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Effect of seasonal changes in the pathways of methanogenesis on the $\delta^{13}C$ values of pore water methane in a Michigan peatland

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Abstract. The $\delta^{13}C$ value of pore water methane produced in a Michigan peatland varied by 11‰ during the year. This isotopic shift resulted from large seasonal changes in the pathways of methane production. On the basis of mass balance calculations, the $\delta^{13}C$ value of methane from CO_2 reduction (average = -71.4 ± 1.8‰) was depleted in ^{13}C compared to that produced from acetate (-44.4 ± 8.2‰). The dissolved methane at the site remained heavy (approximately -51‰) during most of the year. Tracer experiments using ^{14}C-labeled CO_2 indicated that during January 110 ± 25% of the methane was produced by CO_2 reduction. Because of low-methane production rates during the winter, this ^{13}C-depleted methane had only a slight effect on the isotopic composition of the methane pool. In early spring when peat temperatures and methane production rates increased, the $\delta^{13}C$ value of the dissolved methane in shallow peat was influenced by the isotopically light methane and approached -61‰. Peat incubation experiments conducted at 15°C in May and June (when the peat reaches its maximum temperature) indicated that an average of 84 ± 9% of the methane production was from acetate and had an average $\delta^{13}C$ value of -48.7 ± 5.6‰. Rising acetate concentrations during April-May (approaching 1 mmol L^{-1}(mM)) followed by a rapid decrease in acetate concentrations during May-June reflected the shift toward methane production dominated by acetate fermentation. During this period, dissolved methane in shallow peat at the site returned to heavier values (approximately -51‰) similar to that produced in the incubation experiments.

1. Introduction

Wetlands contribute approximately 100-200 Tg of methane to the atmosphere each year representing about 20-40% of total methane sources [*Cicerone and Oremland*, 1988; *Fung et al.*, 1991]. Northern peatlands, an important component of these wetland sources, contribute as much as 35 Tg of methane to the atmosphere each year, approximately 1/6 to 1/3 of total wetland sources [*Fung et al.*, 1991]. These estimates rely heavily on stable isotope mass balance models that use representative $\delta^{13}C$ and flux values from known sources [*Fung et al.*, 1991]. However, spatial and temporal variability of both flux and $\delta^{13}C$ values of methane from peatlands and other wetlands [*Martens et al.*, 1986; *Burke et al.*, 1988, 1992; *Chanton and Martens*, 1988; *Crill et al.*, 1988a, b; *Yavitt et al.*, 1988; *Blair et al.*, 1993; *Martens et al.*, 1992; *Kelley et al.*, 1992; *Bartlett and Harriss*, 1993] add considerable uncertainty to these budgets. Therefore determining the underlying controls on the stable isotopic composition of biogenic methane is critical to understanding how variability of $\delta^{13}C$ values affects methane budgets which rely on stable isotopic mixing models.

Published $\delta^{13}C$ values of methane from different peatlands range from -46‰ [*Martens et al.*, 1992] to -82‰ [*Lansdown et al.*, 1992]. Seasonal $\delta^{13}C$ variations observed within individual peatlands range from 0‰ [*Lansdown et al.*, 1992] to 13‰ [*Kelley et al.*, 1992]. In the absence of oxidation effects, changes in pathways of methane production occurring in wetland soils and sediments have been suggested as a mechanism for these isotopic shifts [*Martens et al.*, 1986; *Burke et al.*, 1988; *Kelley et al.*, 1992; *Martens et al.*, 1992; *Burke et al.*, 1992; *Shannon and White*, 1996]. Methane from acetate is generally thought to be enriched in ^{13}C compared to that from CO_2 reduction [*Whiticar et al.*, 1986], so seasonal changes in the utilization of these two substrates should affect the $\delta^{13}C$ value of methane produced from them. However, this link between seasonal changes in the $\delta^{13}C$ value of methane and changes in pathways has not been confirmed with direct rate measurements of CO_2 reduction and aceticlastic methanogenesis within a peat soil, and only a few studies [*Blair et al.*, 1993; *Alperin et al.*, 1992; *Sugimoto and Wada*, 1993] utilizing methanogenic rate measurements and isotopic data have determined the $\delta^{13}C$ values of methane produced from CO_2 and acetate.

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In the current study, a peatland in Michigan, "Buck Hollow Bog," was used to determine the $\delta^{13}C$ values of methane produced individually from CO_2 and acetate and to test the hypothesis (through direct rate and isotopic measurements) that changes in mechanisms of methane production may be responsible for isotopic variations. Several years of pore water chemistry and methane flux studies have been conducted at this site [*Shannon*, 1993; *Shannon and White*, 1994; *Shannon et al.*, 1996; *Shannon and White*, 1996]. As the peat begins to warm in early spring, acetate concentrations increase from less than 20μM to over 500 μM. Later in the spring, as the peat continues to warm, the acetate concentrations decrease rapidly [*Shannon and White*, 1996], followed by an increase in the flux of isotopically heavy methane [*R. D. Shannon and J. R. White*, unpublished data, 1996]. These data suggest an increase in the production of methane derived from acetate during the spring. Therefore this site provided an ideal environment to study changes in pathways of methane production and its effect on the $\delta^{13}C$ values of biogenic methane. The experimental approach combined rate measurements of CO_2 reduction and aceticlastic methanogenesis (using ^{14}C-labeled tracers) with isotopic determinations of the $\delta^{13}C$ values of the ΣCO_2 pool and produced methane. The specific goals of this project were (1) to determine the $\delta^{13}C$ values of methane produced from CO_2 and acetate, (2) to compare the amount of methane produced from acetate and CO_2 during the winter and summer, and (3) to perform mass balance calculations based on the results of peat incubation experiments to predict the $\delta^{13}C$ value of the dissolved methane pool during different times of the year. This study is the first to confirm pathway changes as an important mechanism affecting the seasonal variation in $\delta^{13}C$ values of biogenic methane in a natural freshwater system.

2. Methods

2.1. Site Description and Sampling

Peat and pore water 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]. Bog pore waters were obtained every 3-5 weeks from November 1993 through June 1994. Pore waters were sampled to a depth of 40-45 cm below the peat surface with Hesslein-type diffusion "peepers" [*Hesslein*, 1976] fitted with 0.2 μm pore size Gelman HT-200 membrane for determination of acetate concentrations and stable carbon isotopic analysis of dissolved methane. Pore waters were collected from peepers with glass syringes and stored at 4°C until analyzed as described below. Peat temperatures at 10 cm below the peat surface were measured with a thermocouple connected to a data logger; the data logger was programmed to sample peat temperature every 10 min and record the hourly mean temperature. Further description of Buck Hollow Bog and field sampling protocols are available from *Shannon* [1993] and *Shannon and White* [1996].

2.2. Rates of Total Methane Production, CO_2 Reduction, and Acetate Fermentation

The top 40 cm of the cores were used for rate measurements since acetate concentrations vary seasonally in this interval [*Shannon and White*, 1996]. The section of core was 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 were removed so that the sediment could be drawn into the syringes. Twenty milliliters 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 samples were stoppered, crimped, and injected with either 25-μL of ^{14}C-HCO_3^- (5,000,000 disintegrations per minute (dpm)) 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 desired rate measurement. At evenly spaced intervals, individual samples were sacrificed to produce a time series of CO_2 reduction rate, acetate fermentation rate, 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 peat 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 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 turned over during CO_2 reduction rate measurements and between 5 and 66% of the added tracer was turned over during acetate fermentation rate measurements.

Tracer-determined rates of methane production conducted at the in situ temperatures did not vary with incubation time (Figure 1). 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, $\alpha = 1.12$, and, for aceticlastic methanogenesis, $\alpha = 1.09$. 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 gives the methane production rate.

2.3. Fraction of Methane Production From CO_2 and Acetate

In the present study, 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; *Sandbeck and Ward*, 1981; *Lovley and Klug*, 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; *Sandbeck and Ward*, 1981; *Lovley and Klug*,

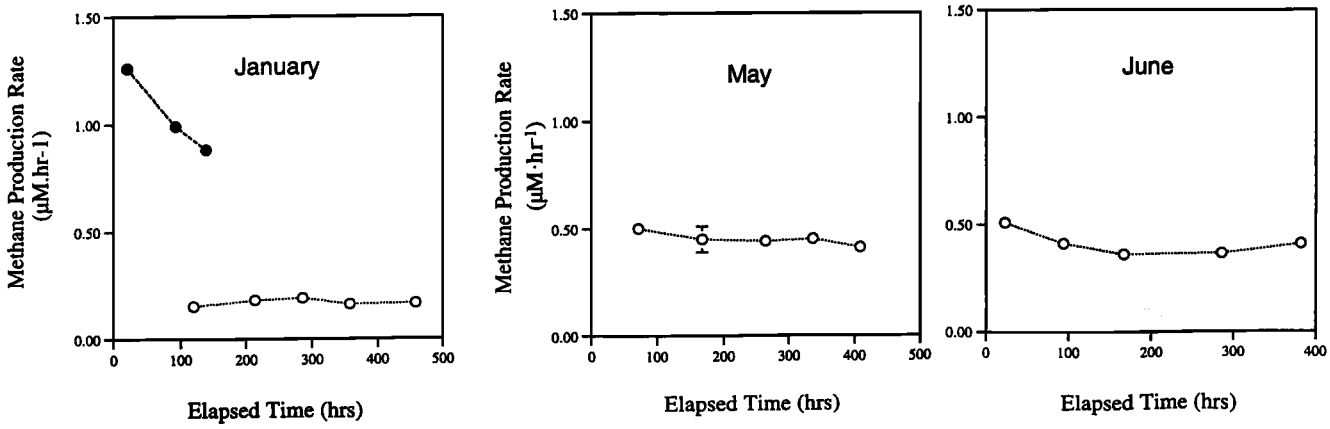

Figure 1. Time series of ^{14}C-tracer determined rates of CO_2 reduction for Buck Hollow Bog peat incubation experiments. For all experiments, open circles are CO_2 reduction rate at ambient temperature, and filled circles are January CO_2 reduction rate at 25°C. All points are the average of replicate samples except for the January experiment at 25°C where points represent only one measurement. Error bars are 1 standard deviation of the mean. Where error bars cannot be seen, they are smaller than the symbol.

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 percentage of methane production from acetate by difference. The fraction of methane production from CO_2 reduction was calculated from the ^{14}C tracer-determined rate of CO_2 reduction and the total methane production rate described above. The remaining methane production was assumed to be from acetate fermentation. 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.*, [1987], the term acetate fermentation (aceticlastic methanogenesis) may include any other methane production pathways where a methyl group is transferred from a substrate.

2.4. Concentrations of ΣCO_2 and Acetate

Samples identical to those used for rate determination 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 incubation experiments using ion chromatography as reported by *Shannon and White* [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.

2.5. The $\delta^{13}C$ Values of Methane and ΣCO_2

Gas samples for isotopic analysis were obtained from either gas extracted from vials following laboratory incubation experiments or field pore water collected from peepers. Methane samples were prepared for mass spectrometer analysis on a methane combustion vacuum line. The gas was flushed out of the vial and then passed through a magnesium perchlorate column (to remove water) and a liquid nitrogen trap (to remove CO_2) before being quantitatively combusted in a CuO/Pt column at 790°C. The water from the combusted methane was removed by a heated liquid nitrogen trap (-120°C); the CO_2 from the combusted methane was collected in a liquid nitrogen trap (-196°C). After removal of the carrier gas by vacuum, the CO_2 was transferred to a breakseal vial.

Gas samples for ΣCO_2 isotopic analysis were prepared in a similar fashion. After acidification of the samples (~1-5 ml) with 0.2 ml of 1M H_3PO_4, the gas was purged though a column containing magnesium perchlorate and the heated liquid nitrogen trap (-116°C) to remove water. The CO_2 was then collected in a liquid nitrogen trap (-196°C). The carrier gas was removed by vacuum and the CO_2 gas transferred to a breakseal vial. The $\delta^{13}C$ values of $CO_{2(aqueous)}$ and ΣCO_2 were assumed to be the same in Buck Hollow peat since at *p*H 4.2 (the approximate in situ *p*H) the majority of the ΣCO_2 pool is $CO_{2(aq)}$.

The purified samples were analyzed for ^{13}C and ^{12}C abundances on either a Finnigan MAT Delta E isotope ratio mass spectrometer in Neal Blair's laboratory at North Carolina State University or on a Finnigan MAT 252 isotope ratio mass spectrometer at University of North Carolina, Chapel Hill. All error measurements provided in the text are ± 1 standard deviation of the mean.

3. Results

3.1. Laboratory Studies: Pathways of Methane Production and $\delta^{13}C$ Values of Produced Methane

During an experiment conducted in January at the in situ temperature of 4°C, CO_2 reduction accounted for 110 ± 25% of total methane production determined by measuring methane concentration increase versus time. In an experiment conducted on this peat at an elevated temperature (25°C), 50 ± 9% of the methane was derived from CO_2 reduction; aceticlastic methanogenesis contributed the balance of 50 ± 15% (Table 1). In the incubation experiment performed on peat collected in May (15°C in situ temperature), 17 ± 3% of the methane production was from CO_2 with the balance (83 ± 16%) from acetate (Table 1). Similar results were obtained for the peat collected in June and incubated at the in situ temperature of 15°C; 15 ± 2% of the methane production came from CO_2, and 85 ± 7% was derived from acetate (Table 1).

The $\delta^{13}C$ value of methane produced in the May incubation experiment was -49.0 ± 10.3‰; the $\delta^{13}C$ value of methane produced in June was -48.4 ± 4.2‰ (Table 2). These values were calculated from the total methane production rate, the initial methane concentrations, and the initial and final $\delta^{13}C$ values of methane in the incubation experiments. The $\delta^{13}C$ value of methane produced in the January experiment was not determined because of high variability of methane concentrations during the end of the incubation experiment. Initial concentrations of acetate in the incubation experiments were 368 μM in January, 467 μM in May, and 187 μM in June.

3.2. Field Data: Acetate Concentrations and $\delta^{13}C$ Values of Pore Water Methane

The $\delta^{13}C$ values of pore water methane in the surface sediments of Buck Hollow Bog ranged from -50 to -61‰ (Figure 2a). The methane was enriched in ^{13}C (approximately -51 ‰) throughout the late fall and winter months and became progressively lighter, approaching -61‰ during the early spring (from approximately March through May). Dissolved

Table 1. Results of Michigan Peat Incubation Experiments

Month (Temperature)	Total Methane production	CO_2 Reduction Rate	Acetate Fermentation Rate	CO_2 Reduction, %	Acetate Fermentation, %
January (4°C)	0.18 ± 0.02 (*n*=6)	0.20 ± 0.04 (*n*=6)	-0.02 ± 0.04	110 ± 25	-10 ± 22
January (25°C)	1.97 ± 0.28 (*n*=5)	0.99 ± 0.10 (*n*=3)	0.98 ± 0.	50 ± 9	50 ± 15
May (15°C)	2.72 ± 0.34 (*n*=20)	0.46 ± 0.05 (*n*=10)	2.26 ± 0.34	17 ± 3	83 ± 16
June (15°C)	2.79 ± 0.15 (*n*=20)	0.41 ± 0.06 (*n*=10)	2.38 ± 0.16	15 ± 2	85 ± 7

Total methane production rate was determined by methane concentration increase versus time. Rates of CO_2 reduction were determined with ^{14}C-labeled tracers. Acetate fermentation rate was determined by the difference between total methane production rate and the CO_2 reduction rate. All rates are $\mu M\ hr^{-1}$; "n" is number of replicate samples used to determined rates (all errors are ± 1 standard deviation of the mean). All temperatures were the in situ temperatures and experimental incubation temperature except the January experiment at 25°C.

methane returned to heavier values (approximately -52‰) in early June (Figure 2a). During the same period, acetate concentrations, generally remaining below 20 μM for the late fall and early winter months, began to accumulate in the shallow pore waters during late March and reached maximum values (> 1000 μM) in late May. Acetate concentrations then rapidly decreased to <20 μM before mid-June (Figure 2b).

4. Discussion

4.1. Effect of Temperature on Pathways of Methane Production

Plots of CO_2 reduction rates versus incubation temperatures for the four experiments conducted in this study indicated an exponential relationship (Figure 3a). These results are similar to those obtained for CO_2 reduction and total methane production in incubation experiments utilizing White Oak River, North Carolina sediments [*Avery*, 1996]. However, plots of aceticlastic methanogenesis rates versus incubation temperatures for the four experiments conducted in this study did not show an exponential relationship. Aceticlastic methanogenesis rates were higher in May and June at 15°C than in January at 4°C (Figure 3b; Table 1). However, the rate measured at 25°C in January was lower than that for May and June at 15°C. These results may indicate a temperature optimum for aceticlastic methanogenesis in this peat between 15°-25°C (i.e., aceticlastic methanogenic rates may peak in the 15 to 25°C range). This is consistent with the results of *Svensson* [1984] which showed that aceticlastic methanogens growing in peat from a cold climate had a temperature optimum of about 20°C.

An alternative explanation for low rates of aceticlastic methanogenesis during the experiment conducted during January at 25°C would be seasonal differences in microbial populations. *Vogels et al.* [1988] showed that doubling times

Table 2. Rate and $\delta^{13}C$ Values of Methane From May and June Buck Hollow Peat Experiments and Calculated $\delta^{13}C$ Values of Methane Produced in the Experiments

Month	MPR	Time	$CH_{4(new)}$	$CH_{4(init.)}$	$CH_{4(tot.)}$	$\%CH_{4(new)}$
May	2.72 ± 0.34 (*n*=3)	408	1.11 ± 0.13	0.24 ± 0.02 (*n*=3)	1.35 ± 0.13	0.82 ± 13
June	2.79 ± 0.15 (*n*=4)	383	1.07 ± 0.05	0.28 ± 0.02 (*n*=3)	1.35 ± 0.06	0.79 ± 5

Month	$\delta^{13}C$ of $CH_{4(initial)}$	$\delta^{13}C$ of $CH_{4(final)}$	$\delta^{13}C$ of $CH_{4(produced)}$
May	-62.9 ± 0.1 ‰ (*n*=3)	-51.5 ± 0.9 ‰ (*n*=3)	-49.0 ± 10.3 ‰
June	-64.5 ± 1.1 ‰ (*n*=3)	-51.7 ± 0.0 ‰ (*n*=2)	-48.4 ± 4.2 ‰

"MPR" is the total methane production rate calculated from methane concentration versus time plots ($\mu M\ hr^{-1}$); "Time" is experimental incubation time (hr.); "$CH_{4(new)}$" is the amount of methane produced during the experiment (MPR x Time, units are mM); "$CH_{4(init.)}$" is the initial methane concentration (mM); "$CH_{4(tot.)}$" is total methane concentration at the end of the experiment ($CH_{4(new)}$) + $CH_{4(init.)}$); "$\%CH_{4(new)}$" is the percentage of the total methane that was produced during the experiment ($CH_{4(tot.)}/CH_{4(new)}$); "$\delta^{13}C$ of $CH_{4(initial)}$ and $\delta^{13}C$ of $CH_{4(final)}$"are the $\delta^{13}C$ values of methane measured at the beginning and end of the experiment, respectively; $\delta^{13}C$ of $CH_{4(produced)}$ is the $\delta^{13}C$ value of the methane produced in the experiment calculated with the following equation: $\delta^{13}C$ of $CH_{4(final)} = (\%CH_{4(new)}(\ \delta^{13}C$ of $CH_{4(produced)}\ ) + [(1 - \%CH_{4(new)})](\ \delta^{13}C$ of $CH_{4(initial)})$; "*n*" is the number of individual determinations of the given parameter. All errors are ± 1 standard deviation of the mean.

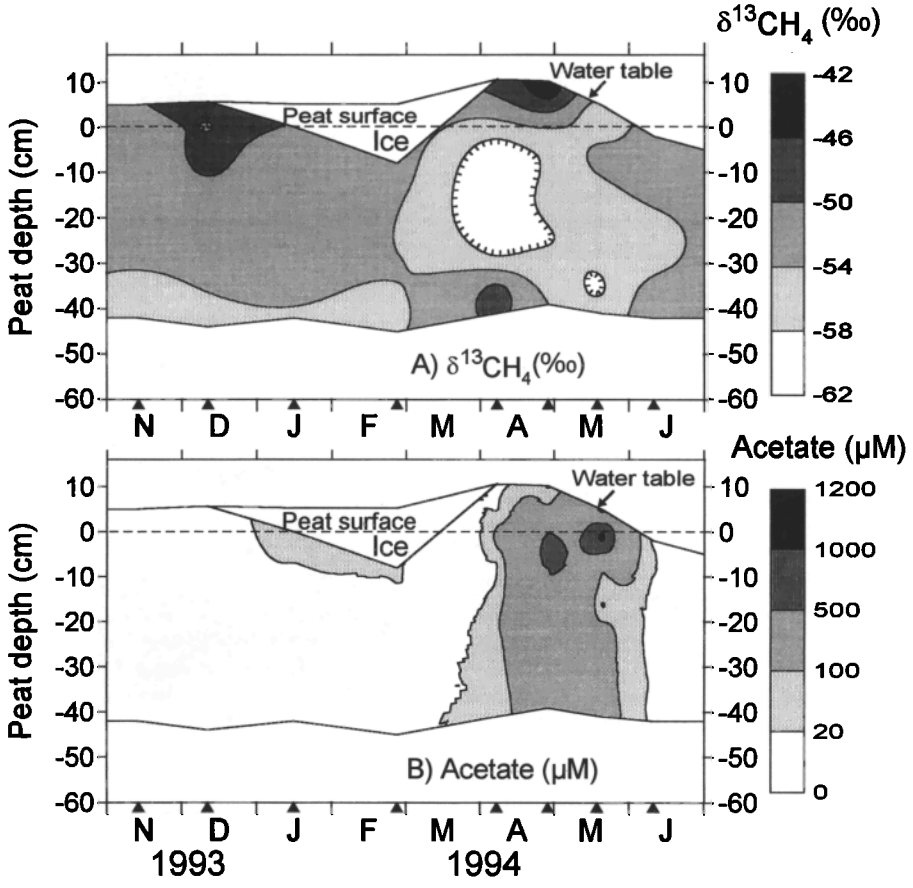

Figure 2. Isopleth plots of (a) stable carbon isotopic values of methane ($\delta^{13}C$, ‰) and (b) acetate concentrations (μM) in Buck Hollow Bog pore waters. The top solid lines represent the changing water level with time; bottom solid lines represent the bottom cell of each pore water sampler.

for acetate utilizing methanogens were longer than those for CO_2 reducers. During January, the concentrations of acetate in Buck Hollow were typically < 20 μM, and rates of aceticlastic methanogenesis were also low. Therefore an active population of aceticlastic methanogens may not have been established during the winter. This seems likely since the concentrations of acetate continued to rise well into the spring [*Shannon and White*, 1996](Figure 2b, Figure 4). By late May and June, an efficient community of aceticlastic methanogens had probably been established. Declining pore water acetate concentrations during this period [*Shannon and White*, 1996](Figure 2b, Figure 4) indicate the presence of a population of aceticlastic methanogens capable of consuming more acetate than was produced. This phenomenon has also been observed in the marine sediments of Cape Lookout Bight, North Carolina. After the depletion of pore water SO_4^{-2}, methanogenesis (using the newly available substrates CO_2/ H_2 and acetate) was dominated by CO_2 reduction until the aceticlastic population had time to become established [*Albert et al.*, 1991; *Alperin et al.*, 1992]. In the current study, the differential response of CO_2 and acetate consuming methanogens to temperature may be caused by different temperature optima and/or seasonal population dynamics. The different responses should ultimately have an effect on the $\delta^{13}C$ values of biogenic methane, provided that the $\delta^{13}C$ values of methane from the two substrates are different.

4.2. The $\delta^{13}C$ Values of Methane Derived From CO_2 and Acetate

The $\delta^{13}C$ value of methane from CO_2 reduction and acetate fermentation in Buck Hollow Bog were determined from the results of the May and June peat incubation experiments, assuming that CO_2 and acetate were the only two methanogenic substrates. The $\delta^{13}C$ value of the ΣCO_2 pool was -7.9 ± 0.5‰ (n=5) for May and -6.8 ± 0.5‰ (n=5) for June. The fractionation factor (α) for CO_2 and methane calculated for the White Oak River, North Carolina (α = 1.069 ± 0.0014 in *Avery* [1996]) was used for this calculation, as it is similar to values reported for CO_2 reduction in sediments from Cape Lookout Bight, North Carolina, (α = 1.060 in *Blair et al.* [1993]) and a peatland in Washington state (α = 1.073 ± 0.007 in *Lansdown et al.* [1992]). The $\delta^{13}C$ value of methane was calculated using the following equation

$$\alpha = \frac{\delta^{13}CO_2 + 1000}{\delta^{13}CH_4 + 1000} \quad (1)$$

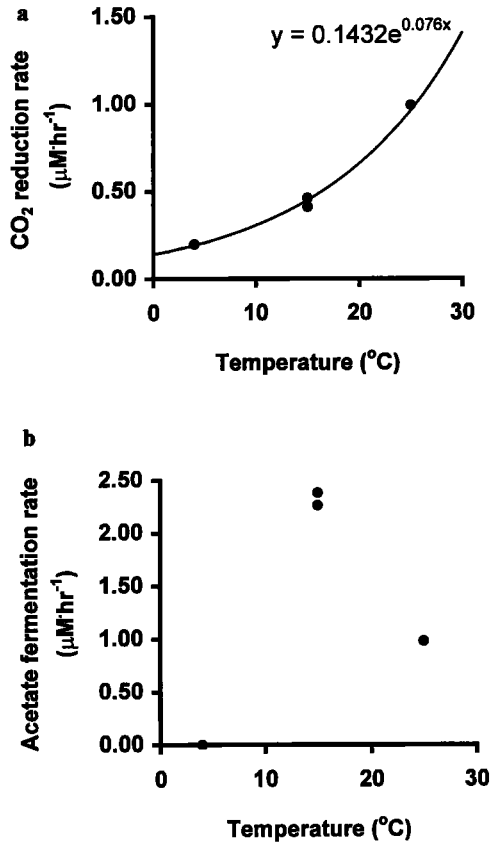

Figure 3. Plots of (a) the CO_2 reduction rate for Michigan peat (Buck Hollow Bog) versus incubation temperature and (b) acetate fermentation rates (calculated by difference) versus incubation temperature. Samples are the following: One sample at 4°C was collected in January and incubated at ambient temperature; two peat samples at 15°C were collected during May and June and incubated at ambient temperature; one sample at 25°C was collected in January and incubated at an artificially elevated temperature.

where $\delta^{13}CO_2$ is the $\delta^{13}C$ value of the ΣCO_2 pool and $\delta^{13}CH4$ is the $\delta^{13}C$ value of methane produced from CO_2. Substituting known values into equation (1), the $\delta^{13}CH4$ for May and June were -71.9 ± 1.3‰ and -70.9 ± 1.3‰, respectively. We calculated the $\delta^{13}C$ of methane from acetate from the following equation:

$$\delta^{13}CH_4 = (f c)(\delta mc) + (f a)(\delta ma) \quad (2)$$

where $\delta^{13}CH_4$ is the $\delta^{13}C$ value of methane produced in the experiments (May or June)(Table 2); $f c$ and $f a$ are the fraction of methane produced from CO_2 and acetate, respectively (Table 1); and δmc and δma are the $\delta^{13}C$ values of methane from CO_2 and acetate, respectively (δmc calculated above). Solving for δma using data for May yielded a value of -44.3 ± 15.2‰. The δma for June was -44.5 ± 6.4‰. The $\delta^{13}C$ values of methane produced from CO_2 and acetate are very similar to those obtained in other studies (Table 3).

4.3. Seasonal Changes in $\delta^{13}C$ Values of Pore Water Methane

Shifts in the $\delta^{13}C$ values of pore water methane of Buck Hollow Bog can be explained by changes in the production and consumption of acetate [*Shannon and White*, 1996] as well as by differences in the absolute and relative rates of CO_2 reduction and aceticlastic methanogenesis. The seasonal patterns in these parameters can be divided into three distinct time periods including fall-winter, early spring, and late-spring, which are discussed separately below

4.3.1. Fall-winter. The peat temperature during the late fall was 5°C and steadily decreased to about 0°C by late February (Figure 4). Sediment incubation experiments during January (4°C) showed that methane production rates were very low (0.18 ± 0.02 $\mu M hr^{-1}$) and were dominated by CO_2 reduction (Table 1). Tracer-determined rates of CO_2 reduction accounted for 110 ± 25% of the total methane production. Therefore the $\delta^{13}C$ value of methane produced during the winter should be very light assuming the $\delta^{13}C$ values of methane produced from CO_2 during the winter were similar to that produced from CO_2 during the May and June incubation experiments. However, the $\delta^{13}C$ values of dissolved methane from bog pore waters during this period ranged from -50 to -52.5‰ (Figure 2a; Figure 4). These values are very similar

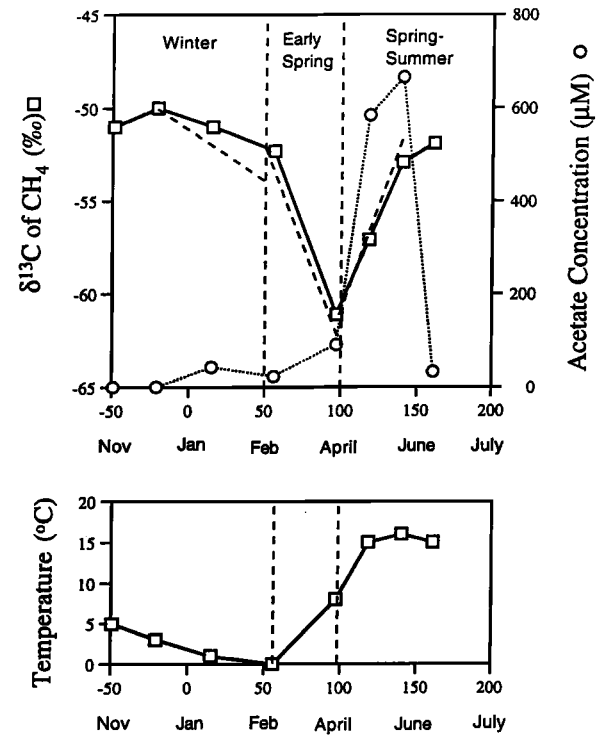

Figure 4. (top) Acetate concentrations (open circles) and $\delta^{13}C$ values (open squares) of dissolved methane in pore waters of sediments in Buck Hollow Bog. (bottom) Peat temperature. Dotted lines are predicted changes in the $\delta^{13}C$ values on dissolved methane pool based on ^{14}C-tracer rates and methane pool size.

Table 3. The $\delta^{13}C$ Values of Methane Produced From CO_2 and Acetate From Various Studies.

Study	Environment	$\delta^{13}C$ value $CH_{4(CO_2)}$, ‰	$\delta^{13}C$ value $CH_{4(acetate)}$, ‰
Alperin et al., [1992]	Coastal Marine	-62	-39 to -37*
Lansdown et al., [1992]	Peatland	-73 ± 4	N/A
Sugimoto and Wada, [1993]	Rice Paddy Soil	-77 to -60	-43 to -30
Blair et al., [1993]	Coastal Marine	-57.7 to -62.	-43 ± 10
N.E. Blair (personal communication, 1996)	Freshwater Estuary	N/A	-61
Avery [1996]	Freshwater Estuary	-71.9 ± 2.2	N/A
This study	Peatland (May)	-71.9 ± 1.3	-43.8 ± 11.9
	Peatland (June)	-70.9 ± 1.3	-44.5 ± 5.4

Both coastal marine sites were Cape Lookout Bight, North Carolina; Both freshwater estuary sites were the White Oak River, North Carolina; the *Lansdown et al.*, [1992] peatland was Kings Lake Bog in Washington state; and the rice paddy soil was a rehydrated organic manure from a rice field in Konosu, Japan.

* This value was for acetate not produced from autotrophic acetogenesis. Extremely ^{13}C-depleted methane (less than -115‰) was produced from autotrophically produced acetate during this experiment.

to the $\delta^{13}C$ values of methane produced during the May and June incubation experiments (Table 2), when rates of methane production (this study) and methane emissions [*Shannon and White*, 1994] were at maximum values. This suggests that methane produced during the winter had little effect on the $\delta^{13}C$ value of the methane pool which was produced during previous warm months. The slight trend toward lighter methane (approximately -2.5‰ from December to February, Figure 4) during this period may have been due to small additions of ^{13}C-depleted methane produced during the winter.

To test this hypothesis, we estimated the effect of winter-produced methane on the $\delta^{13}C$ value of the dissolved methane pool and compared it to the field observations. Assuming that the methane produced during this period (from CO_2 reduction) had a $\delta^{13}C$ value similar to that produced from CO_2 reduction during the May and June incubation experiments (-71.4± 1.8‰), a dissolved methane pool concentration for the fall-winter of 1000 μM [*Shannon and White*, 1996], a methane production rate of 0.14 μM hr^{-1} (predicted for 0°C by the exponential relationship for CO_2 reduction and temperature; Figure 3a), and a 75-day period (from approximately December to February when the isotopic shift in the $\delta^{13}C$ values of the methane pool was observed; Figure 2a and Figure 4), the methane produced during the winter (252 μM) would comprise 20% of the final pool (assuming mixing of the original pool with newly produced methane and a loss of methane from each source in proportion to their final concentrations). The $\delta^{13}C$ of the methane pool at the end of this period was calculated as follows:

$$\delta^{13}CH_4 = (\not{f}\,\text{old})(\delta\text{old}) + (\not{f}\,\text{new})(\delta\text{new}) \quad (3)$$

$$\delta^{13}CH_4 = -54.2 \pm 0.4‰,$$

where $\delta^{13}CH_4$ was the calculated $\delta^{13}C$ value of methane at the end of the period; δold was the $\delta^{13}C$ value of methane at the beginning of the period (December $\delta^{13}C$ value = -50.0‰; Figure 4); δnew was the $\delta^{13}C$ value of methane produced during the winter; $\not{f}$new was the fraction of new methane produced during the 75-day period (20% or 0.2, see calculation above); and $\not{f}$old was the fraction of old methane (originally present at the beginning of the 75-day period) present at the end of the 75-day period (80% or 0.8). This calculation predicted a $\delta^{13}C$ value of methane at the end of winter of -54.2 ± 0.4‰, which was similar to the $\delta^{13}C$ value observed in the field (-52.5‰; Figure 4) supporting the idea that changes in the $\delta^{13}C$ values of the methane observed in the field resulted from a small addition of ^{13}C-depleted methane produced during the winter. It should be noted that the concentration of the dissolved methane pool was largest during this period due to its decreased efflux from the soil [*Shannon and White*, 1996]. This decreased flux, coupled with low production rates, made the $\delta^{13}C$ value of the methane pool very insensitive to the isotopic composition of methane produced during this period. Therefore, on the basis of these calculations and the observed trends in the field data, the isotopic composition of methane present in the sediments during the winter was most likely controlled by methane produced during the preceding warmer months and was only slightly affected by that produced during the winter.

4.3.2. Early spring. During this period (~ 50 days), the $\delta^{13}C$ value of the methane pool in Buck Hollow Bog became depleted in ^{13}C by approximately 11.0‰ (Figure 4). The temperature increased rapidly from about 0° to 10°C. Acetate concentrations began to increase because of higher production coupled with low rates of consumption [*Shannon and White*, 1996] (Figure 2b). Concentrations of acetate continued to rise rapidly well into the spring, indicating an underdeveloped acetate-utilizing methanogenic community during the spring. Therefore it is not likely that aceticlastic methanogenesis contributed significantly to methane production during this period. The ^{13}C depletion of the methane pool observed in the field data was most likely a result of an increase in methane production mainly from CO_2 reduction. We tested this hypothesis by performing mass balance calculations to

determine the effect of methane produced during this period on the $\delta^{13}C$ value of the methane pool.

On the basis of the exponential relationship between temperature and CO_2 reduction rate for this peat (Figure 3a), the rate of CO_2 reduction doubled during this period (0.0°C = 0.14 μM hr^{-1}; 10.0°C = 0.30 μM hr^{-1}). At 5°C, the average temperature during the early spring, the CO_2 reduction rate was 0.21 μM hr^{-1}. With this production rate, 252 μM of methane was produced during the 50-day period. Using the approximate dissolved pore water methane concentration during April (300 μM; *Shannon and White* [1996]), the newly produced methane comprised approximately 46% of the final methane pool (same mixing assumptions as above). Assuming the methane produced during this time was dominated by CO_2 reduction with a $\delta^{13}C$ value similar to that calculated for the winter (-71.4 ± 1.8‰), the $\delta^{13}C$ of the methane pool at the end of early spring was calculated using equation (3) as before, where $\delta^{13}CH_4$ was the predicted $\delta^{13}C$ value of the methane pool at the end of the period; δold was the $\delta^{13}C$ value of the methane pool at the beginning of the period (-52.5‰; Figure 4); δnew was the calculated $\delta^{13}C$ value of methane produced during winter (-71.4 ± 1.8‰); fnew was the fraction of new methane produced during the 50-day period (46% or 0.46, see calculation above); and f old was the fraction of old methane (originally present at the beginning of the 50-day period) present at the end of the 50-day period (54% or 0.54). The predicted $\delta^{13}C$ value of methane at the end of early spring was -62.7 ± 1.0‰, which was similar to the $\delta^{13}C$ value observed in the field (-61‰; Figure 4). Therefore the $\delta^{13}C$ shift observed during the early spring can be explained by increased production of ^{13}C depleted methane (mainly from CO_2 reduction) during this period.

4.3.3. Late spring and summer. During the late spring, the $\delta^{13}C$ of the methane pool in the shallow peat returned to heavy values of approximately -52‰ (Figure 2a; Figure 4) suggesting the addition of ^{13}C-enriched methane produced from acetate. This represents a methane pool ^{13}C enrichment of approximately 10‰ during a 1 month period. A previous study of this peatland by *Shannon and White* [1996] showed a dramatic decrease in pore water acetate concentrations followed by an increase in methane flux during this time, also suggesting increased rates of aceticlastic methanogenesis. Isotopic and rate data from our incubation experiments confirmed the hypothesis that acetate was an important substrate during this period. Incubation experiments conducted in May and June at 15°C showed that methane production was dominated by aceticlastic methanogenesis (Table 1). The $\delta^{13}C$ values of methane produced in these incubation experiments were heavy (Table 2) and similar to field values observed during this period (-52‰). The flux of methane was also at its maximum [*Shannon and White*, 1994] indicating a flushing of the sediments with newly produced methane and a return to heavier $\delta^{13}C$ values of the methane pool.

We calculated the effect of methane produced during this period on the $\delta^{13}C$ value of the methane pool and compared it to the +10‰ shift observed in the field data. The amount of methane produced during the 30-day period from mid-April to mid-May was 1958 ± 245 μM, based on a total methane production rate of 272 ± 0.43 μM hr^{-1} during this period (Table 1). With a dissolved methane pool of 300 μM [*Shannon and White*, 1996], the methane produced during this period comprised 87 ± 14% of the final methane pool (same mixing assumptions as sections 4.3.1 and 4.3.2). We then calculated the predicted $\delta^{13}C$ of the methane pool at mid-May (the end of this period) using equation (3), with values of δold = -61‰ (field value of dissolved methane mid-April), δnew = -49.0 ± 10.3‰ (dissolved methane value for the May incubation experiment), fnew = 0.87 or 87% (fraction of methane produced during the 30-day period), and fold = 0.13 or 13% (fraction of methane present at the beginning of the 30-day period). The predicted $\delta^{13}C$ value of the methane pool at the end of this period was -50.6 ± 13.4‰, which is similar to the field $\delta^{13}C$ value of methane after the 30-day period (-52‰). Therefore, during the spring and summer, the addition of ^{13}C-enriched methane, produced mainly from acetate, returned the $\delta^{13}C$ values of the dissolved methane pool to heavier values.

5. Summary and Conclusions

This study is the first to quantitatively show, through direct rate and stable carbon isotopic measurements, that changes in pathways of methane production controlled the $\delta^{13}C$ values of methane produced in a peat soil. Methane production pathway in this peat changed from CO_2 reduction in the winter to predominantly acetate fermentation during the late spring, resulting in an 11‰ shift in the $\delta^{13}C$ values of dissolved methane. The $\delta^{13}C$ values of the methane pool predicted by the incubation experiments agreed well with field observations, demonstrating the usefulness of combining laboratory experiments and field observations to study microbial processes occurring in sediments. Similar quantitative approaches are necessary to understand the underlying controls on the isotopic composition of biogenic methane in order to provide a basis for modeling seasonal methane flux from peatlands and other wetland environments. By better understanding these temporal variations, we can improve the database for wetland source strengths which in turn will help to better constrain global atmospheric methane budgets.

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