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Control of carbon mineralization to CH_4 and CO_2 in anaerobic, *Sphagnum*-derived peat from Big Run Bog, West Virginia

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Abstract. The mineralization of organic carbon to CH_4 and CO_2 in *Sphagnum*-derived peat from Big Run Bog, West Virginia, was measured at 4 times in the year (February, May, September, and November) using anaerobic, peat-slurry incubations. Rates of both CH_4 production and CO_2 production changed seasonally in surface peat (0–25 cm depth), but were the same on each collection date in deep peat (30–45 cm depth). Methane production in surface peat ranged from 0.2 to 18.8 μmol mol(C)$^{-1}$ hr^{-1} (or 0.07 to 10.4 μg(CH_4) g^{-1} hr^{-1}) between the February and September collections, respectively, and was approximately 1 μmol mol(C)$^{-1}$ hr^{-1} in deep peat. Carbon dioxide production in surface peat ranged from 3.2 to 20 μmol mol(C)$^{-1}$ hr^{-1} (or 4.8 to 30.3 μg(CO_2) g^{-1} hr^{-1}) between the February and September collections, respectively, and was about 4 μmol mol(C)$^{-1}$ hr^{-1} in deep peat. In surface peat, temperature the master variable controlling the seasonal pattern in CO_2 production, but the rate of CH_4 production still had the lowest values in the February collection even when the peat was incubated at 19 °C. The addition of glucose, acetate, and H_2 to the peat-slurry did not stimulate CH_4 production in surface peat, indicating that CH_4 production in the winter was limited by factors other than glucose degradation products. The low rate of carbon mineralization in deep peat was due, in part, to poor chemical quality of the peat, because adding glucose and hydrogen directly stimulated CH_4 production, and CO_2 production to a lesser extent. Acetate was utilized in the peat by methanogens, but became a toxin at low pH values. The addition of SO_4^{2-} to the peat-slurry inhibited CH_4 production in surface peat, as expected, but surprisingly increased carbon mineralization through CH_4 production in deep peat. Carbon mineralization under anaerobic conditions is of sufficient magnitude to have a major influence on peat accumulation and helps to explain the thin (< 2 m deep), old (> 13,000 yr) peat deposit found in Big Run Bog.

Introduction

Sphagnum-dominated wetlands are characterized by long-term accumulations of detrital organic carbon commonly referred to as peat. This situation arises because the input of carbon from organic matter production has exceeded the amount of carbon mineralized through organic matter decomposition. It is generally assumed that carbon mineralization of *Sphagnum*-derived peat is inhibited by the anaerobic, acidic conditions found in the wetland (Clymo 1965, 1983).

Carbon mineralization in anaerobic aquatic sediments has been reviewed by (Nedwell 1984); only the mechanisms that are operative in wetlands are described here (Fig. 1). The first step in the decomposition of peat is hydrolysis by fermentative bacteria which produces a variety of end products including ace-

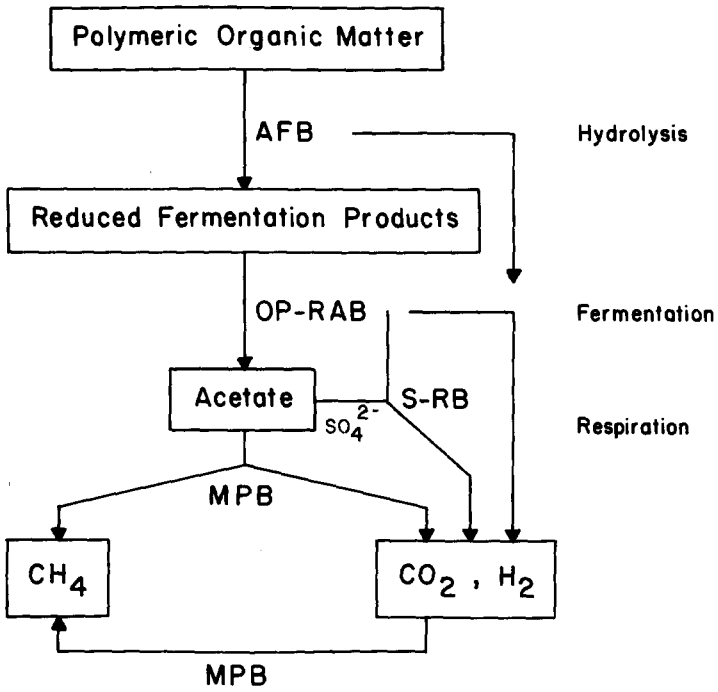

Fig. 1. Schematic representation of carbon mineralization from the decomposition of organic matter under anaerobic conditions in a freshwater wetland. This schematic is not inclusive of all the potential mineralization pathways. AFB denotes acid forming bacteria, OP-RAB denotes obligate proton-reducing acetogenic bacteria, S-RB denotes sulfate-reducing bacteria, and MPB denotes methane producing bacteria.

tate, H_2, and CO_2. These end products become substrates for terminal respirations, such as sulfate reduction, and/or CH_4 production. When sulfate reduction is the dominant terminal process, acetate and H_2 are preferentially utilized by sulfate-reducing bacteria (Mechalas 1974, Billen 1982, Kristjansson *et al.* 1982) and CH_4 production should be inhibited. Under conditions of limited SO_4^{2-} availability, CH_4 production becomes the terminal process of carbon mineralization (Ward and Winfrey 1985), thereby producing equimolar amounts of CO_2 and CH_4 (Froelich *et al.* 1974). While methanogens have been isolated from acidic *Sphagnum*-derived peat (Williams and Crawford 1983), laboratory studies suggest that they metabolize best at near-neutral pH of the environment (Wolfe and Higgins 1979).

This paper examines carbon mineralization through CH_4 production and CO_2 production pathways in peat from Big Run Bog (39°0′N, 79°35′W), a *Sphagnum*-dominated wetland in the Appalachian Mountains of West Virginia. Since Big Run Bog receives water and nutrients from the surrounding watershed, it is physiographically a minerotrophic fen and not a true ombrotrophic bog. The water table is at or near the surface most of the year. Oxygen content of the

water drops from 4 to $< 0.5\,mg\ L^{-1}$ (limit of detection) within 20 cm of the surface. Surface water has an average pH of 4.0 and a SO_4^{2-} concentration of $50\,\mu mol\ L^{-1}$ (Wieder 1985). Structurally and functionally Appalachian wetlands are similar to their circumboreal counterparts (Gore 1983), but by comparison have thinner deposits of more highly decomposed peat (Cameron 1968), despite being older (Watts 1979) and having more net primary production (Wieder and Lang 1983). In combination these characteristics suggest that the mineralization of carbon in organic matter decomposition has a major influence on peat accumulation in Big Run Bog.

The specific objectives of this study were to evaluate the importance of temperature and substrate quality of the peat in controlling the rate of carbon mineralization. We used peat collected at different times of the year and incubated under controlled temperature conditions. The influence of substrate quality was examined by amending the peat prior to incubation with either glucose (principle product of organic matter hydrolysis), acetate, H_2/CO_2, SO_4^{2-}, or MoO_4^{2-} (an inhibitor of sulfate reduction; Banat and Nedwell 1984).

Methods

Sampling

Peat was collected in September 1984, November 1984, February 1985, and May 1985 from the *Sphagnum-Eriophorum* community in Big Run Bog; see Wieder (1985) for a description of the peat and water chemistry of this community. On each date, 3 cores were randomly taken using a 50 cm tall, 30 cm diameter plastic cylinder that was open at both ends and pushed downwards into the peat until the top was flush with the peat surface. Compaction of the peat within the cylinder was minimal. After removing the green *Sphagnum* biomass, the peat was sectioned into 6 separate depth intervals (0–5, 5–10, 10–15, 20–25, 30–35, and 40–45 cm beneath the surface). Peat from the 3 cores from each depth interval was combined and placed into an air-tight plastic container and transported back to the laboratory for processing within 4 hr after collection.

Laboratory incubations

Our approach was to incubate at prescribed temperatures a peat-slurry with or without specific chemical amendments and then measure carbon mineralization as the rate of CH_4 and CO_2 production. Slurries ensure uniform distribution of an amendment but have been observed to reduce the rate of carbon flow in aquatic sediments (Jorgensen 1978, Jones and Simon 1984). Although we did not test this observation, the use of slurries probably has less of an influence on carbon flow in peat than in sediments because peat is 90% water by volume and

is an amorphous substrate unlike structured sediments. Pore water collected from 10–20 cm below the wetland surface was maintained in an O_2 free container until used for the slurry.

For each depth interval replicate slurries were prepared separately in 250 mL Erlenmeyer flasks. While the bulk peat was maintained under a constant stream of N_2, a 75 cm^3 subsample of peat was taken and placed into a flask followed by the addition of pore water to give a final volume of 125 cm^3 . Each flask was capped with a rubber stopper that was fitted with an aluminum-crimped stopper for sampling the head space gas, and immediately degassed 3 times with N_2 to ensure anaerobic conditions. This procedure is a modification of the technique described by Balch *et al.* (1979) for handling methanogens. All flasks were incubated without shaking.

On each collection date 3 replicate flasks from each of the 6 depth intervals were incubated at the ambient temperature of the surface peat measured on the day of collection, *i.e.*, 19 °C for September 1984, 12 °C for November 1984 and May 1985, and 4 °C for February 1985. Another set of 3 replicate flasks were incubated at a constant temperature of 19 ° ± 2 °C. In the wetland during the September collection, ambient temperature decreased from 19 °C at the surface to 15 °C at the 15 cm depth, thereafter remaining constant with increasing depth. On the other 3 collection dates, the temperature range was less than 2 °C between surface and deep peat.

In one experiment, peat from each of the 6 depth intervals from the September collection was amended with glucose at 10 mg(C) $flask^{-1}$. In a second experiment, peat from the November collection was amended with acetate (*i.e.*, $NaC_2H_3O_2$) at 4 mg(C) $flask^{-1}$. The acetate amendment was investigated along with a concomitant pH adjustment because organic acids may be toxic to anaerobic bacteria, especially when present in the undissociated form (Wolin 1969). Peat from each of the 5–10, 20–25 and 40–45 cm depth intervals was divided into 3 subsamples. The pH of the first subsample was lowered from the field pH value of 4.45 to a pH of 3.1 with 0.1 M HCl. Then one-half of this subsample was dispensed into 3 replicate flasks containing the acetate amendment. The remaining one-half of the 3.1 pH subsample was dispensed into 3 replicate flasks without any acetate addition and was used as a control. Using 0.1 M NaOH, the pH of the second subsample of peat was raised to a pH of 6.4, a value greater than the pK of acetic acid (4.75). As above, one-half of the subsample was dispensed into 3 replicate flasks containing the acetate amendment; the remaining one-half was divided into 3 flasks and served as a control for the pH effect. A third subsample of the peat was treated similarly with an acetate amendment but without any pH adjustment. For both experiments, flasks were gently agitated initially and then incubated at 19 ° ± 2 °C.

The effect of H_2 as a limiting substrate for carbon mineralization was investigated by adjusting the head space atmosphere in each of 3 replicate flasks containing peat from each of the 6 depth intervals from the November collection to a gas phase mixture of 90% N_2, 8% H_2, and 2% CO_2. These flasks were shaken periodically during the incubation period to enhance mixing of the head space gas with the peat. The flasks were incubated at 19 ° ± 2 °C. As a part of

this experiment the pH of the peat in each treatment and control flask was measured prior to and after incubation to determine if the CO_2 addition altered the bicarbonate buffering equilibrium.

Carbon flow through the sulfate reduction pathway was evaluated using SO_4^{2-} amendments and a MoO_4^{2-} amendment. Sulfate was added at 3 different concentrations to determine whether CH_4 production decreased and CO_2 production increased with increasing SO_4^{2-} concentration. Molybdate was used to inhibit sulfate reduction. Peat from each of the 6 depth intervals from the May collection was placed into 12 flasks. The sulfate concentration in 3 replicate flasks was adjusted with Na_2SO_4 to a final concentration of 0.1 mmol L^{-1}, in another 3 flasks to 1 mmol L^{-1}, and in another 3 flasks to 5 mmol L^{-1}. Peat in the last 3 flasks was adjusted to a MoO_4^{2-} concentration of 2 mmol L^{-1} using Na_2MoO_4. The flasks were gently agitated and then incubated at 19° ± 2 °C; the unamended flasks, which had 0.05 mmol L^{-1} background SO_4^{2-} and which were incubated at 19° ± 2 °C, served as the control.

Measurements

Gas chromatography was used to measure changes in the CH_4 and CO_2 concentrations in the head space of the flasks. In each experiment, 1.0 mL gas sample was taken from each flask at a 24–48 hr interval over a 10 day period. Component gases were separated with a Poropak Q column and detected by thermal conductivity. Gas concentrations in the head space generally increased linearly with time. Following the incubation period, the dry mass of the peat in the slurry was determined gravimetrically.

Rates of CH_4 production and CO_2 production typically are expressed per unit of material, *i.e.*, per gram of peat. However, we used the carbon content of the peat since carbon is the material from which CH_4 and CO_2 are produced. Carbon contents of representative subsamples of peat from the different depths were determined on a Coleman C–O–H analyzer.

Carbon quantities of peat (mol(C) $g(peat)^{-1}$) for each depth interval are; 0.0375, 0–5 cm; 0.0325, 5–10 cm; 0.0291, 10–15 cm; 0.0283, 20–25 cm; 0.0266, 30–35 cm; and 0.0241, 40–45 cm.

Results and discussion

Effect of temperature on unamended peat

Rates of both CH_4 production and CO_2 production in peat incubated at ambient temperature varied with date, but the effect was not the same at all depths as indicated by a significant date by depth interaction in the two-way ANOVA (Table 1A). Generally the rates of CH_4 production and CO_2 production from each of the 4 sampling intervals in the top 25 cm of peat increased from the lowest values in the February collection to maximum values in the September

Table 1. Results of the two-way ANOVA showing the effects of collection date and peat depth on rates of CH_4 production and CO_2 production. Incubation temperatures were: (A) ambient peat temperature on the date of collection, and (B) 19 °C.

Product	Source	df	MS	F
A.				
CH_4	Date	3	496.2	191.9*
	Depth	5	245.5	95.0*
	Date × Depth	15	57.7	22.3*
	Error	48	2.6	
CO_2	Date	3	205.8	48.9*
	Depth	5	224.6	53.4*
	Date × Depth	15	29.7	7.1*
	Error	48	4.2	
B.				
CH_4	Date	3	250.8	47.5*
	Depth	5	492.3	93.1*
	Date × Depth	15	35.0	6.6*
	Error	48	5.3	
CO_2	Date	3	9.2	1.6
	Depth	5	526.9	9.9*
	Date × Depth	15	3.5	0.6
	Error	48	5.7	

*$p < 0.001$

and November collections (Fig. 2). In contrast, the rate of carbon mineralization in peat from each of the 2 deepest sampling intervals was relatively invariant across the 4 collection dates. Williams and Crawford (1984) also reported that seasonal fluctuations in temperature in a *Sphagnum* wetland in Minnesota had a greater effect on CH_4 production in surface peat than in deep peat.

Low temperature had less of an impact on CO_2 production than on CH_4 production (Fig. 2). In peat from the February collection, the rate of CO_2 production was about 4 μmol mol(C)$^{-1}$ hr^{-1} across all depth intervals, whereas the rate of CH_4 production was < 0.5 μmol mol(C)$^{-1}$ hr^{-1}. In peat from the May collection, carbon flow through the CO_2 production pathway was still greater than through the CH_4 production pathway at each depth interval, but by the September collection the amount of carbon flow through each production pathway was nearly equal.

The incubation of peat from each collection date at 19 °C allowed us to eliminate the effect of temperature in controlling carbon mineralization and to evaluate other factors. The rate of CH_4 production in surface peat (0–25 cm depth) still exhibited an increase from the February to May to September collections (Fig. 3). By combining the results from Figs. 2 and 3, it appears that CH_4 production in surface peat in winter must be limited by other factors besides low temperature. One possibility is the presence of a low population of methanogens which limits CH_4 production; alternatively reduced activity of hydrolytic organisms that convert polymeric organic matter to fermentable

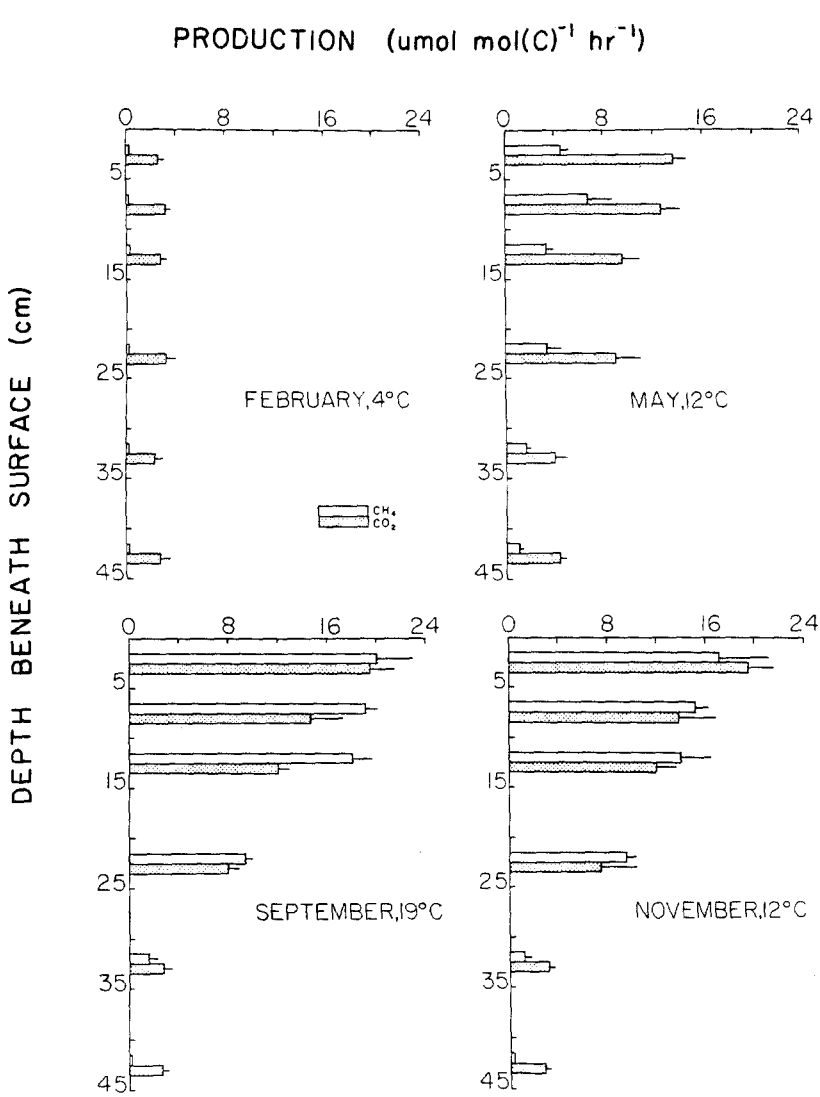

Fig. 2. Mean rates of CH_4 production and CO_2 production in peat. Temperatures represent conditions in the field on the date of collection and in the subsequent laboratory incubations. Line segments represent 1 standard error.

substrates may limit the activity of the methanogenic bacteria. The persistent seasonal pattern in CH_4 production was not observed in peat from the 2 deepest sampling intervals (Table 1B, Fig. 3). Here, CH_4 production rates in peat incubated at 19 °C were low regardless of the date of collection.

In contrast, eliminating the effect of temperature eliminated the seasonal pattern in CO_2 production in peat from each depth interval (Table 1B, Fig. 3). That is peat from the February and May collections produced essentially the

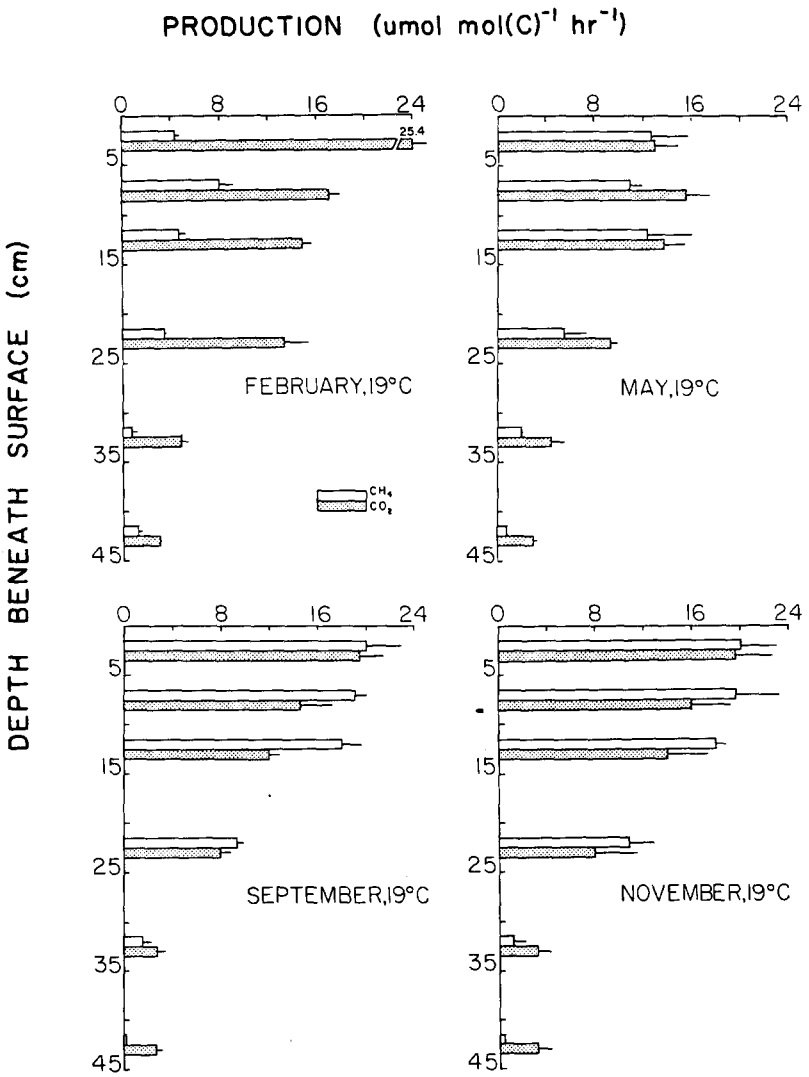

Fig. 3. Mean rates of CH_4 production and CO_2 production in peat. All samples were incubated at 19 °C. Line segments represent 1 standard error.

same amount of CO_2 as peat from the September and November collections, indicating that the organic matter was as equally decomposable by CO_2-producing bacteria in the winter as in the summer. Benner *et al.* (1986) also have shown that temperature was the major environmental factor responsible for seasonal differences in the rate of hydrolysis of salt marsh peat.

Effects of amendments with organic substrates and hydrogen

Anaerobic degradation of glucose directly results in CO_2 production, as well as producing substrates for sulfate reduction and CH_4 production (Fig. 1). The rate of CH_4 production in peat from the top 3 sampling depths was not significantly affected by the addition of glucose, but in peat from the 20–25, 30–35, and 40–45 cm depth intervals the relative increase was 1.7-, 6.1-, and 9.0-fold, respectively (Table 2). The glucose amendment increased the rate of CO_2 production at all depths, but the effect was significant only in peat from the 20–25 and 30–35 cm depths (1.5- and 4.3-fold increases, respectively; Table 2). Consequently the rates of CH_4 production and CO_2 production in near surface peat (0–15 cm depth) probably are not limited by the availability of substrates derived from glucose degradation. Deep peat is, however, of a poorer organic chemical quality, which apparently is responsible for the low rates of carbon mineralization especially through the CH_4 production pathway. Presumably the added glucose in deep peat was utilized by obligate proton-reducing acetogenic bacteria (Fig. 1), producing acetate that was directly utilized by methanogenic bacteria. This would account for the increase in the molar CH_4:CO_2 ratio from < 0.6:1 in the unamended deep peat to 1:1 in peat with glucose.

Acetate is a substrate for CH_4 production, but may also be metabolized to CO_2 by sulfate-reducing bacteria without producing CH_4 (Fig. 1). Methane production rates were markedly inhibited by the acetate amendment in peat from the 3 different sampling intervals incubated at the field pH and when the pH was lowered to 3.1 (Table 3). However, when the pH was increased to 6.4, the addition of acetate stimulated CH_4 production over that in unamended peat 2.2- and 1.6-fold for the 0–5 and 20–25 cm depths, respectively. The pH adjustment of the peat independent of the acetate amendment gave contrary responses. When the pH was lowered to 3.1, very low rates of CH_4 production resulted compared to that in unamended peat at the field pH; however, when the pH was

Table 2. Effect of the glucose amendment on rates of CH_4 production and CO_2 production. Numbers are means of 3 replicates ± 1 standard error in parentheses. A paired t-test was used to compare production rates between control and amended flasks.

Depth Interval (cm)	Production (μmol mol (C)$^{-1}$ hr^{-1})			
	CH_4		CO_2	
	Control	Amended	Control	Amended
0–5	20.2(2.9)	18.2(4.3)	19.4(2.2)	20.3(2.4)
5–10	18.8(0.8)	20.9(2.1)	14.5(2.9)	16.7(4.8)
10–15	18.1(1.4)	18.8(3.3)	11.8(0.7)	12.4(1.7)
20–25	9.3(0.4)	16.2(1.8)*	7.9(0.9)	11.8(0.7)**
30–35	1.5(0.4)	9.2(1.0**	2.7(0.5)	11.7(0.4)***
40–45	0.3(0.1)	2.7(0.2)***	2.4(0.5)	2.4(0.5)

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$

Table 3. Effect of the acetate amendment on rates of: (A) CH_4 production, and (B) CO_2 production. Numbers are means of 3 replicates. A paired t-test was used to compare production rates at each pH condition.

Depth interval (cm)	CH_4 Production (μmol mol (C)$^{-1}$ hr^{-1})					
	pH = 3.1		pH = 4.45		pH = 6.40	
	Control	Amended	Control	Amended	Control	Amended
A.						
0–5	4.1	0.2***	20.2	0.2***	12.5	27.7***
20–25	3.0	0.3***	9.3	1.5***	9.3	15.0*
30–35	0.6	0.5	1.5	0.4*	2.6	1.9
Depth interval (cm)	CO_2 Production (μmol mol (C)$^{-1}$ hr^{-1})					
	pH = 3.1		pH = 4.45		pH = 6.40	
	Control	Amended	Control	Amended	Control	Amended
B.						
0–5	17.1	16.5	19.4	5.5***	21.6	20.8
20–25	7.0	5.5	7.9	6.1	9.0	10.0
30–35	3.5	2.6	2.7	5.2**	3.8	4.6

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$

increased to 6.4, CH_4 production was not significantly increased over that at pH 4.45. In general, the acetate amendment and the pH adjustment had little effect on the rate of CO_2 production in peat from each of the 3 depth intervals (Table 3).

With regard to CH_4 production, the results of the acetate amendment confirm the findings of Wolin (1969) who reported that organic acids in low pH environments are toxic to many microorganisms. The undissociated forms of organic acids can permeate cytoplasmic membranes and acidify the cytoplasm. Presumably in studies where acetate amendments stimulated CH_4 production (*cf.* Svensson 1984), the pH of the environment was greater than the pK of acetic acid. The inhibitory effect of the acetate amendment only applies to CH_4-producing bacteria in the peat since there was not a similar decrease in CO_2 production (Table 3).

Because CH_4 production in peat from the 30–35 cm depth was stimulated by the glucose amendment but not by the acetate amendment, we evaluated the possibility that CH_4 production in the deepest peat was limited by H_2. In peat from the top 3 sampling depths, the H_2/CO_2 amendment did not significantly affect CH_4 production; however, the rate of CH_4 production in peat from both the 20–25 and 30–35 depths was significantly increased by the amendment (Table 4). At the 40–45 cm depth, the production rate in the treatment flasks was 2-fold greater than in the control flasks, although the effect was not statistically significant. The results of these experiments imply that CH_4 production in the

Table 4. Effect of the H_2 /CO_2 amendment on rates of CH_4 production and CO_2 production. Numbers are means of 3 replicates ± 1 standard error in parentheses. A paired t-test was used to compare production rates between control and amended flasks.

Depth interval (cm)	Production (μmol mol $(C)^{-1}$ hr^{-1})			
	CH_4		CO_2	
	Control	Amended	Control	Amended
0–5	17.2 (3.8)	25.4 (2.6)	19.5 (2.0)	10.9 (1.8)**
5–10	15.2 (2.0)	16.1 (3.4)	13.8 (3.0)	6.6 (0.5)*
10–15	14.7 (2.6)	17.7 (3.7)	12.0 (1.4)	13.6 (1.8)
20–25	9.7 (0.8)	17.7 (1.0)***	7.6 (3.0)	4.8 (2.5)
30–35	1.2 (0.6)	2.0 (0.3)***	3.2 (0.5)	2.1 (0.7)
40–45	0.2 (0.1)	0.4 (0.5)	2.8 (0.4)	4.3 (0.5)

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$

deepest peat proceeds primarily through the CO_2-reduction pathway (Fig. 1), and that the rate is limited, in part, by the availability of H_2.

The H_2/CO_2 amendment significantly inhibited the rate of CO_2 production in peat from the 0–5 and 5–10 cm depths, but thereafter had no effect on CO_2 production throughout the remainder of the depth profile. In none of these experiments was there any measurable change in the pH due to the addition of CO_2. It is possible that in surface peat the H_2/CO_2 amendment created unfavorable thermodynamic conditions for the acid forming bacteria and resulted in less CO_2 production directly from fermentation (Sorensen *et al.* 1981, Jones *et al.* 1982).

Effects of sulfate and molybdate amendments

Methane production generally decreased with increasing concentrations of SO_4^{2-}; the trend was most evident in peat from the 0–25 cm depth (Fig. 4). In deep peat (30–45 cm depth), however, we observed a 4-fold increase in the rate of CH_4 production in the 0.1 mmol L^{-1} SO_4^{2-} treatment, and rates higher than in the control in the other SO_4^{2-} treatments. In general, the rate of CO_2 production was stimulated by SO_4^{2-} addition (Fig. 4).

Sulfate concentrations are low in most freshwater environments so that sulfate-reducing bacteria are limited by SO_4^{2-} availability. When SO_4^{2-} is added, carbon flow through the sulfate reduction pathway is stimulated in lieu of carbon flow through the CH_4 production pathway (Ward and Winfrey 1985). The results shown in Fig. 4 generally confirm this classic scenerio, even though we did not always observe a proportional increase in CO_2 production to exactly compensate for the decrease in CH_4 production. It is also important to note that both CH_4 production and CO_2 production in deep peat were stimulated by an increase in pore water SO_4^{2-} concentration. Winfrey and Zeikus (1977) and Zaiss

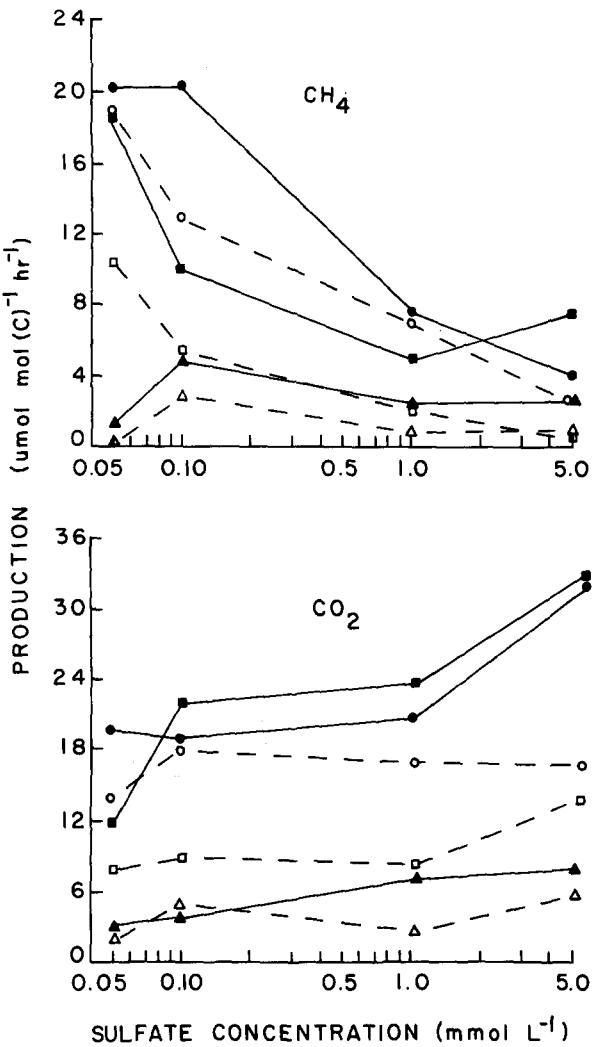

Fig. 4. Effect of increasing SO_4^{2-} concentration on the rate of CH_4 production and CO_2 production in peat from 6 different depth intervals; ●–●, 0–5 cm; ○–○, 5–10 cm; ■–■, 10–15 cm; □–□, 20–25 cm; ▲–▲, 30–35 cm; △–△, 40–45 cm.

(1981) both have reported that the inhibition of CH_4 production by SO_4^{2-} addition in freshwater sediments could be reversed by the addition of acetate or H_2. Possibly the addition of SO_4^{2-} to deep peat started a cascade of anaerobic reactions that lead to an increase in the production of such substrates. For example, the stimulation of sulfate reduction could have consumed H_2 or some other reducing equivalents, thereby creating more favorable thermodynamic conditions for the fermentation of organic matter and an increase in the production of substrates that stimulated concomitant methanogen activity.

The addition of MoO_4^{2-} had virtually no effect on either CH_4 production or CO_2 production in peat from each of the depth intervals (Table 5). These results are contrary to others who have reported that in marine sediments, adding MoO_4^{2-} inhibits CO_2 production through the sulfate reduction pathway (Banat and Nedwell 1984) and stimulates CH_4 production (Oremland and Taylor 1978). However, the results in Table 5 should not be taken to mean that sulfate reduction is unimportant in peat from Big Run Bog because studies with $^{35}SO_4^{2-}$ show substantial sulfate reduction activity (Wieder and Lang manuscript). It is possible that the sulfate-reducing bacteria in low-SO_4^{2-} environments are not inhibited by MoO_4^{2-} like those in high-SO_4^{2-} environments.

Ecological relevance

Comparison with other ecosystems. The CH_4 production rate in September for the 0–5 cm depth interval expressed on a dry mass basis is 10.4 $\mu g(CH_4)$ g^{-1} hr^{-1}. Comparable values for laboratory incubations of *Sphagnum*-derived peat from the surface of Minnesota and Swedish wetlands are 3.6 and 1.1 $\mu g(CH_4)$ g^{-1} hr^{-1}, respectively (Williams and Crawford 1984, Svensson 1984, respectively). A representative value for rice paddy sediments is 0.5 $\mu g(CH_4)$ g^{-1} hr^{-1} (Holzapfel-Pschorn *et al.* 1985), for an anerobic mineral soil, 0.08 $\mu g(CH_4)$ g^{-1} hr^{-1} (Bollag and Czlonkowski 1973), and for marine sediments, $< 0.02\,\mu g(CH_4)$ g^{-1} hr^{-1} (Griffiths *et al.* 1983). Even when compared on a per unit of carbon basis, the maximum CH_4 production rate in this study is about 2-fold greater than the maximum value for anoxic sediments in hypereutrophic Knaack Lake (Phelps and Zeikus 1984). Based on these representative values, it appears that CH_4 production at Big Run Bog far exceeds that reported for other natural ecosystems.

Controls on carbon mineralization. Temperature was the master variable controlling the seasonal pattern in carbon mineralization in *Sphagnum*-derived peat

Table 5. Effect of the molybdate amendment on rates of CH_4 production and CO_2 production. Numbers are means of 3 replicates ± 1 standard error in parentheses. A paired t-test was used to compare production rates between control and amended flasks.

Depth interval (cm)	Production (μmol mol $(C)^{-1}$ hr^{-1})			
	CH_4		CO_2	
	Control	Amended	Control	Amended
0–5	20.2 (2.92)	17.7 (1.97)	19.4 (2.15)	15.7 (2.43)
5–10	18.8 (0.80)	14.0 (1.07)	14.5 (2.88)	17.9 (1.02)
10–15	18.1 (1.44)	24.1 (2.83)	11.8 (0.70)	12.6 (0.14)
20–25	9.3 (0.37)	6.1 (0.84)*	7.9 (0.85)	5.8 (0.73)*
30–35	1.5 (0.40)	1.5 (0.12)	2.7 (0.50)	2.3 (0.20)
40–45	0.3 (0.09)	1.3 (0.41)	2.4 (0.53)	3.9 (1.24)

*$p < 0.05$

from the surface of Big Run Bog, especially through the CO_2 production pathway. However, temperature alone could not account for the seasonal pattern in carbon flow through the CH_4 production pathway (Figs. 2 and 3). Our results confirm those of Kelly and Chynoweth (1981) and Svensson (1984) who reported that CH_4 production will not respond to an increase in temperature if some other environmental factor is limiting production. In both of those studies, the availability of organic substrates or H_2 limited CH_4 production. It is unlikely that a similar control was responsible in this study because fermentation occurred in the peat from the winter collection incubated at 19 °C, and the laboratory amendment experiments failed to stimulate CH_4 production when the effect of low temperature was eliminated.

There are at least 2 factors, besides temperature, that might account for the low rate of carbon flow through the CH_4 production pathway in surface peat in the winter. First, a low population size of methanogens might exist in the winter which gradually increases throughout the spring, reaching a maximum size in the late fall. Second, CH_4 production in the winter might be limited by a substrate other than those products of glucose degradation such as methylamines (cf. Oremland *et al.* 1982, King 1984). Although we did not study 'noncompetitive' substrates in CH_4 production, they may play an important role in *Sphagnum*-derived peat.

The low rate of carbon mineralization in deep peat, as well as the observation that carbon mineralization in deep peat was insensitive to temperature, was not unexpected. *Sphagnum* is composed almost entirely of relatively labile polyuronic acids and contains no lignin. Consequently, hydrolysis and carbon mineralization of fresh *Sphagnum* litter in surface peat proceeds rapidly, leaving behind the recalcitrant residue in the peat. Peat from the 30–45 cm depth in Big Run Bog is relatively old (ca. 6500 yr B.P., our unpublished data) and highly decomposed, therefore the recalcitrant residues predominate.

Carbon mineralization in a wetland ecosystem. Deep peat deposits occur in *Sphagnum*-dominated wetlands in northern latitudes (Heinselman 1970, Chambers 1983, Foster and Glaser 1986) and the depth of the peat decreases with decreasing latitude (Wieder 1985) due, in part, to climate (Damman 1979). For instance, the maximum peat temperature in late summer in Big Run Bog is much greater than the 8 to 16 °C in Swedish bogs (Svensson and Rosswall 1984) and *ca* 11 °C in British bogs (Heal and Smith 1978). In addition, Big Run Bog becomes snow-free earlier in the year than *Sphagnum*-dominated wetlands in more northern latitudes, and remains snow-free longer into the fall. While these conditions favor an increased rate of net primary production in Big Run Bog (Wieder and Lang 1983), apparently they also are responsible for a relatively high rate of carbon mineralization through organic matter decomposition. We evaluated the ecological significance of decomposition under anaerobic conditions by combining data on CH_4 production and CO_2 production with information on bulk density of peat (Wieder 1985) to calculate carbon flow on an area basis. This calculation gives values of 1.0 and 2.1 g (C) m^{-2} day^{-1} for CH_4

production and CO_2 production, respectively, at 12 °C (early summer conditions). Taken together, these values approximately equal the 3.3 g (C) m^{-2} day^{-1} reported for the production of the dominant *Sphagnum* species in Big Run Bog (Wieder and Lang 1983). There appears to be a delicate balance between carbon input in production and carbon mineralization in peat in Big Run Bog.

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References

Balch, W.E., G.E. Fox, L.J. Magrum, C.R. Woese and R.S. Wolfe. 1979. Methanogens: reevaluation of a unique biological group. Microbiological Reviews 43: 260–296.

Banat, I.M. and D.B. Nedwell. 1984. Inhibition of sulphate reduction in anoxic marine sediment by group VI anions. Estuarine Coastal and Shelf Science 18: 361–366.

Benner, R., A.E. Maccubbin and R.E. Hodson. 1986. Temporal relationship between the deposition and microbial degradation of lignocellulosic detritus in a Georgia salt marsh and the Okefenokee Swamp. Microbial Ecology 12: 291–298.

Billen, G. 1982. Modelling the processes of organic matter degradation and nutrients recycling in sedimentary systems, pp. 15–20, In: D.B. Nedwell, C.M. Brown (eds.), Sediment Microbiology. Academic Press, London.

Bollag, J.M. and S.T. Czlonkowski. 1973. Inhibition of methane formation in soil by various nitrogen-containing compounds. Soil Biology and Biochemistry 5: 673–678.

Cameron, C.C. 1968. Peat, pp. 136–145, In: Mineral Resources of the Appalachian Region. US Geological Survey Professional Paper 580.

Chambers, F.M. 1983. Three radiocarbon-dated pollen diagrams from upland peats north-west of Merthyr Tydfil, South Wales. Journal of Ecology 71: 475–487.

Clymo, R.S. 1965. Experiments on breakdown of *Sphagnum* in two bogs. Journal of Ecology 53: 747–757.

Clymo, R.S. 1983. Peat, pp. 159–224, In: A.J.P. Gore (ed.), Mires: Swamp, Bog, Fen, and Moor. Elsevier Scientific Publishers, Amsterdam.

Damman, A.W.H. 1979. Geographic patterns in peatland development in eastern North America, pp. 42–57, In: Proceedings of the International Symposium on Classification of Peat and Peatlands. International Peat Society. Hyytiala, Finland.

Foster, D.R. and P.H. Glaser. 1986. The raised bogs of south-eastern Labrador, Canada: classification, distribution, vegetation and recent dynamics. Journal of Ecology 74: 47–71.

Froelich, P.N., G.P. Klinkhammer, M.L. Bender, N.A. Leudtke, G.R. Heath, D. Cullen, P. Daupin, D. Hammond, B. Hartman and V. Maynard. 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et Cosmochimica Acta 43: 1075–1090.

Gore, A.J.P. 1983. Ecosystems of the world, Mires: Swamp, Bog, Fen and Moor. Elsevier, New York, pp. 440.

Griffiths, R.P., B.A. Caldwell, W.A. Broich and R.Y. Morita. 1983. Microbial processes relating to carbon cycling in southeastern Bering Sea sediments. Marine Ecology Progressive Series 10: 265–275.

Heal, O.W. and R.A.H. Smith. 1978. Production ecology of British moors and montane grasslands. Springer-Verlag, Berlin. pp. 426.

Heinselman, M.L. 1970. Landscape evolution, peatland types, and the environment in the Lake Agassiz Peatlands Natural Area, Minnesota. Ecological Monographs 40: 235–261

Holzapfel-Pschorn, A., R. Conrad and W. Seiler. 1985. Production, oxidation and emission of methane in rice paddies. FEMS Microbiology Ecology 31: 343–351.

Jones, J.G. and B.M. Simon. 1984. Measure of microbial turnover of carbon in anoxic freshwater sediments: cautionary comments. Journal of Microbiological Methods 3: 47–55.

Jones, J.G., B.M. Simon and S. Gardener. 1982. Factors affecting methanogenesis and associated anaerobic processes in the sediment of a stratified lake. Journal of General Microbiology 128: 1–11.

Jorgensen, B.B. 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. Geomicrobiology Journal 1: 11–27.

Kelly, C.A. and D.P. Chynoweth. 1981. The contributions of temperature and of the input of organic matter in controlling rates of sediment methanogenesis. Limnology and Oceanography 26: 891–897.

King, G.M. 1984. Utilization of hydrogen, acetate, and 'noncompetitive' substrates by methanogenic bacteria in marine sediments. Geomicrobiology Journal 3: 275–306.

Kristjansson, J.K., P. Schonheit and R.K. Thauer. 1982. Different *Ks* values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. Archives fur Microbiology 131: 278–282.

Mechalas, B.J. 1974. Pathways and environmental requirements for biogenic gas production in the ocean, pp. 12–25, In: I.R. Kaplan (Ed.), Natural Gases in Marine Sediment. Plenum Press, New York.

Nedwell, D.B. 1984. The input and mineralization of organic carbon in anaerobic aquatic sediments. Advances in Microbial Ecology 7: 93–131.

Oremland, R.S., L.M. Marsh and S. Polcin. 1982. Methane production and simultaneous sulfate reduction in anoxic, salt marsh sediments. Nature 296: 143–145.

Oremland, R.S. and B.F. Taylor. 1978. Sulfate reduction and methanogenesis in marine sediments. Geochimica et Cosmochimica Acta 42: 209–214.

Phelps, T.J. and J.G. Zeikus. 1984. Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake. Applied and Environmental Microbiology 48: 1088–1995.

Sorensen, J., D. Christensen and B.B. Jorgensen. 1981. Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediments. Applied and Environmental Microbiology 42: 5–11.

Svensson, B.H. 1984. Different temperature optima for methane formation when enrichments from acid peat are supplemented with acetate or hydrogen. Applied and Environmental Microbiology 48: 389–394.

Svensson, B.H. and T. Rosswall. 1984. In situ methane production from acid peat in plant communities with different moisture regimes in a subarctic mire. Oikos 43: 341–350.

Ward, D.M. and M.R. Winfrey. 1985. Interactions between methanogenic and sulfate-reducing bacteria in sediments. Advances in Aquatic Microbiology 3: 141–179.

Watts, W.A. 1979. Late Quaternary vegetation of central Appalachia and the New Jersey coastal plain. Ecological Monographs 49: 427–469.

Wieder, R.K. 1985. Peat and water chemistry at Big Run Bog, a peatland in the Appalachian Mountains of West Virginia, USA. Biogeochemistry 1: 277–302.

Wieder, R.K. and G.E. Lang. 1983. Net primary production of the dominant bryophytes in a *Sphagnum*-dominated wetland in West Virginia. The Bryologist 86: 278–284.

Williams, R.T. and R.L. Crawford. 1983. Microbial diversity of Minnesota peatlands. Microbial Ecology 9: 201–214.

Williams, R.T. and R.L. Crawford. 1984. Methane production in Minnesota Peatlands. Applied and Environmental Microbiology 47: 1266–1271.

Winfrey, M.R. and J.G. Zeikus. 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Applied and Environmental Microbiology 33: 275–281.

Wolfe, R.S. and I.J. Higgins. 1979. Microbial biochemistry of methane — a study in contrasts. International Review of Biochemistry 21: 267–353.

Wolin, M.J. 1969. Volatile fatty acids and the inhibition of *Escherichia coli* growth by rumen fluid. Applied Microbiology 17: 83–87.

Zaiss, U. 1981. Seasonal studies of methanogenesis and desulfurication in sediments of the River Saar. Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene, Abt. I. Originale C-2: 76–89.