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Methane Metabolism in a Temperate Swamp

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Comparisons between in situ CH_4 concentration and potential factors controlling its net production were made in a temperate swamp. Seasonal measurements of water table level and depth profiles of pH, dissolved CH_4, CO_2, O_2, SO_4^{2-}, NO_3^-, formate, acetate, propionate, and butyrate were made at two adjacent sites 1.5 to 2 m apart. Dissolved CH_4 was inversely correlated to O_2 and, in general, to NO_3^- and SO_4^{2-}, potential inhibitors of methanogenesis. At low water table levels (August 1992), maximal CH_4 (2 to 4 μM) occurred below 30 cm, whereas at high water table levels (October 1992) or under flooded conditions (May 1993), CH_4 maxima (4 to 55 μM) occurred in the top 10 to 20 cm. Higher CH_4 concentrations were likely supported by inputs of fresh organic matter from decaying leaf litter, as suggested by high acetate and propionate concentrations (25 to 100 μM) in one of the sites in fall and spring. Measurements of potential CH_4 production (and consumption) showed that the highest rates generally occurred in the top 10 cm of soil. Soil slurry incubations confirmed the importance of organic matter to CH_4 production but also showed that competition for substrates by nonmethanogenic microorganisms could greatly attenuate its effect.

Methane (CH_4) production has received much attention due not only to its importance as a terminal step in anaerobic organic matter degradation but also to its potentially significant role in climatic change and atmospheric chemistry (5). The recent and continuing increase in atmospheric CH_4 concentration (about 1% per year [5]) has prompted more detailed studies of the sources (anthropogenic and natural) and factors controlling its release to the atmosphere.

Wetlands are an important source and potential reservoir of CH_4 (4, 6), but the factors controlling its production and emission from these environments are not fully understood. Studies have shown that water table depth, temperature, and pH can have a major impact on CH_4 production and release in wetlands (9, 10, 22, 23, 36, 37). These parameters alone, however, do not explain the variability in methane production in different wetlands. A better understanding of the microbial processes leading to production (and consumption) of CH_4 in these environments is necessary.

The physiology of methanogens and the chemical factors involved in CH_4 production are well known (42) and have been studied in pure culture and a variety of different environments. However, observations made in one environment are not always applicable to others due to the complexity of the different microbial and chemical processes which may occur. For example, competitive inhibition of methanogenesis by sulfate reduction occurs in many different natural habitats (34) but not always in peat lands (45). The presence of microbially utilizable carbon and of various electron acceptors (O_2, NO_3^-, and SO_4^{2-}) is likely to be an important control of methanogenesis in natural environments.

We examined in situ availability of substrates and the distribution of electron acceptors in a temperate peat swamp in an attempt to determine how the chemistry and microbiology of the site affected methane production. Measurements were obtained in the summer, fall, and spring in two sites to determine both temporal and spatial variability. Laboratory incubations with slurried peat soil were carried out to determine the effects of various chemical supplements on methanogenesis.

MATERIALS AND METHODS

Site description. The study was done in a wooded, temperate basin swamp in Mont St. Hilaire, Quebec, Canada (45°33′N, 73°08′W). Vegetation consisted largely of birch and hemlock (*Betula alleghaniensis* and *Tsuga canadensis*) (22), with some beech and maple representation. Soil at the study site consisted of highly degraded peat (H7 on the von Post scale), with an organic content of >80% by weight (22). Measurements were made at two sites (A and B) 1.5 to 2 m apart. There were no obvious visual differences between the sites except for 5-cm-higher elevation at site B. Inorganic nutrients such as NH_4^+, NO_3^-, and SO_4^{2-} are supplied by both groundwater and throughfall (rainwater passing through the canopy) at respective concentrations of 34 to 37, 65 to 175, and 32 to 190 μM in throughfall and 20 to 60, 0 to 25, and 100 to 200 μM in groundwater (1, 21).

Depth profiles. A group of three acrylic equilibration samplers (60 by 10 by 1.5 cm) (11) was inserted in the ground, within a 30-cm radius, in each site for 13 to 15 days. Before placement, the equilibrator wells (1 cm apart; 4 ml) were filled with deoxygenated, deionized water and covered with a 0.45-μm-pore-size membrane (Tuffryn HT-450; Gelman Sciences, Inc., Ann Arbor, Mich.). The samplers were transported to the sites in a sealed, deoxygenated, water-filled container to prevent atmospheric contamination. After equilibration, the samplers were removed from the ground and immediately (<15 min) sampled to minimize loss of dissolved gases to the atmosphere (11). Measurements were made in the late summer (12 to 26 August 1992), early fall (15 to 29 October 1992), and early spring (28 April to 10 May 1993). The samplers were placed in the same locations during the different samplings.

One of the three samplers at each site was used to determine dissolved CH_4 and inorganic C (mostly CO_2). Well contents were removed with a repeating Cornwall syringe and injected

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into evacuated 14-ml serum vials (14) sealed with grey butyl stoppers and aluminum crimps. Phosphoric acid (10 μl) in the vials halted microbial activity (pH <3) and converted HCO_3^- to CO_2. Dissolved gases were allowed to equilibrate overnight, and the headspace was sampled (0.2 to 0.3 ml) by pressure lock syringe for gas chromatographic analysis (26). Gas remaining in the aqueous phase was calculated by using solubility coefficients (39, 43).

A second sampler was used for pH and ion analyses. Samples were drawn into glass syringes with 22-gauge needles, in which the dead volume was filled with degassed, deionized water (14). The pH was determined with a glass electrode in a closed system to avoid changes from equilibration with the atmosphere (14). Subsequently, sulfate, nitrate, nitrite, acetate, propionate, and butyrate concentrations were determined simultaneously on the same water samples by capillary electrophoresis (Quanta 4000; Waters, Millipore). The components were separated in a 60-cm (75-μm inside diameter) fused silica column, using 5 mM chromate buffer with an osmotic flow modifier (CIA-Pak, OFM Anion-BT; Waters), at a voltage of 20 kV and detected by UV (254 nm). Components were identified by migration time comparison with standards. The detection limit was about 2 μM for organic acids and 0.5 to 1 μM for inorganic anions.

Samples from a third sampler were collected in the same way, and dissolved O_2 was analyzed directly in the syringe by a modification of the Winkler method (33). After sampling, the needles were replaced with small rubber stoppers, and 0.2 ml of Winkler reagents was injected into the syringe, thus avoiding contact of the sample with the atmosphere. Samples were titrated within 2 h in the laboratory. Contamination with atmospheric O_2 during handling was determined on blank syringes (4 ml of deoxygenated water) treated in parallel with the sample syringes. The results were corrected for background contamination (about 30 to 50 μM O_2).

The possibility of biofilm formation affecting the results obtained with equilibrators has been considered (16). Whereas some evidence was found that H_2 measurements may be unreliable, tests concluded that CH_4 concentrations are not affected (3, 16).

Laboratory incubations. Potential rates of methane production and consumption with depth in the soil were determined at each sampling time. Cores were obtained in 3.5-cm-inside diameter, clear plastic tubes and sliced (0 to 2, 2 to 4, 4 to 6, and 12 to 14 cm) within 2 days of retrieval. Individual slices were slurried under N_2 with an equal volume of groundwater. Aliquots (5 to 10 ml) of the slurry were dispensed into 58-ml serum bottles which were capped with grey butyl stoppers and aluminum crimps. The bottles were evacuated (15 min) and backfilled three times to remove dissolved gases from the pore water. Bottles used to determine potential methanogenesis were backfilled with prepurified N_2 or He, while those used to determine potential methanotrophy were backfilled with room air and a 1% final concentration of CH_4. Anaerobic samples were incubated statically, while aerobic samples were shaken at 250 rpm. All samples were incubated in the dark at in situ temperature. Total CH_4 in the bottles was monitored for 6 days, and production or consumption within this period was used as a relative measure of these two processes for different depths in the soil column.

The effect of different supplements on methanogenesis was determined with soil obtained in bulk from the top 10 cm. Samples were stored in sealed mason jars under N_2 (4°C) until use (within 30 to 60 days of collection). Soil was slurried and 30 to 50 ml was dispensed into 58- or 180-ml serum vials, as described above, and incubated at 15°C. Slurries were supplemented with the potential methanogenic substrates methanol, methylamine, dimethylamine, trimethylamine, acetate, and formate (1 mM final concentration each) and H_2 (0.81 to 81.1 kPa). Molybdate (1 mM), an inhibitor of sulfate reduction, was added to examine the effect of this process on methanogenesis. Chloroform (100 μM) was added as an inhibitor of methanogenesis (30). Headspace CO_2 and CH_4 contents were determined as described above, and H_2 was measured with a reduction analyzer by the conversion of HgO to Hg (32). Slurry aliquots (0.5 ml) were removed periodically by syringe and needle (16 gauge), and pore water was obtained by microcentrifugation. Filtered pore water (0.45-μm pore size; Millipore) was analyzed for inorganic anions and short-chain organic acids by capillary electrophoresis, as described above.

In one experiment, slurries were supplemented with different concentrations of leaf leachate (1, 10, and 100 mM C) in order to provide substrates similar to those occurring in situ. Fallen leaves (500 g), collected at the study site, were submerged in 750 ml of distilled water for 1 week (20°C). The resulting leachate was sequentially filtered with GF/D, GF/C, and 0.45-μm-pore-size Millipore filters. The pH was adjusted to 5.5 and the solution was again filtered (0.45-μm pore size). Leachate and groundwater were added to soil to give the desired C concentration while a soil/liquid ratio of 1:1 was maintained. Carbon content in the leachate was estimated as 50% of dry weight. Methanol and ethanol contents in the leachate were determined with a gas chromatograph equipped with a 6-ft (1 ft = 30.48 cm) Chromosorb 101 packed column and flame ionization detector. The oven temperature was controlled to rise from 100 to 115°C at 4°C/min. The detection limit was 50 μM.

RESULTS

Depth profiles. Depth distributions of the measured chemical components of peat pore water varied between sites and sampling time (Fig. 1). Both sites A and B showed progressively anaerobic conditions, decreasing concentrations of NO_3^- and SO_4^{2-}, and increasing CH_4 concentrations from summer (1992) to spring (1993). Differences between sites were most apparent in the levels and distribution of CH_4 and organic acids. Levels of CO_2 (not shown) were similar between sites and seasons, being near atmospheric levels at the top of the soil column and increasing to 1 to 3 mM below 10 cm. Values of pH (not shown) ranged from 5.2 to 5.4 in the summer to 5.75 to 6.45 in the fall and spring of 1992 to 1993, with the higher values in the top 10 cm of the soil column. Soil temperatures (10-cm depth) were 15, 7, and 10°C in the summer, fall, and spring, respectively.

In the summer (Fig. 1A), the water table dropped from 20 to >50 cm below ground level during the equilibration period and strongly influenced the profiles obtained in both sites A and B. Levels of ions equilibrated in the sampler cells above groundwater level are considered to represent concentrations in soil pore fluid. Aerobic conditions, created by drawdown of atmospheric O_2, occurred to 30-cm depth. The highest CH_4 concentrations occurred below 30 cm, corresponding to the disappearance of O_2. High nitrate concentrations (up to 150 μM) were observed in the oxygenated zone, suggesting conditions supporting nitrification. Nitrate and sulfate also accumulated in aerobic slurry incubations (1), indicating nitrifying and sulfide oxidizing potential in the soil. Interestingly, however, both NO_3^- and SO_4^{2-} showed minima at between 2- and 7-cm depth in sites A and B, indicating a zone of high assimilation activity or the existence of microsites with sufficiently low O_2 tension for denitrification and SO_4^{2-} reduction to occur.

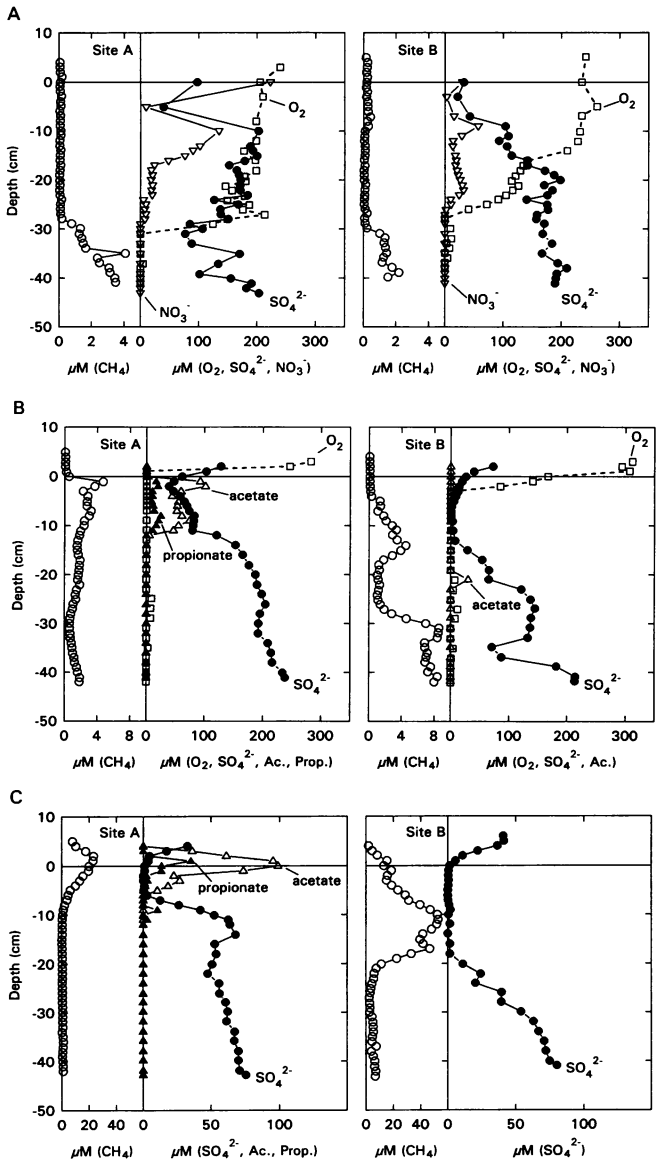

FIG. 1. Depth profiles of chemical components in peat interstitial water measured in August 1992 (A), October 1992 (B), and May 1993 (C). Ground level (0-cm depth) is the interface of the litter layer and underlying soil.

In the fall (Fig. 1B), the water table remained stable (3-cm depth) over the equilibration period. A mat of dense, wet leaf litter 3 to 5 cm thick covered the ground at both sites A and B. These conditions resulted in complete or near complete depletion of O_2 immediately below ground. In site A, the highest CH_4 concentrations occurred at 1- and 7-cm depths, in contrast to the summer profile (Fig. 1A). High concentrations of acetate and propionate (up to 100 μM) were observed in the top 10 cm of the peat column, with maxima corresponding to those of CH_4. Curiously, despite its proximity to site A, we did not detect a similar accumulation of organic acids immediately below the litter mat in site B. Furthermore, CH_4 maxima in site B occurred deeper in the peat column (13- and 30-cm depths) and were of greater magnitude than in site A.

In the spring (Fig. 1C), both sites were covered by 20 cm of water and a 5- to 10-cm-thick mat of decaying leaf material. Anoxic conditions existed throughout the entire peat column as well as in the overlying litter mat (Fig. 1C, sites A and B). Pore water CH_4 concentrations were the highest yet observed, being 5 to 20 times higher than summer and fall values (Fig. 1A and B). Again, unexpected and striking differences occurred between sites despite similar environmental conditions. In site A, the CH_4 maximum again corresponded to organic acid maxima and occurred at the top of the peat column and into the litter mat. In contrast, in site B, a much broader (and greater) methane maximum was observed lower in the peat column (10 cm) and no organic acids were detected. In both sites, minima in SO_4^{2-} concentration corresponded to CH_4 maxima, suggesting that SO_4^{2-} was a controlling factor of in situ methanogenesis. Spring SO_4^{2-} levels (100 μM) were only one-third of the summer and fall values (Fig. 1A and B), possibly reflecting a decrease in SO_4^{2-} input by groundwater and an increase in its assimilation or reduction. Formate and butyrate were not detected at any time.

TABLE 1. Potential methane production rates of anaerobic soil slurries

Depth (cm)	Methane production rate (nmol cm^{-3} day^{-1})[a]					
	Summer (26 Aug.)		Fall (29 Oct.)		Spring (10 May)	
	Site A	Site B[b]	Site A	Site B	Site A	Site B
0–2	0.41		0.81	0.22	0.70	0.10
2–4	1.43		0.39	0.24	0.73	0.08
4–6	0.93		0.48	0.70	0.12	0.09
12–14	0.04		0.33	0.22	0.08	0.06

[a] Rates were calculated at 6 days of incubation.
[b] Not done.

Potential CH_4 production and consumption. Potential rates of CH_4 production and consumption were determined for different depths in sites A and B in an attempt to explain the chemical depth profiles (Fig. 1A to C) in terms of these microbial activities. In site A, the highest potential production rates occurred in the top portions of the soil column (0 to 6 cm) (Table 1). Production rates in site B, however, were more homogeneous with depth and generally lower than in site A, which is inconsistent with the observed in situ CH_4 profiles (Fig. 1A to C). However, the maximal summer rates in site A at 2- to 6-cm depth corresponded to the zone low in the methanogenic inhibitors, NO_3^- and SO_4^{2-}, observed in situ (Fig. 1A). Seasonal rate differences were in part due to the different incubation temperatures (9).

TABLE 2. Potential methane consumption rates of aerobic soil slurries

Depth (cm)	Methane consumption rate (μmol cm^{-3} day^{-1})[a]					
	Summer (26 Aug.)		Fall (29 Oct.)		Spring (10 May)	
	Site A	Site B[b]	Site A	Site B	Site A	Site B
0–2	2.43[c]		0.31	0.01	0.23	0.21
2–4	1.59[d]		0.43	0.37	0.32	0.17
4–6	0.84		0.85	0.25	0.27	0.29
12–14	0.01		0.11	0.05	0.002	0.93

[a] Rates were calculated at 6 days of incubation.
[b] Not done.
[c] Calculated at 3 days due to complete CH_4 consumption.
[d] Calculated at 5 days due to complete CH_4 consumption.

TABLE 3. Effect of inhibitors (1 mM) of methanogenesis and sulfate reduction on production (and consumption) rates of various chemical components in anaerobic soil slurries

Date	Treatment	Production rate (μmol liter of slurry^{-1} day^{-1}) (± 1 SD)[a]				
		CH_4	CO_2	H_2	SO_4^{2-}	Acetate
09-92	Control	0.32 ± 0.02	201 ± 30	0.076 ± 0.026	−17.1 ± 5.1	ND[b]
	$+MoO_4^{2-}$	0.69 ± 0.1	157 ± 37	0.036 ± 0.005	−0.4 ± 5.1	ND
10-92	Control	0.79 ± 0.05	191 ± 20		−14.1 ± 0.8	ND
	$+CHCl_3$	0.03 ± 0.01	235 ± 11		−13.8 ± 0.6	ND
	$+MoO_4^{2-}$	1.51 ± 0.14	197 ± 13		−0.8 ± 1.4	ND
05-93	Control	2.38 ± 0.09	127 ± 37	0.069 ± 0.002	−1.4 ± 0.2[c]	0.20 ± 0.17
	$+CHCl_3$	0.00 ± 0.01	126 ± 30	0.072 ± 0.01	−1.9 ± 0.1[c]	0.18 ± 0.07

[a] Calculated at 13, 8, and 4 days of incubation for 09-92, 10-92, and 05-92 dates, respectively. SO_4^{2-} consumption was calculated at 4 to 6 days due to depletion.
[b] ND, not detected.
[c] Initial SO_4^{2-} concentration was very low (<12 μM).

No consistent pattern of potential CH_4 consumption rates was observed (Table 2). The highest rates were observed in peat which had been oxygenated (site A, 0 to 2 and 2 to 4 cm, summer; Fig. 1A) and which, interestingly, also showed high methanogenic potential (Table 1). However, soil usually under anoxic conditions also showed high oxidation potential (e.g., site B, 12 to 14 cm, spring; Table 2). Unlike methanogenesis, potential methanotrophic activities were similar in sites A and B (Table 2).

Effect of inhibitors on slurries. CH_4 production in soil slurries (containing 80 to 130 μM SO_4^{2-}) was enhanced more than two-fold when sulfate reduction was completely inhibited by 1 mM molybdate (Table 3; Fig. 2), indicating that sulfate reduction was attenuating methanogenesis. Interestingly, the CH_4 production rate in the control (no molybdate) flask did not increase after depletion of SO_4^{2-} to a low, steady-state level (≈9 μM, Fig. 2), as would be expected since inhibition by sulfate reduction should cease. This observation suggests the possibility that rapid S cycling between reduced S and sulfate competes with methanogenesis for reducing power. Despite obvious effects on sulfate reduction and CH_4 production, molybdate addition had no significant effect on CO_2, H_2, or acetate dynamics (Table 3).

Chloroform (100 μM) inhibited CH_4 but not CO_2 production rates (Table 3), confirming selective inhibition of methanogenesis. However, no measurable accumulation of potential methanogenic substrates (H_2, CO_2, and acetate) resulted from the inhibition of CH_4 production within the incubation period (Table 3), in contrast to what has been observed in other studies (17, 30).

Effect of C supplements on methanogenesis. A natural source of organic C, added in the form of leaf leachate, stimulated CH_4 production by soil slurry (Fig. 3). The effect of 10 and 100 mM leachate C additions, although vigorous, was short-lived, lasting only 1 week. The degree of stimulation of CH_4 production was similar for both of these supplements despite a 10-fold difference in added C. A lower concentration of leachate C (1 mM) showed little initial effect but was clearly enhancing methanogenesis as late as 25 days of incubation (Fig. 3).

Capillary electrophoretic and gas chromatographic analyses (data not shown) showed that the leachate contained significant concentrations of ethanol (1.5 mM), methanol (0.49 mM), formate (0.15 mM), acetate (4.2 mM), and propionate (2.8 mM), compounds which can, directly or indirectly, be substrates for methanogens (42). Other compounds which can contribute to CH_4 production (e.g., organic polymers, methylamines, and sugars) were not measured.

Formate and acetate, substrates which can be used directly by many methanogens, showed a time-dependent stimulation of methanogenesis (Fig. 4). Both compounds markedly and rapidly enhanced CH_4 production, but only in the short term. This short-lived effect was due to their consumption by other processes (Fig. 4). Consequently, only a small fraction of their carbon (2.6% of formate C and 0.7% of acetate C) was converted to CH_4.

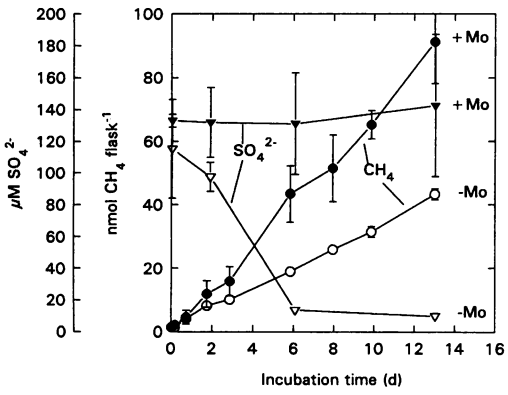

FIG. 2. Concentrations of CH_4 (○, ●) and SO_4^{2-} (▽, ▼) in anaerobic peat slurries in the presence (closed symbols) and absence (open symbols) of 1 mM sodium molybdate. Data are means of duplicates ± 1 standard deviation. d, days.

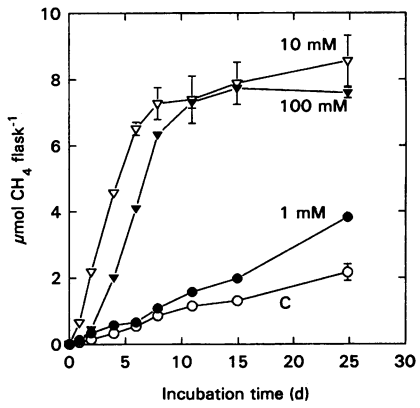

FIG. 3. Effect of leaf leachate supplements on methane production by anaerobic peat slurries. Additions of 1 (●), 10 (▽), and 100 (▼) mM leachate C were compared with a control (○). Data are means of duplicates ± 1 standard deviation. d, days.

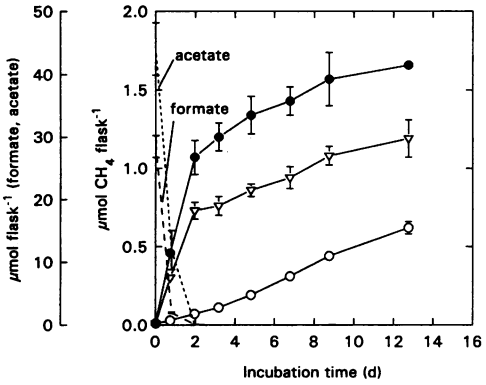

FIG. 4. Production of CH_4 in anaerobic peat slurries supplemented with 1 mM formate (●) or acetate (▽) relative to a nonsupplemented slurry (○). Consumption of the organic acids is indicated by the dashed lines. Levels of the acids in the control flask are too low to be seen on the graph. Data are means of duplicates ± 1 standard deviation. d, days.

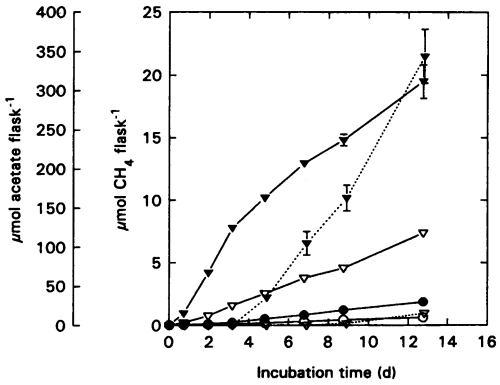

FIG. 6. Effect of supplements of 0.81 (●), 8.1 (▽), and 81.1 (▼) kPa of H_2 on CH_4 (solid lines) and acetate (dotted lines) production in anaerobic peat slurries relative to a control (no additions) (○). Carbon dioxide was added with H_2 at a ratio of 4:1 H_2/CO_2. Data are means of duplicates ± 1 standard deviation.

Several "noncompetitive" substrates (methylated amines and methanol), believed to be primarily methanogenic substrates (5, 28), were also tested, and of these, only methanol significantly enhanced methanogenesis (Fig. 5). The increase was linear over the 13-day incubation, in contrast to the formate and acetate effects, but accounted for a conversion of only 6% of added methanol C to CH_4. We found that methanol enhanced methanogenesis in soil from a 0- to 2-cm depth, but not from a 12- to 14-cm depth (1), which is consistent with a methanogenic population adapted to a natural methanol source from decaying leaf matter at the top of the soil column.

Effect of H_2 supplements. Hydrogen supplements enhanced both methanogenesis and sulfate consumption (assimilation and/or reduction) in soil slurries, but concentrations much higher than steady state (<1 Pa) were required to give a significant effect (Fig. 6; Table 4). At high concentrations of H_2 (≥8 kPa), chemolithotrophic acetogenesis (18) became an important process, with 2 to 20 times more acetate than methane produced. The onset of acetogenesis caused a decrease in CH_4 production rate, indicating competition between the two processes (Fig. 6). However, prior to acetogenesis, rates of H_2 consumption were higher than could be accounted for by methanogenesis and sulfate consumption (Table 4), indicating other anaerobic H_2-consuming reactions. No significant acetate accumulation was observed with addition of 0.8 kPa H_2 or under endogenous H_2 concentrations.

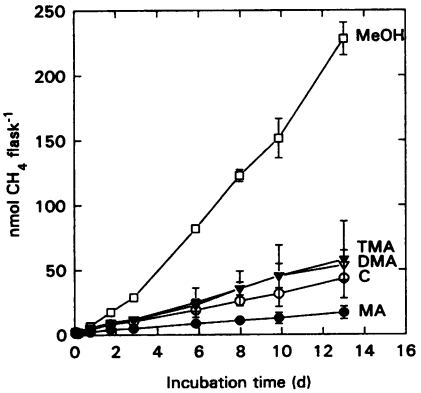

FIG. 5. Production of CH_4 in anaerobic peat slurries in the presence of 1 mM (final concentration) methylamine (MA; ●), dimethylamine (DMA; ▽), trimethylamine (TMA; ▼), or methanol (MeOH; □). A control flask received no addition (C; ○). Data are means of duplicates ± 1 standard deviation.

DISCUSSION

Our results demonstrate the significant combined effects of hydrology, chemistry, and microbiology on net CH_4 production and content in a wetland soil. Water table position determines O_2 tension in the soil column, thus controlling the occurrence of CH_4 production and oxidation. Not surprisingly, several studies found it an important regulator of CH_4 flux (7, 10, 23, 24). However, other studies (25) found a variety of factors which can also affect CH_4 flux and obscure the role of water table level (24). We found a clear relationship between water table level and profiles of CH_4 and O_2 content in the soil column. These profiles, as well as the high potential CH_4 consumption rates in the summer core, further support the previous conclusion (22, 23) that CH_4 oxidation contributes to low CH_4 fluxes at low water table levels in this swamp. The observed CH_4 profiles generally corresponded to the pattern of emission previously measured at this site (23). The absence of aquatic plants and low dissolved CH_4 concentration suggest that plant-mediated flux (31) and bubble ebullition are less important than diffusion in controlling CH_4 flux at this site.

TABLE 4. Effect of H_2 supplements on rates of CH_4 production and H_2 and SO_4^{2-} consumption in anaerobic soil slurry (October 1992)

H_2 added (kPa)	Production rate (μmol liter of slurry^{-1} day^{-1}) (± 1 SD)[a]		
	CH_4	SO_4^{2-}	H_2
0.0	0.8 ± 0.2	−67 ± 14	
0.81	2.1 ± 0.2	−80 ± 3	−66 ± 37
8.1	11.8 ± 1.1	−88 ± 4	−322 ± 71
81.1	69.6 ± 5.2	−110 ± 2	−3,125 ± 660

[a] Calculated at 3 days (CH_4 and H_2), while activity was linear, and before onset of acetogenesis (see Fig. 6). SO_4^{2-} consumption was calculated at 2 days due to rapid disappearance.

Oxygenation of the soil column can lead to changes in peat pore water chemistry which may influence net production and distribution of CH_4 even after O_2 depletion. For example, nitrification and sulfide oxidation can, as we observed, generate high concentrations of NO_3^- and SO_4^{2-} in the soil. Higher levels of NO_3^- and SO_4^{2-}, inhibitors of methanogenesis (2, 34, 36), may help to maintain low in situ CH_4 by retarding its production when anaerobic conditions resume. Evidence for this effect was obtained with incubated samples from the summer core, in which the highest CH_4 production rates occurred from depths where in situ concentrations of SO_4^{2-} and NO_3^- showed distinct minima. This effect may partly explain the strong hysteresis in CH_4 flux observed for soil columns in which the water table level was manipulated (24).

The mechanism of inhibition of methanogenesis by NO_3^- (and other N oxides) is not clear (2, 36). However, inhibition by sulfate is attributed to competition for H_2 and acetate between methanogens and the more efficient sulfate reducers (19, 34) and depends on the relative amounts of substrates and SO_4^{2-}. The inverse in situ SO_4^{2-} and CH_4 profiles seen in the spring indicated an inhibitory effect of SO_4^{2-} on methanogenesis, as was confirmed by slurry incubations with molybdate. Previously regarded as important mainly in high-sulfate environments (34), this phenomenon has been reported in freshwater sediment (19) and wetland soil (36, 45) with sulfate concentrations similar to those here (100 to 200 μM). Some wetland studies have reported variable effects of sulfate reduction on methane production (44, 45). SO_4^{2-} may be a more important inhibitor of methanogenesis than NO_3^- in this swamp due to its larger pool size and constant supply by groundwater.

One of the most important chemical factors regulating methanogenesis in natural systems, other than the presence of alternate electron acceptors, is the availability of metabolizable organic C (13). Higher rates of methanogenesis and CH_4 content in wetland soils are correlated to higher levels of extractable, labile organic C (44) as well as to easily decomposed types of vegetation (27). In our study site, yearly leaf fall is the major source of fresh, labile, organic C (23). The effect of this C on microbial activity was evident in the corresponding maxima of in situ CH_4 and litter-derived organic acid content in site A. The low SO_4^{2-} and high CH_4 concentrations, as well as potential CH_4 production and consumption rates, indicated higher microbial activity in the upper 10 to 20 cm of peat, consistent with a source of labile organic C at the surface.

Anaerobic incubations with leaf leachate confirmed an initial stimulation of methanogenesis by leaf litter but also showed a potential for its attenuation with time. Accumulation of metabolic end products of organic matter degradation, such as organic acids and NH_3 (12, 40, 45), may have been responsible for this late occurring inhibition. Inhibition of gas production by volatile and nonvolatile metabolites was observed in anaerobic incubations of forest soils (20). Exchange of metabolites with the atmosphere and groundwater may offset the effect of inhibitory compounds in nature (20, 40). However, low rates of methanogenesis in our site (<2.5 μmol liter^{-1} day^{-1}) could be due to accumulated inhibitory compounds in the soil. Indeed, soil from this swamp inhibited methanogenesis in a peat from a different region (8).

When compared with another nearby basin swamp, our site showed lower CH_4 flux (1.2 versus 4.2 g m^{-2} year^{-1}) and significantly lower potential CH_4 production (4 versus 89 nmol g^{-1} day^{-1}) (23). Our results show that this is due in part to the apparent domination of anaerobic C mineralization by processes other than methanogenesis. We calculate that methanogenesis accounted for only 0.3 to 3.7% of C mineralized in slurry incubations. These values are lower than previous reports for wetlands in which the bulk of anaerobic C flow was shown to be through sulfate reduction (35, 36, 38). Such a minor role for methanogenesis is contrary to current concepts of anaerobic C flow in freshwater systems, where it is expected to dominate.

The domination of anaerobic metabolism by other processes was illustrated by the high nonmethanogenic H_2 consumption, a lack of accumulation of CH_4 precursors upon specific inhibition of methanogenesis, and very low conversion of formate and acetate C supplements to CH_4 (only 10 and 1.5% of the expected, assuming one CH_4 molecule produced per one acetate or four formate molecules consumed [42]). In contrast, for another peat type, we observed that >85% of the expected acetate C was converted to CH_4 (1). Furthermore, of the traditional non-competitive substrates, only methanol was utilized by swamp methanogens. The linearity of the observed increase in CH_4 production may indicate that methanol is primarily a methanogenic substrate in this swamp, despite the fact that it has been shown to support sulfate reduction in some environments (15, 36). The slow conversion of methanol-C to CH_4 was perhaps indicative of inhibition of the methanogenic population by components in the soil.

It is not clear which process(es) controls anaerobic C mineralization in this swamp. The observed net rates of sulfate disappearance (14 to 67 μmol liter of slurry^{-1} day^{-1}) indicate that sulfate reduction alone cannot account for the high rate of anaerobic CO_2 production. However, rapid cycling of S has been implicated in greater than expected anaerobic C mineralization by sulfate reduction in low-sulfate wetlands (38). We found millimolar quantities of reduced inorganic S in the soil (1) which could support such a cycle and possibly explain the lack of response of CH_4 production rate to the depletion of soluble SO_4^{2-} in anaerobic soil slurries. Nevertheless, the lack of effect of sulfate reduction inhibition on acetate, H_2, and CO_2 levels suggests that it was a minor process in anaerobic metabolism in the soil. The high CO_2 production observed could have been due to a variety of processes including reduction of iron, manganese, and S^0 (464 to 1,068 μmol liter^{-1} in situ), fermentation reactions, and even chemical equilibration of bicarbonate. Nitrate levels were too low for any significant contribution to anaerobic C mineralization in slurry incubations.

A significant observation of this study was the striking difference in chemical profiles between the two sites, A and B. Natural variability in wetland systems is not uncommon. Large variations in CH_4 emissions between wetland types and within wetlands have been reported (7, 41) due in large part, but not completely (7), to hydrology, temperature, and nutrient considerations. However, the scale of the distance between measurements is usually large (several meters to kilometers), and we did not expect such a large difference between sites so close (<2 m) and under identical conditions of hydrology and source of fresh organic C (see Results). It does not seem likely that uneven distribution of organic matter could explain the high spatial variability of organic acid concentration in our site, as it can in rice paddy soil (29). Rothfuss and Conrad (29) concluded that a homogeneous microbial population operating at highly variable rates contributed to the large spatial chemical variability in a paddy soil. Such a mechanism might also explain our observations. For example, the high concentrations of organic acids in site A, but not in site B, in the fall and spring may indicate a dominance of fermentative over respiratory processes in site A, but not in site B. We speculate that, along with differences in hydrology and chemical characteristics, heterogeneity in microbial activity may also contribute to the spatial variability of CH_4 production and emission in wetlands.

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