Pathways of anaerobic carbon cycling across an ombrotrophic–minerotrophic peatland gradient

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Abstract

Peatland soils represent globally significant stores of carbon, and understanding carbon cycling pathways in these ecosystems has important implications for global climate change. We measured aceticlastic and autotrophic methanogenesis, sulfate reduction, denitrification, and iron reduction in a bog, an intermediate fen, and a rich fen in the Upper Peninsula of Michigan for one growing season. In 3-d anaerobic incubations of slurried peat, denitrification and iron reduction were minor components of anaerobic carbon mineralization. Experiments using ^{14}C-labeled methanogenic substrates showed that methanogenesis in these peatlands was primarily through the aceticlastic pathway, except early in the growing season in more ombrotrophic peatlands, where the autotrophic pathway was dominant or codominant. Overall, methane production was responsible for 3–70% of anaerobic carbon mineralization. Sulfate reduction accounted for 0–26% of anaerobic carbon mineralization, suggesting a rapid turnover of a very small sulfate pool. A large percentage of anaerobic carbon mineralization (from 29% to 85%) was unexplained by any measured process, which could have resulted from fermentation or possibly from the use of organic molecules (e.g., humic acids) as alternative electron acceptors.

Peatland soils contain an estimated 462×10^{15} g of carbon worldwide, approximately one-third of the terrestrial soil carbon pool (Maltby and Immirzi 1993). Much of this soil carbon exists below the water table, where it is subject to anaerobic microbial decomposition, which generates carbon dioxide (CO_2) and methane (CH_4) as end products. Both CO_2 and CH_4 are important greenhouse gases and combined are responsible for ~80% of the radiative forcing from all greenhouse gases (Ramaswamy et al. 2001). Although they occupy <3% of the terrestrial land surface (Bridgham et al. 2001), peatlands are currently responsible for approximately 8% of the global CH_4 flux (Bartlett and Harriss 1993). If future global change increases the release of CO_2 and/or CH_4 from peatlands to the atmosphere, it could accelerate ongoing climate change associated with these greenhouse gases (Bridgham et al. 1995; Gorham 1995).

Under anaerobic conditions, the decomposition of organic carbon to CO_2 and CH_4 is carried out by a consortium of microbes. Complex organic polymers are initially degraded by fermenting bacteria to yield a few simple products, which are subsequently used by methanogens to produce CH_4. In freshwater wetlands, the dominant methanogenic substrates are thought to be dihydrogen (H_2) and CO_2, which are used by autotrophic methanogens, and acetate, which is converted to CH_4 and CO_2 by aceticlastic methanogens (Conrad 1989). Methanogens compete for substrates generated by upstream fermentation (i.e., acetate and H_2) with microbial processes that couple the decomposition of organic matter with the reduction of alternative inorganic electron acceptors and generate CO_2 as a respiratory byproduct [i.e., denitrification, manganese [Mn(IV)] reduction, iron [Fe(III)] reduction, and sulfate reduction]. From a thermodynamic perspective, these electron acceptors should be used in a predictable sequence defined by their energetic yields [in order of decreasing yield: nitrate, Mn(IV), Fe(III), and sulfate], with CH_4 production occurring only after these alternative inorganic electron acceptors have been depleted (Conrad 1989). However, heterogeneity in many wetland ecosystems enables these processes to occur simultaneously (Wieder et al. 1990; Yavitt and Lang 1990; Vile et al. 2003a), with the relative contribution of these mineralization pathways ultimately determining the amount of CO_2 and CH_4 produced anaerobically. Given the much greater global warming potential of CH_4 as compared to CO_2 on a mass basis (Ramaswamy et al. 2001), the ratio at which these gases are produced during anaerobic respiration in wetlands is of critical importance.

A number of environmental controls over microbial respiration have been well studied in wetlands (reviewed by Segers 1998). For example, the importance of watertable level, temperature, pH, soil carbon quality, and nutrients in

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controlling anaerobic mineralization of organic carbon to CO_2 and/or CH_4 in peatlands has been well established (e.g., Blodau 2002). However, the relative importance of the various microbial pathways that contribute to anaerobic carbon mineralization in peatlands [i.e., denitrification, Fe(III) reduction, Mn(IV) reduction, sulfate reduction, and methanogenesis] have received considerably less attention (e.g., Yavitt and Lang 1990; Shannon and White 1996; Vile et al. 2003*a*). Similarly, the relative contributions of aceticlastic methanogenesis and autotrophic methanogenesis to CH_4 production in peatlands are only beginning to be understood (e.g., Avery et al. 2002; Duddleston et al. 2002). In fact, to our knowledge, all of these pathways have never been measured simultaneously in the same peatland.

A better understanding of these interacting microbial processes may provide valuable insights into how peatlands will respond to future global change. For example, Hines et al. (2001) demonstrated that the majority of methanogenesis in Alaskan peatlands was from the autotrophic pathway, resulting in pooling of acetate under anaerobic conditions. They hypothesized that a warming-induced initiation of aceticlastic methanogenesis would dramatically increase the total CH_4 production from these ecosystems. Vile et al. (2003*a*) demonstrated that atmospheric sulfur deposition (resulting from industrial activity) increased carbon flow through the sulfate reduction pathway and suppressed rates of CH_4 production in ombrotrophic peatlands. A similar pattern of inhibition of CH_4 production by anthropogenic sulfur pollution appears common to wetlands in Europe and North America (Gauci et al. 2004). Thus, understanding the relative contribution of various microbial pathways to anaerobic carbon mineralization in peatlands can have important implications in the global carbon cycle.

Peatlands exist along an ombrotrophic–minerotrophic gradient ranging precipitation-fed (ombrotrophic) bogs to predominantly groundwater-fed (minerotrophic) fens. Although this gradient is defined predominantly by degree of groundwater influence, factors such as pH, plant community structure and productivity, alkalinity, and soil carbon quality also change dramatically along this gradient (e.g., Szumigalski and Bayley 1996; Bridgham et al. 1996, 1998). For example, potential anaerobic carbon mineralization tends to increase along the ombrotrophic–minerotrophic gradient (Bridgham et al. 1998), reflecting an increase in soil carbon quality. The proportion of anaerobic carbon mineralization as CH_4 may also increase by several orders of magnitude along this gradient (Bridgham et al. 1998; van Hulzen et al. 1999; Keller and Bridgham unpubl. data), suggesting that the pathways of anaerobic carbon flow differ among peatland types.

Here, we quantify the pathways of anaerobic carbon mineralization across one growing season in a bog, an intermediate fen, and a rich fen in the Upper Peninsula of Michigan. These wetlands are typical of northern peatlands in the area and represent an ombrotrophic–minerotrophic peatland gradient. Using ^{14}C-labeled methanogenic substrates, we measured the relative importance of autotrophic and aceticlastic methanogenesis in these systems. In a second experiment, we estimated the contribution of denitrification, Fe(III) reduction, sulfate reduction, and methanogenesis to total anaerobic carbon mineralization in the same three peatlands. The combination of these experiments represents one of the most complete anaerobic carbon budgets for peatland ecosystems to date.

Materials and methods

Study sites—For this study, we sampled a bog, an intermediate fen, and a rich fen at the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan (46°N, 89°W). These sites are representative of peatlands in the area and have been described previously (Kellogg and Bridgham 2003; Iversen 2004). Vegetation at the bog was dominated by bryophytes, primarily *Sphagnum* spp., and ericaceous shrubs such as *Vaccinium oxycoccus* (L.), *Rhododendron groenlandicum* (Oeder) Kron & Judd, and *Chamaedaphne calyculata* (L.) Moench. Graminoids, including *Carex oligosperma* Michx. and *Scheuchzeria palustris* (L.), were also present in the bog. The intermediate fen was dominated by *Carex* spp., *Eriophorum vaginatum* (L.), *E. virginicum* (L.), and ericaceous shrubs including *R. groenlandicum* and *C. calyculata*. Alder, *Alnus incana rugosa* (Du Roi) R.T. Clausen (henceforth *A. rugosa*), and an incomplete ground layer of *Sphagnum* spp. were also present at the intermediate fen. Graminoids (*Calamagrostic canadensis* [Michx.] P. Beauv. and *Carex* spp.) dominated the rich fen, although *A. rugosa* and *Salix* spp. were also present. The average soil pH across the growing season was 3.89 in the bog (range 3.74–3.99), 4.77 in the intermediate fen (range 4.70–4.85), and 5.86 in the rich fen (range 5.78–5.94).

Sampling protocol—Replicate soil cores were extracted from each peatland approximately monthly from 21 May 2004 through 15 September 2004. Cores were collected using polyvinyl chloride tubes (10-cm diameter, ~30-cm depth) guided into the peat with a sharp, serrated knife to minimize compaction. Upon extraction, cores were immediately capped on the bottom to maintain in situ watertable levels and reduce oxygen (O_2) leakage into the soil. Cores were transported to the laboratory at the University of Notre Dame (Notre Dame, Indiana) in open coolers and stored at ambient temperature in a solarium for a maximum of 72 hours until processing. The average watertable depth and soil temperature at -10 cm below the peat surface were recorded in each peatland on each sampling date. Soil temperatures (averaged across all three peatlands) were 11.0°C, 17.6°C 18.8°C, 16.0°C, and 17.5°C for monthly sampling dates from May to September, respectively.

In the laboratory, cores were processed in a glove box filled with a nitrogen (N_2) atmosphere to maintain anaerobic conditions. From each core, the 10-cm section directly below the average watertable depth was used for biogeochemical rate measurements (described in the *Methanogenic pathways* and *Pathways of anaerobic carbon mineralization* sections below). Large roots, woody material, and green vegetation were quickly removed from these depth sections in the glove box. Approximately 20 g of field-moist peat was added to 120-mL serum bottles and

slurried with 20 mL of pore water collected from each peatland. Pore water was filtered through a Buchner funnel filled with glass wool to remove large organic material and bubbled with N_2 for at least 10 minutes before creating the peat slurries. All peat slurries were then bubbled vigorously with N_2 for an additional 10 minutes to ensure anaerobic conditions and subsequently were sealed with gray butyl septa.

We used peat from below the watertable depth for the experiments because anaerobic conditions and the associated anaerobic microbial consortium were likely already established in these depth sections. Thus, measured rates reflect in situ anaerobic carbon cycling processes except to the extent that short storage times and the creation of slurries altered these processes. An inherent trade-off in this approach is that the depth of peat used on each sampling date varied slightly, and seasonal patterns, as well as comparisons among peatlands, may be confounded as belowground productivity, litter and carbon quality, and associated microbial processes change with depth. However, measured watertable levels were fairly consistent within each peatland, likely minimizing potential depth effects. Watertable levels ranged from -4 to -15 cm in the bog, -2 to -10 cm in the intermediate fen, and -10 to -15 cm in the rich fen across all sampling dates (Fig. 1).

Methanogenic pathways—Cores collected in May, July, and September were used to estimate the relative contribution of aceticlastic and autotrophic methanogenesis to total CH_4 production. Five cores were collected from each peatland, and after processing (described above) six slurries were created from each peat core on each sampling date. All slurries were incubated in the dark for 3 days at the average soil temperature measured in all three peatlands, with the exception of bog peat from the May sampling date, which was incubated for 4 days to ensure sufficient CH_4 production.

From each peat core, four slurries were used to measure rates of total CO_2 and CH_4 production. Headspace samples were analyzed approximately daily for CO_2 and CH_4 simultaneously using a Varian 3600 gas chromatograph equipped with a thermal conductivity detector (for CO_2) and a flame ionization detector (for CH_4). Each sample was shaken vigorously before injection to remove gas bubbles trapped within the peat slurry. Total CH_4 and CO_2 were calculated using Henry's Law, adjusting for solubility, temperature, and pH (Greenberg et al. 1992; Drever 1997). CH_4 and CO_2 production rates were calculated using the accumulation of gases through time. Rates of CO_2 production were linear ($r^2 > 0.85$, typically $r^2 > 0.95$) in all cases. Across all sampling dates, only four samples (out of 180 total slurries) exhibited nonlinear CH_4 production ($r^2 < 0.85$). These nonlinear samples were excluded from subsequent analysis, and the CH_4 production rates were the average of the remaining three slurries from each core. On each sampling date, cores were treated as independent replicates ($n = 5$) within each peatland type, and CO_2 and CH_4 production rates were the average of rates of the four slurries created from each core. Additional peat samples
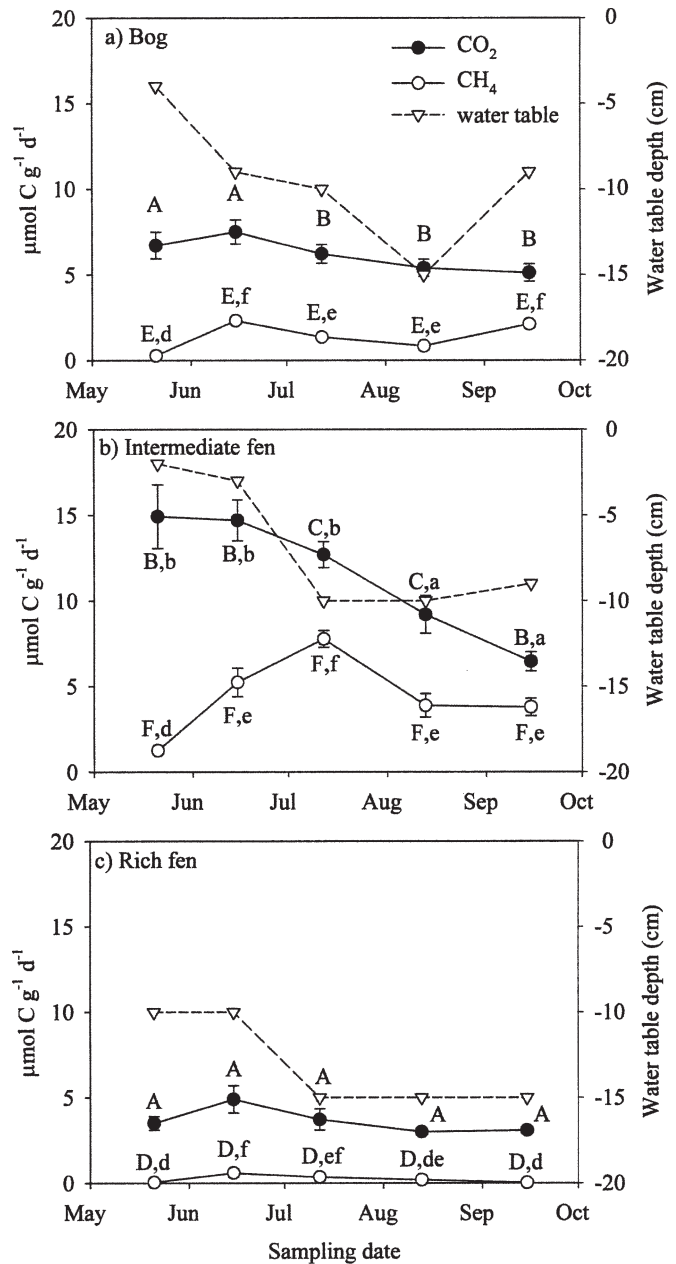

Fig. 1. Rates of anaerobic CO_2 production and CH_4 production (± 1 SE) measured for one growing season in (a) bog peat, (b) intermediate fen peat, and (c) rich fen peat. Within each sampling date, peatland types with different uppercase letters differed significantly. Differences among sampling dates within each peat type are indicated by different lower case letters. The letters a–c indicate differences in CO_2 production, and d–f indicate differences in CH_4 production with letters later in the alphabet indicating higher rates. The depth of the watertable below the peat surface is indicated by the dashed line.

were used to calculate percent moisture by drying at 60°C for 48 h.

Simultaneously, ^{14}C-labeled methanogenic substrates were used to estimate CH_4 production rates from the autotrophic and aceticlastic pathways. The remaining two peat slurries from each core were injected with either 40 μL

of ^{14}C-HCO$_3^-$ (7,500,000 dpm in deionized water; MP Biomedicals) or 20 μL of 2-^{14}C-acetate (250,000 dpm in deionized water; MP Biomedicals) to estimate rates of autotrophic and aceticlastic methanogenesis. Radiotracers were added to the sealed slurries as point injections, followed by vigorous shaking. The addition of radiotracers added <1.1 μmol of acetate and <200 μmol of bicarbonate to the slurries, which likely represent minimal changes to the acetate and ΣCO_2 pools.

At the end of the 3–4-d incubations, ^{14}CH$_4$ produced from the added tracers was collected using a system similar to those described previously (Avery et al. 2002). Biological activity in the slurries was stopped by injecting 5 mL of 5 mol L^{-1} NaOH. After the addition of NaOH, the slurries were shaken vigorously for 5 min to facilitate the conversion of the ΣCO_2 pool to CO_3^{-2} and release any trapped CH$_4$ bubbles. Subsequently, the headspace was purged using a steady stream of N$_2$ (50 mL min^{-1}) for 1 h. The gas first passed through two 20-mL serum bottles containing 10 mL of 2 mol L^{-1} NaOH to remove any ^{14}C-labeled CO_2 not trapped in the high-pH solution. The gas stream subsequently passed through a CuO column heated to 700°C in a Thermolyne 21100 tube furnace (Barnstead Thermolyne) to oxidize the headspace CH$_4$ to CO_2, which was trapped in two additional 20-mL serum bottles containing 10 mL of 2 mol L^{-1} NaOH. Four milliliters of each NaOH trap containing oxidized CH$_4$ was added to 12 mL of ScinitSafe Plus 50% liquid scintillation cocktail (Fischer Scientific), which was counted on a Beckman LS 5000TD liquid scintillation counter (Beckman Coulter) after at least 8 h of equilibration in the dark. Between 0.01% and 11.1% of the added ^{14}C-labeled HCO$_3^-$ was converted to CH$_4$ during the autotrophic methanogenesis rate measurements, and between 0.06% and 41.1% of the added acetate tracer was converted to CH$_4$ during aceticlastic methanogenesis rate measurements.

Autotrophic methanogenesis rates were calculated as $R = aC\alpha/Atg$, where R is the rate of autotrophic methanogenesis, A is the amount of ^{14}C-labeled HCO$_3^-$ added, a is the amount of ^{14}C-labeled CH$_4$ recovered after the incubation, C is the ΣCO_2 pool size, t is the incubation time, and g is the dry mass of peat in the slurry. For autotrophic methanogenesis, $\alpha = 1.12$ and is the fractionation factor for CH$_4$ production from ^{14}C relative to ^{12}C (Avery et al. 2002). The size of the ΣCO_2 pool was calculated based on nontracer estimates of the total CO_2 pool (corrected for solubility and pH, as described above). An inherent limitation of this approach is that any ^{14}C-labeled HCO$_3^-$ that was converted to acetate and subsequently to ^{14}CH$_4$ was classified as CH$_4$ derived from the autotrophic pathway. Thus, our estimates of autotrophic methanogenesis should be considered maximum rates of this process.

Aceticlastic methanogenesis rates were calculated in a similar manner, with $\alpha = 1.06$ (Avery et al. 2002). However, when rates of aceticlastic methanogenesis were the dominant pathway of CH$_4$ production (>50% of total CH$_4$ production as calculated by difference as described below), the sum of tracer-determined CH$_4$ production accounted for an average of 43% ± 7% (mean ± 1 SE) of total CH$_4$ production measured in nonlabeled slurries (compared to an average of 112% ± 7% when aceticlastic methanogenesis was responsible for <50% of the total CH$_4$ production). Thus, it appears that aceticlastic CH$_4$ production rates measured in this manner were not reliable for quantitative analyses. Previous research has also demonstrated problems with tracer-determined rates of aceticlastic methanogenesis (summarized in Avery et al. 2002). Therefore, we estimated the fraction of CH$_4$ production from the autotrophic pathway using labeled ^{14}CO$_3^-$ incubations, and the fraction of CH$_4$ production from acetate was determined by the difference (Avery et al. 2002). This approach assumes that autotrophic and aceticlastic methanogenesis are the only possible pathways of CH$_4$ production in these systems, which is generally accepted in freshwater ecosystems (Conrad 1989).

At the end of the incubations, two unlabeled slurries were filtered through Whatman glass-fiber GFF filters, and the resulting pore water was used to measure concentrations of acetate, sulfate, and nitrate using ion chromatography. Samples were frozen until analysis on a Dionex DX500 ion chromatography system equipped with an AES suppressor and ED50 electrochemical detector (Dionex Corporation). Samples were analyzed using a Dionex Ionpac AS11 column with an Ionpac AG11 guard column and a 12 mmol L^{-1} NaOH eluent.

Pathways of anaerobic carbon mineralization—To estimate the relative contribution of methanogenesis, sulfate reduction, denitrification, and Fe(III) reduction to total anaerobic carbon mineralization, five cores were collected from each peatland in June, August, and September (cores collected in September were the same cores used to estimate methanogenic pathways). From each core, four slurries were created anaerobically and incubated as described above. Two untreated slurries were used to measure rates of total CH$_4$ and CO_2 production in 3-day incubations. Once again, cores were treated as independent replicates for statistical analyses. At the end of the incubations, pore water was filtered through Whatman glass-fiber GFF filters. Pore water from one slurry from each peat core was frozen until analysis for acetate, sulfate, and nitrate on the ion chromatograph as described above.

Sulfate reduction was measured in an additional slurry from each peat core using the selective inhibitor sodium molybdate (Oremland and Capone 1988). Although likely more accurate, the use of ^{35}SO$_4$ to estimate sulfate reduction was not logistically possible in the context of this experiment. Sodium molybdate was added to the slurries at a final concentration of 10 mmol L^{-1} followed by vigorous shaking. Rates of total CO_2 and CH$_4$ production during the 3-day incubations were calculated using gas chromatography, as described above. The difference in CO_2 production between sodium molybdate–inhibited slurries and unamended slurries was attributed to sulfate reduction. In many cases, sodium molybdate also inhibited methanogenesis in the slurries (Oremland and Capone 1988). Thus, we corrected the difference in CO_2 production between inhibited and unamended slurries for the amount of CO_2 that would have been produced by

aceticlastic methanogenesis using the percentage of CH_4 from acetate as determined in the radiotracer experiments (described above). Because we did not use ^{14}C-tracers on the June and August sampling dates, the percent of CH_4 from acetate was the average of the percentages in the preceding and following months. The percent of CH_4 from acetate in September was calculated based on the September radiotracer measurements. Sulfate reduction rates were therefore the difference in CO_2 production between molybdate-inhibited and unamended slurries that could not be attributed to CO_2 from aceticlastic methanogenesis.

Fe(III) reduction rates were estimated using pore water from one untreated slurry of each peat core by measuring the concentration of reduced Fe(II) at the end of the incubations. Fe(II) was measured by adding 0.5 mL of filtered pore water to 2 mL of 0.1% ferrozine and 50 mmol L^{-1} HEPES buffer solution (pH = 7.0) and measuring the absorbance at 562 nm (Lovley and Phillips 1986). This approach assumes that all Fe(II) present at the end of the incubation accumulated during the incubation time, and thus represents a maximum of Fe(III) reduction. We are not able to rule out the possibility that rapid recycling of Fe(II) to Fe(III) was taking place in our slurries, leading to a significant underestimate of the role of Fe(III) reduction. However, given the anaerobic nature of the slurries and the low nitrate concentrations in peat soils, we assume that the re-oxidation of Fe(II) was likely minimal in this experiment. A ratio of 1 mol carbon mineralized to 4 mol Fe(III) reduced was used to estimate carbon equivalents of iron reduction (Roden and Wetzel 1996). As Mn(IV) concentrations in peat soils are typically lower than Fe(III) concentrations (e.g., Gorham and Janssens 2005), we assumed that Mn(IV) reduction was insignificant in these systems.

Denitrification rates were estimated in the fourth slurry from each core using the acetylene block technique (Mosier and Klemedtsson 1994) by the addition of 12 mL of acetylene (generated from calcium carbide) to the slurry headspace, followed by vigorous shaking. Slurries used to measure denitrification also contained chloramphenicol (5 mmol L^{-1}) to limit de novo synthesis of denitrification enzymes (Smith and Tiedje 1979). Denitrification samples were incubated in the dark at the same temperature as other incubations. After ~4 hours, 4 mL of headspace was removed from the slurries and stored in vacutainers until N$_2$O analysis on a Varian 3600 gas chromatograph equipped with an electron capture detector. We assumed linear accumulation of N$_2$O during the course of these short incubations and calculated total N$_2$O production using Henry's law to correct for solubility and temperature (Weiss and Price 1980). Denitrification rates were expressed as carbon equivalents using a ratio of 3/4 moles of carbon mineralized per 1 mole of N$_2$O produced from denitrification (Schlesinger 1997).

The estimates of denitrification and Fe(III) reduction were appropriate to confirm the a priori hypothesis that Fe(III) reduction and denitrification rates would be negligible in the anaerobic carbon budget of the peatlands. Previous research has assumed minimal rates of denitrification and Fe(III) reduction in peatlands because of the highly organic nature of peat soils (with very low iron concentrations) and anaerobic and acidic conditions that limit nitrate availability (Wieder et al. 1990; Yavitt and Lang 1990; Vile et al. 2003*a*).

We were thus able to create an anaerobic carbon budget for each peatland. Total anaerobic carbon mineralization was defined as the sum of CO_2 and CH_4 produced from unamended slurries. This definition underestimates actual rates of mineralization if fermentation intermediates (e.g., acetate, lactate, and methanol) accumulate instead of being fully mineralized to CO_2 and CH_4 (a more complete explanation of this potential underestimate is included in the Discussion section). Carbon equivalents of CH_4 production, Fe(III) reduction, denitrification, and sulfate reduction were subtracted from this total, and the difference was defined as "unexplained carbon." The carbon equivalents from CH_4 production also included CO_2 produced from aceticlastic methanogenesis, which was calculated using the percentage of CH_4 from acetate as determined in the radiotracer experiments (described above).

Statistical analyses—Cores were extracted at least several meters apart on each sampling date from 32-m^2 portions of the three peatlands; thus, we assumed that individual cores were independent replicates for biogeochemical measurements. All statistical analyses were conducted using SAS version 9.1 (SAS Institute). On each sampling date, differences in CO_2 production rates and CH_4 production rates among peatland types were analyzed using one-factor analysis of variances (ANOVAs) with peatland type as the between subject variable (GLM procedure). Following significant differences among peat types ($p < 0.05$), pairwise comparisons were determined using Fisher's tests of least significant difference ([LSD] $\alpha = 0.05$). To investigate seasonal patterns within each peatland type, biogeochemical rates were analyzed using one-factor ANOVAs with the sampling date as the between-subject variable (GLM procedure). In cases where there were significant differences among sampling dates ($p < 0.05$), pairwise comparisons among sampling dates were determined using Fisher's tests of LSD ($\alpha = 0.05$). All data were tested for normality and log-transformed where the transformation resulted in a significant improvement in overall distribution.

Results

CO_2 production rates ranged from 5.1 μmol g^{-1} d^{-1} to 7.5 μmol g^{-1} d^{-1} in bog peat, 6.5 μmol g^{-1} d^{-1} to 14.9 μmol g^{-1} d^{-1} in intermediate fen peat, and 3.0 μmol g^{-1} d^{-1} to 4.9 μmol g^{-1} d^{-1} in rich fen peat (Fig. 1). Within each sampling date, the highest anaerobic CO_2 production rates were in peat from the intermediate fen, the lowest rates were in peat from the rich fen, and rates in peat from the bog peat were intermediate; however, there were not always significant difference among all peat types (Fig. 1). In peat from the bog and rich fen, there were no significant differences in CO_2 production rates among

sampling dates (ANOVA, $p = 0.07$ and $p = 0.14$, respectively; Fig. 1). CO_2 production rates decreased through the growing season in peat from the intermediate fen (ANOVA, $p = 0.0002$; Fig. 1).

CH_4 production was a smaller component of anaerobic carbon mineralization in all peatland types and ranged from 0.3 μmol g^{-1} d^{-1} to 2.3 μmol g^{-1} d^{-1} in peat from the bog, 1.3 μmol g^{-1} d^{-1} to 7.8 μmol g^{-1} d^{-1} in peat from the intermediate fen, and 0.04 μmol g^{-1} d^{-1} to 0.6 μmol g^{-1} d^{-1} in peat from the rich fen (Fig. 1). Regardless of sampling date, CH_4 production decreased from intermediate fen to bog to rich fen (Fig. 1). CH_4 production rates peaked in the early to mid growing season (June or July sampling dates) and tended to decrease during the remainder of the growing season (Fig. 1).

Autotrophic methanogenesis dominated CH_4 production in the bog early in the growing season (94% of total CH_4 production), but was less important in July and September (34–39%; Fig. 2). In the intermediate fen, both aceticlastic and autotrophic methanogenesis were equally important early in the growing season. However, autotrophic methanogenesis was less important later in the growing season in the intermediate fen, contributing only 10% in July and 13% in September (Fig. 2). Regardless of sampling date, autotrophic methanogenesis was a minor component of CH_4 production in the rich fen, although the percent of total CH_4 production from the autotrophic pathway increased from 3% in May to 20% in September (Fig. 2). As contributions of aceticlastic methanogenesis were calculated by difference, aceticlastic CH_4 production mirrored patterns of autotrophic CH_4 production (Fig. 2).

Denitrification and Fe(III) reduction were minor components of anaerobic carbon mineralization in all samples. Less than 0.2 μmol C g^{-1} d^{-1} was mineralized through denitrification, which amounted to ≤1% of the total anaerobic carbon mineralization on all but one sampling date. Denitrification was responsible for ~7% of anaerobic carbon mineralization in the rich fen in September, likely because of low rates of overall anaerobic carbon mineralization (Fig. 1). Fe(III) reduction contributed <0.04 μmol C g^{-1} d^{-1} to anaerobic carbon mineralization (≤1% of the total) across all sampling dates. Although these processes were included in the anaerobic carbon budgets, they were consistently minor components, and statistical analyses are not presented here.

The contribution of sulfate reduction to anaerobic carbon mineralization did not differ among sampling dates in any peat type ($p = 0.26–0.32$). Sulfate reduction was responsible for 6–26% of anaerobic carbon mineralization in the bog, 0–9% in the intermediate fen, and for 5–23% in the rich fen (Fig. 3).

Carbon mineralized through methanogenic pathways (including CO_2 produced through aceticlastic methanogenesis) ranged from 21% to 45% of the total anaerobic carbon mineralization in the bog, with the highest contribution occurring in September and the lowest contribution in August (Fig. 3). The contribution of CH_4 production to total carbon mineralization in the intermediate fen increased through the growing season from 44% in June to 70% in September (Fig. 3). In contrast, the contribution of
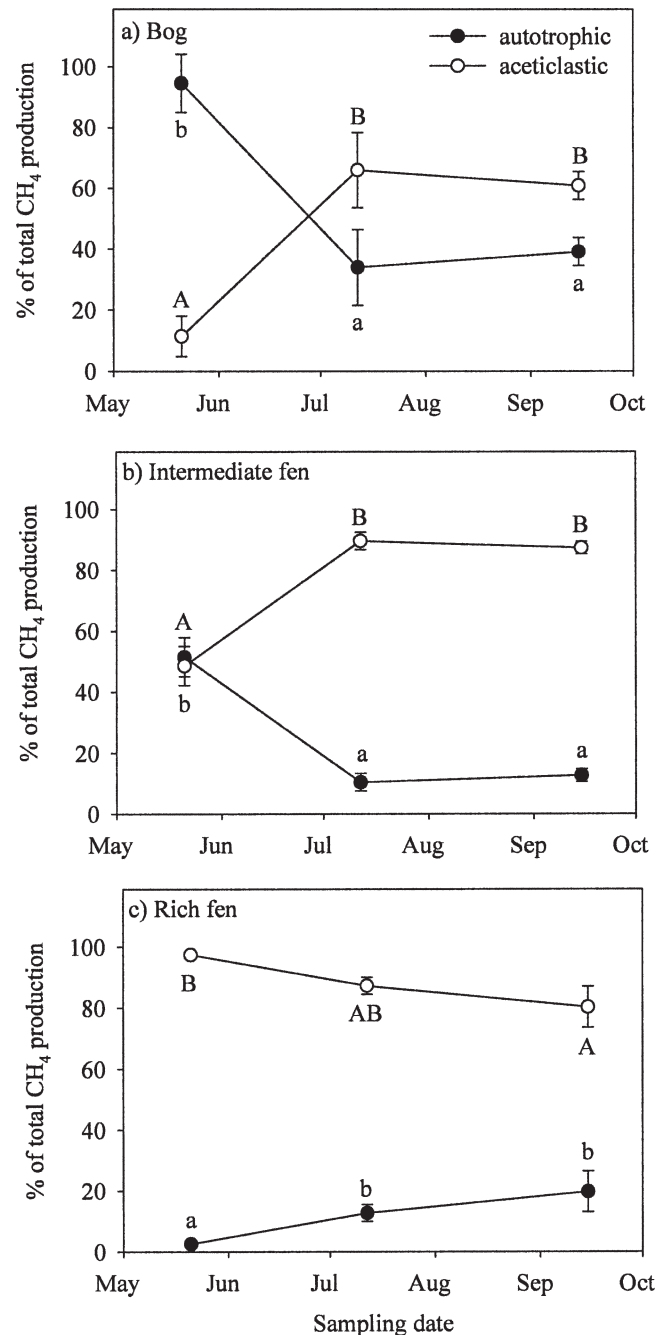

Fig. 2. Percent of total CH_4 production (± 1 SE) as aceticlastic and autotrophic methanogenesis across one growing season in (a) bog peat, (b) intermediate fen peat, and (c) rich fen peat. Note that the percent from aceticlastic methanogenesis was calculated by difference. Within each peat type, significant differences among sampling dates are indicated by different uppercase letters (for aceticlastic methanogenesis) or different lowercase letters (for autotrophic methanogenesis).

methanogenesis decreased from 24% to 3% through the growing season in the rich fen (Fig. 3).

Between 48% and 56% of the total carbon mineralized in the bog could not be explained by any of the pathways measured in this experiment ("unexplained" in Fig. 3). Similarly, large percentages of mineralized carbon were
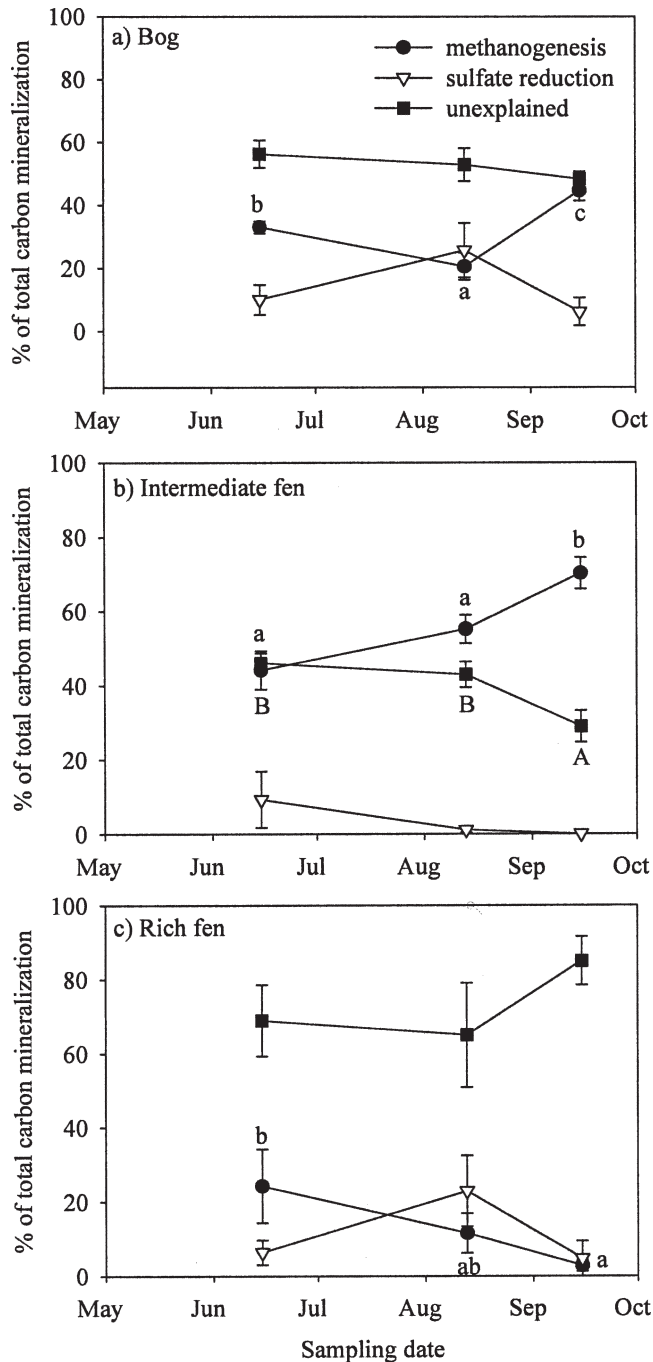
Fig. 3. Percent of total anaerobic carbon mineralization (±1 SE) as methanogenesis and sulfate reduction in (a) bog peat, (b) intermediate fen peat, and (c) rich fen peat. The contribution of methanogenesis also includes CO_2 produced via aceticlastic methanogenesis. The percent of total anaerobic carbon mineralization as denitrification and Fe(III) reduction are not shown because their contributions were generally minor. Total anaerobic carbon mineralization was calculated as the sum of CO_2 production and CH_4 production. "Unexplained" is the mineralized carbon that could not be explained by any measured process. Within each peat type, significant differences among sampling dates are indicated by different lowercase letters (for methanogenesis) or uppercase letters (for unexplained carbon).

unexplained in the intermediate fen (29–46%) and rich fen (65–85%). The unexplained fraction of mineralized carbon did not did change across the growing season in the bog or rich fen peat, but decreased in the intermediate fen peat (Fig. 3).

Acetate was present in all slurries after the incubations in this experiment. Concentrations ranged from 201.2 μmol L^{-1} to 532.3 μmol L^{-1} in the bog, 99.8 μmol L^{-1} to 343.7 μmol L^{-1} in the intermediate fen, and 39.8 μmol L^{-1} to 359 μmol L^{-1} in the rich fen (Fig. 4). In the bog and rich fen, acetate concentrations exhibited a strong peak on the July sampling date. Acetate concentrations in the intermediate fen generally decreased through the growing season. By comparison, concentrations of sulfate and nitrate ions were much lower and were often at or near the detection limits of 2.1 μmol L^{-1} for sulfate and 3.5 μmol L^{-1} for nitrate (Fig. 4).

Discussion

The goal of this project was to quantify the pathways of anaerobic carbon mineralization in three peatlands, representing an ombrotrophic–minerotrophic gradient, across one growing season. There were a number of tradeoffs associated with our detailed measurements of multiple potential anaerobic respiratory pathways in multiple peatlands. First, we limited our sampling regime to a single wetland of each peatland type, and the results of our experiments may not generalize to other peatlands. Second, although samples were collected and processed anaerobically, our ^{14}C-labeling and selective inhibition incubations required root-free, slurried peat, and, to this extent, our rates may not represent in situ processes. However, we used the shortest incubation times possible in this experiment to limit the depletion of labile carbon pools and turnover of associated microbial populations. Finally, although they are frequently used in ecological studies, there are a number of potential challenges associated with the use the selective inhibitors to measure biogeochemical processes. Thus, while we acknowledge the potential limitations of our experimental design, we suggest that our measurements are reasonable estimates of the pathways of anaerobic carbon mineralization in peatland ecosystems.

Anaerobic carbon mineralization—The total carbon mineralized anaerobically (as CO_2 and CH_4) was highest in the intermediate fen and lowest in the rich fen on all sampling dates (Fig. 1). The low rates of anaerobic carbon mineralization measured in the rich fen were surprising because carbon quality, and thus mineralization potential, generally increases along the ombrotrophic–minerotrophic peatland gradient (Valentine et al. 1994; Bridgham et al. 1998). Previous research using these three peatlands revealed that during 5-week laboratory incubations, rich fen peat had a higher anaerobic carbon mineralization potential and a lower CO_2 to CH_4 ratio than the bog and intermediate fen peats (Keller and Bridgham unpubl. data). Consistently low watertable levels in the rich fen (Fig. 1) likely increased the frequency of aerobic conditions at this site, which could have led to a rapid loss of labile carbon
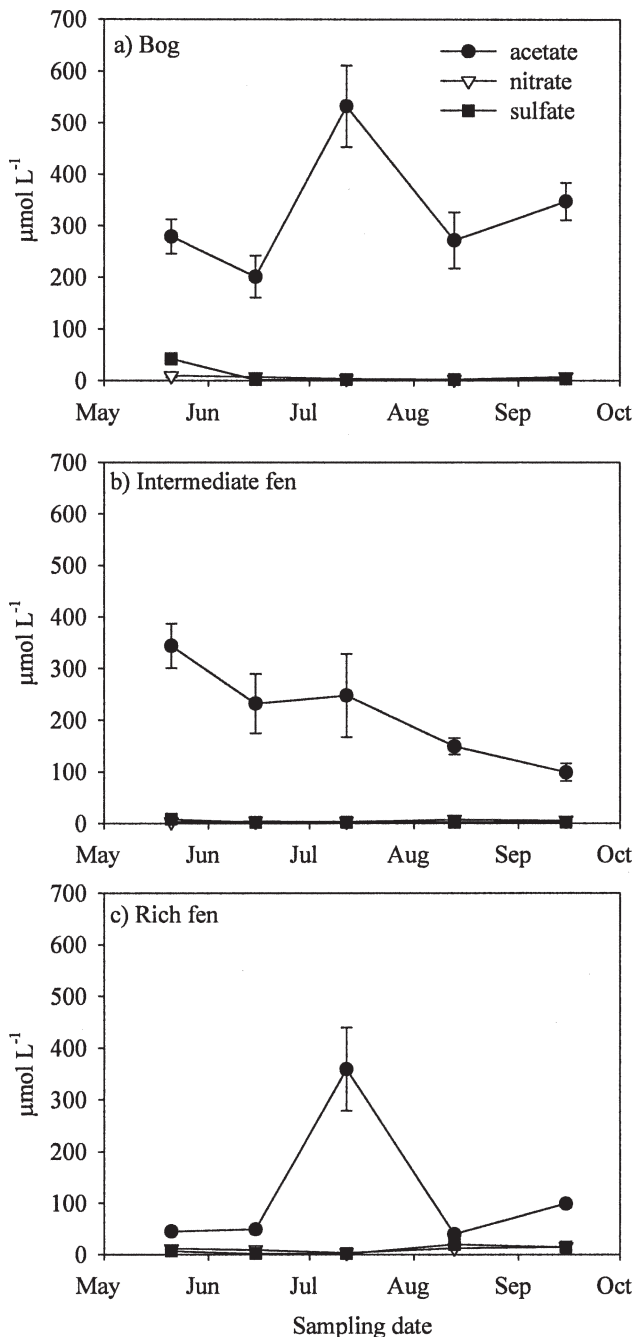
Fig. 4. Mean concentration (±1 SE) of anions measured in post-incubation pore water in (a) bog peat, (b) intermediate fen peat, and (c) rich fen peat.

substrates by aerobic respiration, ultimately resulting in lower carbon quality available for anaerobic decomposition.

Because a number of environmental variables changed concomitantly during the course of our experiments, it is difficult to isolate the mechanisms responsible for seasonal shifts in anaerobic carbon mineralization rates. For example, watertable levels (and the depth of peat sampled) changed through the growing season (Fig. 1). Incubation temperatures also differed among sampling dates (ranging

from 11.0°C to 18.8°C), as did the phenology of vegetation in each site (Keller unpubl. data). To minimize the importance of seasonal shifts on microbial respiration rates, we focus primarily on the relative contribution of various processes (e.g., aceticlastic vs. autotrophic methanogenesis) to overall anaerobic carbon mineralization in the remainder of this discussion.

Methanogenic pathways—Overall, aceticlastic methanogenesis was the dominant CH_4 production pathway (>60% of total CH_4 production) on most sampling dates in these peatlands. Autotrophic methanogenesis was an important pathway in more ombrotrophic peatlands, especially early in the growing season where it was dominant (94% of the total CH_4 production) in the bog and co-dominant (51% of the total CH_4 production) in the intermediate fen (Fig. 2). To the extent that our measurements of autotrophic methanogenesis could be overestimates because of the conversion of the ^{14}C-labeled HCO_3^- to acetate and subsequently to CH_4 by aceticlastic methanogens, it is possible that aceticlastic methanogenesis was even more dominant in these peatlands.

Most studies suggest that autotrophic methanogenesis is dominant in ombrotrophic peatlands (e.g., Duddelston et al. 2002; Horn et al. 2003), with aceticlastic methanogenesis becoming more important in minerotrophic peatlands (Kelly et al. 1992; Chasar et al. 2000). Our results generally support this pattern, with the highest contribution of autotrophic methanogenesis occurring in the bog on all sampling dates (Fig. 2).

Our results also suggest that the relative contribution of autotrophic and aceticlastic methanogenesis varies seasonally, especially in the bog and intermediate fen where autotrophic methanogenesis dominated early in the growing season followed by a dominance of aceticlastic methanogenesis beginning in the late spring and continuing through the summer (Fig. 2). This pattern is similar to seasonal dynamics of CH_4 production observed in bogs from southern Michigan; however, in those systems, the onset of aceticlastic methanogenesis rapidly depleted acetate pools, resulting in a switch back to autotrophic methanogenesis in summer months (Shannon and White 1996; Avery et al. 2002). By contrast, despite the importance of aceticlastic methanogenesis on most sampling dates in this experiment (Fig. 2), acetate was still present at the end of the incubations in our study (Fig. 4). Inputs of labile carbon in the form of root exudates have been hypothesized to favor aceticlastic methanogenesis in the rooting zone of peatlands, with rates of autotrophic methanogenesis increasing with depth as labile root exudates become less available (e.g., Chasar et al. 2000). All samples used in our study came from active rooting zones (Keller unpubl. data), and it is possible that root exudates sustained acetate availability during the course of the growing season.

It is important to note that by calculating the rates of aceticlastic methanogenesis by difference, we assume that acetate and CO_2/H_2 are the only substrates used to generate CH_4 in these peatlands. Whereas these two methanogenic pathways are thought to dominate CH_4 production in

freshwater ecosystems (Conrad 1989), methanogens are known to use additional substrates (e.g., carbon monoxide, formate, some alcohols, and methlyated compounds such as methanol) under laboratory conditions (Zinder 1993). Coles and Yavitt (2002) have also hypothesized the potential for direct conversion of ethanol to CH_4 by methanogens in forested peat ecosystems. Any CH_4 produced through these alternative pathways would have been classified as aceticlastic methanogenesis using our experimental approach.

Pathways of anaerobic carbon mineralization—Denitrification and Fe(III) reduction appear to be very limited pathways of anaerobic carbon mineralization in all three peatlands (typically <1% of the total anaerobic carbon mineralized, Table 1). Denitrification is usually a small component of anaerobic carbon cycling in peatlands (Francez et al. 2000), and the low rates measured in this study are not surprising considering low nitrate availability (Fig. 4) and low soil pH values, which can inhibit nitrification (Lång et al. 1993). In contrast to wetland systems with mineral soils where Fe(III) reduction can be a major component of the anaerobic carbon cycle (e.g., Roden and Wetzel 1996), Fe(III) reduction is assumed to be low in organic peat soils because of the low availability of Fe(III) in these systems (Wieder and Lang 1986; Blodau et al. 2002).

Although there are few studies that have investigated the role of sulfate reduction in freshwater peatlands, there is a growing consensus that this process can be an important pathway of anaerobic carbon mineralization in peatland ecosystems despite low porewater sulfate concentrations (e.g., Wieder et al. 1990; Yavitt and Lang 1990). Our results support this emerging pattern and show that sulfate reduction was responsible for up to 23% of mineralized carbon in the three peatlands investigated (Fig. 3). Although we did not measure initial sulfate concentrations, the low concentrations of sulfate measured in post-incubation pore water (Fig. 4) are typical of peatlands and suggest that sulfate is able to rapidly cycle between reduced and oxidized forms in peatland ecosystems (Wieder and Lang 1988; Vile et al. 2003a,b).

Perhaps the most striking pattern in our results was the consistently high percentage of anaerobic carbon mineralization (from 29% to 85%), which could not be explained by any measured process (Fig. 3). It is possible that methodological artifacts are responsible for a portion of this unexplained carbon. For example, if despite the use of standard anaerobic techniques oxygen was present at the start of our incubations, aerobic respiration could account for a portion of the unexplained CO_2. However, any oxygen present in our slurries would have likely been rapidly consumed, and this pattern does not explain the linear accumulation of CO_2 during the course of 3-day laboratory incubations. Further, our calculations show that from 35% to 162% of the oxygen that would have been present under fully aerobic conditions would be necessary to account for the unexplained carbon in our slurries (data not shown). Whereas trace amounts of oxygen may have remained at the start of our incubations, it seems unlikely

Table 1. Pathways of anaerobic carbon mineralization in peatlands.

Wetland type	Location	Percent of anaerobic carbon mineralization (%)					Citation
		Methanogenesis	Sulfate reduction	Denitrification*	Fe(III)/Mn(IV) reduction*	Unexplained	
Bog	Michigan	21–45	6–26	≤1	≤1	48–56	this study
Intermediate fen	Michigan	44–70	0–9	≤1	≤1	29–46	this study
Rich fen	Michigan	3–24	5–23	<1–7	<1	65–85	this study
Sphagnum-bog	Maryland	1–3	6–52	nd	nd	47–92	Yavitt and Lang (1990)†
Sphagnum-eriophorum	West Virginia	14–37	8–19	nd	nd	55–67	Yavitt and Lang (1990)†
Sphagnum-shrub	West Virginia	6–26	8–33	nd	nd	42–86	Yavitt and Lang (1990)†
Sphagnum-forest	West Virginia	16–22	1–41	nd	nd	37–83	Yavitt and Lang (1990)†
Polytrichum-shrub	West Virginia	6–20	2–100	nd	nd	0–78	Yavitt and Lang (1990)†
Sedge meadow	West Virginia	9	<1–11	nd	nd	80–90	Yavitt and Lang (1990)†
Bog	West Virginia	24	64	nd	nd	12	Wieder et al. (1990)
Bog	Maryland	6	38	nd	nd	56	Wieder et al. (1990)
Bog	Alberta	<1	1	nd	nd	99	Vile et al. (2003a)
Bog	Czech Republic	≤1	27–36	nd	nd	64–73	Vile et al. (2003a)

* nd, not determined, assumed to be zero.
† Ranges include values from the 0–5-cm and 20–25-cm depth increments within each peatland.

that such large amounts of oxygen would have been present. Finally, the consistently linear nature of methanogenesis (a strictly anaerobic process) during the course of our incubations, suggests that our slurries were effectively anaerobic.

Another potential source of error is the possibility that the use of sodium molybdate inherently, and drastically, underestimates rates of sulfate reduction in freshwater peatlands. Although we cannot rule out this possibility, we note that even after doubling our estimates of sulfate reduction, from 27% to 80% of anaerobic carbon mineralization remains unexplained by any measured process. Finally, to evaluate the possibility that variability associated with our measured processes was responsible for the unexplained carbon, we summed the standard errors from each measured process [i.e., CH_4 production, sulfate reduction, Fe(III) reduction, and denitrification] in each peatland type on each sampling date. The sum of errors explained from 7% to 25% of the unexplained carbon, suggesting that this unexplained portion was not caused solely by variability of the measured processes.

Thus, we suggest that a large portion of anaerobic carbon mineralization in peatlands is the result of a process (or processes) not measured in our experiment. This appears to be a common pattern among studies that have constructed anaerobic carbon budgets using a variety of methodologies (including $^{35}SO_4$ tracers to measure sulfate reduction). In all cases, a significant portion of anaerobically mineralized carbon cannot be attributed to CH_4 production or inorganic alternative electron acceptor processes (Table 1).

Vile et al. (2003*a*) suggested that this unexplained carbon results from fermentation that uses organic molecules as both electron acceptors and donors. This hypothesis has also been used to explain the high CO_2 to CH_4 ratios seen in peatland studies (Valentine et al. 1994; Bridgham et al. 1995). Previous studies have shown that fermentation intermediates can accumulate in peatlands, although acetate is usually the dominant fermentation product found in these systems (Shannon and White 1996; Blodau et al. 2002). In our experiment, acetate was present in post-incubation slurry pore water on all sampling dates (Fig. 4); however, we did not measure the rates of accumulation of acetate or any other fermentation intermediates (e.g., propionate, butyrate, formate). Thus, we may be underestimating the true rates of carbon mineralization in our experiment if some portion of carbon is partially mineralized to fermentation intermediates that accumulate in the system.

In Alaskan peatlands, acetate, not CO_2 or CH_4, has been shown to be the dominant end product of anaerobic carbon mineralization (Hines et al. 2001; Duddelston et al. 2002). There is indirect evidence suggesting that a similar pattern can occur in these Michigan peatlands. The post-incubation acetate concentrations measured in this experiment likely reflect pore-water acetate concentrations in the field, as it would require unrealistically fast anaerobic decomposition and/or acetogenesis to produce these high acetate concentrations during our incubations. Assuming that post-incubation acetate measurements represent field pore

water concentrations, than the bog and rich fen exhibited a clear peak in acetate concentrations on the July sampling date (Fig. 4). If we assume that the net accumulation of acetate between the June and July sampling dates was linear, acetate would have been accumulating at rates of 25.5 μmol C g^{-1} d^{-1} in the bog and 7.9 μmol C g^{-1} d^{-1} in the rich fen. These rates are higher than CO_2 and CH_4 production rates in the bog and fen peat on any sampling date (Fig. 1), suggesting that acetate can be the dominant anaerobic mineralization end product in these systems for extended periods. Interestingly, the total rates of anaerobic carbon mineralization (as CO_2 and CH_4 production) were not high enough to explain the decline in acetate concentrations in the bog and rich fen peat following the July peak (Fig. 4). This suggests that either we failed to capture a peak in CO_2 and CH_4 production or that acetate may have other sinks in these systems, possibly through migration to aerobic portions of the peat profile where it would be rapidly consumed or through losses as dissolved organic carbon.

The accumulation of fermentation products cannot be responsible for the unexplained carbon mineralization observed in this experiment (Table 1), as they were not accounted for in our estimates of carbon mineralization (i.e., the sum of respired CH_4 + CO_2). If fermentation is responsible for this missing carbon, it must be as a result of CO_2 released during fermentation and not the accumulation of fermentation products per se. Assuming that the fermentation of a 6-carbon substrate yields solely acetate and CO_2, than 1 mole of CO_2 would be produced for each mole of acetate generated by fermentation. High rates of acetate accumulation seen in some peatlands suggest that fermentation can account for the unexplained carbon mineralization during at least some periods. However, the acetate accumulation rate was only high enough to account for a significant amount of the unexplained carbon mineralization from the June to July sampling in our experiment, suggesting that other processes dominated during other parts of the growing season. It should be noted that the presence of acetogenic bacteria could further reduce the contribution of fermentation to total anaerobic CO_2 production by reducing CO_2 to acetate (Drake et al. 2002).

Although the role of fermentation in peatland mineralization merits further study, we suggest that the use of organic electron acceptors could also be responsible for a large part of the unexplained anaerobic carbon mineralization in peatlands (Table 1), as has been suggested previously (Segers 1998). Lovley et al. (1996) demonstrated that humic acids can act as organic alternative electron acceptors, and bacteria capable of reducing humic substances have been isolated from a number of environments, including many wetlands (Coates et al. 1998). This process could be particularly important in the organic soils of peatland ecosystems that have very high humic acid concentrations, although, to our knowledge, the use of humic acids as organic alternative electron acceptors has never been experimentally demonstrated in peatland ecosystems.

Despite measuring what were assumed to be the dominant pathways of anaerobic carbon mineralization

(denitrification, Fe(III) reduction, sulfate reduction, and methanogenesis), from 29% to 85% of the total anaerobic carbon mineralization was unexplained in our experiments. This range is comparable to estimates of unexplained mineralized carbon in previous peatland studies (Table 1) and suggests that additional pathways of anaerobic carbon mineralization (e.g., fermentation and/or humic acid reduction) are extremely important, though understudied, in freshwater peatlands. Additional studies of anaerobic carbon cycling in peatland ecosystems are necessary to explain this missing carbon. Such studies will likely help in providing a more mechanistic understanding of how peatlands will respond to ongoing global change.

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