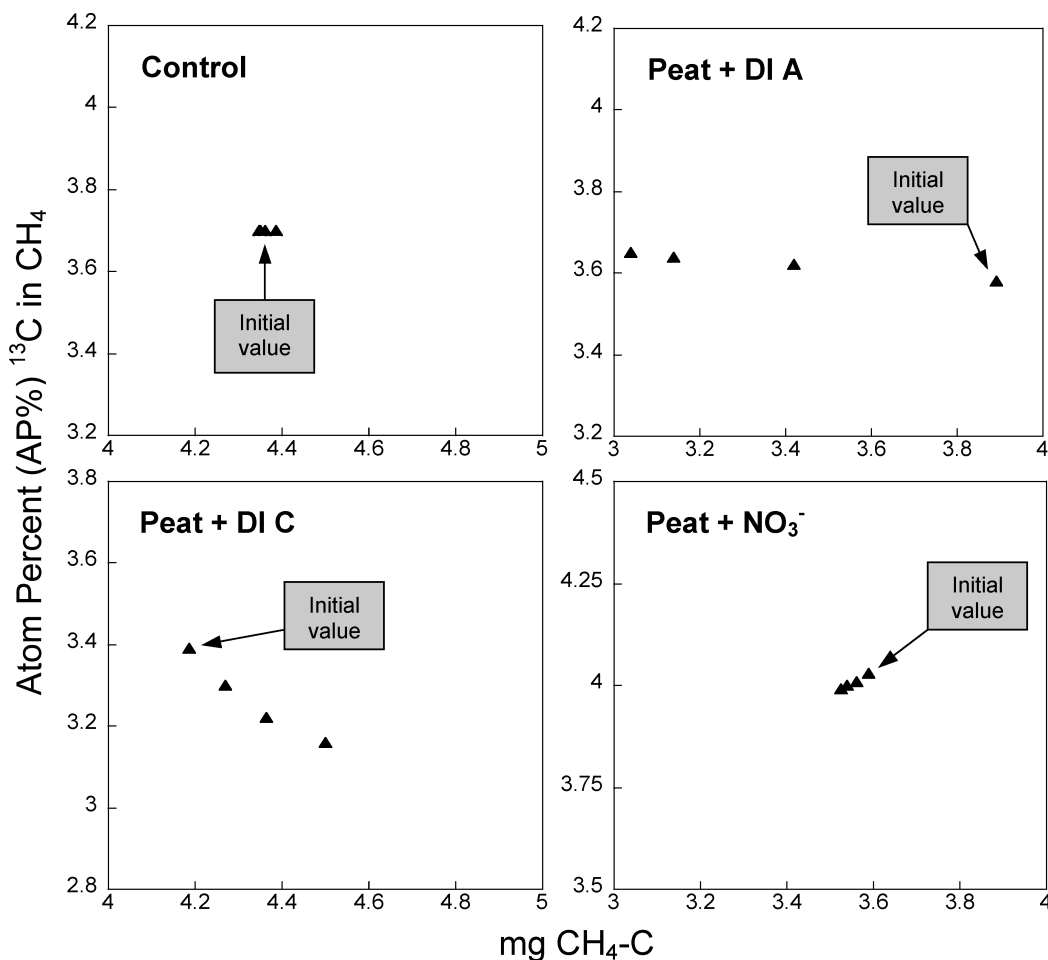


FIG. 3. Results from stable isotope pool-dilution experiment with *Typha*-derived Michigan hollow peat with DI, NO₃⁻, and control (sterilized blanks) treatments. Each graph represents an individual incubation from Table 2. Triangles represent time point measurements (t = 0, 60, 120 and 180 hours) of AP% ¹³C in CH₄ and mass of CH₄-C (mg) over an incubation period of 180 hours. Initial values correspond to t = 0.

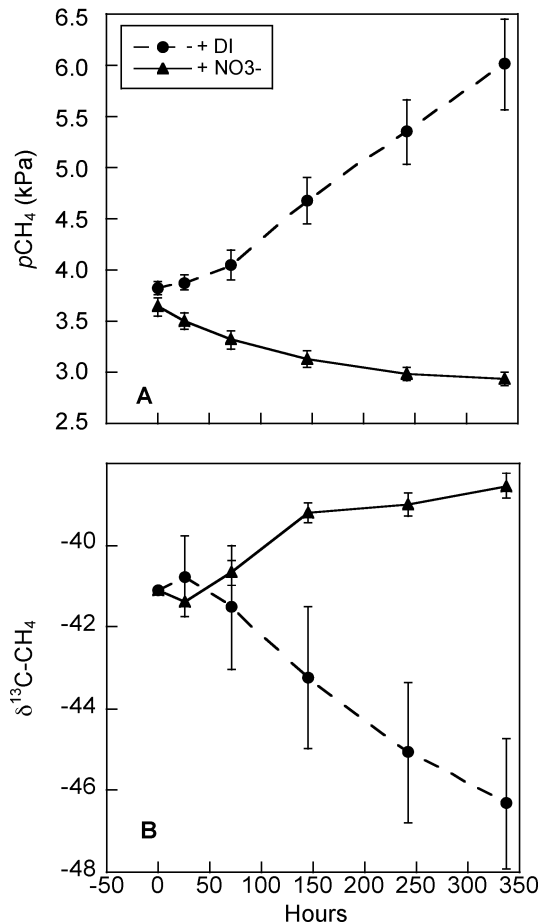


FIG. 4. Analysis of natural abundance $\delta^{13}\text{C-CH}_4$ in the headspace of anoxic CH_4 incubations of Michigan Hollow peat amended with nitrate (NO_3^-) as a methanogenic inhibitor and de-ionized water (DI- H_2O) as a control. **A** is the mean ($n = 6$) headspace CH_4 concentration \pm SE as a function of time, and **B** is the mean ($n = 6$) $\delta^{13}\text{C-CH}_4$ PDB \pm SE as a function of time.

CH_4 in headspace). This pattern contrasted consistent measurements of positive net CH_4 flux in replicates receiving no CH_4 additions. This suggests that the process is indeed biological, and that increased CH_4 consumption rates are associated with the growth of a population(s) of anaerobic CH_4 oxidizers and not decreased CH_4 production in response to methanogenic substrate depletion.

Net vs. Gross Processes

Inhibition experiments without CH_4 enrichment demonstrated that methanogenesis and AOM occurred simultaneously in both *Carex* and *Typha*-derived peat. DI- H_2O controls consistently exhibited a mean net CH_4 production rate of 15.99 ± 4.68 $\text{nmol kg}^{-1} \text{ s}^{-1}$ in *Carex*-derived peat and 4.09 ± 1.73 $\text{nmol kg}^{-1} \text{ s}^{-1}$ in *Typha*-derived peat, whereas additions of BES resulted in significant ($p < 0.05$) mean net CH_4 oxidation rates of -1.85 ± 1.02 $\text{nmol kg}^{-1} \text{ s}^{-1}$ (*Carex*) and -4.15 ± 2.05 $\text{nmol kg}^{-1} \text{ s}^{-1}$ (*Typha*). Additions of NO_3^- also resulted in net oxidation rates

of 1.82 ± 1.66 $\text{nmol kg}^{-1} \text{ s}^{-1}$ (*Carex*) and -2.38 ± 1.84 $\text{nmol kg}^{-1} \text{ s}^{-1}$ (*Typha*). The stable isotope pool-dilution technique confirmed simultaneous methanogenesis and AOM (Table 2). Two of the samples with DI- H_2O added showed net CH_4 production, whereas the other sample showed net CH_4 oxidation. Despite net CH_4 production in two of the samples, stable isotope pool-dilution calculations indicate that more CH_4 was produced than accumulated in the headspace, with up to 65% of gross CH_4 production being consumed (Table 2 (bolded columns)). The DI- H_2O sample with net CH_4 oxidation consumed $4\times$ more CH_4 than was internally produced. One sample with NO_3^- showed little measured change in mass but a decrease in $\delta^{13}\text{C-CH}_4$. The blank sample showed no significant change in CH_4 -C mass or $\delta^{13}\text{C-CH}_4$. In addition, plots of atom percent (AP%) $^{13}\text{C-CH}_4$ against CH_4 -C mass over time (Figure 3) showed expected trajectories as depicted in our conceptual model.

Based on a third method, patterns of natural abundance $\delta^{13}\text{C-CH}_4$ in relation to headspace $[\text{CH}_4]$ as a function of time showed an expected decrease in the $\delta^{13}\text{C-CH}_4$ as the CH_4 pool was diluted with isotopically light CH_4 from methanogenesis (Figures 4a and 4b). In contrast, the samples with NO_3^- added as a methanogenic inhibitor exhibited an increase in the $\delta^{13}\text{C-CH}_4$ as net CH_4 consumption occurred, providing evidence for C isotope fractionation during AOM.

Electron Acceptor

We expanded our use of the enrichment time-series to examine the long-term effects of repeated CH_4 additions on AOM. *Carex*-derived peat samples ($n = 16$) that were incubated in an enriched CH_4 headspace (1.5 kPa) for 10 days, in order to attain maximum net oxidation rates, were then incubated for 3 consecutive 5-day periods with either DI- H_2O (control; $n = 8$) or NO_3^- (inhibitor; $n = 8$). The headspace was flushed and new CH_4 (1.5 kPa) was added at the end of each incubation period. Over the three incubation periods (15 days), net CH_4 production rates increased significantly ($p < 0.05$) from -4.08 ± 0.45 to 2.54 ± 0.18 $\text{nmol kg}^{-1} \text{ s}^{-1}$ in the DI- H_2O -treatment and from -5.22 ± 0.98 to 1.26 ± 0.29 $\text{nmol kg}^{-1} \text{ s}^{-1}$ in the NO_3^- treatment. Thus, high rates of AOM in *Carex*-derived peat were not sustained in the long term, suggesting a potential "exhaustion" of terminal electron acceptor(s) and a lack of electron acceptor "replenishment" in a closed system. Additionally, anoxic incubations of peat porewater from Michigan Hollow ($n = 15$) consumed 361.3 ± 37.5 $\mu\text{Moles l}^{-1}$ of CH_4 on average over an 8-day period. This indicated that the process can be supported outside of the peat matrix and that the terminal electron acceptor was available in the porewater. Moreover, the same pattern of electron acceptor "exhaustion" was seen in the porewater samples. These results led us to conduct a series of experiments using additions of potential terminal electron acceptors to stimulate or maintain net rates of AOM.

The addition of Fe(III), as a potential electron acceptor, to anoxic incubations of *Carex*-derived peat had no apparent effect

TABLE 3

Mean (\pm SE) calculations from Michigan Hollow peat ($n = 3$, blanks; $n = 9$, DI; $n = 12$, Fe) isotope pool-dilution experiment with Fe(III) addition and CH₄ enrichment (~ 1 kPa headspace [CH₄]).

Treatment	N	Net headspace flux (ng hr ⁻¹)	Gross consumption (ng hr ⁻¹)	Gross production (ng hr ⁻¹)	Calculated mass produced (mg) ^{2,3}	Measured mass produced or consumed (mg) ³	% of production consumed
Control ⁴	3	0.89 (0.62)	0.0 (0)	0.00 (0)	0.00 (0.00)	0.09 (0.06)	–
DI	9	7.46 (0.54)	11.75 (1.29)	19.20 (1.74)	2.13 (0.22)	0.72 (0.05)	65.2% (1.9%)
Fe(III)	11	7.06 (0.53)	11.02 (0.71)	18.08 (1.19)	1.97 (0.14)	0.68 (0.05)	65.4% (1.0%)

¹Treatment means for samples that had a $r^2 > 0.6$ for headspace CH₄ flux and ¹³C flux.

²Based on a $\delta^{13}\text{C-CH}_4$ of -60‰ for CH₄ production.

³Calculated for the entire incubation time of 96 hours.

⁴Small amount of production measured in controls due to slight GC drift.

Fe(III) and DI treatments are not significantly different. Bolded columns emphasize the discrepancy between measured versus calculated values, which were used to calculate % of production consumed. Controls are empty sterilized jars.

on net CH₄ production in incubations with or without CH₄ enrichment. Measurements of Fe(II) and Fe(III) concentrations in *Carex*-derived peat porewater following 96 hours of incubation ($n = 12$) indicated that nearly all of the 50 mM Fe addition was reduced to Fe(II) over the incubation period. Nevertheless, there was no apparent effect on CH₄ dynamics and no significant increase in net CO₂ production. Using the stable isotope pool-dilution model, an additional experiment with Fe(III) and ¹³C-CH₄ enrichment showed that Fe(III) had no apparent effect on the cycling of CH₄ or the fractionation of C isotopes (Table 3). Pool-dilution calculations did support our previous results that AOM occurred simultaneously with CH₄ production (Table 3).

Other Sites

Sphagnum (Bog lake and Junction Fen) and *Sphagnum/Carex* (Alborn Fen)-derived peat from north-central Minnesota, incubated with a CH₄-enriched (~ 1 kPa) headspace, showed highly variable responses to additions of DI-H₂O, NO₃⁻, and Fe(III) (Table 4). Only surface peat from Junction Fen showed net CH₄ oxidation in the DI controls. With the exception of the deep sample at Bog Lake, additions of NO₃⁻ significantly ($p < 0.05$) suppressed methane production compared to DI controls and in most cases resulted in net CH₄ oxidation. Fe(III) significantly

($p < 0.05$) stimulated net CH₄ production compared to DI controls in surface samples at Bog Lake and Junction Fen. Net CH₄ production was suppressed by Fe(III) additions in the deep peat at Junction Fen, but did not significantly alter CH₄ cycling in Alborn Fen or the deep peat at Bog lake.

In peat from Sweden, AOM was more associated with peat depth than with trophic status, yet CH₄ was cycled at significantly greater rates in the minerotrophic site than in the drier, more ombrotrophic sites. No significant net AOM was measured in intermediate and ombrotrophic samples. Mean CH₄ production rates were -5.0 ± 22.3 (surface) and 12.5 ± 8.0 (deep) nmol kg⁻¹ s⁻¹ in the intermediate peat and 25.7 ± 5.2 (surface) and 7.5 ± 17.3 (deep) nmol kg⁻¹ s⁻¹ in the ombrotrophic peat. In the minerotrophic site, younger surface peat had a mean net CH₄ production rate of 57.1 ± 16.3 nmol kg⁻¹ s⁻¹ while a mean net CH₄ consumption rate of -82.8 ± 28.3 nmol kg⁻¹ s⁻¹ was measured in deep older peat. However, stable isotope pool-dilution calculations for individual peat samples revealed a more complicated pattern. A significant portion of gross production ($\sim 35\%$) was still lost to AOM in surface minerotrophic peat. Surface peat from the dry ombrotrophic site showed little activity at all, but calculations estimated $\sim 30\times$ more CH₄ was consumed than produced. Deep sample CH₄ dynamics also showed disparities

TABLE 4

Mean (\pm SE) anoxic CH₄ production/consumption rates (nmol kg⁻¹ s⁻¹) for Minnesota peat incubations ($n = 4$) amended with DI-H₂O, NO₃⁻, or Fe(III).

Treatment	Bog Lake		Junction Fen		Alborn Fen	
	Surface	Deep	Surface	Deep	Surface	Deep
DI	20.8 (9.9)	10.0 (13.8)	-13.4 (3.8)	62.5 (9.1)	28.0 (7.7)	-1.5 (0.7)
NO ₃ ⁻	-21.0 (5.9)	2.4 (8.2)	-22.1 (4.2)	-18.0 (4.1)	-4.0 (6.7)	-15.4 (6.2)
Fe(III)	66.4 (14.8)	7.3 (7.2)	60.5 (26.4)	20.8 (4.8)	18.6 (7.6)	-2.1 (0.5)

All headspaces were enriched with CH₄ (~ 1 kPa).

between measured and predicted CH_4 production and suggested that despite low apparent net flux rates, CH_4 might be turning over rapidly in these sites. Underestimates of CH_4 production in the minerotrophic samples further suggested that C isotope fractionation during AOM is significant. Nevertheless, the data from Sweden does not provide evidence for generalizable patterns with respect to peat depth or across the trophic gradient.

Site vs. Electron Acceptor

Michigan Hollow peat exhibited net consumption in the SO_4^{2-} and Fe(III) treatments compared to DI-controls and sterilized peat, with the highest significant ($p < 0.05$; compared to DI-controls) mean rate of consumption in the SO_4^{2-} treatment (Fig. 5). Stordalen peat had the highest rates of CH_4 production, with no apparent consumption or significant treatment effect. In Alborn Fen peat, the SO_4^{2-} and Fe(III) treatments were significantly different ($p < 0.05$) than the DI-control but not significantly different from sterile peat.

The isotope pool-dilution results from Michigan Hollow agree with the headspace CH_4 production/consumption data. Both DI treatment samples showed net CH_4 production yet a significant portion of the CH_4 produced was consumed (Table 5 (bolded columns)). The SO_4^{2-} and Fe(III) treatment samples that exhibited net consumption had calculated gross consumption rates 5x that of production rates. Alborn Fen samples, with the exception of the sterile control and one SO_4^{2-} sample, showed low rates of net CH_4 production. The importance of AOM appeared to be small in most samples and the overall effect of SO_4^{2-} on net flux was related to suppression of gross CH_4 production. Stordalen samples possessed a similar pattern, yet AOM was quantitatively more important. DI and Fe(III) treatments showed net CH_4 production, and pool-dilution calculations over-

estimated the amount of CH_4 produced indicating some AOM. Although SO_4^{2-} -amended samples exhibited net production, calculations imply that AOM was important in that treatment (Table 5). In one sample, pool-dilution underestimated the amount of CH_4 produced, suggesting a fractionation effect from AOM.

DISCUSSION

Evidence for AOM in Peatlands

AOM in marine sediments (e.g., Boetius et al. 2000) consumes from 20-100 (Reeburgh 1989) to 300 (Hinrichs and Boetius 2002) $\text{Tg CH}_4 \text{ yr}^{-1}$, despite having very slow rates. Measured rates of AOM in freshwater systems by Grossman et al. (2002), Murase and Kimura (1994c), Smith et al. (1991) and Smith et al. (1993) are similarly slow, with high rates in the range of 0.1-10 $\text{pmol kg}^{-1} \text{ s}^{-1}$. The *in vitro* rates we present here for peat-forming wetlands are generally in the range of 1-100 $\text{nmol kg}^{-1} \text{ s}^{-1}$. This does not suggest that AOM is that much more important in peatlands, only that AOM rates reflect the relatively higher $p\text{CH}_4$ found in many anoxic peat soils.

The fact that AOM has not been reported in past laboratory studies of CH_4 dynamics in freshwater peat soils is not surprising and could be explained by inherent methodological limitations. Most laboratory assays begin with little or no CH_4 in the headspace and AOM is less favorable under low $[\text{CH}_4]$ (Sørensen et al. 2001). These conditions do not reflect *in situ* conditions where aqueous peatland porewater CH_4 concentrations can exceed 30 kPa (Smemo and Yavitt 2006). We suspected AOM was occurring in previous assays measuring CH_4 production without CH_4 additions where headspace CH_4 concentrations reached a threshold and oxidation rates began to exceed production rates (Smemo 2003), and Malek and Weismann (1988) reported a similar pattern of cyclic shifts from net anoxic CH_4 production to net anoxic consumption. The relationship between AOM rates and $[\text{CH}_4]$ shown here ($r^2 = 0.81$) exceeds what we would expect from a simple demonstration of Fick's law and is further refuted by our demonstration of AOM exhaustion over time. Although others have shown that AOM rates vary with $[\text{CH}_4]$ (Iversen et al. 1987; Smith et al. 1991), we took a different approach and examined the effect of $[\text{CH}_4]$ on CH_4 production in samples displaying net production. Because methanogenesis does not exhibit end-product inhibition (Zehnder and Brock 1980), increasing headspace $[\text{CH}_4]$ should have no effect on production rates. Our results showed this was not the case, suggesting that decreased net production was caused by increased gross consumption rates.

No anaerobic CH_4 oxidizer has yet been isolated or grown in pure culture (Valentine and Reeburgh 2000). Nevertheless, we were able to create an enrichment time-series for an anaerobic CH_4 oxidizer(s) in intact Michigan Hollow peat samples, although the actual manner in which CH_4 is being used is unclear. Studies in marine systems have shown that CH_4 can be used as an electron donor in sulfate-reduction or as an organic carbon source used for structural growth (Michaelis et al. 2002).

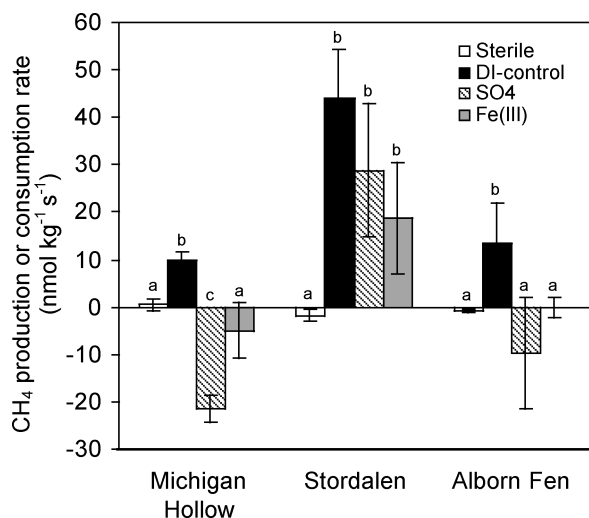


FIG. 5. Effect of electron acceptors (SO_4^{2-} , Fe(III)) vs. sterilized peat and DI- H_2O controls on mean ($n = 4$) net CH_4 production or consumption rates \pm SE for anoxic incubations of Michigan Hollow (*Carex*-derived), Alborn Fen, and Stordalen (minerotrophic) peat. Bars with different letters within site are significantly different at $p < 0.05$.

TABLE 5

Results and calculations from across site stable isotope pool-dilution experiment using alternate electron acceptors (SO₄²⁻ and Fe(III)) and peat from Michigan hollow, Alborn Fen (Minnesota), and Stordalen (minerotrophic).

Site	Treatment ¹	Net headspace flux (ng hr ⁻¹)	Gross consumption (ng hr ⁻¹)	Gross production (ng hr ⁻¹)	Calculated mass produced (mg) ^{2,3}	Measured mass produced or consumed (mg) ³	% of production ⁴ consumed
Michigan							
Hollow	⁵ Sterile control	-0.64	0.00	0.00	-0.05	-0.04	—
	DI	4.48 (0.58)	-6.99 (0.87)	1 1.47 (0.29)	1.26 (0.06)	0.45 (0.05)	63.7% (5.3)
	SO ₄	-6.65 (2.13)	-7.79 (2.42)	1.14(0.29)	0.15(0.03)	-0.67 (0.23)	535.8% (75.2)
	Fe(III)	-3.31 (2.09)	-7.33 (2.22)	4.02 (0.13)	0.47(0.01)	-0.34 (0.23)	172.2% (48.0)
Stordalen							
	Sterile control	-1.07	0.00	0.00	0.01	-0.01	—
	DI	3.22 (0.72)	-1 .08 (0.72)	4.31 (0.01)	0.47 (0.00)	0.35 (0.08)	26.5% (17.5)
	SO ₄	2.89 (1.48)	0.57 (0.99)	2.32 (0.49)	0.27 (0.06)	0.33 (0.17)	-15.9% (37.7)
	Fe(III)	1 .55 (0.62)	-0.86 (0.99)	2.41 (0.55)	0.25 (0.05)	0.16 (0.06)	40.4% (10.8)
Alborn							
	DI	1.71 (0.23)	-0.36 (0.06)	2.06 (0.17)	0.27 (0.02)	0.18 (0.03)	18.7% (4.7)
	SO ₄	-1.35(2.31)	-2.54(2.11)	1.19(0.20)	0.14 (0.00)	-0.13 (0.23)	195.6% (163.6)
	Fe(III)	1 .28 (0.07)	-0.15(0.27)	1 .43 (0.20)	0.15 (0.01)	0.13 (0.02)	9.3% (19.0)

All headspaces were enriched with CH₄ (~1 kPa). Values are mean calculations (n = 2, treatments; n = 1, controls) with SE in parentheses. Bolded columns emphasize the discrepancy between measured versus calculated values, which were used to calculate % of production consumed. Sterilized peat and DI-H₂O additions were both used as controls.

¹Treatment means for samples that had a r² ≥ 0.6 for headspace CH₄ flux and ¹³C flux.

²Based on a δ¹³C-CH₄ of -60‰ for CH₄ production.

³Calculated for the entire incubation time of 106 hours.

⁴Negative values due to measured mass greater than calculated mass.

⁵Small amount of production or consumption measured in controls likely due to slight GC and MS drift, and/or incomplete kill.

Aerobic methanotrophy in freshwater systems leads to the incorporation of CH₄-C into microbial biomass (Hanson and Hanson 1996). Data for freshwater systems is limited, but correlative evidence suggests that a significant fraction of CH₄ is being oxidized to CO₂ (Smemo 2003), yet further experiments with ¹⁴C are warranted to trace the flow of CH₄.

Gross vs. Net Processes

One common approach to separating gross and net processes is to use chemical inhibitors to impede one process while measuring another. We used both NO₃⁻ and BES to inhibit methanogenesis and were successful at measuring increased net CH₄ oxidation rates when compared to controls, or in many cases net oxidation compared to net CH₄ production in controls. Our inhibition experiments provided useful insights. Nevertheless, the physiology and biochemistry of anaerobic CH₄ oxidizers is not known and we are uncertain whether the inhibitors acted specifically on anaerobic methanotrophs or if CH₄ production was completely inhibited. Given the complex structure of peat soil and the spatial variability of microbial populations, it was difficult to believe that inhibition would be 100% successful. Hence, we may still have underestimated rates of AOM. Anaerobic

methanotrophs and methanogens also might be phylogenetically and ecologically similar, thus confounding the use of biochemical inhibitors. Zehnder and Brock (1979) first hypothesized that AOM is carried out by methanogens "running in reverse," and current evidence from marine systems has shown that the organism responsible for the process is closely related to modern methanogens (Boetius et al. 2000; Valentine and Reeburgh 2000; Hallam et al. 2004). Furthermore, Kruger et al. (2003) demonstrated that an enzyme similar to but distinct from methyl coenzyme M reductase is abundant in natural populations of anaerobic methane oxidizers. BES might specifically inhibit this enzyme.

Our inhibition experiments were in agreement with Alperin and Reeburgh (1985), suggesting that AOM does occur when methanogenic inhibitors are added. It is possible that the 'biochemical machinery' used by methanogens to produce CH₄ is different from that involved in CH₄ oxidation, and that Coenzyme M may not be involved in the process. This would explain why we measured significant oxidation even when BES was added to incubations. Results from NO₃⁻ addition experiments also demonstrated that an organism could carry out the process without being affected by de-nitrification intermediates,

suggesting that a methanogen could still be responsible for AOM if these toxic intermediates interfere with enzymes involved in CH_4 production (Roy et al. 1999) but not those involved in the oxidation process.

Previous studies in freshwater systems have used mass balance approaches (Smith et al. 1993; Murase and Kimura 1994c; Grossman et al. 2002), natural abundance stable isotope techniques (Grossman et al. 2002), and transfer of C isotopes from CH_4 to CO_2 (Panganiban et al. 1979; Smith et al. 1991) to estimate the occurrence and rate of AOM. The stable isotope pool-dilution technique that we utilized estimates gross consumption rates directly, and our study has been the first to quantify co-occurring CH_4 consumption and methanogenesis in an anoxic peat soil. The discrepancy between the measured change in headspace CH_4 concentration and the calculated rates for gross production can only be reconciled by either AOM or unrealistic ^{13}C isotope discrimination during methanogenesis ($\alpha \approx -2$). Previous studies have found ^{13}C fractionation from methanogenesis ranging from $\alpha = 1.007$ to 1.08, depending on the carbon source and metabolic pathway (Valentine et al. 2004). These observations further support the conclusion that the CH_4 is being consumed biologically.

Initially, we assumed no measurable C fractionation during AOM in peat soils, yet we provide evidence to the contrary (Figures 3 and 4). Reports from the literature have shown less isotopic discrimination during AOM than for methanogenesis (e.g., Grossman et al. 2002). Values for aerobic CH_4 oxidizing bacteria are in the range of -15 to -24% (Coleman et al. 1981; Quay et al. 1988), whereas estimates for AOM were $\sim -13\%$ in a landfill leachate plume (Grossman et al. 2002), -2 to -14% in marine and brackish sediments (Whiticar and Faber 1986), and -8.8% in marine sediments (Alperin et al. 1988). In contrast, Smith et al. (1993) found that AOM in the water column of an Antarctic lake resulted in no discernible enrichment of the $^{13}\text{CH}_4$ pool. Our results agree qualitatively with Grossman (2002) and Alperin (1988), with an average C isotope discrimination of -12.4% ($\pm 1.5\%$; $N = 6$). This estimate was based on the relationship between the change in CH_4 mass and CH_4 isotopic composition over time that was illustrated in our conceptual model (Fig. 1). Fractionation estimates were then back-calculated using the isotope mixing-model. However, we report this number with caution given the uncertainties in our assumptions such as the exact $\delta^{13}\text{C}-\text{CH}_4$ of CH_4 produced. We used an average $\delta^{13}\text{C}-\text{CH}_4$ of CH_4 produced value of -60% (Quay et al. 1988; von Fischer and Hedin 2002). This assumption is discussed below in the methodological considerations section.

Electron Acceptor

We demonstrated significant AOM in Michigan Hollow peat incubated as a closed system with constant CH_4 additions. When net rates of AOM reached a threshold, continuous CH_4 additions yielded decreasing net oxidation rates and ultimately net production. This suggests that the process can be electron acceptor

limited if the electron acceptor is not replenished. In freshwater wetlands, redox status can change temporally on scales ranging from minutes to years, while climatic factors and underlying hydrology can influence the supply of potential electron acceptors. It is possible that environmental conditions conspire to provide an annual and/or seasonal mechanism for electron acceptor depletion and replenishment, thus explaining the spatial and temporal AOM patterns that we observed.

We originally hypothesized that NO_3^- was the terminal electron acceptor in AOM because increased oxidation rates were observed when NO_3^- was added. This made sense thermodynamically, and recent evidence has suggested that AOM is linked to denitrification (Islas-Lima et al. 2004; Raghoebarsing et al. 2006). However, our stoichiometric calculations indicate that NO_3^- additions were not sufficient to account for the total amount of CH_4 consumed in our experiments. The measured increase in AOM rates was due in part to inhibition of CH_4 production and resulted in net oxidation. In addition, NO_3^- availability would be ephemeral in most peatlands due to fluctuating hydrology, rapid denitrification, and lack of nitrification under anoxic conditions.

The importance of Fe as a terminal electron acceptor for organic C mineralization in anoxic aquatic systems has been shown in a variety of studies (e.g., Scott et al. 1998). Zehnder and Brock (1980) were the first to suggest that AOM might be carried out via a metal-oxide electron acceptor such as Fe or Mn. Michigan Hollow porewater has high concentrations of visible flocculated Fe(III) in the spring and early summer during wet years. We also observed that CH_4 could be oxidized anaerobically in Fe(III) rich porewater from Michigan Hollow, but that the process weakened over time as the color of the porewater changed from orange to gray. However, we were unable to stimulate AOM with additions of amorphous Fe(III). The Fe(III) we added to our incubations of Michigan Hollow peat was clearly reduced in the peat, yet it was not necessarily microbially reduced. Peat is an electron-rich environment, and Fe(III) could accept electrons from the peat and be chemically reduced at a rapid rate, thereby explaining the observed Fe-reduction. Fe additions in experiments from other sites showed similar results. In the case of Bog Lake and Junction Fen surface peat, Fe(III) additions apparently stimulated CH_4 production. Iron plays an important role in the biochemistry of methanogens (Jarrell and Kalmokoff 1988). Our addition might have alleviated a trace-metal limitation on methanogenesis (Basiliko and Yavitt 2001). Overall, we feel that our results exclude the possibility that Fe(III) is the terminal electron acceptor in AOM.

Lack of support for the Fe(III) hypothesis convinced us to revisit SO_4^{2-} as a potential electron acceptor in freshwater systems. This is logical considering the importance of SO_4^{2-} -dependent AOM in marine systems (Valentine 2002), and many studies (e.g., Dise and Verry 2001; Granberg et al. 2001; Gauci et al. 2002) have shown decreased CH_4 emission rates as a result of SO_4^{2-} addition. Results from our experiment using incubations of peat from different sites, along with additions of potential electron acceptors, demonstrated that SO_4^{2-} additions significantly

reduced net CH₄ flux in Michigan Hollow peat and had a measurable but non-significant effect in Alborn peat (Figure 5; Table 5). This evidence was suggestive, however the stable isotope calculations clearly demonstrated that the primary effect of SO₄²⁻ was to reduce gross CH₄ production, which confirms the conclusions of SO₄²⁻ addition studies mentioned before. Moreover, SO₄²⁻ concentrations in most freshwater systems are assumed to be insufficient to support AOM (Alperin and Reeburgh 1984).

Because we were unable to identify the terminal electron acceptor in our experiments, further studies are clearly warranted. The role of NO₃⁻ as potential electron in peat soils is still uncertain and other possibilities still exist. For example, an organic molecule derived from organic matter decay could serve as an electron acceptor in peat soils. Decomposition products, such as humic acids, do function as electron acceptors in certain environments and can be recycled (Lovley et al. 1996; Scott et al. 1998). Such humic acids should be readily available in organic C-rich peatland ecosystems. Therefore, this potential mechanism deserves further attention.

Other Sites

Results from the two trophic gradients suggest that AOM is quantitatively more important in highly minerotrophic systems than in less minerotrophic systems. Across the US gradient, *Carex*-derived Michigan Hollow peat had consistently higher and more sustainable AOM rates than did the less minerotrophic peat from sites in Minnesota. The Swedish gradient also suggested that net AOM and overall CH₄ cycling were greater in the minerotrophic site than in the intermediate and ombrotrophic sites, but with an additional pattern related to peat depth. However, we found no clear patterns when comparing intermediate and ombrotrophic systems and we therefore are unable to support our initial hypothesis or make sweeping generalizations regarding wetland trophic status and AOM rates. The initial hypothesis stated that AOM would be quantitatively more important in minerotrophic wetlands due to the supply of inorganic electron acceptors. On the other hand, if an organic matter decay product were responsible (e.g., quinones; Scott et al. 1998), then *Carex*-dominated systems (more minerotrophic) with rapid rates of decomposition would support the process while *Sphagnum*-dominated systems that possess more recalcitrant plant material would not. In more closed systems, tight internal cycling via decomposition and sharp redox gradients might sustain the process at the low rates we observed. As the mechanisms for AOM in peatland soils become clearer, this hypothesis needs to be revisited.

Methodological Considerations

Because so little is known about AOM in peat soils, and we started with observational evidence, some methodological discussion is needed. Overall, these pitfalls do not detract from our primary thesis that AOM occurs in freshwater peatlands, may exert influence on CH₄ emissions, and therefore deserves more attention.

First, our method of transferring gases might not have accurately controlled for the production of CO₂ and other gases that can affect CH₄ partial pressure and ultimately dilute headspace [CH₄]. We feel these changes were sufficiently accounted for, but small errors could have affected our calculations. Second, we were unable to confirm our rate estimates in relation to potential electron acceptor availability. Neither SO₄²⁻ nor NO₃⁻ additions used in our experiments were sufficient to account for the AOM rates we measured. Therefore, the process is either linked to more than one electron acceptor or electron acceptors are regenerated internally. Considering the thermodynamic constraints on AOM, rate overestimates are a plausible explanation.

In contrast, some measured rates may be underestimates. The stable isotope pool-dilution technique developed by von Fischer and Hedin (2002) was developed for measuring aerobic CH₄ oxidation in soils and involves known values of isotopic discrimination for that process. Isotopic discrimination during AOM is not well constrained, particularly in non-marine systems, so we assumed no fractionation during AOM for estimation purposes. This would tend to underestimate the actual rates instead of overestimate. We also feel that the AOM rates from our inhibition experiments are underestimates because it is doubtful that inhibition of methanogenesis was complete.

In our stable isotope calculations, we assumed that CH₄ production would have a δ¹³C value of ~-60‰. We realize that this value can vary in time and space depending on the dominant methanogenic pathway. However, methanogenesis is dominated by the acetogenic pathway in Michigan Hollow (Dettling et al. 2006), and varying this number by 10‰, based on range of values reported by Chanton et al. (2005), resulted in very small changes (~1%) in our calculations. The results reported here are therefore not very sensitive to this assumption.

Whenever CH₄ was added to incubations, CH₄ measurements were corrected for headspace losses due to diffusion and dissolution of CH₄ into peat porewater (total peat volume) by using a version of Henry's law (Flett et al. 1976) and Bunsen solubility coefficient's (Yamamoto et al. 1976); yet, this diffusive flux was very small relative to the CH₄ consumed in many of the assays. Therefore, diffusive processes and peat porewater equilibration cannot account for the measured decreases in headspace CH₄ concentration. Some diffusion into the peat mass could be fostered by the jar shaking process, but this effect is likely countered by the CH₄ production within the peat.

CONCLUSIONS AND IMPLICATIONS

The results presented here demonstrate that AOM may be an important component of the biogeochemical cycle of CH₄ in Michigan Hollow peat soil and might function to decrease CH₄ emissions. Additional experiments demonstrated that this process is not novel to Michigan Hollow, but is in fact widespread across a variety of different peatland ecosystems. AOM was spatially and temporally variable, and it seasonally may consume excess CH₄ during periods of low CH₄ production. The exact

electron acceptor involved in the process is not clear, but it is unlikely that AOM was carried out via the reduction of Fe(III) or SO_4^{2-} . We cannot reject NO_3^- as a potential electron acceptor based on the evidence presented here, and recent work by Raghoebarsing et al. (2006) suggests that this mechanism merits further attention. However, humic acids also may serve as important electron acceptors in such C-rich ecosystems. Despite the apparent ubiquity of AOM across peatlands, the process appears to be quantitatively more important in minerotrophic systems that are not *Sphagnum*-dominated. More studies clearly are needed to elucidate the mechanisms, electron acceptor(s) and organism(s) involved.

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