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Analysis of methane production pathways in a riparian wetland of a temperate forest catchment, using δ₁³C of pore water CH₄ and CO₂

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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, Nobuhito Ohte, Keisuke Koba, Atsuko Sugimoto, and Makoto Tani

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To clarify how hydrological processes affect biogenic methane (CH$_4$) production and emission from soil surfaces, we analyzed the $\delta^{13}$C of CH$_4$ and CO$_2$ and chemical constituents dissolved in groundwater at a wetland in the headwater catchment of a temperate forest in Japan. We estimated the contribution of acetate fermentation using the $\delta^{13}$C isotope mass balance of dissolved CH$_4$ and CO$_2$. CH$_4$ production pathways (e.g., acetate fermentation and carbonate reduction) changed temporally and spatially with hydrologically controlled redox conditions. The proportion of methanogenesis attributable to acetate fermentation usually decreased with temperature, suggesting that carbonate reduction dominated under conditions of high CO$_2$ concentration. In particular, the groundwater table and summer temperatures were key controlling factors in the interannual and intra-annual changes in CH$_4$ production pathways, controlling oxygen supply and consumption and, therefore, redox conditions in the soil. Under high temperature and high water table conditions during summer, the soil was strongly reduced and the proportion of carbonate reduction increased. Acetate fermentation also increased episodically, resulting in sporadic increases in $\delta^{13}$C-CH$_4$. The calculated acetate contribution obviously decreased in periods of low water table and high temperature when the soil surface was relatively oxic, implying deactivation of acetoclastic methanogenesis under oxic conditions. Thus, hydrological processes control the supply of these electron donors and acceptors and therefore play an important role in determining the relative proportions of CH$_4$-producing pathways. Our results also indicate that an increase in acetate contribution under highly reducing conditions stimulates CH$_4$ production and emission from the soil surface.


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; Brüinlich 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-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-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

Figure 1. (a) Location of KEW. (b) Topographic map of KEW and the locations of wetlands. Shading indicates riparian wetland areas. (c) Locations of the observation plots. Each number indicates an observation plot (1: KW2-edge, 2: KW2-center, 3: KW2-downstream).
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. CH$_4$ production pathways can change drastically on small temporal and spatial scales in such riparian wetlands. Thus, for a more reliable estimate of the CH$_4$ budget on a larger scale, such as an entire forest catchment, an understanding of CH$_4$ production mechanisms, including production pathways, is strongly required.

In this study, we used the $\delta^{13}$C isotope mass balance of pore water CH$_4$ and CO$_2$ [Sugimoto and Wada, 1993] to determine temporal and spatial changes in CH$_4$ 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 CH$_4$ 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 m$^2$) 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 average of values obtained from 2000 to 2005. From June to August.

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 CH$_4$ 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 CH$_4$ production in the soil and CH$_4$ 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 CH$_4$ and CO$_2$) and isotopic composition of $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-CO$_2$ 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 perennial 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 CH$_4$ and CO$_2$ 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, CH$_4$, and CO$_2$ were conducted. Detailed information on the methods and water chemical constituent results were given by Itoh et al. [2007].

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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean annual air temp. ($^\circ$C)</td>
<td>12.9</td>
<td>13.9</td>
<td>13.0</td>
<td>13.2$^b$</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>

$^a$ Data from Itoh et al. [2007].
$^b$ Average of values obtained from 2000 to 2005.
$^c$ From June to August.
Dissolved CH\textsubscript{4} and CO\textsubscript{2} concentrations were determined within 8 h of sampling by multiple equilibrations with a headspace of ultra high purity (UHP) helium [McAuliffe, 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\textsubscript{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\textsubscript{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\textsubscript{2} concentrations in water samples were also measured in situ from June 2003 to August 2005 using a portable pCO\textsubscript{2} meter (CGP-1, DKK-TOA, Japan [Ohte et al., 1995]). GC measurements of dissolved CO\textsubscript{2} concentrations were carried out from February 2005 and confirmed the data obtained by in situ dissolved pCO\textsubscript{2} measurements.

Carbon isotopic compositions of dissolved CH\textsubscript{4} and CO\textsubscript{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\textsubscript{4} or CO\textsubscript{2} concentrations, such as stream and surface water.

### 2.3. 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).

\[ \delta^{13}C \] analyses were also carried out on soil samples. The samples were dried in an oven at 40°C for 48 h prior to


3. Results

3.1. C and N Concentrations and $\delta^{13}$C of Wetland Soils

$^{[16]}$ C and N contents, the C/N ratio, and $\delta^{13}$C 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 $\delta^{13}$C values ranged between −29.8 and −28.3‰, and there was no obvious trend in $\delta^{13}$C with depth or plot (Table 2).

3.2. CH$_4$ and CO$_2$ Concentrations and Carbon Isotopic Composition

$^{[17]}$ CH$_4$ and CO$_2$ concentrations in groundwater increased with temperature at all three plots (Figures 2c, 3c, 5).

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**Table 2.** C and N Content, C/N Ratio, and $\delta^{13}$C of Litter and Soil at Each Sampling Depth

<table>
<thead>
<tr>
<th>Plot</th>
<th>Depth (m)</th>
<th>C(%)</th>
<th>N(%)</th>
<th>C/N</th>
<th>$\delta^{13}$C(‰)</th>
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</thead>
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<tr>
<td>Litter</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KW2-edge</td>
<td>0</td>
<td>46.4</td>
<td>1.05</td>
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<td></td>
<td>0.10</td>
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<td>0.226</td>
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<td></td>
<td>0.25</td>
<td>1.13</td>
<td>0.131</td>
<td>25.0</td>
<td>−28.0</td>
</tr>
<tr>
<td>KW2-center</td>
<td>0</td>
<td>10.4</td>
<td>0.547</td>
<td>20.1</td>
<td>−29.0</td>
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<tr>
<td></td>
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<td>0.617</td>
<td>18.6</td>
<td>−29.3</td>
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<td></td>
<td>0.20</td>
<td>7.25</td>
<td>0.384</td>
<td>19.2</td>
<td>−29.0</td>
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<tr>
<td></td>
<td>0.30</td>
<td>2.30</td>
<td>0.114</td>
<td>19.0</td>
<td>−28.5</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.65</td>
<td>0.0316</td>
<td>20.4</td>
<td>−27.9</td>
</tr>
<tr>
<td></td>
<td>0.70</td>
<td>1.29</td>
<td>0.0630</td>
<td>19.2</td>
<td>−28.2</td>
</tr>
<tr>
<td>KW2-downstream</td>
<td>0</td>
<td>1.73</td>
<td>0.0753</td>
<td>22.2</td>
<td>−28.6</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.237</td>
<td>0.0967</td>
<td>24.6</td>
<td>−28.5</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>5.53</td>
<td>0.229</td>
<td>24.3</td>
<td>−28.5</td>
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<tr>
<td></td>
<td>0.30</td>
<td>6.30</td>
<td>0.280</td>
<td>22.5</td>
<td>−28.5</td>
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<td>4.27</td>
<td>0.220</td>
<td>19.6</td>
<td>−28.1</td>
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<td>0.70</td>
<td>2.06</td>
<td>0.100</td>
<td>20.7</td>
<td>−28.2</td>
</tr>
</tbody>
</table>

$^{13}$C analysis. $\delta^{13}$C was determined using a mass spectrometer (Delta plus XP, Thermo Electron) coupled with an elemental analyzer (Flash EA, Thermo Electron).

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**Figure 3.** Seasonal variations in (a) soil temperature at KW2-downstream, (b) monthly precipitation measured at the nearby meteorological station, (c) groundwater CO$_2$ concentration and water table level, and (d) $\delta^{13}$C of CO$_2$ in each sampling plot. The shaded areas indicate low temperature (<10°C) periods.
and 5). The $\delta^{13}$C of pore water CH$_4$ usually increased from summer to autumn at KW2-edge and KW2-downstream. At KW2-center, variation was relatively small in summer (Figure 2d). CH$_4$ 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, CH$_4$ 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]. CO$_2$ in groundwater increased in high temperature periods and was highest in summer 2005, when the surface soil was drier than usual (Figure 3c). $\delta^{13}$C-CH$_4$ 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 $\delta^{13}$C-CO$_2$ value was $-4.1\%$ (Table 3). Rapid increases in CH$_4$ and CO$_2$ concentrations, $\delta^{13}$C-CH$_4$, and $\delta^{13}$C-CO$_2$ 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.

[16] At KW2-center, CH$_4$ 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 ($<10^\circ$C), when it decreased dramatically in other plots (Figure 2c). In this plot, mean and maximum CO$_2$ in groundwater were higher than those in the other plots. CO$_2$ was also highest at 0.30 m, where the soil C concentration was highest (Table 2 and Figure 3c). The range of $\delta^{13}$C-CH$_4$ values was much larger than in any other plot (Figure 2d and Table 3). That is, we observed lower $\delta^{13}$C-CH$_4$ 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. $\delta^{13}$C-CH$_4$ 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 $\delta^{13}$C-CO$_2$ was seen in the high temperature period, but this trend was unclear in the other two plots. Comparisons of the vertical distributions of CH$_4$, CO$_2$, and $\delta^{13}$C-CO$_2$ 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 CH$_4$ concentration was observed below 0.20 m. Seasonal (low in late winter and high in late summer) and yearly variations in CH$_4$ were clearer than in the other two plots. Maximum groundwater CH$_4$ and CO$_2$ 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 $\delta^{13}$C-CH$_4$ (Figure 2d) and $\delta^{13}$C-CO$_2$ (Figure 3d) were also much lower in 2003, especially at 0.10 m. For example, maximum $\delta^{13}$C-CO$_2$ was much lower in 2003 (~10.9%o at 0.50 m, 18 September 2003) than in 2004 (~4.0%o at 0.30 m, 21 August 2004) and 2005 (~3.6%o at 0.50 m, 15 September 2005). $\delta^{13}$C-CO$_2$ in groundwater showed clear seasonal changes, ranging from ~27 to ~4%, and increased dramatically in high temperature periods (Figure 3d). $\delta^{13}$C-CH$_4$ showed a similar seasonal change, though not as obvious as $\delta^{13}$C-CO$_2$ (Figure 2d). In this plot, no obvious vertical difference in $\delta^{13}$C-CH$_4$ was observed (Figure 2d). $\delta^{13}$C-CH$_4$ 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 CH$_4$ and CO$_2$ Dynamics

[21] The measured soil $\delta^{13}$C at KW2 ranged from $-27.9$ to $-29.3\%$ (Table 2). The measured $\delta^{13}$C 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). CO$_2$ is produced as a by-product of respiration under both oxic and anoxic conditions in soils. CO$_2$ produced from the metabolism of lactate is
depleted in $^{13}$C by as much as 26% [Smejkal et al., 1971]. Also, CO$_2$ respired 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$_2$ respired 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 by-product 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 $-5\%$ [Charman et al., 1994] or $+9\%$ [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$_4$ = 48.4% on 28 June 2004 at 0.70 m; 50.8% on 10 September 2004 at 0.10 m; −49.5% on 8 November 2004 at 0.20 m; and −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$_4$ = 51.6% at 0.10 m on 7 October 2004 and 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. 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$^a$

<table>
<thead>
<tr>
<th>Plot</th>
<th>Depth (m)</th>
<th>$n$</th>
<th>$F$</th>
<th>$p$</th>
<th>$r^2$</th>
<th>$n$</th>
<th>$F$</th>
<th>$p$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KW2-edge</td>
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<td>32</td>
<td>21.7</td>
<td>&lt;0.001</td>
<td>0.41</td>
<td>33</td>
<td>22.7</td>
<td>&lt;0.001</td>
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<td></td>
<td>0.25</td>
<td>42</td>
<td>5.67</td>
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<td>0.12</td>
<td>41</td>
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<td>18</td>
<td>46.1</td>
<td>&lt;0.001</td>
<td>0.73</td>
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<td>0.137</td>
<td>0.09</td>
<td>26</td>
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<td>0.495</td>
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<td>24</td>
<td>2.06</td>
<td>0.164</td>
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<td>KW2-center</td>
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<td>40</td>
<td>191</td>
<td>&lt;0.001</td>
<td>0.83</td>
</tr>
</tbody>
</table>

$^a$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$_2$.

[25] 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 Kug, 1983b; Conrad and Claus, 2005]. The fractional Ac contribution is expressed by $F_{\text{Ac}}$ as:

$$F_{\text{Ac}} = \frac{\text{CH}_4 \text{ from acetate}}{\text{CH}_4 \text{ from CO}_2 + \text{CH}_4 \text{ from acetate}}$$  \hspace{1cm} (2)

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

$$\delta^{13}\text{CH}_4(\text{Ac})F_{\text{Ac}} + \delta^{13}\text{CH}_4(\text{CO}_2)(1 - F_{\text{Ac}}) = \delta^{13}\text{CH}_4$$ \hspace{1cm} (3)

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).

[27] 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 $\delta^{13}\text{C}$ of 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:

$$\ln \alpha_c = (23.0/T) - 0.022$$ \hspace{1cm} (4)

[28] 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 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$_4$ $(Ac) = -35\%$ 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 $\alpha_{c2}$ [ln $\alpha_{c2} = (23/T) - 0.0015$], 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 $-35\%$ [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

![Figure 8. Relationships between soil temperature and $F_{Ac}$ at (a) KW2-edge, (b) KW2-center, and (c) KW2-downstream.](image)

### 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>$\alpha_{c2}$</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>0.30</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>Center</td>
<td>0.10</td>
<td>$\alpha_{c1}$</td>
<td>47.5 (35.8 – 58.4)</td>
<td>54.2 (41.8 – 65.9)</td>
<td>64.6 (51.3 – 77.1)</td>
</tr>
<tr>
<td></td>
<td>0.20</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>0.30</td>
<td>$\alpha_{c1}$</td>
<td>30.9 (6.8 – 66.9)</td>
<td>36.6 (8.1 – 81.3)</td>
<td>46.2 (10.3 – 107)</td>
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<tr>
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<td>$\alpha_{c1}$</td>
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<td>31.3 (24.7 – 45.4)</td>
<td>39.8 (32.3 – 59.1)</td>
</tr>
<tr>
<td></td>
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<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>0.10</td>
<td>$\alpha_{c2}$</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>
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<td>$\alpha_{c2}$</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>
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<td>49.4 (32.9 – 56.9)</td>
<td>59.0 (39.3 – 67.2)</td>
</tr>
<tr>
<td>Downstream</td>
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<td>45.3 (6.2 – 57.6)</td>
<td>51.4 (7.1 – 65.3)</td>
<td>60.5 (8.4 – 76.9)</td>
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<td></td>
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<td>$\alpha_{c1}$</td>
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<td>38.0 (18.6 – 53.8)</td>
<td>48.6 (25.9 – 72.9)</td>
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<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>
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<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>
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<tr>
<td></td>
<td>0.70</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>
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<td>53.2 (41.5 – 66.1)</td>
<td>60.9 (42.4 – 76.4)</td>
</tr>
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<td>53.8 (39.1 – 70.1)</td>
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<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>
</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 $\alpha_{c1}$ 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 $\alpha_{c1}$, 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-C$_4$ [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 availability 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-C$_4$ and $\delta^{13}$C-CO$_2$, with arrows indicating temporal changes in $\delta^{13}$C-C$_4$ and $\delta^{13}$C-CO$_2$, from an incubation experiment of rice paddy soil at different temperatures [Fey et al., 2004]. Arrow 1 is from the results of experiments conducted under the condition that CO$_2$ reduction solely contributed to methanogenesis. Arrow 2 is provided under the condition that acetate fermentation was the dominant form of methanogenesis. Figure 9b shows the data at KW2-downstream in summer 2004 labeled by sampling month.

**Figure 9.** Crossplot of $\delta^{13}$C data from CH$_4$ and CO$_2$ in groundwater at KW2-downstream. The dashed lines give the apparent fractionation between CO$_2$ and CH$_4$. Arrows indicate temporal change in $\delta^{13}$C-C$_4$ and $\delta^{13}$C-CO$_2$ during an incubation experiment using rice paddy soil at different temperatures [Fey et al., 2004]. Arrow 1 is from the results of experiments conducted under the condition that CO$_2$ reduction solely contributed to methanogenesis. Arrow 2 is provided under the condition that acetate fermentation was the dominant form of methanogenesis. Figure 9b shows the data at KW2-downstream in summer 2004 labeled by sampling month.
fermentation requires more time to be activated than does \( \text{CO}_2 \) reduction, in agreement with Vogels et al. [1988], who showed that doubling times for acetoclastic methanogens were longer than those for \( \text{CO}_2 \)-reducing methanogens.

In addition to the effect of increased temperature, episodic increases in \( F_{\text{Ac}} \) were also observed in low temperature periods (e.g., KW2-downstream, 0.50 m: \(-53.3^\circ\)C, 9 March 2006), implying that there is another factor influencing \( F_{\text{Ac}} \), such as supply of acetate or competition for acetate with another acetate utilizing, 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 \( \text{SO}_4^- \) [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 \( \mu\text{M} \) [Lovley and Klug, 1983a]. In our wetland, the \( \text{SO}_4^- \) concentration was usually low in summer at all plots (\( \approx 0 \) [Itoh et al., 2007]). In particular, \( \text{SO}_4^- \) was lowest at all plots in the hot and wet season of 2004. This agrees with the increase in \( \text{CH}_4 \) production. Also, \( \text{SO}_4^- \) was higher in rainy (2003; \( \approx 50 \mu\text{M} \) 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.

\( F_{\text{Ac}} \) 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).

The relationship between \( ^{13}\text{C-CH}_4 \) and \( ^{13}\text{C-CO}_2 \) 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.

Conrad et al. [1989] and Chin and Conrad [1995] showed that the contribution of carbonate reduction decreased when temperature decreased (from 30\(^\circ\)C to 10\(^\circ\)C) and that \( \text{CH}_4 \) 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 \( ^{13}\text{C-CH}_4 \) and (therefore, calculated \( F_{\text{Ac}} \)) in deeper soil were observed only in the mild winter of 2004/2005 (Figure 2d). The reported range of \( ^{13}\text{C-CH}_4 \) from biogenic sources is \(-11\) to \(-86\%\) [Quay et al., 1988] and that from peatlands is \(-46\%\) [Martens et al., 1992] to \(-83\%\) [Lansdowen et al., 1992]. The low \( ^{13}\text{C} \) 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\(^\circ\)C; average value from 1996 to 2005 was 5.1\(^\circ\)C). In addition, such a large decrease in \( ^{13}\text{C-CH}_4 \) 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 \( \text{CH}_4 \) production remained much higher from late summer to autumn 2004 than in other years (Figure 2b). This may have induced the large depletion of \( ^{13}\text{C-CH}_4 \) and \( F_{\text{Ac}} \), especially at KW2-center where water exchange is slow.

### 4.3. Relationship Between Changes in Contribution of Acetate Fermentation and \( \text{CH}_4 \) Flux

At KW2-downstream, the patterns of \( ^{13}\text{C-CH}_4 \) and \( ^{13}\text{C-CO}_2 \) 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 \( \text{CH}_4 \) emission from the soil surface with increased \( \text{CH}_4 \) concentration in the surface groundwater in summer 2004. These observations suggest that \( \text{CH}_4 \) emission from the soil surface results from increased \( \text{CH}_4 \) production and accumulation in soil. The activation of acetate fermentation under highly reducing conditions probably contributed to this large \( \text{CH}_4 \) 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 \( \text{CH}_4 \) flux, and then a sharp decrease in acetate concentration in freshwater peatlands in southern Michigan, USA [Shannon and White, 1996].

Our results indicate that detecting increases in acetate fermentation using isotopic signatures can provide information on changes in \( \text{CH}_4 \) production and emission in summers with different redox conditions. Large variations in \( \text{CH}_4 \) 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.

Schematics of the effects of changes in environmental conditions (e.g., precipitation, water table, and runoff) on \( \text{CH}_4 \) 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 \( \text{CH}_4 \) 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$_4^{2-}$/C$_0^{2-}$ 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.

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