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<td>Author(s)</td>
<td>Itoh, Masayuki; Ohte, Nobuhito; Koba, Keisuke; Sugimoto, Atsuko; Tani, Makoto</td>
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Kyoto University
Analysis of methane production pathways in a riparian wetland of a temperate forest catchment, using $\delta^{13}$C of pore water CH$_4$ and CO$_2$

Masayuki Itoh, Nobuhiro Ohte, Keisuke Koba, Atsuko Sugimoto, and Makoto Tani

1. Introduction

Methane (CH$_4$) is a key greenhouse gas, and its infrared radiative heating effect is 26 times greater than that of carbon dioxide on a mole-per-mole basis [Lelieveld and Crutzen, 1992]. Soil functions as both a main source and a main sink of CH$_4$. In anoxic environments such as wetland soils, CH$_4$ is produced by methanogenic bacteria that are active only under anoxic and strongly reducing conditions [Takai, 1970; Schütz et al., 1989]. In contrast, CH$_4$ is usually oxidized by methanotrophic bacteria in oxic soils. Over the last 150 years, the mixing ratio of CH$_4$ in the atmosphere has more than doubled [Etheridge et al., 1998], and improved estimates of the strength of each source and sink are of high importance. However, much uncertainty regarding the CH$_4$ production and consumption mechanisms in soils still leads to uncertainty in estimating the levels of atmospheric CH$_4$.

Because biospheric sources of CH$_4$ are highly variable, stable isotope ratios of CH$_4$ have been used to constrain the global CH$_4$ budget, as microbe-produced CH$_4$ has a significantly different isotopic signal than CH$_4$ from other sources [Whiticar, 1999]. In particular, the $^{13}$C compositions of CH$_4$ in background tropospheric air and of the major CH$_4$ sources have added further constraint to the individual CH$_4$ source strengths by isotope mass balance using the $\delta^{13}$C value of each source [Stevens and Rust, 1982; Cicerone and Oremland, 1988; Stevens and Engelkemeir, 1988; Whalen et al., 1989; Quay et al., 1991; Lowe et al., 1994; Gupta et al., 1996; Bräunlich et al., 2001; Fletcher et al., 2004] and have revealed that 70–80% of atmospheric CH$_4$ is of biogenic origin, with natural wetlands as the largest source [Bartlett and Harris, 1993; Khalil and Shearer, 1993]. However, estimating the representative $\delta^{13}$C-CH$_4$ of each source...
remains challenging because $\delta^{13}$C-$\text{CH}_4$ values are highly variable, especially in rice paddies and wetlands [Quay et al., 1991], reflecting the multiple processes involved in CH$_4$ production and consumption in these ecosystems. For example, methanogenesis from carbonate results in a larger fractionation against $^{13}$C and, thus, lower $\delta^{13}$C-$\text{CH}_4$ values than methanogenesis from acetate [Games et al., 1978; Krzycki et al., 1987; Gelwicks et al., 1994]. However, previous studies have shown that the fractionation factors vary with site and conditions (e.g., reviewed by Conrad [2005]). More data under various environmental conditions are required for the explicit determination of fractionation factors.

Among the various CH$_4$ sinks and sources, forests are assumed to be a major sink of atmospheric CH$_4$ by microbial oxidation in aerobic soils [Reeburgh et al., 1993; IPCC, 2001]. However, Itoh et al. [2005, 2007] suggested that wet riparian areas in forests can function as ‘hot spots’ of CH$_4$ emission and that these ‘hot spots’ can also significantly affect the total budget of trace gas emissions on larger scales, such as whole forest ecosystems. Soil hydrological conditions in forest catchments are spatially variable. Riparian wetlands in small headwater catchments are characterized by high CH$_4$ production, and production and emission rates are strongly affected by changes in hydrological processes and temperature [Itoh et al., 2007]. This hydrological variability can also affect CH$_4$ production pathways that mainly consist of acetate fermentation [Zeikus et al., 1975] and carbonate reduction [Takai, 1970; Crill and Martens, 1986; Martens et al., 1986; Burke et al., 1988; Schütz et al., 1989]. Redox conditions in such wetland soils
change on a shorter temporal scale with hydrological conditions (precipitation patterns and water movement in soil [Mitsch and Gosselink, 2000; Itoh et al., 2007]) than in ombrotrophic wetlands, where most previous work has been conducted. CH₄ production pathways can change drastically on small temporal and spatial scales in such riparian wetlands. Thus, for a more reliable estimate of the CH₄ budget on a larger scale, such as an entire forest catchment, an understanding of CH₄ production mechanisms, including production pathways, is strongly required.

In this study, we used the δ¹³C isotope mass balance of pore water CH₄ and CO₂ [Sugimoto and Wada, 1993] to determine temporal and spatial changes in CH₄ production pathways and to understand what processes control pathway changes in riparian wetlands. In addition, we considered the effects of climate differences, such as characteristic precipitation patterns in Asian monsoon climates, on CH₄ production pathways.

2. Materials and Methods

2.1. Site Description and Hydrological and Biogeochemical Features

We studied forested wetlands in the Kiryu Experimental Watershed (KEW; 35°N, 136°E; 190–255 m above sea level; 5.99 ha), located in southeastern Shiga Prefecture, central Japan (Figure 1). The KEW comprises about 99.3% forest floor, with 0.67% (400.6 m²) distinct wetland riparian zones. The wetland studied here, Kiryu Wetland 2 (KW2), is located upstream of a check dam constructed across the mainstream of the watershed about 100 years ago to prevent soil erosion. There are other natural wetlands in KEW, but all are located in riparian zones along streams. The wetland soils are either always submerged or periodically submerged. The entire watershed is on a base of weathered granitic rock with an abundance of albite. In the 1960s, Japanese cypress (Chamaecyparis obtusa) was planted on the hillslope over the watershed. Although several tree species (Eurya japonica, Alnus japonica, Clethra barvinservis, Evodiopanax innovans, and Rhus trichocarpa) and sphagnum grow in and around the wetlands, vegetation in the wetlands is sparse, probably because of occasional sediment transport with surface flow.

Precipitation was measured at a meteorological station within the watershed. Mean annual air temperature and precipitation were recorded during the observation period (Table 1). Surface soil temperatures at depths of 0.02 and 0.10 m were also continuously monitored (Figure 2a) at each groundwater-sampling plot. The water levels in KW2 were measured in wells installed at each groundwater-sampling plot. We also installed a capacitance water level sensor and data recorder (CR-10x, Campbell, USA) 0.50 m from the KW2-downstream plot.

Table 1. Mean Annual Air Temperature and Annual and Summer Precipitation in the Kiryu Experimental Watershed

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mean annual air temperature (°C)</td>
<td>12.9</td>
<td>13.9</td>
<td>13.0</td>
<td>13.2ᵇ</td>
</tr>
<tr>
<td>Annual precipitation (mm year⁻¹)</td>
<td>1946.8</td>
<td>1796.8</td>
<td>1150.5</td>
<td>1574.1</td>
</tr>
<tr>
<td>Summer precipitation (mm)</td>
<td>804.0</td>
<td>500.3</td>
<td>474.0</td>
<td>550.6</td>
</tr>
</tbody>
</table>

aData from Itoh et al. [2007].

bAverage of values obtained from 2000 to 2005.

The study site is affected by the Asian monsoon system, which usually results in a summer rainy season. Variation was observed in summer (June, July, and August) precipitation; a large amount of precipitation was observed in 2003, and less in 2004 and 2005 (Table 1 and Figure 2b). Summer precipitation accounted for 146.0, 90.9, and 86.1% of the mean annual precipitation (1574.1 mm for 1996–2005) in 2003, 2004, and 2005, respectively.

Strongly reducing conditions (e.g., activation in denitrification and manganese-, iron-, and sulfate-reductions) were formed in high temperature periods in all three sampling plots [Itoh et al., 2007]. However, relatively dissolved oxygen (DO)-rich stream and subsurface flow from the hillslope due to heavy precipitation induced more oxic conditions than usual in the surface and bottom layers of wetlands in 2003. In contrast, a decrease in the water table formed oxic conditions in the surface soil layer in summer 2005. In these summers, the CH₄ concentration in the surface soil was much lower than in the summer of 2004, when the water table and soil temperature were high. These changes in redox conditions, depending on hydrologic conditions, strongly affect CH₄ production in the soil and CH₄ emission from the soil surface [Itoh et al., 2007].

2.2. Groundwater Collection and Analysis

Pore water samples were collected vertically to measure the concentration of CH₄ and CO₂ and isotopic composition of δ¹³C-CH₄ and δ¹³C-CO₂ and water chemistry in each of three observation plots, KW2-edge (0.55 m soil depth), KW2-center (1.77 m), and KW2-downstream (1.38 m; Figure 1c). KW2-edge was near the hillslope and KW2-downstream was along a perennia stream (Figure 1c). The surface soils of all three sampling plots were silty with much undecomposed litter. We used double-walled pore water samplers [Itoh et al., 2007] which collected pore water without degassing and high decompression. The pore water samplers were placed at KW2-edge (at soil depths of 0.10 and 0.25 m), KW2-center, and KW2-downstream (at 0.10, 0.20, 0.30, 0.50, and 0.70 m). Pore water samples were injected into 20-, 30-, or 50-mL pre-evacuated vials for the measurement of dissolved CH₄ and CO₂ concentrations and their carbon isotope ratios without exposure to the atmosphere, and into plastic bottles for other chemical analyses; the vials and plastic bottles were stored in a cooler (around 4°C) in the field. Surface water (KW2-edge and KW2-center) and stream water (KW2-downstream) were also sampled.

In situ measurements, including pH and electrical conductivity (EC), and laboratory measurements of dissolved components including DO, CH₄, and CO₂ were conducted. Detailed information on the methods and water chemical constituent results were given by Itoh et al. [2007].
Dissolved CH$_4$ and CO$_2$ concentrations were determined within 8 h of sampling by multiple equilibrations with a headspace of ultra high purity (UHP) helium [McAullife, 1971]. The headspace was prepared in a vial by replacing sample water with He (>99.999% purity). The vials were vigorously shaken for 2 min to drive gases from the pore water into the headspace. The headspace gas was withdrawn using a gas-tight syringe, and CH$_4$ concentration was determined using a gas chromatograph (GC; GC-14BPF, Shimadzu, Japan) equipped with a flame ionization detector (FID [Itoh et al., 2007]). For CO$_2$ concentration measurements, samples of the same gas were collected from the headspace and injected into a GC (GC-8APT, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD [Itoh et al., 2007]). Dissolved CO$_2$ concentrations in water samples were also measured in situ from June 2003 to August 2005 using a portable pCO$_2$ meter (CGP-1, DKK-TOA, Japan [Ohte et al., 1995]). GC measurements of dissolved CO$_2$ concentrations were carried out from February 2005 and confirmed the data obtained by in situ dissolved pCO$_2$ measurements.

Carbon isotopic compositions of dissolved CH$_4$ and CO$_2$ were analyzed using a gas chromatograph/combustion/isotope ratio mass spectrometer (GCCMS) MAT 252 equipped with an HP G1530A system [Sugimoto, 1996] at the Center for Ecological Research at Kyoto University. Because of the detection limit, isotopic measurement could not be conducted on samples with low CH$_4$ or CO$_2$ concentrations, such as stream and surface water.

### Soil Sampling and Analysis

Mineral and organic soil samples were collected in triplicate in each plot in January 2006. Topsoil and 10–20-cm interval samples underlying the thin litter layer (0–1 cm thick) were collected from the surface to depths of 0.25 m (KW2-edge) or 1.00 m (KW2-center and KW2-downstream). The depth of organic soil was approximately 0.15, 0.40, and 0.50 m in KW2-edge, KW2-center, and KW2-downstream, respectively. Soils were sieved through a 2-mm mesh sieve to remove coarse fragments and then homogenized. The total C and total N concentrations of soil samples were measured using the combustion method [Bremner, 1996] in an NC-analyzer (Sumigraph NC-900, Sumigraph Co., Japan).

δ$^{13}$C analyses were also carried out on soil samples. The samples were dried in an oven at 40°C for 48 h prior to...
13C analysis. 13C was determined using a mass spectrometer (Delta plus XP, Thermo Electron) coupled with an elemental analyzer (Flash EA, Thermo Electron).

3. Results

3.1. C and N Concentrations and 13C of Wetland Soils

[16] C and N contents, the C/N ratio, and 13C of litter and soil at each sampling depth are shown in Table 2. At KW2-edge and KW2-center, litter accumulates in the surface soils because of slow degradation under wet and anoxic conditions, resulting in higher soil C concentrations at the surface than in the bottom layer. At KW2-center, in particular, the upper soil layer contained much more organic matter than the bottom layer or the other plots. At KW2-downstream, soil C concentration was highest at 0.30 m. At all sampling plots, soil 13C values ranged between 29.8 and 28.3‰, and there was no obvious trend in 13C with depth or plot (Table 2).

3.2. CH4 and CO2 Concentrations and Carbon Isotopic Composition

[17] CH4 and CO2 concentrations in groundwater increased with temperature at all three plots (Figures 2c, 3c, 3d).

Figure 3. Seasonal variations in (a) soil temperature at KW2-downstream, (b) monthly precipitation measured at the nearby meteorological station, (c) groundwater CO2 concentration and water table level, and (d) 13C of CO2 in each sampling plot. The shaded areas indicate low temperature (<10°C) periods.
and 5). The δ¹³C of pore water CH₄ usually increased from summer to autumn at KW2-edge and KW2-downstream. At KW2-center, variation was relatively small in summer (Figure 2d). CH₄ production generally increased with temperature; however, Figures 2a and 2c suggest a time lag between temperature change and methanogenic activity (approximately 1–2 months).

[15] At KW2-edge, CH₄ was higher at 0.10 m than at 0.25 m, except in summer 2005 when the water table dropped (Figure 2c), suggesting that the most reducing conditions were usually at 0.10 m [Itoh et al., 2007]. CO₂ in groundwater increased in high temperature periods and was highest in summer 2005, when the surface soil was drier than usual (Figure 3c). δ¹³C-CH₄ values at 0.10 m were slightly higher than those at 0.25 m in the hot and wet summer of 2004 and lower in low temperature periods (Figure 2d). In this plot, the maximum δ¹³C-CO₂ value was −4.1% (Table 3). Rapid increases in CH₄ and CO₂ concentrations, δ¹³C-CH₄, and δ¹³C-CO₂ in groundwater were observed at the beginning of summer (Figures 2 and 3). This indicates that microbial activity increased rapidly with temperature in this plot.

[19] At KW2-center, CH₄ in groundwater was highest at 0.30 m through the sampling period (Figure 2c and Table 3) and maintained a high level even in low temperature periods (<10°C), when it decreased dramatically in other plots (Figure 2c). In this plot, mean and maximum CO₂ in groundwater were higher than those in the other plots. CO₂ was also highest at 0.30 m, where the soil C concentration was highest (Table 2 and Figure 3c). The range of δ¹³C-CH₄ values was much larger than in any other plot (Figure 2d and Table 3). That is, we observed lower δ¹³C-CH₄ values in low temperature periods (e.g., −83.0‰ at 0.30 m depth, 9 March 2005; −85.0‰ at 0.50 m and −89.1‰ at 0.70 m, 9 February 2005; Figure 2d and Table 3) and higher values in high temperature periods (e.g., −41.9‰ at 0.20 m, 10 September 2004), relative to other plots. δ¹³C-CH₄ was highest at 0.20 m in summer 2004, and no clear vertical trend was seen in other summers (Figure 2d). A temporal increase in δ¹³C-CO₂ was seen in the high temperature period, but this trend was unclear in the other two plots. Comparisons of the vertical distributions of CH₄, CO₂, and δ¹³C-CO₂ between the summers of 2004 and 2005 (Figures 2c, 3c, and 3d) all showed convex distributions, peaking at 0.30 m in both summers.

[20] At KW2-downstream, no clear vertical distribution pattern in CH₄ concentration was observed below 0.20 m. Seasonal (low in late winter and high in late summer) and yearly variations in CH₄ were clearer than in the other two plots. Maximum groundwater CH₄ and CO₂ in this plot were much lower in 2003 than in 2004 and 2005, especially at 0.10 m, indicating less microbial activity at low temperatures and high levels of DO in the rainy summer of 2003 (Figures 2c and 3c [Itoh et al., 2007]). Maximum δ¹³C-CH₄ (Figure 2d) and δ¹³C-CO₂ (Figure 3d) were also much lower in 2003, especially at 0.10 m. For example, maximum δ¹³C-CO₂ was much lower in 2003 (−10.9‰ at 0.50 m, 18 September 2003) than in 2004 (−4.0‰ at 0.30 m, 21 August 2004) and 2005 (−3.6‰ at 0.50 m, 15 September 2005). δ¹³C-CO₂ in groundwater showed clear seasonal changes, ranging from −27 to −4‰, and increased dramatically in high temperature periods (Figure 3d). δ¹³C-CH₄ showed a similar seasonal change, though not as obvious as δ¹³C-CO₂ (Figure 2d). In this plot, no obvious vertical difference in δ¹³C-CH₄ was observed (Figure 2d). δ¹³C-CH₄ increased dramatically in the summers of 2004 (at all depths) and 2005 (especially at 0.30 m).

4. Discussion

4.1. Effects of Temperature and Hydrology on Changes in CH₄ and CO₂ Dynamics

[21] The measured soil δ¹³C at KW2 ranged from −27.9 to −29.3‰ (Table 2). The measured δ¹³C of leaves ranges from −24.6 to −28.9‰ in cypress [Matsuo, 2003], which is the major vegetation in KEW, and from −33.3 to −32.5‰ in forest floor vegetation (Eurya japonica, N. Matsuo, personal communication). CO₂ is produced as a by-product of respiration under both oxic and anoxic conditions in soils. CO₂ produced from the metabolism of lactate is
depleted in $^{13}$C by as much as 26% [Smejkal et al., 1971]. Also, CO$_2$ resired during heterotrophic microbial metabolism is 3.4% depleted in $^{13}$C relative to the glucose used as the carbon source [Blair et al., 1985]. Negative isotopic enrichment occurs during the decomposition process, whatever the substrate [Mary et al., 1992]. The $\delta^{13}$C of CO$_2$ resired by decomposing litter and soil organic matter in KEW is expected to be lower than the $\delta^{13}$C of soil organic matter (= -29% in surface soil at KW2; Table 2), which is similar to the $^{13}$C of cypress. However, we observed much heavier $\delta^{13}$C-CO$_2$ values in groundwater than in soil, litter, and vegetation. At KW2-edge and KW2-downstream, $\delta^{13}$C-CO$_2$ dramatically increased in the summer when dissolved CH$_4$ concentrations were high (Figures 2c and 3d). At KW2-center, $\delta^{13}$C-CO$_2$ was also much higher than soil $\delta^{13}$C (Table 3). Processes that might induce such an increase in $\delta^{13}$C-CO$_2$ are (1) preferential use of $^{12}$CO$_2$ as a substrate for CH$_4$ by carbonate reduction or (2) $^{13}$C-enriched CO$_2$ as a byproduct of methanogenesis via acetate fermentation under anoxic conditions [Sugimoto and Wada, 1993]. CO$_2$ produced in this manner, i.e., by acetate fermentation, is strongly enriched in $^{13}$C and may have a $\delta^{13}$C value as high as $-5\%_{\text{o}}$ [Charman et al., 1994] or $+9\%_{\text{o}}$ [Waldron et al., 1999].

The significant positive regression of CH$_4$ concentration on $\delta^{13}$C-CO$_2$ (Figures 4 and 5 and Table 4) in all plots suggests that much of the CH$_4$ produced originates from carbonate reduction. Heavy $\delta^{13}$C-CO$_2$ from acetate fermentation may also contribute to CO$_2$ enrichment. The fact that the CO$_2$ concentration does not decrease in this zone indicates that CO$_2$ is continuously added by organic remineralization to the pool in the methanogenic zone, although $^{13}$C enrichment by methanogenesis does occur. Here, the increase in the proportion of carbonate reduction with temperature is confirmed by an increase in the apparent $\alpha$ in high temperature periods (Figures 6, 7, and 8; discussed below). These results support the idea that carbonate reduction becomes dominant in high temperature periods. As for carbonate (CO$_2$/H$_2$) reduction, although we have no data on hydrogen concentrations in groundwater, hydrogen concentration can increase with soil temperature by stimulating litter decomposition [Sugimoto and Fujita, 2006].

A less obvious increase in $\delta^{13}$C-CO$_2$ in the rainy summer of 2003 at KW2-downstream (Figure 3d) was attributed to lower CH$_4$ production than in a typical summer because of the DO supplied by increased water flow [Itoh et al., 2007]. These results show that preferential use of $^{12}$CO$_2$ for methanogenesis can drastically change $\delta^{13}$C-CO$_2$. Although the coefficients were not statistically significant at the deep zone of KW2-center, where CH$_4$ concentration was high throughout the year (Table 4), our results suggest that CO$_2$ contributed largely to CH$_4$ production in our site and residual CO$_2$ was enriched with $^{13}$C during this process.
In addition, changes in both temperature and hydrologic conditions (rainfall and the water table) played important roles in the variations in CH$_4$ and CO$_2$ concentrations and $\delta^{13}$C-CO$_2$. However, acetate fermentation must affect CH$_4$ production, and we consider the effects of acetate contributions to methanogenesis in the next section.

4.2. Acetate Contribution Determined by Isotope Mass Balance

[24] Similar seasonal variations in $\delta^{13}$C of CO$_2$ and CH$_4$ indicated that CO$_2$ contributes much as a substrate for CH$_4$ production. However, $\delta^{13}$C-CH$_4$ varied seasonally and fluctuated more wildly than $\delta^{13}$C-CO$_2$ at KW2-downstream (Figures 2d and 3d). Remarkably high $\delta^{13}$C-CH$_4$ values were occasionally observed in summer (e.g., KW2-downstream: $\delta^{13}$C-CH$_4$: 0.48.4% on 28 June 2004 at 0.70 m; $\delta^{13}$C-CH$_4$: 0.50.8% on 10 September 2004 at 0.10 m; $\delta^{13}$C-CH$_4$: 0.49.5% on 8 November 2004 at 0.20 m; and $\delta^{13}$C-CH$_4$: 0.52.5% on 15 September 2005 at 0.30 m; Figure 2d) when high CH$_4$ production occurred. Also at KW2-edge, heavy $\delta^{13}$C-CH$_4$ values were observed in summer (i.e., $\delta^{13}$C-CH$_4$: 0.51.6% at 0.10 m on 7 October 2004 and $\delta^{13}$C-CH$_4$: 0.57.3% at 0.25 m on 12 October 2005). The crossplots of $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-CO$_2$ are shown in Figures 6a–6c, with the apparent fractionation between CO$_2$ and CH$_4$ calculated by the

![Figure 5](Figure 5. Relationships between soil temperature and CH$_4$ concentration and $\delta^{13}$C-CH$_4$ at (a) KW2-edge, (b) KW2-center, and (c) KW2-downstream.)

Table 4. Results of Regression Analyses Between CH$_4$ Concentration and $\delta^{13}$CH$_4$ or $\delta^{13}$CO$_2$ at Each Sampling Depth

<table>
<thead>
<tr>
<th>Plot</th>
<th>Depth (m)</th>
<th>$\delta^{13}$CH$_4$</th>
<th>$\delta^{13}$CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KW2-edge</td>
<td>0.10</td>
<td>52</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>42</td>
<td>5.67</td>
</tr>
<tr>
<td>KW2-center</td>
<td>0.10</td>
<td>18</td>
<td>4.81</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>26</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>26</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>26</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>0.70</td>
<td>24</td>
<td>0.479</td>
</tr>
<tr>
<td>KW2-downstream</td>
<td>0.10</td>
<td>33</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>39</td>
<td>25.3</td>
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<td>0.30</td>
<td>42</td>
<td>60.9</td>
</tr>
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<td>0.50</td>
<td>39</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>0.70</td>
<td>39</td>
<td>27.5</td>
</tr>
</tbody>
</table>

*Values of $F$ and $p$ are observed variance ratios and probabilities in the analyses, respectively.*
where $\delta^{13}\text{CO}_2$ is the $\delta^{13}\text{C}$ value of the CO$_2$ pool at the sampling time and $\delta^{13}\text{CH}_4(\text{CO}_2)$ is the $\delta^{13}\text{C}$ value of CH$_4$ from CO$_2$.

Our results indicate that acetate fermentation, which has a smaller isotopic fractionation than carbonate reduction, affected the change in apparent $\alpha$ in our wetland. Here, we calculate the acetate contribution ($F_{\text{Ac}}$) to CH$_4$ production by means of isotope mass balance [Sugimoto and Wada, 1993]. In this calculation, only two substrates, CO$_2$ and acetate, are considered for methanogenesis, because CH$_4$ from other substrates such as methanol and trimethylamine has so far not been found to play a major role in freshwater environments [Lovley and Klug, 1983b; Conrad and Claus, 2005]. The fractional Ac contribution is expressed by $F_{\text{Ac}}$ as:

$$ F_{\text{Ac}} = \frac{\text{CH}_4 \text{ from acetate}}{\text{CH}_4 \text{ from CO}_2 + \text{CH}_4 \text{ from acetate}} $$

The isotope mass balance for produced CH$_4$ is expressed by the following equation:

$$ \delta^{13}\text{CH}_4(\text{Ac})F_{\text{Ac}} + \delta^{13}\text{CH}_4(\text{CO}_2)(1 - F_{\text{Ac}}) = \delta^{13}\text{CH}_4 $$

where $\delta^{13}\text{CH}_4(\text{Ac})$ and $\delta^{13}\text{CH}_4(\text{CO}_2)$ are the $\delta^{13}\text{C}$ values of CH$_4$ produced from acetate and CO$_2$, respectively, and $\delta^{13}\text{CH}_4$ is that of CH$_4$ produced during the indicated period. When the $\delta^{13}\text{C}$ values of CH$_4$, $\delta^{13}\text{CH}_4(\text{Ac})$, and $\delta^{13}\text{CH}_4(\text{CO}_2)$ are obtained, $F_{\text{Ac}}$ can be calculated from this equation. $\delta^{13}\text{CH}_4(\text{CO}_2)$ was calculated using equation (1).

Because we have no data on $\delta^{13}\text{C}$ of acetate and CH$_4$ from acetate, we used possible $\delta^{13}\text{C}$ values of CH$_4$ from acetate ranging from $-44$ to $-27\%_o$, considering the values obtained from previous studies [e.g., Sugimoto and Wada, 1993; Avery et al., 1999; Nakagawa et al., 2002], and $\delta^{13}\text{C}$ data from soil collected from KW2 (Table 2). During acetate fermentation, CH$_4$ is produced primarily from the methyl carbon of acetate [Pine and Barker, 1956; Krzycki et al., 1982]. Sugimoto and Wada [1993] incubated Japanese rice paddy field soil ($\delta^{13}\text{C} = -26.5\%_o$) with BES (a methanogenesis inhibitor) and measured the $\delta^{13}\text{C}$ of both methyl carbon and carboxyl carbon of acetate. The $\delta^{13}\text{C}$ of methyl carbon ranged from $-36$ to $-30\%_o$, which was lower than that of carboxyl carbon ($-21$ to $-15\%_o$). From this and the $\delta^{13}\text{C}$ of our wetland soils (approximately $-29\%_o$), we assume $\delta^{13}\text{CH}_4(\text{Ac}) = -35\%_o$. We must also assume values for the $13\text{C}$ fractionation coefficient during CO$_2$ reduction to CH$_4$ ($\alpha_c$). When assuming the $\alpha_c$ value, the temperature dependence of $\alpha_c$ [e.g., Whiticar et al., 1986; Whiticar, 1999; Conrad, 2005] is considered. Blair et al. [1993] found that $\alpha_c$ in marine sediment decreased with increasing temperature according to:

$$ \ln \alpha_c = (23.0/T) - 0.022 $$

A similar relationship was found for methanogenesis cultures [Botz et al., 1996], $\ln \alpha_c = (29/T) - 0.030$. We used the slope of equation (4) with the soil temperature range of KEW (0°C to 23°C at a depth of 0.10 m at KW2-downstream). As a first approximation, we assumed $\ln \alpha_c = (23/T) - 0.022$ according to the value of 1.06–1.07 based on a review of

![Figure 6. Crossplots of $\delta^{13}\text{C}$ data from CH$_4$ and CO$_2$ in groundwater at (a) KW2-edge, (b) KW2-center, and (c) KW2-downstream. The dashed lines give the apparent fractionation between CO$_2$ and CH$_4$ ($\alpha$) calculated by the ratio of ($\delta^{13}\text{C-CO}_2 + 1000$)/($\delta^{13}\text{C-CH}_4 + 1000$). From this, the apparent $\alpha$ was widely distributed from 1.032 to 1.083 and was usually between 1.04 and 1.06. This range is lower than reported values for $^{13}\text{C}$ fractionation during CO$_2$ reduction to CH$_4$ ($\alpha_c = 1.06–1.07$) according to a review of experimentally determined $\alpha_c$ of natural wetlands [Conrad, 2005]. Here,

$$ \alpha_c = \frac{\delta^{13}\text{CO}_2 + 1000}{\delta^{13}\text{CH}_4(\text{CO}_2) + 1000} $$


Figure 7. Seasonal variation in (a) soil temperature and (b) $F_{Ac}$ (acetate contribution to methanogenesis) in each sampling plot. The shaded areas indicate low temperature (<10°C) periods.
experimentally determined $\alpha_c$ of natural wetlands [Conrad, 2005], resulting in a range of 1.068 at 23°C to 1.075 at 0°C ($\alpha_{c1}$) for $\alpha_c$. Next, if the $F_{Ac}$ result was negative, we assumed a larger $\alpha_c$ value in order to obtain positive $F_{Ac}$ results ($\alpha_{c2}$).

[29] Figure 7b shows the seasonal variation of $F_{Ac}$ calculated from the above equations, assuming $\alpha_{c1}$ and $\delta^{13}CH_4 (Ac) = -33.5\%$ for KW2-edge and KW2-downstream. Table 5 shows the possible ranges of $F_{Ac}$ with varying parameters. For KW2-center, however, calculated $F_{Ac}$ values using $\alpha_{c1}$ in winter 2004 were negative for depths of 0.20 and 0.30 m regardless of the $\delta^{13}CH_4 (Ac)$ value (Table 5). Therefore, we assumed $\alpha_{c2}$ [In $\alpha_{c2} = (23/T) - 0.0015$], resulting in a range of 1.079 at 23°C to 1.086 at 0°C, and used these values to calculate $Ac$ for the three plots (Figure 7b and Table 5).

[30] Larger $F_{Ac}$ values result from larger $\alpha_c$ (at most 14.5% given the same $\delta^{13}CH_4 (Ac)$ and $\delta^{13}CH_4 (Ac)$ (at most 18.5% given the same $\alpha_c$). Also, smaller $\alpha_c$ and larger $\delta^{13}CH_4 (Ac)$ values result in wider ranges of $F_{Ac}$ (Figure 7b and Table 5). This indicates that the setting of these values changes the result significantly. However, the seasonal trend of $F_{Ac}$ was characteristic in each plot and did not change with changes to the parameters. As mentioned above, for purposes of discussion, here we used the results based on an appropriate $\delta^{13}CH_4 (Ac)$ value for KEW of $\sim -35\%$ [Sugimoto and Wada, 1993]. In addition, some attention should be paid to the expected systematic change in $\delta^{13}CH_4 (Ac)$ with temperature, although the degree of change is unknown because this has not been studied in detail. The range of possible $\delta^{13}CH_4 (Ac)$ is most

Table 5. Mean, Minimum, and Maximum Ac Contributions Calculated Using Equation (3)$^a$

<table>
<thead>
<tr>
<th>Plot</th>
<th>Depth (m)</th>
<th>$\alpha_c$</th>
<th>$\delta^{13}CH_4 (Ac) = -27$</th>
<th>$\delta^{13}CH_4 (Ac) = -35$</th>
<th>$\delta^{13}CH_4 (Ac) = -44$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge</td>
<td>0.10</td>
<td>$\alpha_{c1}$</td>
<td>33.1 (22.3 – 43.1)</td>
<td>39.5 (26.5 – 52.9)</td>
<td>50.6 (33.6 – 71.1)</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>$\alpha_{c1}$</td>
<td>38.2 (22.5 – 51.9)</td>
<td>44.7 (27.2 – 59.8)</td>
<td>55.4 (35.5 – 72.1)</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>$\alpha_{c2}$</td>
<td>44.1 (34.8 – 53.6)</td>
<td>51.0 (40.1 – 63.1)</td>
<td>62.0 (48.5 – 78.9)</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>$\alpha_{c2}$</td>
<td>47.5 (35.8 – 58.4)</td>
<td>54.2 (41.8 – 65.9)</td>
<td>64.6 (53.1 – 77.1)</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>$\alpha_{c1}$</td>
<td>34.6 (24.7 – 53.4)</td>
<td>40.3 (28.9 – 61.7)</td>
<td>49.6 (35.6 – 74.7)</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>$\alpha_{c1}$</td>
<td>30.9 (6.8 – 66.0)</td>
<td>36.6 (8.1 – 81.3)</td>
<td>46.2 (10.3 – 107)</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>$\alpha_{c1}$</td>
<td>26.4 (20.5 – 37.7)</td>
<td>31.3 (24.7 – 45.4)</td>
<td>39.8 (32.3 – 59.1)</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>$\alpha_{c1}$</td>
<td>32.9 (16.2 – 42.3)</td>
<td>38.7 (19.0 – 48.9)</td>
<td>48.2 (23.6 – 59.2)</td>
</tr>
<tr>
<td></td>
<td>0.70</td>
<td>$\alpha_{c1}$</td>
<td>38.3 (27.2 – 50.6)</td>
<td>44.4 (31.3 – 58.7)</td>
<td>54.1 (37.7 – 71.5)</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>$\alpha_{c1}$</td>
<td>44.2 (35.8 – 59.9)</td>
<td>50.3 (40.8 – 67.7)</td>
<td>59.3 (49.0 – 75.1)</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>$\alpha_{c1}$</td>
<td>41.9 (9.8 – 72.9)</td>
<td>48.2 (11.2 – 85.3)</td>
<td>58.0 (13.5 – 105)</td>
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<tr>
<td></td>
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<td>44.2 (0.4 – 56.3)</td>
<td>53.4 (0.5 – 69.1)</td>
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<tr>
<td></td>
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<td>$\alpha_{c2}$</td>
<td>43.1 (28.8 – 50.3)</td>
<td>49.4 (32.9 – 56.9)</td>
<td>59.0 (39.3 – 67.2)</td>
</tr>
<tr>
<td></td>
<td>0.70</td>
<td>$\alpha_{c2}$</td>
<td>45.3 (6.2 – 57.6)</td>
<td>51.4 (7.1 – 65.3)</td>
<td>60.5 (8.4 – 76.9)</td>
</tr>
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<td>31.9 (14.9 – 43.6)</td>
<td>38.0 (18.6 – 53.8)</td>
<td>48.6 (25.9 – 72.9)</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>$\alpha_{c1}$</td>
<td>37.7 (22.2 – 52.0)</td>
<td>44.1 (27.5 – 62.6)</td>
<td>54.8 (33.7 – 81.5)</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>$\alpha_{c1}$</td>
<td>37.3 (21.5 – 51.7)</td>
<td>43.8 (26.8 – 59.8)</td>
<td>54.8 (37.0 – 72.5)</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>$\alpha_{c1}$</td>
<td>36.7 (16.2 – 57.3)</td>
<td>43.0 (20.0 – 65.9)</td>
<td>53.4 (27.3 – 79.1)</td>
</tr>
<tr>
<td></td>
<td>0.70</td>
<td>$\alpha_{c1}$</td>
<td>37.8 (22.1 – 57.0)</td>
<td>44.3 (26.4 – 67.9)</td>
<td>55.1 (33.9 – 86.6)</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>$\alpha_{c2}$</td>
<td>46.4 (35.2 – 56.5)</td>
<td>53.2 (41.5 – 66.1)</td>
<td>60.9 (42.4 – 76.4)</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>$\alpha_{c2}$</td>
<td>47.1 (34.5 – 60.2)</td>
<td>53.8 (39.1 – 70.1)</td>
<td>64.2 (45.8 – 86.0)</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>$\alpha_{c2}$</td>
<td>47.0 (36.7 – 58.4)</td>
<td>53.8 (43.6 – 66.1)</td>
<td>64.5 (55.4 – 77.6)</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>$\alpha_{c2}$</td>
<td>46.4 (32.0 – 63.0)</td>
<td>53.1 (38.0 – 71.0)</td>
<td>63.4 (47.9 – 82.8)</td>
</tr>
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<td></td>
<td>0.70</td>
<td>$\alpha_{c2}$</td>
<td>46.2 (35.6 – 64.2)</td>
<td>53.0 (41.2 – 74.1)</td>
<td>63.5 (49.9 – 89.7)</td>
</tr>
</tbody>
</table>

$^a$Data obtained from June 2003 to June 2006 at KW2-edge and KW2-downstream, and from August 2004 to June 2006 at KW2-center.
likely within the ranges reported in Table 5. Therefore, in our
discussion, this effect would be covered by the ranges used for
calculating $F_{Ac}$ with various parameters.

[31] Mean acetate contributions during sampling periods
varied between 30 and 44% with $\alpha_{c2}$ and 44 and 54% with
$\alpha_{c2}$ (Figure 7b and Table 5). As mentioned above, the
calculated $F_{Ac}$ at KW2-center fell below 0% in winter
2004/2005 with $\alpha_{c2}$, indicating that $\alpha_{c2}$ was more appropriate
for this plot. At KW2-edge, $F_{Ac}$ increased in late summer
2004 and decreased from winter to summer 2005 (Figure 7b).
At KW2-center, $F_{Ac}$ was usually stable expect in winter
2004/2005 (±0%, Figure 7b). At KW2-downstream, the
seasonal trend was a lower $F_{Ac}$ in high temperature periods
(40.3% with $\alpha_{c1}$ and 52.7% with $\alpha_{c2}$, averaged from 0.10 m
to 0.70 m) and higher $F_{Ac}$ in low temperature periods
(45.3% with $\alpha_{c1}$ and 54.4% with $\alpha_{c2}$); however, $F_{Ac}$ showed
episodic increases especially in summer 2004 when the
most reducing conditions were established (Figure 9b) [Itoh
et al., 2007]. These episodic and short-lived increases
suggest that acetate is easily exhausted, probably due to its
availability. This episodic increase in $F_{Ac}$ was supported by
various results. First, incubation experiments showed that
active consumption of added acetate induces a rapid increase
in $\delta^{13}$C-CH$_4$ [Sugimoto and Wada, 1993]; second, field
observations showed rapid decreases in acetate with
increases in temperature [Shannon and White, 1996; Avery
et al., 1999]. Third, pore water acetate concentrations
increased during transition phases between acetate consumption
by SO$_4^{2-}$ reducers and methanogens [Alperin et al.,
1992; Sugimoto and Wada, 1993], suggesting a high avail-
ability of acetate. On the other hand, $F_{Ac}$ clearly decreased at
KW2-edge and downstream in the dry summer of 2005.

[32] Figure 9a shows a crossplot of $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-
CO$_2$, with arrows indicating temporal changes in $\delta^{13}$C-CH$_4$
and $\delta^{13}$C-CO$_2$, from an incubation experiment of rice paddy
soil at different temperatures [Fey et al., 2004]. Using $^{13}$C-
labeled bicarbonate, the incubation experiment found CH$_4$
production to be solely the result of CO$_2$ reduction at high
temperature (50°C) [Fey and Conrad, 2000; Fey et al.,
2001]. Arrow 1 is provided from the results of experiments
conducted under the same conditions, suggesting that $\alpha_c =
1.073$ [Fey et al., 2004]. The results of Fey et al. [2004] also
suggest that acetate with heavy $\delta^{13}$C initially increased and
then decreased with increases in CH$_4$ concentration and
$\delta^{13}$C-CH$_4$ at temperatures of 10–37°C, hence arrow 2. This
suggests that when acetate fermentation is dominant, only
$\delta^{13}$C-CH$_4$ becomes heavier. Chan et al. [2005] also showed
larger $\alpha_c$, i.e., 1.0864–1.0885 and 1.0811–1.0892, for lake
sediment cores sampled in May and August, respectively, by
using an inhibitor of acetotrophic methanogenesis (CH$_3$F).
The $\alpha_c$ values shown by Chan et al. [2005] are much larger
than the apparent $\alpha_c$ in the present study. Figure 9b shows the
summer 2004 data labeled by sampling month. In
summer 2004, when CH$_4$ production dramatically in-
creased, both $\delta^{13}$C-CO$_2$ and $\delta^{13}$C-CH$_4$ increased simulta-
aneously in early summer, followed by a sharp increase only
in $\delta^{13}$C-CH$_4$. According to the results of Fey et al. [2004],
the simultaneous increase in $\delta^{13}$C-CO$_2$ and $\delta^{13}$C-CH$_4$
indicates that CO$_2$ reduction usually is dominant in periods
of high temperature, and the contribution from acetate
fermentation is variable. This is shown in the linear rela-
tionship between $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-CO$_2$, composed of
arrows 1 and 2 in Figure 9a. This increase in the propor-
tion of carbonate reduction is also suggested by the increase in
apparent $\alpha_c$ in this season. The outliers (high $\delta^{13}$C-CH$_4$)
from this linear relationship suggest the sporadic and short-
lived increase in acetate fermentation (shown as arrow 2 in
Figure 9a). It is unlikely that increases in $\delta^{13}$C-CH$_4$ due to
re-oxidation of CH$_4$ [Roslev and King, 1994, 1996] oc-
curred in this period under low DO conditions [Itoh et al.,
2007]. Furthermore, only two of nine values with $\delta^{13}$CH$_4$
above −55‰ are from 0.10 m (CH$_4$ oxidation may be
expected to occur because oxidation is usually observed
from the soil surface downward), while during the same
period, high CH$_4$ concentrations were measured up to that
depth. Thus, our results suggest that the unusually high
$\delta^{13}$C-CH$_4$ values observed in high temperature periods were
due to increases in acetate fermentation under high soil
temperature (Figures 8b and 8c). This implies that acetate
fermentation requires more time to be activated than does CO₂ reduction, in agreement with Vogels et al. [1988], who showed that doubling times for acetoclastic methanogens were longer than those for CO₂-reducing methanogens.

[33] In addition to the effect of increased temperature, episodic increases in F_ac were also observed in low temperature periods (e.g., KW2-downstream, 0.50 m: \(-53.3\%\), 9 March 2006), implying that there is another factor influencing F_ac, such as supply of acetate or competition for acetate with another acetate utilizer, e.g., sulfate-reducing bacteria (SRB) that are active under almost the same reducing conditions as methanogenesis. SRB may compete for acetate with acetoclastic methanogens under mildly reducing conditions (with some SO₂⁻ [e.g., Winfrey and Zeikus, 1977; Schönheit et al., 1982]). This phenomenon is usually observed in marine sediments, but can also occur in freshwater sediments; SRB can only outcompete methanogenesis when sulfate concentrations are increased to 60 \(\mu\)M [Lovley and Klug, 1983a]. In our wetland, the SO₂⁻ concentration was usually low in summer at all plots (≈0 [Itoh et al., 2007]). In particular, SO₂⁻ was lowest at all plots in the hot and wet season of 2004. This agrees with the increase in CH₄ production. Also, SO₂⁻ was higher in rainy (2003; ≈50 \(\mu\)M at 0.10 m) and dry (2005 at 0.50 m) seasons; under such conditions, the activity of SRB may be higher, thereby reducing acetate fermentation. Under highly reducing conditions, SRB activity becomes limited by a decrease in available sulfate; thus, acetate may become available for methanogenesis under highly reducing summer conditions.

[34] F_ac decreased more in the dry summer of 2005 than in 2004 at KW2-downstream (Figure 7b; summer to fall 2005), indicating that acetate fermentation may be inhibited by a decrease in acetate supply with low water transport in a low precipitation summer. In fact, the water table in the entire KW2 area was much lower in summer 2005 than in 2004 (Figure 2c).

[35] The relationship between \(\delta^{13}\)C-CH₄ and \(\delta^{13}\)C-CO₂ was unclear at KW2-center (Figure 6b), where strong reducing conditions were maintained during the year. A low level of water exchange with streams indicated by a small hydraulic head [Itoh et al., 2007] and high soil C concentration (Table 2) in this plot may affect the stable contribution of acetate fermentation.

[36] Conrad et al. [1989] and Chin and Conrad [1995] showed that the contribution of carbonate reduction decreased when temperature decreased (from 30°C to 10°C) and that CH₄ is mainly produced from acetate. Our results indicating a higher acetate contribution in periods of low temperature agree with their report. However, much lower levels of \(\delta^{13}\)C-CH₄ (and therefore, calculated F_ac) in deeper soil were observed only in the mild winter of 2004/2005 (Figure 2d). The reported range of \(\delta^{13}\)C-CH₄ from biogenic sources is \(-41\) to \(-86\%\) [Quay et al., 1988] and that from peatlands is \(-46\%\) [Martens et al., 1992] to \(-83\%\) [Lansdown et al., 1992]. The low \(\delta^{13}\)C values observed here usually fell in these ranges; however, we do not know why such low values were observed only in the winter of 2004/2005. This winter was warmer than normal, especially in December (monthly average air temperature at meteorological station was 6.3°C; average value from 1996 to 2005 was 5.1°C). In addition, such a large decrease in \(\delta^{13}\)C-CH₄ was not observed in the much colder winter of 2005/2006 (Figure 2a), suggesting that some factor other than temperature affects this phenomenon. Although statistical analysis showed that hydrological conditions such as rainfall [including the antecedent precipitation index (API)], runoff, and water table are not directly related to this phenomenon (data not shown), we assume that acetate becomes exhausted under conditions of high methanogenic activity, even in early winter 2004, because CH₄ production remained much higher from late summer to autumn 2004 than in other years (Figure 2b). This may have induced the large depletion of \(\delta^{13}\)C-CH₄ and F_ac, especially at KW2-center where water exchange is slow.

4.3. Relationship Between Changes in Contribution of Acetate Fermentation and CH₄ Flux

[37] At KW2-downstream, the patterns of \(\delta^{13}\)C-CH₄ and \(\delta^{13}\)C-CO₂ differed each summer (Figures 2d and 3d) depending on reducing conditions controlled by precipitation, runoff, water table, and thus DO (Table 1 [Itoh et al., 2007]). As described above, based on isotope signatures, the calculated contribution of acetate fermentation usually decreased in high temperature periods; however, it episodically increased under the highly reducing conditions in summer 2004 (Figures 7b and 9b). Itoh et al. [2007] reported a dramatic increase in CH₄ emission from the soil surface with increased CH₄ concentration in the surface groundwater in summer 2004. These observations suggest that CH₄ emission from the soil surface results from increased CH₄ production and accumulation in soil. The activation of acetate fermentation under highly reducing conditions probably contributed to this large CH₄ emission. A similar pattern was observed in KW2-edge in summer 2004. These results are consistent with previous observations of high acetate concentrations during early spring, followed by a large CH₄ flux, and then a sharp decrease in acetate concentration in freshwater peatlands in southern Michigan, USA [Shannon and White, 1996].

[38] Our results indicate that detecting increases in acetate fermentation using isotopic signatures can provide information on changes in CH₄ production and emission in summers with different redox conditions. Large variations in CH₄ production pathways, as described above, are probably more applicable in regions with large variations in temperature and precipitation, such as the Asian monsoon region. In such regions, the methanogenic pathway can vary widely in spatial and temporal scale with variations in redox conditions. Our observations indicate that the amount of acetate available for methanogenesis is controlled by supply, and therefore both water movement and microbial consumption are important factors.

[39] Schematics of the effects of changes in environmental conditions (e.g., precipitation, water table, and runoff) on CH₄ dynamics (formation pathways, groundwater concentrations, and emissions from soil surfaces) in summer are presented in Figure 10. Under “low temperature” and “high water table” conditions (e.g., the rainy summer of 2003 at our study site), less-reduced conditions than normal for summer were maintained by (1) increased oxygen supply from more frequent incoming water due to high runoff and precipitation and (2) low oxygen consumption due to low microbial activity. In such years, the acetate contribution to CH₄ production is stable and methanogenic activity from
acetate is low. The amount of CH$_4$ produced and retained in groundwater is also low; consequently, CH$_4$ emissions from the soil surface are small (Figure 10).

[40] In a highly reducing summer, under conditions of “high temperature” and “high water table” (e.g., 2004 at our study site), CH$_4$ production from both carbonate reduction and acetate fermentation becomes active, and the proportion of carbonate reduction is generally increased under high CO$_2$ concentrations. In addition, acetate fermentation episodically increases under high microbial activity, resulting in heavier $^{13}$C-CH$_4$. A large quantity of CH$_4$ is retained in surface soils and contributes to high CH$_4$ emissions from the soil surface.

[41] Under “high temperature” and “low water table” conditions that usually occur in low-precipitation summers (e.g., 2005 at our study site), water table depletion induces the formation of an aerobic (oxic) layer in surface soils. On the other hand, highly reducing conditions form in the bottom layer because of the decreased oxygen levels caused by the reduced water movement [Itoh et al., 2007]. The proportion of acetate fermentation decreases, probably because of low water movement under such conditions. CH$_4$ emissions from the soil surface are suppressed because of low anoxic microbial activity in the aerobic surface layer. Re-oxidation of CH$_4$ may occur in this layer. Our results suggest that changes in the CH$_4$ production pathway are influenced by changes in hydrologically controlled redox conditions and, consequentially, affect emissions from the soil surface.

5. Conclusions

[42] Our results show that changes in environmental conditions, especially hydrological conditions (water table and temperature), affect the CH$_4$ production pathways in riparian wetlands in temperate forests because hydrological conditions control the spatial and temporal variation in redox conditions. Under “high water table” and “low temperature” conditions (rainy summers with high levels of runoff), high levels of DO create more oxic conditions, and the proportion of carbonate reduction and acetate fermentation is stable and activity levels are low. In contrast, in a highly reducing summer with “high water table” and “high temperature” conditions, the proportion of carbonate reduction increases with soil temperature, with episodic increases in acetate fermentation. In such a period, the decreased SRB activity associated with limited SO$_4^{2-}$/CO$_3$ levels can also induce acetate fermentation. This induces higher CH$_4$ production and CH$_4$ accumulation in soil and, consequently, high CH$_4$ emissions from the soil surface. In drier summers with “low water table” and “high temperature” conditions, the contribution of acetate fermentation is lower probably due to oxic conditions and decreased acetate supply. Acetate fermentation also decreases sharply in winter following a highly reducing summer. These patterns indicate that the amount of acetate available for methanogenesis is controlled by supply, and both water movement and microbial consumption are important factors regulating this supply.

[43] The results of this study demonstrate the effects of hydrological changes in wetlands on changes in CH$_4$ production pathways. Our findings indicate the importance of considering hydrologic effects when assessing CH$_4$ production in wetlands where redox conditions vary on shorter temporal and smaller spatial scales. In addition, our results that acetate fermentation was highly variable compared to carbonate reduction suggest that a more detailed understanding of what processes affect acetate supply and consumption is required.

[44] Acknowledgments. We thank anonymous reviewers for their valuable comments on an earlier draft of this manuscript; N. Matsuo (Mie University) for providing data; Y. Kosugi (Kyoto University) and

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**Figure 10.** Schematics of the effects of environmental changes on CH$_4$ dynamics in summer. The thickness of an arrow indicates its degree of contribution.
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References


Conrad, R., and P. Claus (2005), Contribution of methanol to the production of methane and its δ13C-isotopic signature in anoxic rice field soil, Biochemistry, 73, 381–393.


