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Controls on methane production in a tidal freshwater estuary and a peatland: methane production via acetate fermentation and CO_2 reduction

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Abstract. Rates of total methane production, acetate fermentation and CO_2 reduction were compared for two different wetland sites. On a per-liter basis, sediments from the White Oak River estuary, a tidal freshwater site in eastern North Carolina, had an annual methane production rate (53.3 mM yr^{-1}) an order of magnitude higher than that of Buck Hollow Bog (5.5 mM yr^{-1}), a peatland in Michigan. Methane was produced in the White Oak River site on an annual basis by both acetate fermentation (72%) and CO_2 reduction (28%) in a ratio typical of freshwater methanogenic sites. Competition for acetate by non-methanogenic microorganisms in Buck Hollow peat limited methane production from acetate to only a few months a year, severely impacting annual methane production rates. However, when acetate was available to the methanogens in the peat during early spring, the percentage of methane production from acetate fermentation (84%) and CO_2 reduction (16%) and rates of total methane production were similar to those of the White Oak River sediments at the same temperature. Rates of CO_2 reduction and acetate fermentation conducted at both sites at various temperatures showed that Buck Hollow peat methane production was also limited by a colder temperature regime as well as differences in the response of the CO_2 reducing and aceticlastic methanogens to temperature variations.

Introduction

Increases in the tropospheric concentration of methane, and its potential for global warming as a greenhouse gas, have generated considerable research during the past few decades. Studies of biogenic methane from wetlands, which contribute roughly 20–40% of total methane sources to the atmosphere (Cicerone and Oremland 1988; Fung et al. 1991), have been the focus of much of these efforts. Many studies have focused on determining the flux of methane from representative wetlands in an attempt to quantify wetland source strengths. However, quantifying wetland flux based on these measurements has proven to be a difficult task given the spatial and temporal variability in fluxes from individual wetlands and wetland types (see review by Bartlett and Harriss (1993)). Several laboratory and field studies have at-

tempted to understand factors controlling methane fluxes by examining the controls on methane production within wetland soils and sediments. These studies include the effects of several variables on methane production including water table position (Moore and Dalva 1993; Kelley et al. 1995; Sigren et al. 1997a), competition for substrates between methanogens and other bacterial populations (Cappenberg 1974; Senior et al. 1982; Lovley et al. 1982; Lovley and Klug (1983, 1986); Kuivila et al. 1989), the response to temperature variations of methanogenic populations using acetate and CO_2/H_2 as substrates (Svensson 1984; Conrad et al. 1987; Conrad and Babbel 1989; Schulz et al. 1997), and sediment composition (Yavitt and Lang 1990; Sigren et al. 1997b). With a few exceptions, studies comparing rates of methane production via acetate fermentation and CO_2 reduction versus field flux measurements are limited (Crill and Martens 1986; Kuivila et al. 1990; Lansdown et al. 1992). In order to better understand the flux of methane from wetlands, it is important to understand the controls and limitations on methane production in different wetland environments taking into account the factors described above.

The purpose of the current study was to quantitatively compare the influence of several factors possibly controlling methane production in two very different wetland environments. We examined the overall effects of temperature regime, the specific responses of CO_2 reducing and acetate fermenting bacteria to temperature variations, and substrate competition on methane production in these two wetlands. The two sites utilized in this study were a tidal freshwater sediment in eastern North Carolina (White Oak River) and an ombrogenous peatland in Michigan (Buck Hollow). These two sites provided different temperature regimes, geochemical settings, and organic substrates in which to compare controls on methane production rates. Previous studies had also determined seasonal methane flux rates from both sites so that comparisons could be made with the methane production data.

Methods

Study sites

The section of the White Oak River examined in this study (Station GI) has been previously described by Martens and Goldhaber (1978) and Kelley (1988, 1993), Chanton et al. (1989), Kelley et al. (1990, 1995). Briefly, it is a tidal freshwater section of an eastern North Carolina estuary located approximately 20-km from the Atlantic Ocean. The diurnal tides are approximately 70 cm in amplitude (Chanton and Martens 1988) with water depths ranging from about 5 cm to 1 m. The sediments have been previously described as Dorovan muck (Barnhill 1992). The sediments, which deposit over a sandy bottom, contain approximately 18% organic carbon which consists largely of macrophyte detritus. The main flora contributing organic material to the sediments are the submerged macrophytes *Ceratophyllum* and *Najas*, as well as several blue-green algal species (Kelley 1993).

White Oak River Estuary sediments used in incubation experiments were collected monthly between February 1992 and February 1993. Samples were obtained by combining material from 3–5 cores (9-cm diameter, 50-cm long, Plexiglas). Core tubes were gently inserted into the sediments, capped on both ends with rubber stoppers and then carefully withdrawn. Cores were kept at in situ temperature during transport to the laboratory in Chapel Hill, NC and processed within 48 hours of collection. The top 10-cm of sediments were used for incubation experiments because the majority of methane production occurs in this depth interval (Kelley 1993).

Peat samples for all experiments were obtained from Buck Hollow Bog, located in southern Michigan (42°27′ N, 84°01′ W) on the Edwin S. George Reserve, a University of Michigan field station. Buck Hollow Bog is a 1-ha, ombrogenous peatland (mean *p*H = 4.2) formed in a kettle hole depression. Intact cores of the top 60–70 cm of peat from the central portion of the bog were collected in January, May, and June of 1994 and stored on ice for transport to the lab. Core sections were taken from below the surface of the water table to avoid the effects of methane oxidation (Shannon and White 1994). The top 40 cm of the cores were used for rate measurements. Further description of Buck Hollow Bog and field sampling protocols are available from Shannon (1993) and Shannon and White (1996). The peat was comprised primarily of *Sphagnum* moss fibers and occasional remains of *Chamaedaphne calyculata.*

Rates of total methane production, CO_2 reduction, and acetate fermentation

The core sections were extruded and combined in a covered 4-L beaker which was constantly purged with a stream of O_2-free N_2. The sediments were homogenized by gently stirring with a glass rod for approximately 2 min. Samples were then drawn into 60-mL catheter tip syringes which had the ends removed to provide an opening approximately 1 cm in diameter. Some large pieces of plant material in the peat samples were removed so that the sample could be drawn into the syringes. Twenty milliliter aliquots of homogenized sediment were injected into 50-mL (63-mL capacity) serum vials which were flushed with O_2-free N_2. The vials were then purged with the N_2 gas for an additional 2 min to assure an O_2-free headspace. The sample vials were sealed with stoppers, crimped, and injected with either 25-μL of ^{14}C-HCO_3^- (5,000,000 disintegrations per minute (dpm) 55 mCi mmol^{-1}) for CO_2 reduction and total methane production rate determination or 10-μL of 2-^{14}C-acetate (125,000 dpm) for aceticlastic methanogenesis and total methane production rate measurement. Tracers were added to sediments as point injections. A minimum of 18 vials were prepared for each experiment.

The sediments were incubated in the dark at a constant temperature from several minutes to several weeks, depending on the predicted optimum time frame for the rate measurement. At evenly spaced intervals, individual samples were sacrificed to produce a time series of methane production rate by CO_2 reduction or acetate fermentation, and total methane production rate (based on increase in methane concentration with time). A 5-mL aliquot of 5N NaOH was injected into the

samples to stop all biological activity and convert the ΣCO_2 pool to CO_3^{-2}. The samples were vigorously agitated on a shaker for 5 min to facilitate dissolution of the ΣCO_2 pool and release the moderately insoluble methane gas to the headspace. The mechanical shaker removed more than 95% of dissolved methane (additional extractions (3–5) produced no more than 5% of the total removed). A 25-μL aliquot of headspace was injected into a gas chromatograph (Shimadzu Mini 2, Porapak Q column) equipped with a flame ionization detector to determine the total amount of methane present in the vial (i.e., all methane present at the start of the incubation and that produced during the incubation). Methane concentrations in incubation experiments increased linearly with time (typical $r^2 > 0.8$) except for a single measurement in which peat collected in January was incubated at the in situ temperature of 4 °C. In this case, the methane increased linearly for the first 12 days (days 5, 9, and 12) and then leveled off (days 15 and 19). Therefore only the data from the first 12 days from this experiment were used to calculate methane production and CO_2 reduction rates. Methane concentrations did not increase throughout the incubation period when samples were autoclaved, indicating that the methane produced in the experiments was biogenic.

After measuring methane concentration, ^{14}C-labeled methane produced from the added tracers was collected by purging the headspace through a stripping rig similar to that described by Crill and Martens (1986). The gas was passed through an ascarite column to remove any ^{14}C-labeled CO_2 not trapped in the high-*p*H solution. The methane was then combusted on a CuO column at 800 °C to form CO_2 which was then trapped in a scintillation cocktail (2-L ScintiVerse II [Fisher Scientific], 500-mL B-phenethylamine, 500-mL methanol). The cocktail containing the ^{14}C-labeled CO_2 (from combusted ^{14}C-labeled methane) was counted on a Beckman LS 6800 liquid scintillation counter. Tests showed that under these oxidation conditions, 99.9% of the methane was oxidized to CO_2 (Chanton and Martens 1988). Generally greater than 90% of the labeled substrate added to sediments and processed through this stripping line was recovered in CO_2 reduction incubation experiments (Hoehler 1998). Counts from blank samples (prepared identically to experimental samples but not injected with tracers) were similar to background counts (< 70 dpm) and were low compared to typical count recoveries (> 1000 dpm). Between 1 and 10% of the added ^{14}C-tracer was converted to methane during CO_2 reduction rate measurements and between 5 and 66% of the added tracer was converted to methane during acetate fermentation rate measurements.

Tracer-determined rates of methane production conducted at the in situ temperatures did not vary with incubation time (Avery et al. 1999a). The rate of methane production from CO_2 or acetate was calculated from $R = aC\alpha/At$, where R is the rate of methane production (from CO_2 or acetate), A is the amount of ^{14}C-labeled substrate added, a is the amount of ^{14}C-labeled substrate converted to methane, C is the pool size of the substrate (see ΣCO_2 and acetate concentration methods below), t is the elapsed time after injection of the ^{14}C label, and α is the fractionation factor for methane production from ^{14}C relative to ^{12}C. For CO_2 reduction, α = 1.12, and for aceticlastic methanogenesis, α = 1.06. These fractionation factors are twice the ^{13}C isotope effect reported *by* Blair et al. (1993) for CO_2 reduction and

by Blair and Carter (1992) for acetate fermentation. Doubling the ^{13}C isotope effect is based on statistical-thermodynamic theory as reported by Stern and Vogel (1971). Total methane production rates were determined by plotting total methane present in the vials versus elapsed incubation time; the slope of this line gave the methane production rate.

Fraction of methane production from CO_2 and acetate

Acetate concentrations in White Oak River sediment incubation experiments were below the detection limit (2 μM, G. Avery, unpublished data) so rates of acetoclastic methanogenesis could not be determined during these experiments by traditional ^{14}C-tracer techniques. Therefore, the rates of acetate fermentation were calculated by the difference between the total methane production rate and the CO_2 reduction rate.

In the peat incubation experiments, the sum of the tracer determined rates of CO_2 reduction and acetate fermentation agreed well with the total methane production rate (the sum of the tracer-determined rates = 117 ± 22% of the total methane production rate) when tracer-determined rates of acetate fermentation were negligible compared to CO_2 reduction rates (i.e., most of the methane production was from CO_2 reduction). When the tracer-determined rates of acetate fermentation indicated that a significant amount of methane production was from acetate, the sum of the tracer-determined rates accounted for only 30–39% of the total methane production rate. This suggested that the acetate fermentation rates measured in this study may not have been reliable for quantitative determinations.

Problems with tracer-determined acetate fermentation rates have also been observed in previous studies. These problems have included inconsistencies with measured rates of other sediment processes (Shaw et al. 1984; Ansbaek and Blackburn 1980; Christensen and Blackburn 1982; Schutz et al. 1989), oxidation of the ^{14}C-labeled methyl group to CO_2 (e.g., Winfrey and Zeikus (1979) and Sandbeck and Ward (1981), Lovley et al. (1982)), a small and variable pool size (Winfrey and Zeikus 1979), and incorporation of the labeled acetate into sediments or biomass (Winfrey and Zeikus 1979; Christensen and Blackburn 1982; Shaw et al. 1984). In several methanogenic pathway studies (e.g., Winfrey and Zeikus (1979) and Sandbeck and Ward (1981), Lovley et al. (1982), Conrad and Babbel (1989)), the fraction of methane production from CO_2 reduction was determined from labeled $^{14}CO_2$ experiments, and the percentage of methane production from acetate was determined by difference.

Owing to potential problems with the ^{14}C-labeled acetate fermentation rates measured in the current study, we also calculated the rates of methane production from acetate for the peat experiments by difference. The rate of methane production from acetate fermentation was calculated from the ^{14}C tracer-determined rate of CO_2 reduction and the total methane production rate described above. It should be noted that calculating the rate of acetate fermentation by difference assumes that no other methanogenic substrates are contributing to methane production. Therefore in this study, similar to the approach used by Whiticar et al. (1986), the term

acetate fermentation (aceticlastic methanogenesis) may include any other methane production pathways where a methyl group is transferred from a substrate.

Concentrations of ΣCO_2, acetate and H_2

Samples identical to those used for rate determinations were prepared for ΣCO_2 measurements (see rate measurements in section 2.2). At the end of the incubation period, a 5 mL aliquot of 5M H_2SO_4 was injected into the vials to convert ΣCO_2 to CO_2(g). The vials were then placed on a shaker to facilitate the transfer of CO_2 into the headspace. The CO_2 concentration in the headspace was determined by a gas chromatograph (Shimadzu GC-14A, Porapak R column) equipped with a thermal conductivity detector. ΣCO_2 measurements were conducted immediately after addition of the acid because preliminary experiments indicated that storage of filtered pore water resulted in a loss of ΣCO_2.

Pore water acetate concentrations were determined at the beginning of the peat incubation experiments using ion chromatography as reported by Shannon et al. (1996). Briefly, measurements were made on a Dionex 4500 ion chromatography system utilizing a Dionex AS1 ion exclusion column with suppression. The eluent was 1-mM octanesulfonic acid with 2% 2-propanol; the suppresser regenerant was 5-mM tetrabutylammonium hydroxide. The detection limit was 20 μM.

H_2 concentrations were determined on sediment samples in serum vial described above. The measurement was performed identically to the method of Hoehler et al. (1994). Briefly, after equilibration of H_2 between the sediment and headspace, an aliquot of headspace was analyzed for H_2 partial pressure via chromatographic separation and HgO-reduction detection using a Trace Analytical RGA-3.

Results

Methane production rates from White Oak River sediment incubation experiments were determined monthly from February 1992 to January 1993. Total methane production rates ranged from 1.18 $\mu M\,hr^{-1}$ to 11.57 $\mu M\,hr^{-1}$ (Table 1). The average percentage of methane production from acetate fermentation for the twelve experiments was 69 ± 12%. Total methane production rates, CO_2 reduction rates, and acetate fermentation rates increased exponentially with increasing incubation temperature (Figure 1). During three of these experiments, in addition to the in situ temperature incubation experiment, sediments were also incubated over a range of temperatures spanning those encountered in the field. The rates of CO_2 reduction, acetate fermentation, and total methane production for these experiments also increased exponentially with increasing temperatures (Figure 2). In one experiment, where hydrogen concentrations were measured, hydrogen concentrations increased with increasing temperatures (Figure 3). Based on the results of the twelve monthly incubation experiments, the annual methane production rate is estimated at 53.3

Table 1. Monthly methane production rates (MPR) from incubation experiments and in situ temperatures for White Oak River sediments. Acetate fermentation rates were calculated from the difference between measured total methane production rate and CO_2 reduction rates. Here MPR is total methane production rate, CRR is CO_2 reduction rate, AFR is acetate fermentation rate. March and August data from Avery and Martens (1999b).

Month	Temp. (°C)	MPR (μM hr^{-1})	CRR (μM hr^{-1})	AFR (μM hr^{-1})	%AF
Feb.	12.5	3.94	0.87	3.07	78
March	14.0	3.19	1.46	1.73	54
April	19.0	7.80	2.24	5.56	71
May	21.5	11.57	3.41	8.16	71
June	24.0	9.28	1.82	7.46	80
July	27.0	11.46	3.02	8.44	73
Aug.	23.5	11.00	2.46	8.54	78
Sept.	25.0	6.72	2.03	4.69	70
Oct.	14.5	2.95	0.77	2.18	74
Nov.	13.5	1.76	1.07	0.69	39
Dec.	9.5	1.18	0.48	0.70	59
Jan.	8.5	1.69	0.40	1.29	76
					average = 69 ± 12%

mM yr^{-1} with the majority of annual methane production coming from acetate fermentation (72%) (Table 2).

Methane production rates were determined from Buck Hollow peat incubation experiments at in situ temperatures during the winter (January) and spring (May and June). Rates ranged from 0.18 ± 0.02 μM hr^{-1} during January to 2.79 ± 0.15 μM hr^{-1} during June (Table 3). The majority of methane produced in the winter was via CO_2 reduction however, acetate fermentation dominated methane production during the spring (Table 3). The CO_2 reduction rate vs. incubation temperature results for the 3 experiments conducted at in situ temperatures (January 4 °C, May 15 °C, and June 15 °C) and for the January sediments artificially warmed to 25 °C showed an exponential increase in rate with increasing temperature (Figure 1c). Rates of acetate fermentation and total methane production rate vs. temperature displayed a maximum rate at approximately 15 °C (Figures 1a and 1b). The annual methane production rate for Buck Hollow peat was estimated at 5.5 mM yr^{-1} (Table 2) using the total methane production rates measured in January, May and June (Table 3), and calculated CO_2 reduction rates, that were based on the temperature vs. CO_2 reduction relationship, for months that were not measured directly (Figure 1).

Discussion

Annual rates of methane production on a per-liter basis were an order of magnitude higher in sediments of the White Oak River compared to Buck Hollow peat (Ta-

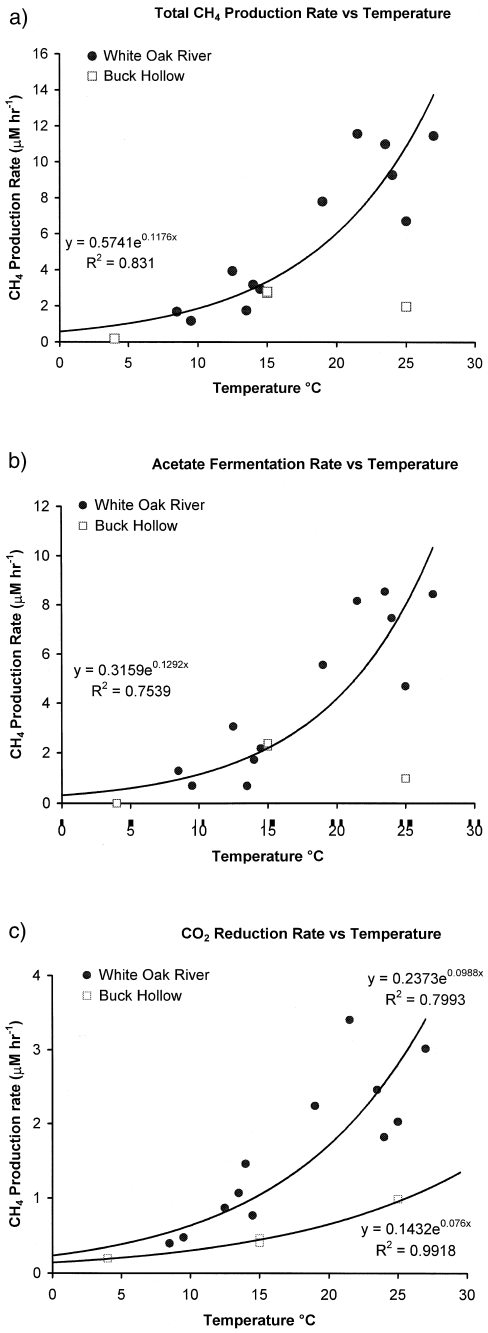

Figure 1. Total methane production rate (A), acetate fermentation rate (B), and CO_2 reduction rate (C) versus temperature for Buck Hollow peat and White Oak River sediment incubation experiments. Here stars are Buck Hollow peat and closed circles are White Oak River sediments. White Oak River sediments were collected on a monthly basis for one year and incubated at in situ temperatures. Buck Hollow peat was collected during May, June, and January and incubated at in situ temperature. The sample at 25 °C was collected in January and incubated at an elevated temperature. Buck Hollow data from Avery et al. (1999a).

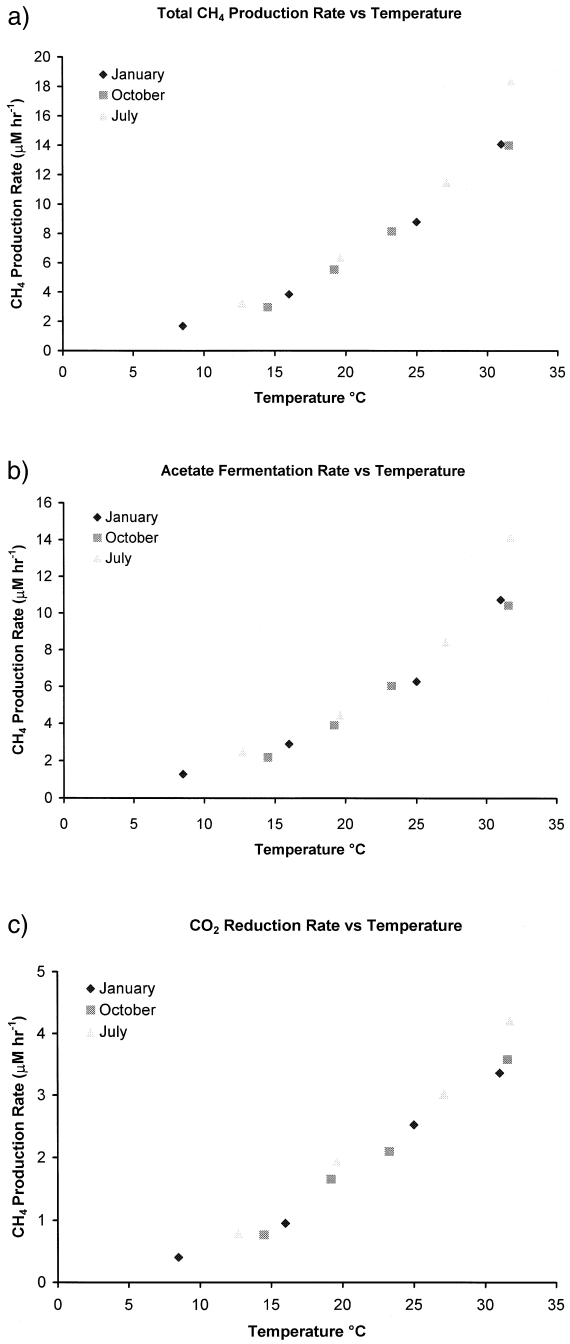

Figure 2. Total methane production rate (A), acetate fermentation rate (B), and CO_2 reduction rate (C), versus temperature for White Oak River Sediments collected during January (solid diamonds), October (solid squares), and July (open triangles), and incubated at various temperatures. CO_2 reduction and total methane production data from Avery and Martens (1999b).

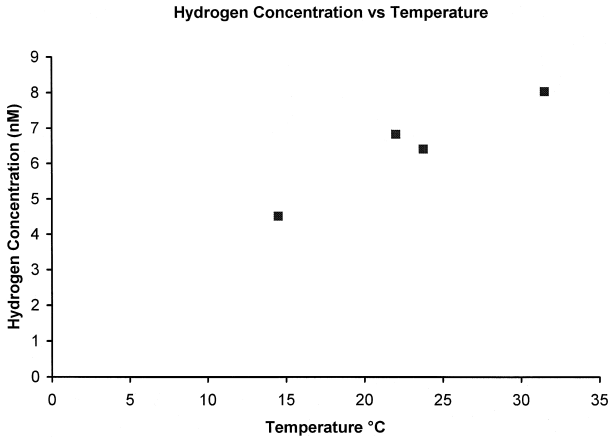

Figure 3. Hydrogen partial pressure versus incubation temperature for White Oak River sediment incubation experiment. The sample is the same October sediment in Figure 2.

Table 2. Annual methane production rate (MPR), CO_2 reduction rate (CRR), acetate fermentation rate (AFR), and percentage of annual methane production from acetate fermentation for White Oak River sediments and Buck Hollow peat.

	White Oak River	Buck Hollow
annual MPR (mM yr^{-1})	53.3	6.7
annual CRR (mM yr^{-1})	14.7	3.3
annual AFR (mM yr^{-1})	38.6	3.4
annual% MPR via acetate fermentation	72%	51%

ble 2). Lower rates of methane production in Buck Hollow peat could have resulted from lower sediment temperatures and therefore lower rates of methane production, or from differences in environmental conditions such competition for methanogenic substrates from other microbial populations. In the discussion that follows, methane production at the two sites is compared, with emphasis on the seasonal occurrence of CO_2 reduction and acetate fermentation and the response of these two pathways of methane production to temperature variations.

Seasonal variations in the occurrence of CO_2 reduction and acetate fermentation

In most freshwater environments, acetate fermentation is thought to be the dominant pathway of methane production with CO_2 reduction accounting for the remainder. The sediments of the White Oak River produced methane from both CO_2 reduction and acetate fermentation year round. The percentage of methane via acetate fermentation (70%) and CO_2 reduction (30%) at this site is typical of freshwater environments where competition for substrates such as SO_4^{2-} is not an important process (Whiticar et al. 1986).

Table 3. Monthly methane production rates (MPR) CO_2 reduction rates (CRR) and acetate fermentation rates (AFR) for Buck Hollow peat. All underlined rates were calculated from temperature vs. CO_2 reduction rate relationship derived from the four peat incubation experiments (see figure 1a). Acetate fermentation rates were assumed to be 0.0 except for May and June and January (indicated by *) where the acetate fermentation rate was determined by the difference between total methane production rate and CO_2 reduction rate. Buck Hollow rate data for January, May and June from Avery et al. (1999a).

Month	Temp. (°C)	MPR (μM hr^{-1})	CRR (μM hr^{-1})	AFR (μM hr^{-1})	%AF
Feb.	0.0	0.14	0.14	0.00	0
March	0.0	0.14	0.14	0.00	0
April	5.0	0.17	0.17	0.00	0
May	15.0	2.72	0.46	2.26*	83*
June	20.0	2.79	0.41	2.38*	85*
July	20.0	0.28	0.28	0.00	0
Aug.	20.0	0.28	0.28	0.00	0
Sept.	20.0	0.28	0.28	0.00	0
Oct.	15.0	0.23	0.23	0.00	0
Nov.	0.0	0.14	0.14	0.00	0
Dec.	0.0	0.14	0.18	0.00	0
Jan.	0.0	0.18	0.14	0.00*	0*

In Buck Hollow peat, tracer determined rates of CO_2 reduction accounted for all the methane production in the wintertime and a portion of total methane production in the two springtime peat incubation experiments. However methane from acetate fermentation only occurred during the May and June experiments after porewater acetate concentrations had increased dramatically. Previous studies employing rate measurements have observed porewater acetate concentrations increasing during transition phases between acetate consumption by SO_4^{2-} reducers and methanogens (Albert et al. 1991; Alperin et al. 1992; Sugimoto and Wada 1993). In a previous study of Buck Hollow peat, Shannon and White (1996) observed high concentrations of acetate during the early spring followed by a large flux of methane and then a sharp decrease in acetate concentrations. This suggested that acetate may be consumed by other microbial populations in the sediments during most of the year rendering it unavailable to methanogens except in early spring or that large amounts of acetate are produced only in the late spring and early summer. The rate measurements from the current study are consistent with either hypothesis. However, SO_4^{2-} reduction may not be the competing process at this site because SO_4^{2-} concentrations are not depleted during the year (Shannon and White 1996).

As discussed above, substrate competition affected acetate fermentation to a greater extent than CO_2 reduction in Buck Hollow peat. Methanogenic studies in environments with varying degrees of competition between SO_4^{2-} reducers and methanogens also support the larger impact of substrate competition on aceticlastic methanogens when compared to CO_2 reducers. For example, Senior et al. (1982) showed that CO_2 reduction was the only pathway for methane production in a salt marsh sediment where the supply of SO_4^{2-} was high. Similarly, in the marine sedi-

ments of Cape Lookout Bight, NC, CO_2 reduction was observed at all depths below the surface layer concurrent with SO_4^{2-} reduction (Crill and Martens 1986). Acetate fermentation on the other hand, was restricted to the deeper sediments where SO_4^{2-} concentration was depleted. Kuivila et al. (1989) showed that acetate fermentation was the predominant pathway of methane production in freshwater environments such as Lake Washington where SO_4^{2-} reduction occurs but to a much lesser extent than in marine environments. However, in the upper surface sediments, where SO_4^{2-} reduction was the dominant process, methane production via CO_2 reduction was greater than that from acetate fermentation. Differences in the ability of acetate fermenting and CO_2 reducing methanogens to compete with other microbial populations may have important implications for methane production in wetland systems. The inability of acetate fermenting methanogens to compete with other microbial processes can severely limit methane production in a freshwater system where the majority of methane is produced via this pathway. The ability of CO_2 reducing methanogens to continue to produce methane in the presence of competitive processes, may insure that methanogenesis is a continous process in many wetland environments.

Response of CO_2 reduction and acetate fermentation to temperature variations

At both sites in this study, CO_2 reduction rates from the incubation experiments increased exponentially with increasing temperatures. For White Oak River sediments, CO_2 reduction rates showed similar responses to temperature variations even though the sediments were collected during different seasons (Figure 2). Furthermore, in all White Oak River sediment and in Buck Hollow peat collected in January but incubated at 25 °C, CO_2 reduction rates continued to increase exponentially at incubation temperatures higher than those normally encountered in the field. These results indicate that CO_2 reduction rates at both sites are limited and controlled by temperature and not by seasonal changes in sediment/peat composition or populations of CO_2 reducing methanogens. In White Oak River sediments incubated over a range of temperatures, the partial pressure of hydrogen was also measured to see if increasing temperatures had an effect on the production of hydrogen and therefore on the CO_2 reduction rate. The hydrogen partial pressures increased with increasing temperatures, supporting this theory (Figure 3) and suggesting that CO_2 reduction is limited at least indirectly by temperature and that CO_2 reducing methanogens at these sites are capable of operating above the normal in situ temperatures.

The exponential curve obtained for CO_2 reduction rate vs. temperature for the White Oak River experiment had a larger slope than the curve obtained for Buck Hollow peat (Figure 1). The lower rates of CO_2 reduction for Buck Hollow peat could have resulted from lower substrate production rates (i.e., H_2), competition for substrates, and/or smaller and less efficient microbial populations of CO_2 reducing methanogens. The production of H_2 via fermentative processes is partially dependent on the quality of the substrate (Burke (1993), and references therein). In the case of Buck Hollow peat, the substrate is considerably older and most likely

more refractory than that of the White Oak River. Competition for H_2 by other microbial populations, as suggested by the acetate data, could further limit H_2 availability although not to the extent to which it affects acetate fermentation rates as described above. Both of the substrate limiting processes described above could lead to a smaller and less efficient population of CO_2 reducing methanogens in Buck Hollow peat. The cumulative and synergistic effects of these factors are most likely responsible for the differences in response of the CO_2 reducing populations of methanogens to temperature variations between these two sites.

As discussed above, acetate fermentation occurred year round in the White Oak River but only during the spring in Buck Hollow peat most likely due to competition for acetate from other microbial processes. Rates of acetate fermentation in the twelve monthly White Oak River sediment incubation experiments increased exponentially with increasing temperatures (Figure 1b). Similar results were obtained for the three White Oak River samples incubated over a range of temperatures (Figure 2b). This pattern was similar to that described for CO_2 reduction, suggesting that acetate fermentation rates at this site are also controlled by temperature and not seasonal changes in the composition of the sediments or microbial populations.

Rates of acetate fermentation in Buck Hollow peat did not display the same pattern. Maximum rates were observed at 15 °C during the May and June peat incubation experiments; rates of acetate fermentation during January at an artificially elevated temperature of 25 °C were lower (Figure 1b). This may indicate an optimum temperature for acetate fermentation less than 25 °C or seasonal changes in the populations of acetate fermenting bacteria. Svensson (1984) showed that acetate fermentation rates were maximum at 20 °C in a cold climate peat, which may explain the lower rates observed at 25 °C in Buck Hollow. Alternatively, since acetate may not be available to methanogens during January, or for several months preceeding January, there may not be an established microbial population during this time to carry out acetate fermentation at an increased rate. In other words, acetate fermentation may be limited by the microbial populations and not the production of substrate or temperature. This theory is consistent with the time lag between porewater acetate concentration increases and increases in methane flux previously observed at this site (Shannon and White 1996). This phenomenon has also been observed in the marine sediments of Cape Lookout Bight, NC. After dissolved SO_4^{2-} was depleted in the sediments, acetate accumulated for several days before acetate fermentation occurred. The time lag between acetate availability and the onset of acetate fermentation was attributed to low populations of acetate fermenting bacteria due to the previous conditions where acetate was not available to the methanogens (Albert et al. 1991; Alperin et al. 1992).

Total methane production

The sediments of the White Oak River produced approximately 8 times more methane than Buck Hollow peat annually on a per-liter basis (Table 2). The majority of annual methane production in the White Oak River was via acetate fermen-

tation due to its year round occurrence and its predominance over CO_2 reduction. In Buck Hollow peat, annual methane production was equally split between the two pathways (Table 2) even though acetate fermentation only occurred during the early spring. It is interesting to note that although acetate fermentation rates responded differently to temperature variations at the two sites, Buck Hollow peat exhibited acetate fermentation rates during the spring at 15 °C comparable to those of the White Oak River at the same temperature (Figure 1). Also, during this time the percentage of methane production via acetate fermentation (84%) was similar to that of the White Oak River and other freshwater environments where methane production is the dominant remineralization process. This suggests that during this period of time, the methanogens in Buck Hollow peat were operating as a methanogenic community not affected by significant competition for substrates. The similarity between White Oak River methane production rates at 15 °C and Buck Hollow methane production rates at 15 °C, when there appears to be no substrate competition, illustrates the importance of substrate competition on limiting methane production in a wetland environment.

Constraints on methane production in buck hollow peat

The constraints on methane production in these two different ecosystems include: in situ temperatures, the response of CO_2 reduction and acetate fermentation to temperature variations, and the prevalence of the two pathways of methane production. Methane production rates and pathways are very similar in the sediments of the White Oak River at 15 °C and in Buck Hollow peat during the spring at 15 °C. Yet, on an annual basis, White Oak River sediments produce almost an order of magnitude more methane than Buck Hollow peat on a per-Liter basis. Quantifying the impact of individual controls on methane production at the two sites in the current study, could be helpful in understanding controls on methane production in other wetland environments. We can semi quantitatively assess the importance each one of these controls has on limiting methane production in Buck Hollow peat by replacing Buck Hollow values for these parameters with White Oak River values. Each parameter will be addressed separately below.

We can assess the effect of a smaller slope for the CO_2 reduction rate vs. temperature curves for Buck Hollow by calculating the annual CO_2 reduction rate for this site using the monthly peat in situ temperatures (Table 3) and the rate vs. temperature curve for the White Oak River (Figure 1). Using the White Oak River curve for Buck Hollow, the calculated annual CO_2 reduction rate for the peat would be 7.7 mM yr^{-1}. When added to Buck Hollows's methane production from acetate fermentation during May and June (3.4 mM yr^{-1}, Table 3), the estimated annual methane production rate would be 11.1 mM yr^{-1}. This is approximately double the observed rate of 6.7 mM yr^{-1} for Buck Hollow (Table 2).

We can estimate the effect of lower monthly in situ temperatures on methane production in Buck Hollow by using the White Oak River monthly temperature data (Table 1) to calculate the CO_2 reduction rates using Buck Hollow's CO_2 reduction rate vs. temperature curve (Figure 1). The acetate fermentation rates in

Buck Hollow peat during May and June can also be estimated at White Oak River temperatures using the temperature vs. acetate fermentation curve for the White Oak River (Figure 1). This assumption is reasonable since acetate fermentation rates in Buck Hollow peat were similar to White Oak River rates at the same temperature during times when there appeared to be no competition for acetate (Figure 1). The annual CO_2 reduction rate for Buck Hollow would approximately double from 3.3 mM yr^{-1} to 5.4 mM yr^{-1}. Annual acetate fermentation rates for May and June combined would increase from 3.4 mM yr^{-1} to 8.8 mM yr^{-1}. The total annual methane production rate would be 14.2 mM yr^{-1} or once again approximately double the measured rate (Table 2).

Finally we can estimate the impact of substrate competition and the limiting of acetate fermentation to only a few months in Buck Hollow peat. Using the acetate fermentation rate vs. temperature curve for the White Oak River and the in situ temperatures for Buck Hollow, the annual acetate fermentation rates for Buck Hollow would be 17.1 mM yr^{-1}, or approximately 6 times the observed rate. When added to the methane production rate for CO_2 reduction, the estimated annual methane production rate would be 20.4 mM yr^{-1}. Therefore, limiting acetate fermentation in Buck Hollow has the largest impact on methane production at this site. Although these calculations are estimates, they serve to illustrate the importance of substrate competition on methane production in wetland environments.

Methane production vs. flux

The timing and magnitude of methane production and flux are tightly coupled at both sites in this study. In Buck Hollow peat, the majority of methane flux is between May and July (Shannon and White 1994) corresponding with the period of time when acetate fermentation is occurring. This agrees well with the methane production rate data which predicts that during this period of time 71% of the annual methane production occurs (Table 3). The annual methane flux per square meter of Buck Hollow peat was calculated using the rate data presented in this study and assuming the top 80 cm of peat was where the majority of methane was produced (Shannon, personal communications, 1998). This calculated flux value based on the laboratory methane production experiments (78 g m^{-2} yr^{-1}) agrees well with fluxes previously measured in the field from this site (66.9–76.3 g m^{-2} yr^{-1}, Shannon and White (1994)). The agreement between methane production rate from this study, and previously reported flux measurements, suggests that methane oxidation was not an important process at this site. This agrees with the findings of Shannon and White (1994) that indicated that methane flux was positively correlated with temperature and not inversely correlated with water table depth at this site during this study. An inverse correlation between methane production and water table level would have indicated that the lowering of the water table increases the amount of oxygen in the peat and therefore the amount of methane oxidation.

Methane transport at station GI in the White Oak River occurs via ebullition and diffusion. Chanton and Martens (1988) obtained 7 bubble flux measurements from this site during the spring and summer of 1985 and 1986. Based on this data, they

estimated the annual ebullitive flux at 20.8 $g\,m^{-2}\,yr^{-1}$. Kelley et al. (1990) estimated the diffusive flux at 56% of the total methane flux at this station. Based on these data, the annual flux of methane from this station would be 47 $g\,m^{-2}\,yr^{-1}$. This is in fairly good agreement with the calculated flux of 62 $g\,m^{-2}\,yr^{-1}$ based on the laboratory experiments and assuming the top 10 cm of sediments was where the majority of methane production occurs (Kelley et al. 1995).

The flux of methane from Buck Hollow peat is similar to that of the White Oak River (table) despite the fact that on a per-liter basis, the sediments of the White Oak river are more productive by almost an order of magnitude. This results from the differences in the depths of methane producing sediments at these two sites. At station GI in the White Oak River, the majority of methane is produced in the top 10 cm (Kelley et al. 1995) whereas in Buck Hollow peat, methane production occurs to approximately 80 cm depth. Therefore, when extrapolating methane production data to flux, it is important to consider the depth of methane producing sediments at a site.

Conclusions

Annual methane production rates on a per liter basis, were an order of magnitude higher in the sediments of the White Oak River estuary compared to Buck Hollow peat. These differences resulted in part from differences in in situ temperatures at the two sites as well as from the response of CO_2 reducing and acetate fermenting microbial populations to these temperature variations. However, the primary factor influencing methane production rates was the presence or absence of acetate fermentation on a year round basis. Methane production at both sites in this study, and possibly in most freshwater environments, is dominated by acetate fermentation. Since substrate competition appears to impact acetate fermentation rates more than CO_2 reduction rates, substrate competition results in a dramatic decrease in methane production rates. This has important implications for methane production at the two sites in the current study as well as in methanogenic freshwater environments in general. When assessing the flux of methane from a given wetland, it is imperative to understand the factors that control the production of methane, especially competition for acetate. The spatial and temporal variations in these competitive processes within a given wetland type should have a significant impact on the methane flux from a given wetland. By better understanding the controls on methane production through laboratory incubation experiments and field observations, we should be better able to predict and quantify the flux of methane from wetland environments to the atmosphere.

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