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Differences in hydrophyte life forms induce spatial heterogeneity of CH₄ production and its carbon isotopic signature in a temperate bog peatland

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Abstract
To clarify the effect of differences in hydrophyte life forms on methane (CH₄) production and its carbon stable isotopic signature (δ¹³C-CH₄), we analyzed CH₄ and carbon dioxide (CO₂) concentrations, their stable carbon isotope values, and chemical constituents dissolved in pore water in a small floating peat bog in Japan. Because eutrophication has modified the surrounding water quality, the bog vegetation on the mat has been, in part, replaced by fen-type vegetation. We hypothesized that differences in hydrophyte habitats affect redox conditions, including dissolved oxygen (DO) in water and therefore the amounts and carbon isotopic values of CH₄ and CO₂ dissolved in pore water. Between the habitats of two Sphagnum species, DO was considerably higher, and CH₄ concentrations were significantly lower in Sphagnum cuspidatum Ehrh. habitats in hollow (DO: 0.62 ± 0.20 mg/L (standard error (SE)) and CH₄: 0.18 ± 0.02 mmol/L) than in Sphagnum palustre L. habitats in hummock (DO: 0.29 ± 0.08 and CH₄: 0.82 ± 0.06) in pore water (10 cm depth). Both DO and CH₄ concentrations in three vascular plant habitats (Rynchospora fauriei Franch., Phragmites australis [reed], and Menyanthes trifoliata L.) in pore water (10 cm depth) were intermediate relative to the two Sphagnum species. However, CH₄ flux in M. trifoliata site was significantly higher than that at both Sphagnum sites, suggesting that the type of gas transport (diffusive or convective via root and stem) affected the depth profile of CH₄ concentrations and its flux. δ¹³C-CH₄ values in pore water also varied among the vegetation types, even within Sphagnum species (e.g., at 10 cm depth, δ¹³C-CH₄: R. fauriei, −55.3 ± 1.8‰ (SE); P. australis, −57.5 ± 1.6‰; M. trifoliata, −56.7 ± 1.5‰ S. cuspidatum, −71.2 ± 1.4‰ and S. palustre, −60.4 ± 0.6‰). Our results suggest that significant differences arise in CH₄ concentration and δ¹³C-CH₄ values among the hydrophyte habitats even within a small peat bog and that change in vegetation relative to trophic conditions can affect CH₄ emissions and associated δ¹³C-CH₄ values.

1. Introduction
Methane (CH₄) is a key greenhouse gas (GHG) that has an infrared radiative heating effect 28 to 34 times greater than that of carbon dioxide (CO₂) on a mass basis over a 100 year time horizon [Intergovernmental Panel on Climate Change (IPCC), 2013]. Based on the inversion of atmospheric measurements of CH₄ from surface stations, global CH₄ emissions for the 2000s are 553 Tg CH₄ yr⁻¹, with a range of 526–569 Tg CH₄ yr⁻¹ [IPCC 2013]. The contribution from natural CH₄ sources (i.e., wetlands, oceans, geologic seepage, termite, and vegetation) is estimated at 215 Tg CH₄ [Schlesinger and Bernhardt, 2013]. In anoxic environments such as wetland soils, CH₄ is produced by methanogenic archaea that are active only under anoxic and strongly reducing conditions [Takai, 1970; Schütz et al., 1989]. In contrast, CH₄ in oxic soils is usually oxidized by methanotrophic bacteria. Improved estimates of the strengths of the various CH₄ sources and sinks are of high importance, as although most sources and sinks of CH₄ have been identified, their relative contributions to atmospheric CH₄ levels are still uncertain [Kirschke et al., 2013].

Among the major natural sources, the single most dominant CH₄ source of the global flux and interannual variability is CH₄ emissions from wetlands (177 to 284 Tg CH₄ yr⁻¹) [IPCC, 2013], and many studies have revealed that various environmental parameters affect CH₄ emissions from natural wetlands, including peatlands, such as soil characteristics, water table depth, and soil temperature (reviewed in Topp and Pattey [1997]). However, estimates of global CH₄ emissions from wetlands are not well constrained. The lack of information on the effects of vegetation types on wetlands is likely...
mosses were shown to be able to oxidize CH$_4$ through symbioses with partially endophytic
in hollows (including
than CH$_4$ from other sources [Whiting and Chanton, 1993], several studies were conducted to understand the effect of trophic status on CH$_4$ dynamics.
Many of them revealed that the CH$_4$ flux was higher in minerotrophic peatlands than in ombrogenous
bogs [Kelley et al., 1992; Chanton et al., 1995; and Bellisario et al., 1999; Hornibrook and Bowes, 2007].
Some studies have focused on vegetation types and cover [King et al., 1998; Van der Nat and Middelburg, 2000; Joabsson and Christensen, 2001; Ström et al., 2005], addressing the effects of vascular
plants on CH$_4$ emission. However, studies examining various types of hydrophyte cover in wetlands
including Sphagnum species are not enough [e.g., Sugimoto and Fujita, 1997, 2006]. Considering that
nutrient condition affects the vegetation cover (e.g., a greater abundance of Sphagnum spp. in
nutrient-poor acidic ombrogenous bogs) and eutrophication can cause changes in vegetation type
[Haraguchi and Matsui, 1990; Shimamura et al., 2006], more detailed information is required regarding
CH$_4$ dynamics in typical vegetation covers.

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 stable carbon isotopic compositions of
CH$_4$ ($\delta^{13}$C-CH$_4$) in background tropospheric air and the major sources of CH$_4$ have further constrained
the individual CH$_4$ source strengths through the isotope mass balance method, using the $\delta^{13}$C value of each source [Bräunlich et al., 2001; Fletcher et al., 2004; reviewed in Dlugokencky et al., 2011].
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, consumption, and transport via plants in
these ecosystems. For example, methanogenesis from carbonate results in a larger fractionation against
$^{13}$C and, thus, more negative $\delta^{13}$C-CH$_4$ values than methanogenesis from acetate [Games et al., 1978; Krzycki et al., 1987; Gelwicks et al., 1994]. Previous studies have shown that the fractionation factors vary depending on location and conditions (e.g., reviewed by Conrad [2005]).
Several studies have reported that acetoclastic methanogenesis is suppressed in some peat ecosystems
and that CH$_4$ from carbonate reduction (H$_2$/CO$_2$) is the dominant pathway [Lansdown et al., 1992; Horn
et al., 2003; Metje and Frenzel, 2005; Prater et al., 2007]. Galand et al. [2005] reported that the highest
portion of CH$_4$ from H$_2$/CO$_2$ was observed from oligotrophic fen followed by the ombrotrophic bog, and
the lowest was observed from the mesotrophic fen based on community studies of methanogen. The
same pattern was supported by pore water $\delta^{13}$C-CH$_4$ values, which were higher in minerotrophic
peatland than in ombrogenous peatland. [Hornibrook and Bowes, 2007]. However, to study the shift in
$\delta^{13}$C-CH$_4$ values with change in trophic status, data from various vegetation types on a single
peatland can add useful information.

Especially, information on the effects of different Sphagnum habitats is totally lacking, although their
life forms and decomposition patterns are species specific. For example, the rate of decomposition of
Sphagnum in hollows (including Sphagnum cuspidatum) was higher than those of Sphagnum in
hummocks [Hogg, 1993], and Johnson and Damman [1991] suggested that the decomposition rate
was faster in S. cuspidatum in hollows than in S. fuscum in hummocks. In addition, Sphagnum
mosses were shown to be able to oxidize CH$_4$ through symbioses with partially endophytic
methanotrophic bacteria [Raghoebarsing et al., 2005]. For this CH$_4$ oxidation, Kip et al. [2010] found
that all mosses collected from pools, lawns, and hummocks were capable of oxidizing CH$_4$ and that
the rate of CH$_4$ oxidation was most pronounced in submerged mosses (pools in hollows). These
results suggest that differences in the dominant Sphagnum species are indicative of the various CH$_4$
dynamics in peat mats.

Therefore, we postulate that vegetative heterogeneity in peatlands assures heterogeneity in CH$_4$ dynamics. If
differences occur in CH$_4$ production pathways, oxidation, and transport processes among the habitats, the
carbon isotope signatures of the CH$_4$ produced should differ. This must be considered to obtain a better
understanding of CH$_4$ dynamics. In this study, we used the $\delta^{13}$C signatures of pore water CH$_4$ and CO$_2$ in a
temperate peatland to determine differences in CH$_4$ dynamics at the sample sites to understand the
processes that control both CH$_4$ concentrations and carbon isotopic values.
2. Materials and Methods

2.1. Site Description and Overview of the Preceding Biogeochemical Observations at the Site

Mizorogaike Pond (35°03′N, 135°50′E; 75 m asl) is a natural pond (0.08 km² in area and 1 km in circumference) located in the northern area of Kyoto City, Japan (Figures 1a and 2). This system contains a floating mat, or bog, in the center of the pond consisting of about 0.05 km² of peat (floating area, 0.03 km²) on which mire vegetation occurs, including some relic species from the last ice age [Haraguchi and Matsui, 1990; Haraguchi, 1991]. The mat edges are connected with the open water. Miki [1929] noted that this floating

![Figure 1](a) Locations of Kyoto City and Mizorogaike Pond. (b) Study sites on the floating mat in Mizorogaike Pond. Circles show the sampling points and habitat types.
The floating mat was about 1.2–1.8 m thick and contained two species of *Sphagnum*: *S. cuspidatum* Ehrh., which inhabited hollows, and *Sphagnum palustre* L., which occurred on hummocks. *S. cuspidatum* and other emergent plants were patchily distributed throughout the hollow sections of the mat [Investigation Group for Mizorogaike Pond in the Research Institute of Plant Biology in Kyoto University, 1981].

Although the bog is floating by gases such as CO₂ and CH₄ derived from the decomposition of organic matter and it shows floating (summer)-sinking (winter) movement compared with the open-water surface level, the amplitude of water-level fluctuation in the location of each hydrophyte community is within 0.1 m throughout the year in most parts of the hollow [Haraguchi, 1991].

Bare hollows did not contain *S. cuspidatum* and were dominated by *Menyanthes trifoliata* L., which was the most dominant species in the mat, except in the hummocks. The *Sphagnum*-rich hollows, which contained *S. cuspidatum*, were distributed along the north side of the floating mat. Some communities of *Rhynchospora fauriei* Franch. also occur in hollows. Habitats of *P. australis* were seen at mat edges that bordered the open water and were slightly flooded. We categorized the typical habitats of the floating mat into five types, described in the next section (Figures 1b and 3).

Haraguchi [1991] investigated the relationship between vegetation and seasonal water-level changes in Mizorogaike Pond, except on the hummocks and suggested that *S. cuspidatum* favors stable water-level conditions that fluctuate near the level at which the community can be submerged. In contrast, the hummocks consist of *S. palustre* and some shrub species that were scattered throughout the mat [Shimizu, 1986]. Haraguchi and Matsui [1990] investigated the chemical properties of water in the hollows and reported that water around *S. cuspidatum* communities had lower pH and electrical conductivity (EC) values than that around *M. trifoliata* communities.

![Figure 2. Seasonal variations in air temperature and precipitation in Kyoto City.](image)

![Figure 3. Schematics of cross-sectional diagram of habitat types on the floating mat in Mizorogaike Pond (summer period).](image)
Sugimoto and Fujita [1997] observed CH₄ flux and δ¹³C of dissolved and bubble CH₄ below the ground surface in Mizorogaike Pond. Their observation were made at three sites with the typical vegetation and characteristic seasonal water-level cycles of the floating mat: (1) a reed site that consisted of *P. australis*; (2) a marsh trefoil site that consisted of *M. trifoliata*; and (3) a *Sphagnum* site that consisted of both *S. cuspidatum* and *S. palustre*. They reported that the averaged CH₄ fluxes from April to October (spring to fall) were highest at the marsh trefoil site (600 mg CH₄ m⁻² d⁻¹) followed by the reed site (387 mg CH₄ m⁻² d⁻¹), and the smallest flux was at the *Sphagnum* site (93 mg CH₄ m⁻² d⁻¹), reflecting the differences in the decomposability of organic matter. δ¹³C-CH₄ values obtained from pore water at 10 cm depth in their study were −64.9 to −52.8‰ at the reed site, −65.0 to −52.5‰ at the marsh trefoil site, and −61.5 to −54.0‰ at the *Sphagnum* site. Sugimoto and Fujita [2006] also reported that the concentration of hydrogen (H₂) in bubble gas in the floating mat that was available for CH₄ production through carbonate reduction increased in summer. These reports provide valuable information on CH₄ dynamics in this peatland; however, the two *Sphagnum* species (*S. cuspidatum* and *S. palustre*) were treated as one community with similar characteristics. As we mentioned previously, a more precise understanding of CH₄ dynamics can be obtained by treating the two *Sphagnum* species separately. Considering their life forms and the rate of decomposition of *Sphagnum*, because it was expected to be higher in hollows (including *S. cuspidatum*) than in hummocks [Hogg, 1993], Johnson and Damman [1991] suggested that decomposition rate was faster in *S. cuspidatum* in hollows than in *S. fuscum* on hummocks.

### 2.2. Distribution of Plant Species and Sampling Points

Figure 3 shows the schematics of the cross-sectional diagram of habitat types on the floating mat in Mizorogaike Pond in summer. We collected pore water samples from five major hydrophyte habitats on the floating mat.

1. *S. cuspidatum*. *S. cuspidatum* is able to grow at the transition between lawn and hummock and can be found growing in the open water, completely submerged. Haraguchi [1991] reported that the greatest number of capitula of *S. cuspidatum* was observed in areas where the water level fluctuations were small and flooding occurred for only a short period. We designated two sampling points as *S. cuspidatum*-dominated sites. One site was located at Point 1 in the northern part of the floating mat and the second was located at Point 2 in the southern part (Figure 1b).

2. *R. fauriei* (vascular) *R. fauriei* grows in bare hollows at the center of the floating mat in Mizorogaike Pond. The distribution of this species is limited to habitats that experience flooding in winter [Haraguchi 1991]. We observed this species at one sampling point (Point 3) in the northern part of the floating mat (Figure 1b).

3. *P. australis* (reed and vascular) *P. australis* is dominant at the margins of the mat. Shoots of *P. australis* open twice per year (June and September). These reed sites are submerged throughout the year, because the roots of the reeds reach the bottom of the pond and anchor the floating mat. We selected two sampling points at the margins of the mat. One was located at Point 4 on the southern edge of the mat and the other was at Point 5 on the western edge (Figure 1b).

4. *M. trifoliata* (vascular) *M. trifoliata* is a clonal plant with a horizontal creeping sympodial rhizome with adventitious roots and leaves at the apex. Live rhizomes of *M. trifoliata* cover almost the entire mat at a depth of approximately 5 cm [Haraguchi 1996]. It shoots twice per year in Mizorogaike Pond, the southern limit of its distribution. The first shoot emerges in mid-April and dies in July, and the second shoot grows from September to October. This species is tolerant of flooding [Coult and Vallance, 1958]. We chose one sampling point (Point 6) at the center of the mat (Figure 1b). Based on the results of Haraguchi [1991], on the Mizorogaike Pond floating mat, *M. trifoliata* is the main vegetation with larger water-level fluctuation (approximately 6 to 10 cm), and it prefers higher water-table conditions (0 to 12 cm) than does *S. cuspidatum*. Sugimoto and Fujita [1997] reported that fluctuation of water levels above the peat surface was less than 10 cm at their *M. trifoliata* site, which is also at the center of the mat and in a similar condition to our sampling point.

5. *S. palustre*. *S. palustre* grows on small hummocks where the water table is below the surface of the mat throughout the year. We set the two sampling points on the hummocks where *S. palustre* plant tissue accumulated to more than 10 cm, and the water-table level was kept below the *Sphagnum* surface in these sites (Figure 3). The depth of the water level never dropped lower than 10 cm from the *Sphagnum* surface during our sampling period. This is probably because of capillary flow of water from the water table to the *Sphagnum* surface [Price, 1996] in addition to floating-sinking movement of the mat. One was located at Point 7 in northern part of the mat and the other was at Point 8 in the southern part (Figure 1b).
For the three species for which samples were collected at two sampling points, *S. cuspidatum*, *P. australis*, and *S. palustre*, we averaged the values of the two samples from each sampling day for analysis.

### 2.3. Water Sample Collection and Analyses

Pore water samples were collected vertically to measure dissolved gases and water chemistry. The pore water samplers were placed at depths of 10 and 40 cm from the soil or *Sphagnum* surface to compare to the results of the same depth sampling by Sugimoto and Fujita [1997]. We used a double-walled sampler screened with numerous vertically aligned 2 mm holes constructed by placing a 100 mL polypropylene bottle inside a wide-mouth, 200 mL polystyrene bottle [Itoh et al., 2007], which collected pore water without degassing or high decomposition and could exclude soil and detritus. Pore water samples were injected into 20 or 30 mL preevacuated vials for the measurement of dissolved CH$_4$ and CO$_2$ concentrations and 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 (−4°C) in the field. In situ measurements, including pH and EC (from August 2005 to August 2006) and laboratory measurements of dissolved components including dissolved O$_2$ (DO), CH$_4$, and CO$_2$ were conducted. For measurements of dissolved oxygen (DO), water samples were collected in 100 mL biological oxygen demand (BOD) bottles and then fixed immediately after sampling. DO was determined following the Winkler method. Measurements of DO were conducted from August 2005 to August 2006.

Samples for CH$_4$ and CO$_2$ concentrations and their carbon isotopic compositions (δ$^{13}$C-CH$_4$ and δ$^{13}$C-CO$_2$) were collected monthly at most sampling points and depths from August 2005 to August 2006. For Point 2, observations were performed from December 2005 to August 2006. Water samples for Points 1, 2, and 4 (for *S. cuspidatum*, *M. trifoliata*, and *S. palustre*, respectively, 10 cm depth) were also taken from October 2006 to December 2007 to compare the dissolved gas components with surface CH$_4$ fluxes. CH$_4$ concentrations were determined using a gas chromatograph (GC; GC-14BPF, Shimadzu, Japan) equipped with a flame ionization detector (FID) and a Porapack Q column (3 mm i.d. (inner diameter) × 2 m, Shinwa Chemical Industry, Japan) using N$_2$ (flow rate, 50 mL min$^{-1}$) as the carrier gas. For dissolved 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 and a Shincarbon T column (2 mm i.d. × 6 m, Shinwa Chemical Industry, Japan) using He (flow rate, 50 mL min$^{-1}$) as the carrier gas [Itoh et al., 2007]. Note that pore water samples were not acidified prior to analysis of dissolved CO$_2$ concentration and δ$^{13}$C-CO$_2$.

Carbon isotopic compositions of dissolved CH$_4$ and CO$_2$ were analyzed using a gas chromatograph/combustion/isotope ratio mass spectrometer (Thermo Finnigan MAT252: Thermo Fisher Scientific, Waltham, MA, USA) equipped with an HP G1530A (Agilent, Santa Clara, CA, USA) GC system [Sugimoto, 1996] at the Center for Ecological Research at Kyoto University. CH$_4$ was separated on a PoraPLOT Q capillary column (0.32 mm i.d. × 25 m) and combusted to CO$_2$ at 940°C in a ceramic reactor containing CuO and Pt wires. The stable isotope ratios are expressed in the standard delta (δ) notation in units of per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB). The analytical precision was better than ±0.2‰ when 44 nmol of CO$_2$ or CH$_4$ was injected.

The water samples collected for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analysis were filtered through 0.45 mm polytetrafluoroethylene membrane filters and stored in glass vials at 4°C. DOC and TDN concentrations were measured using a total organic carbon analyzer with chemiluminescent detection of total nitrogen (TOC-V, TNM-1, Shimadzu, Japan). Samples for measurements of NH$_4^{+}$, NO$_3^{-}$, and SO$_4^{2-}$ concentrations were filtered through 0.20 μm cellulose acetate membrane filters and analyzed by ion chromatography (ICS-90, Dionex, Japan). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and inorganic N (NH$_4^{+}$ + NO$_3^{-}$).

We also collected rainwater from August 2005 to December 2006 using a rainfall collector installed in the center area of the floating mat that consisted of a 5.0 L polypropylene bottle, a polypropylene funnel (180 mm diameter), and a 1.0 mm mesh screen.

### 2.4. Methane Flux Measurements

We measured CH$_4$ flux at Points 1, 6, and 7 (S. cuspidatum, M. trifoliata, and S. palustre, respectively; Figure 1) from October 2006 to December 2007. Three polyvinyl chloride (PVC) static chambers (diameter: 0.26 m;
Plant samples or NH$_4^+$ because lower DOC/DON ratios are indicative of more labile organic matter [Dobbie and Smith 1996]. In our observations, all analyses of individual time series revealed that slope values for CH$_4$ versus time were linear. At each sampling point, the flux was measured only once daily, and the measurements for each observation were performed at approximately the same time during the daytime.

2.5. Plant Tissue Sampling and Analysis

Plant samples were collected for S. cuspidatum, M. trifoliata, S. palustre, R. fauriei, and P. australis in December 2006. Plant samples or S. cuspidatum, M. trifoliata, and S. palustre were collected in quintuplicate. δ$^{13}$C analyses were performed on aboveground sections of the plant tissues. The samples were dried in an oven at 40°C for 48 h prior to δ$^{13}$C analysis. δ$^{13}$C was determined using a mass spectrometer (Delta plus XP, Thermo Electron) coupled with an elemental analyzer (Flash EA, Thermo Electron). The standards were VPDB for δ$^{13}$C. We used DL-alanine as a working standard. The analytical precision was better than ±0.2‰ for δ$^{13}$C. When differences in means were determined to be statistically significant, Tukey’s honestly significant difference test was used.

3. Results

3.1. Environmental Conditions

Daily mean air temperatures and precipitation observed at Kyoto Weather Observatory (5 km southeast of Mizorogaike) are shown in Figure 2. Annual precipitation measurements for 2005, 2006, and 2007 were 954.5, 1582.5, and 1212.5 mm, respectively. Compared to the 10 year average (1373.1 mm for 2000 to 2009), 2006 was the rainiest year and 2005 was the driest during this 10 year period. It is noteworthy that the winters of 2005/2006 and 2006/2007 were the coldest (mean temperature from December to February: 4.5°C) and warmest (6.9°C), respectively, from 2001 to 2010 (10 year average mean temperature from December to February: 5.8°C).

Both pH and EC were lower at the Sphagnum sites (Table 1). EC was low in rainfall. The highest pH in pore water was 5.06 at the 10 cm depth at the edges of the mat (P. australis sites), suggesting that most dissolved carbonate species at all sampling sites existed as CO$_2$ under low pH conditions. NO$_3^-$ concentrations at all sampling sites were almost zero and no significant differences were identified in NO$_3^-$ (10 cm). Significantly higher NH$_4^+$ concentrations were observed at the S. palustre sites for both sampling depths compared to the other habitat types ($t > 3.9$, $p < 0.003$ for 10 cm depth; $t > 5.7$, $p < 0.0001$ for 40 cm depth) with the exception of the M. trifoliata site at the 10 cm depth ($t = 1.8$, $p = 0.40$). NH$_4^+$ concentrations at 10 cm at the M. trifoliata site were significantly higher than at the S. cuspidatum and P. australis sites ($t > 2.9$, $p < 0.047$). SO$_4^{2-}$ was significantly higher at the S. cuspidatum sites at 10 cm than at the S. palustre and R. fauriei sites ($t > 3.2$, $p < 0.022$). No significant differences were found in SO$_4^{2-}$ concentrations (40 cm) among all sampling sites. The DOC concentration was highest at the M. trifoliata site; however, no significant differences was found in DOC concentrations among the sites. The DON concentration was significantly lower at the S. palustre site at 10 cm depth than at the M. trifoliata and P. australis sites ($t > 3.1$, $p < 0.03$). At 40 cm depth, DON concentration was significantly higher at the P. australis site than at all the other sites ($t > 3.4$, $p < 0.01$).

In this study, we used the molar ratio of dissolved C to N (DOC/DON) in pore water to assess the degree of labile C because lower DOC/DON ratios are indicative of more labile organic matter [Melillo et al., 1982; Finzi et al., 1996; Corbett et al., 2013]. At the S. palustre site, the DOC/DON ratio was among the highest at both 10 cm depth ($t > 3.1$, $p < 0.022$) and 40 cm depth ($t > 3.4$, $p < 0.011$; Table 1). The δ$^{13}$C values for plants were
Table 1. Mean (± SE) Values for pH, EC, NH₄⁺, NO₃⁻, SO₄²⁻, DOC, DON Concentrations, and DOC/DON Ratio at Each Sampling Site and Depth

<table>
<thead>
<tr>
<th>Plot</th>
<th>Mean (± SE)</th>
<th>N</th>
<th>bDOC/DON</th>
<th>bDON (mmol/L)</th>
<th>bDOC (mmol/L)</th>
<th>bNO₃⁻ (mg-N/L)</th>
<th>bNH₄⁺ (mg-N/L)</th>
<th>bSO₄²⁻ (mg-S/L)</th>
<th>bEC (μS/cm)</th>
<th>pH</th>
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<td>0.00 ± 0.00</td>
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</tbody>
</table>

DO was lower during higher-temperature periods at the Sphagnum sites. Figure 5 shows the differences in mean (± SE) values of DO (Figures 4a and 4f). DO values at the S. cuspidatum sites were significantly higher than those at the R. fauriei (t = 3.9, p < 0.002), P. australis (t = 3.3, p < 0.014), and M. trifoliata (t = 4.0, p < 0.002) sites at the 10 cm depth (Figure 5a). A significant difference was observed at 40 cm between the S. cuspidatum site and the M. trifoliata site (t > 3.0, p < 0.03, Figure 5a). At the R. fauriei and M. trifoliata sites, which were situated at the center of the floating mat (hollow), DO at the 10 cm depth was lower than that in other parts of the mat (Figure 5a).

3.2. Dissolved CH₄ and CO₂ Concentrations and δ¹³C Values in Pore Water

Generally, dissolved CH₄ and CO₂ concentrations increased in the summer for most of the sites (Figures 4b, 4d, 4g, and 4i). The CH₄ concentration for the P. australis (reed) sites (10 cm, range 0.37–0.54 mmol/L; mean 0.38 mmol/L; 40 cm, range 0.25–0.64 mmol/L; mean 0.49 mmol/L) was generally stable throughout the sampling period (Figure 4b). Among the sites, the CH₄ concentration from the S. cuspidatum sites at the 10 cm depth (range 0.02–0.40 mmol/L; mean 0.18 mmol/L) was significantly lower than that from the P. australis (t = 3.6, p < 0.001), M. trifoliata (range 0.22–0.98 mmol/L, mean 0.67 mmol/L; t = 6.8, p < 0.001), and S. palustre sites (range 0.37–1.31 mmol/L, mean 0.82 mmol/L; t = 10.6, p < 0.001) (Figure 5c and Table 2). In contrast, the CH₄ concentration from the S. palustre habitats was significantly higher than that from any other habitat type at both depths (t > 3.4, p < 0.009 for 10 cm depth; t > 9.4, p < 0.0001 for 40 cm depth) (Figure 5c and Table 2). CO₂ concentration was significantly different at each site compared with other sites at the same sampling depths (Figure 5e and Table 2). However, no significant differences were observed (p > 0.05) between the S. cuspidatum and P. australis sites at both depths, between M. trifoliata and S. palustre at both depths, and between S. cuspidatum and S. palustre at the 40 cm depth (Figure 5e and Table 2). The concentrations of both gases were higher at 40 cm than at 10 cm, and the variations were smaller at 40 cm (Figures 5c and 5e and Table 2).

Variations in δ¹³C-CH₄ were species specific. δ¹³C-CH₄ for the S. cuspidatum sites varied widely and was significantly more negative (10 cm, range −81.6 to −57.7‰, mean −71.2‰; 40 cm, range −80.7 to −65.8‰, mean −73.5‰, t > 6.2, p < 0.0001; Figure 4c, 4h, and 5b and Table 2) than for any other habitat type at both sampling depths (R. fauriei: 10 cm, range −64.6 to −48.5‰, mean −55.3‰; 40 cm, range

−24.3 ± 0.2‰ (standard error (SE)), −28.5‰, −25.1‰, −27.4 ± 0.2‰ (standard error (SE)), and −27.3 ± 0.5‰ (SE) for S. cuspidatum, R. fauriei, P. australis, M. trifoliata, and S. palustre, respectively.
\[ \delta^{13}C_{\text{CO}_2} \text{ also increased in summer at most sites at 10 cm, and variations were small at 40 cm (Figures 4e, 4j, and 5d).} \]

\[ \delta^{13}C_{\text{CH}_4} \text{ values at the } S. \text{ cuspidatum } \text{DO (mg/L)} \]

\[ \delta^{13}C_{\text{CO}_2} \text{ also increased in summer at most sites at 10 cm, and variations were small at 40 cm (Figures 4e, 4j, and 5d).} \]

\[ \delta^{13}C_{\text{CH}_4} \text{ values at the } S. \text{ cuspidatum } \text{DO (mg/L)} \]

\[ \delta^{13}C_{\text{CO}_2} \text{ also increased in summer at most sites at 10 cm, and variations were small at 40 cm (Figures 4e, 4j, and 5d).} \]

\[ \delta^{13}C_{\text{CH}_4} \text{ values at the } S. \text{ cuspidatum } \text{DO (mg/L)} \]

\[ \delta^{13}C_{\text{CO}_2} \text{ also increased in summer at most sites at 10 cm, and variations were small at 40 cm (Figures 4e, 4j, and 5d).} \]

\[ \delta^{13}C_{\text{CH}_4} \text{ values at the } S. \text{ cuspidatum } \text{DO (mg/L)} \]

\[ \delta^{13}C_{\text{CO}_2} \text{ also increased in summer at most sites at 10 cm, and variations were small at 40 cm (Figures 4e, 4j, and 5d).} \]

\[ \delta^{13}C_{\text{CH}_4} \text{ values at the } S. \text{ cuspidatum } \text{DO (mg/L)} \]
and S. palustre (10 cm: range 12.0 to 3.1‰, mean −6.1‰; 40 cm: range 2.6 to 1.7‰, mean −0.3‰) sites were significantly more positive than those in any other habitat type (t > 3.1, p < 0.03) and more positive (t > 5.2, p < 0.0001), respectively, than those at any other habitat type at 10 cm (Figure 5d and Table 2). δ¹³C-CO₂ values in the S. palustre habitats at 40 cm were significantly more positive than those in any other habitat type (t > 15.6, p < 0.0001, Figure 5d).

3.3. CH₄ Flux

CH₄ emissions at the S. cuspidatum sites were consistently low throughout the year (Figure 6). In contrast, CH₄ emissions at the M. trifoliata site were stable at high levels throughout the year. Although we had only five flux sampling occasions, our results show that CH₄ flux at the M. trifoliata site was significantly larger than that at the Sphagnum sites (S. cuspidatum, p < 0.001 and S. palustre, p < 0.005), consistent with the flux data of Sugimoto and Fujita [1997]. The amount of CH₄ emissions we observed at the M. trifoliata site

![Figure 5. Vertical distributions of averaged (±SE) (a) dissolved oxygen, (b) δ¹³C-CH₄, (c) CH₄ concentration, (d) δ¹³C-CO₂ values, and (e) dissolved CO₂ concentration for each hydrophyte type.](image-url)
Table 2. Mean CH$_4$ and CO$_2$ Concentrations and Mean, Minimum, and Maximum Values of $\delta^{13}$C-CH$_4$ and $\delta^{13}$C-CO$_2$ at Each Sampling Site and Depth

<table>
<thead>
<tr>
<th>Plot</th>
<th>Depth (cm)</th>
<th>Mean (± SE) CH$_4$ Concentration (mmol/L)</th>
<th>Mean (± SE) CO$_2$ Concentration (mmol/L)</th>
<th>$\delta^{13}$C-CH$_4$ (%)</th>
<th>$\delta^{13}$C-CO$_2$ (%)</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>mean (± SE) min max</td>
<td>mean (± SE) min max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cuspidatum</td>
<td>10</td>
<td>0.18 ± 0.0 -71.2 ± 1.4 -81.6 ± 0.6 -57.7 ± 0.8</td>
<td>3.67 ± 0.3 -15.2 ± 0.6 -20.8 ± 0.6 -11.9 ± 0.8</td>
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<tr>
<td></td>
<td>40</td>
<td>0.43 ± 0.8 -73.5 ± 0.3 -80.7 ± 0.6 -65.8 ± 0.6</td>
<td>4.44 ± 0.2 -11.0 ± 0.4 -13.1 ± 0.4 -9.3 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. fauriei</td>
<td>10</td>
<td>0.37 ± 0.0 -55.3 ± 1.8 -64.6 ± 0.6 -48.5 ± 0.6</td>
<td>5.81 ± 0.3 -12.7 ± 0.6 -16.7 ± 0.6 -8.9 ± 0.6</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>40</td>
<td>0.37 ± 0.0 -62.2 ± 1.1 -67.2 ± 0.6 -56.6 ± 0.6</td>
<td>6.41 ± 0.4 -10.9 ± 0.4 -12.4 ± 0.4 -8.7 ± 0.4</td>
<td></td>
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<tr>
<td>P. australis</td>
<td>10</td>
<td>0.43 ± 0.0 -57.5 ± 1.6 -67.0 ± 0.6 -47.2 ± 0.6</td>
<td>4.11 ± 0.2 -12.3 ± 0.7 -15.4 ± 0.7 -6.4 ± 0.7</td>
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<tr>
<td></td>
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<td>0.49 ± 0.9 -57.0 ± 1.7 -62.0 ± 0.6 -47.2 ± 0.6</td>
<td>5.14 ± 0.3 -11.9 ± 0.3 -13.5 ± 0.3 -10.4 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. trifoliate</td>
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<td>0.67 ± 0.1 -56.7 ± 1.5 -66.1 ± 0.6 -48.8 ± 0.6</td>
<td>5.40 ± 0.3 -9.3 ± 0.6 -15.3 ± 0.6 -6.6 ± 0.6</td>
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<tr>
<td></td>
<td>40</td>
<td>0.67 ± 0.1 -60.4 ± 1.4 -67.7 ± 0.6 -53.6 ± 0.6</td>
<td>6.41 ± 0.3 -8.7 ± 0.4 -11.2 ± 0.4 -7.2 ± 0.4</td>
<td></td>
<td></td>
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<tr>
<td>S. palustre</td>
<td>10</td>
<td>0.82 ± 0.1 -60.4 ± 0.6 -64.8 ± 0.6 -56.9 ± 0.6</td>
<td>2.73 ± 0.2 -6.1 ± 0.5 -12.0 ± 0.5 -3.1 ± 0.5</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>40</td>
<td>1.26 ± 0.1 -66.0 ± 0.7 -68.1 ± 0.6 -60.5 ± 0.6</td>
<td>3.88 ± 0.1 -0.3 ± 0.5 -2.6 ± 0.1 -1.7 ± 0.1</td>
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(ranging from 449.8 to 994.2 mg CH$_4$ m$^{-2}$ d$^{-1}$) was similar to that observed at the M. trifoliate site in their report. CH$_4$ emissions at the S. palustre sites increased in the summer 2007 (Figure 6). CH$_4$ emissions at the S. palustre sites were one order of magnitude higher than that at S. cuspidatum sites on average (Figure 6).

4. Discussion

4.1. Differences in Redox Conditions and Their Effects on CH$_4$ and CO$_2$ Production

Low NO$_3^-$ and SO$_4^{2-}$ concentrations at all sampling sites indicate the existence of highly reduced conditions, in which existing NO$_3^-$ and SO$_4^{2-}$ are consumed as substrates for denitrification and sulfate reduction (Table 1). Figure 7 shows the relationships between DO and NH$_4^+$ concentrations versus CH$_4$ concentrations. Low CH$_4$ concentrations were observed only when DO was high (Figures 7a and 7c). In addition, significant positive relationships between NH$_4^+$ and CH$_4$ concentrations indicate that CH$_4$ production was higher under highly reduced conditions in which NH$_4^+$ consumption by nitrification was not prevalent (Figures 7b and 7d).

The S. cuspidatum habitats maintained higher DO concentrations than any other habitat type (Figures 4a and 4f). The characteristic location of S. cuspidatum habitats at the boundary of the water surface (Figure 3) must contribute to the high DO content, as oxygen is created by photosynthesis in the surface water. This oxic condition in S. cuspidatum habitats should suppress CH$_4$ production in shallow peat; therefore, CH$_4$ concentrations beneath S. cuspidatum habitats were maintained at lower levels than in other habitats. S. palustre sites on hummocks exhibited different results compared to S. cuspidatum sites, with significantly lower DO concentrations and higher CH$_4$ concentrations at both sampling depths (Figures 5a and 5c and Table 2). Accumulated Sphagnum tissues form dark conditions below the hummock surface. This contributed to prevent photosynthesis below the Sphagnum surface. Therefore, a highly reduced condition was maintained at the water surface without a supply of oxygen, and much CH$_4$ was produced. The CO$_2$ concentration at 10 cm for S. palustre habitats was significantly lower than for the other habitat types ($p < 0.05$ for the P. australis sites and $p < 0.001$ for the other habitats, Figure 5e and Table 2). It is generally known that Sphagnum tissue decomposes very slowly (reviewed by Van Breemen [1995]) because it produces humic acid (Karanen and Ekman, 1982; Rudolph and Samland, 1985) and polysaccharides (Clymo, 1963).

Therefore, production of CH$_4$ and CO$_2$ was thought to be low. Among the Sphagnum species, Johnson and Damman [1991] reported that S. cuspidatum in hollows decayed 1.5 times faster than S. fuscum, which occupied hummocks. In addition, Hogg [1993] compared decay potential (CO$_2$ emission from bog organic matter from Sphagnum) in hollow and hummock species and reported that CO$_2$ emission rates were significantly higher in
hollows in deeper (50–100 cm) layers than those on hummocks. Our results showed results similar to their reports, that is, higher CO2 concentration in pore water from S. cuspidatum habitats (hollows) than in that from S. palustre habitats (hummocks). This was supported by significantly higher DOC/DON ratio in S. palustre habitats than in S. cuspidatum habitats, which suggests DOC in S. palustre habitats was less labile (Table 1). In addition to these data on CO2 conditions in both Sphagnum habitats, our results add information on CH4 dynamics in Sphagnum species both in hollows and on hummocks. We showed that even though both S. cuspidatum and S. palustre belong to the same genus, different redox conditions are formed, and CH4 concentrations in their habitats are also different. This also possibly reflects the contribution of CH4 production from H2/CO2 to the lower CO2 concentrations in S. palustre sites.

Recently, CH4 oxidation in Sphagnum mosses was examined, and Raghoebarsing et al. [2005] and Kip et al. [2010] found that CH4 oxidation was most pronounced in submerged mosses. 13C labeling revealed that CH4-derived carbon was incorporated into plant lipids when moss was submerged, indicating a mutually beneficial symbiosis between Sphagnum mosses and methanotrophs [Kip et al., 2010]. In addition, Larnola et al. [2010] observed 41 Sphagnum species and found that all 41 species exhibited methanotrophic activity, and potential CH4 oxidation was high when the water table level was near the surface. We observed lower CH4 concentrations in the S. cuspidatum habitats (hollows) than in the S. palustre habitats (hummocks), which may be attributable to this methanotrophic activity under submerged conditions. More positive 13CH4 values at shallower depths in both Sphagnum habitats also support the CH4 oxidation and its preferential use of 12CH4.

At the center of the floating mat, where the R. fauriei and M. trifoliate habitats were located (Figure 3), DO was maintained at low levels at 10 cm (Figure 4a). At these sites, CO2 concentrations were significantly higher than at the other sites (p < 0.001) and were similar in these two habitats (Figure 5e). Sugimoto and Fujita [1997] conducted an incubation experiment (measurement of gas production) to discriminate the decomposition
rates of peat at *M. trifoliata*, *P. australis*, and *Sphagnum* habitats and found that the decomposition rate was in the order of *M. trifoliata* > *P. australis* > *Sphagnum*. In their experiments, the gas production rate of the surface peat at *M. trifoliata* site was almost 4 times as great as that at 40 cm depth. We consider that high CO2 concentrations at *M. trifoliata* sites in our observations can be explained by this. Lower DOC/DON ratio at the *M. trifoliata* site at 10 cm depth compared with the other sites suggests the existence of more labile carbon in shallow part of these sites (Table 1). These must contribute higher CH4 production, which explains the fact that the CH4 concentration at 10 cm was signifi cantly higher at the *M. trifoliata* site than at the *R. fauriei* site (p < 0.05), although these habitats were situated adjacent to the center of the mat (Figure 5c). The presence of the lowest DO and the highest CO2 concentration at 10 cm in these habitats indicates that anoxic respiration producing CO2 was dominant in this area. The DO at 40 cm was moderately and greatly higher than that at 10 cm in *M. trifoliata* and *R. fauriei* habitats, respectively (Figure 5a). Considering that the root depth of *M. trifoliata* is not deeper than 10 cm, more decomposition of labile organic matter at 10 cm than at 40 cm in the *M. trifoliata* habitat [Sugimoto and Fujita 1997] can contribute to the formation of such conditions. The larger root depth of *R. fauriei* also could transport oxygen to the deeper layer.

At the edges of the mat, where *P. australis* (reeds) grew, DO, CH4, and CO2 concentrations were at intermediate levels compared to most of the sampling sites (Figure 5). This agreed with the report of Sugimoto and Fujita [1997] that decomposition rates of reeds were intermediate between *M. trifoliata* and *Sphagnum*. They also reported that considerably higher decomposition rates were detected at 10 cm than at 40 cm at the reed site. However, our results showed that the CH4 and CO2 concentrations were higher at 40 cm depth than at 10 cm depth. This can probably be attributed to gas exchange between pore water and the air at shallower zones and/or homogenization of dissolved gas by exchanging pore water and pond water because this habitat is situated at the edges of the floating mat (Figure 3).

4.2. Implications From the $\delta^{13}$C Ratios of CH4 and CO2

Figure 8 shows the clear relationship between CH4 concentration and $\delta^{13}$C-CO2. The distributions of the plots of each species were highly specific; that is, the lowest CH4 concentrations and the most negative $\delta^{13}$C-CO2 were observed at *S. cuspidatum* habitats, and highest CH4 concentrations and the most positive $\delta^{13}$C-CO2 were observed at *S. palustre* habitats. The other species were situated between the two *Sphagnum* species. Such a relationship between CH4 concentration and $\delta^{13}$C-CO2 was reported for seasonal variations of the pore water samples obtained from a Japanese forested wetland under the same climatic conditions [Itoh et al., 2008]. The authors suggested that the $\delta^{13}$C-CO2 increase in correlation with increasing CH4 concentrations in high-temperature periods was due to the process of the preferential use of $^{13}$CO2 as a substrate for CH4 by H2/CO2 reduction [Sugimoto and Wada, 1993]. In our floating bog site, the significant positive regression of CH4 concentration on $\delta^{13}$C-CO2 (Figure 8) was not similar to the results of Itoh et al. [2008] in which the significant positive regression was shown as a seasonal variation in one plot. At our site, a significant positive regression of CH4 concentration on $\delta^{13}$C-CO2...
was observed but as a hydrophyte species-specific distribution in the CH4 concentration—δ13C-CO2 diagram. The measured plant tissue δ13C values in our site ranged from −24.3 to −28.5‰. Considering that CO2 is produced as a byproduct of decomposition of these plant tissues or peats under both oxic and anoxic conditions, CH4 production must contribute high values of δ13C-CO2. These results show that preferential reduction of 12CO2 for methanogenesis can drastically change δ13C-CO2. Under the condition that hydrogen concentration increases in the summer in this floating mat [Sugimoto and Fujita, 2006], it has been suggested that H2/CO2 reduction makes a considerable contribution to CH4 production, especially in the summer.

The CH4 concentration was lower and δ13C-CO2 was more negative at 10 cm depth than at 40 cm in all habitats (CH4, p < 0.001 for both Sphagnum habitats; δ13C-CO2, p < 0.001 for both Sphagnum habitats; and p < 0.05 for the other three habitats). This indicates the possibility of preferential use of 12CH4 by methanotrophs in shallower layer, which produce more negative CO2. This is also supported by δ13C-CH4 values, as discussed in the following.

The crossplots of δ13C-CO2 and δ13C-CH4 are shown in Figure 9, with the apparent fractionation between CO2 and CH4 (α) calculated based on the ratio (δ13C-CO2 + 103)/(δ13C-CH4 + 103). The values of α indicate the magnitude of isotopic separation between coexisting species. The apparent α distribution ranged from 1.034 to 1.073 at our site (Table 3). Whiticar et al. [1986] suggest that CH4 produced primarily by the acetate fermentation pathway in freshwater environments has δ13C values ranging from ~65 to ~50‰, whereas CH4 produced primarily by the CO2 reduction pathway in marine systems has δ13C values ranging from ~110 to ~60‰. Also, from incubation studies, 13C fractionation during CO2 reduction to CH4 (1.025 ≤ αCO2-CH4 ≤ 1.079) is larger than that associated with acetate dissimilation (αacetate-CH4 ≤ 1.021) [Games et al., 1978; Gelwicks et al., 1994; Botz et al., 1996]. The values of α at our sites varied from species to species; that is, wide ranges of values were observed at vascular plant sites and narrow ranges of values in high-α zone at S. cuspidatum and S. palustre sites (Figure 9c and Table 3). High δ13C-CO2 values were observed at highly reduced

![Figure 9. Crossplots of δ13C data from CH4 and CO2 for each habitat type at (a) 10 cm (a), (b) 40 cm, and (c) averaged (C ± SE). The dashed lines indicate the apparent fractionation between CO2 and CH4 (α) calculated by the ratio of (δ13C-CO2 + 103)/(δ13C-CH4 + 103).](image-url)
Table 3. Mean, Minimum, and Maximum Values of $\alpha$ at Each Sampling Site and Depth

<table>
<thead>
<tr>
<th>Plot</th>
<th>Depth (cm)</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
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</tr>
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<td></td>
<td>40</td>
<td>1.068</td>
<td>1.060</td>
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<tr>
<td>R. fauriei</td>
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<td>1.048</td>
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</table>

the reports of Hornibrook and Bowes [2007] and Hornibrook [2009], in which $\delta^{13}$C-$\text{CH}_4$ values in pore water were more positive in minerotrophic peatland than in ombrogenous peatland, and of Galand et al. [2005], in which a larger portion of $\text{CH}_4$ from $\text{H}_2/\text{CO}_2$ was observed from ombrotrophic bog than from the mesotrophic fen in community studies of methanogen. These suggest that $\delta^{13}$C-$\text{CH}_4$ values in a peatland can be shifted with trophic status.

When considering the change in $\alpha$ with depth, increasing $\alpha$ values with depth as a result of decreasing $\delta^{13}$C-$\text{CH}_4$ and increasing $\delta^{13}$C-$\text{CO}_2$ were observed at most sites, with the exception of the P. australis sites (in the same manner as indicated by the arrows in Figure 9c at the Sphagnum sites) (Table 3). Such shifts in $\alpha$ values with increasing depth were reported as the effect of increasing $\text{CH}_4$ from $\text{H}_2/\text{CO}_2$ reduction pathway relative to acetate fermentation [Hornibrook et al., 1997, 2000]. They suggested that, in shallow soils containing an abundance of labile organic carbon, the contribution of the acetate fermentation pathway to $\text{CH}_4$ production was relatively high and that a shift occurred toward carbonate-utilizing methanogenesis with increasing depth as organic matter became increasingly recalcitrant. Such observations that acetoclastic methanogenesis occurs preferably in the upper peat layer and that $\text{CH}_4$ production from $\text{H}_2/\text{CO}_2$ dominates in the deep layers have been previously reported [Popp et al., 1999; Chasar et al., 2000; Kotsyurbenko et al., 2004]. Miyajima et al. [1997] also demonstrated that a decrease in the degradability of organic matter resulted in an enhanced contribution of $\text{CH}_4$ from the $\text{H}_2/\text{CO}_2$ reduction pathway. Our results suggest that the same direction of shift in methanogenic pathway with depth can be found within each hydrophyte habitat, especially in Sphagnum habitats.

In addition, $\delta^{13}$C-$\text{CH}_4$ was more positive at shallower (10 cm) than at greater (40 cm) depth, whereas $\delta^{13}$C-$\text{CO}_2$ was more negative at the shallower depth at both Sphagnum sites. This may be a result of $\text{CH}_4$ oxidation in Sphagnum habitats (as discussed above). Coleman et al. [1981] reported that the carbon isotope effect of $\text{CH}_4$-oxidizing bacteria ranged from 1.013 to 1.025 in culture experiments, and Tyler et al. [1994] reported a similar value of 1.022 ± 0.004 from temperate forest soil. Therefore, $\delta^{13}$C values of $\text{CO}_2$ produced by $\text{CH}_4$ oxidation will have more negative values compared with those of respired $\text{CO}_2$ from other sources. Considering this, more positive $\delta^{13}$C-$\text{CH}_4$ values and more negative $\delta^{13}$C-$\text{CO}_2$ values at shallower depths in Sphagnum habitats can be partly explained by $\text{CH}_4$ oxidation by methanotrophs (Table 4).

As for habitats other than the Sphagnum sites, $\delta^{13}$C-$\text{CH}_4$ values were significantly more negative in the deeper zone (40 cm) than in the shallower zone (10 cm) in R. fauriei habitat ($t > 2.2, p < 0.05$). Chanton [2005], summarizing the $\delta^{13}$C-$\text{CH}_4$ values of diffusive plants (e.g., Peltandra and Oyza), reported that more negative $\text{CH}_4$ is transported and emitted to the atmosphere via plants, and more positive $\text{CH}_4$ is retained in the sediment. Considering that the roots of R. fauriei do not reach as deep as 40 cm, more positive $\delta^{13}$C-$\text{CH}_4$ values at 10 cm depth were thought to be the result of $\text{CH}_4$ diffusion via plants and/or $\text{CH}_4$ oxidation in the rhizosphere. This agrees with the report by Popp et al. [1999], who measured the $\delta^{13}$C-$\text{CH}_4$ in a Carex fen and characterized emitted $\text{CH}_4$ rhizospheric $\text{CH}_4$, and $\text{CH}_4$ below the rhizosphere. They reported that emitted $\text{CH}_4$ was isotopically similar to $\text{CH}_4$ below the rhizosphere (at 50 cm depth), whereas $\text{CH}_4$ within the
larger CH$_4$ emission in their habitat compared with the difference in

S. cuspidatum, and much higher emissions were observed during summer. This must re-

S. palustre, no relationship was observed between dissolved CH$_4$ at 10 cm and emitted CH$_4$,

P. australis. This also agree with the results of Sphagnum sites throughout the sampling period. Gas transport via M. trifoliata must contribute to larger CH$_4$ emission in their habitat compared with the S. palustre habitat. CH$_4$ oxidation within Sphagnum moss (as discussed above) can also contribute to this difference. In addition, lower dissolved CH$_4$

P. australis (reed) sites. This is also in agreement with Chanton [2005], who showed little difference in $^{13}$C-CH$_4$ in sediment CH$_4$ and CH$_4$ emitted during the daytime for convective plants (e.g., Typha and Phragmites). They suggested that the convective through-flow system under daylight does not result in molecular-weight-dependent fractionation of CH$_4$. The fact that rhizomes of reeds can grow below 40 cm in depth also contributes to the homogenous distribution of CH$_4$ and $^{13}$C-CH$_4$ down to the depth of 40 cm. From these results, our isotopic data also reflect the habitat-specific CH$_4$ emission mechanisms at our site.

4.3. Methane Production and Methane Flux

The fact that the M. trifoliata site had higher CH$_4$ emissions than the Sphagnum sites confirms the previous results of Sugimoto and Fujita [1997]. This also agree with the results of Bowes and Hornibrook [2006], who reported that the CH$_4$ flux was an order of magnitude less at a Sphagnum-rich site than at a vascular flora-rich site. The ability of vascular plants to enhance CH$_4$ flux from wetland surfaces is well known [e.g., Thomas et al., 1996; Wassmann and Aulakh, 2000; Kutzbach et al., 2004]. CH$_4$ concentration at 10 cm depth was significantly higher at S. palustre sites than at M. trifoliata sites throughout the sampling period. Gas transport via M. trifoliata must contribute to larger CH$_4$ emission in their habitat compared with the S. palustre habitat. CH$_4$ oxidation within Sphagnum moss (as discussed above) can also contribute to this difference. In addition, lower dissolved CH$_4$

$^{13}$C-CH$_4$ down to the depth of 40 cm. This suggests that accumulated peat can also affect the transport of CH$_4$ produced beneath the S. palustre habitat. This also can induce isotopic fractionation in $^{13}$C-CH$_4$ values during CH$_4$ transportation to the atmosphere. Hornibrook [2009] reported that $^{13}$C values of emitted CH$_4$ from ombrotrophic bogs were more negative than from fens reflecting the differences of CH$_4$ production, CH$_4$ oxidation, and transportation in relationship to trophic status including vegetation. Further study to understand the difference in the $^{13}$C-CH$_4$ values in emitted CH$_4$ among Sphagnum habitats is also needed considering their life forms.

5. Conclusions

Vegetation changes in peatlands will be accompanied by eutrophication and/or climate changes, such as increasing temperatures and higher frequencies of heavy precipitation in the future [e.g., Dai, 2013]. Our results from observations in a small floating bog peatland that has experienced eutrophication suggest that CH$_4$ concentrations and $^{13}$C-CH$_4$ and $^{13}$C-CO$_2$ values in pore water varied greatly among the hydrophyte habitats. This suggests that different plant life forms can affect CH$_4$ production by controlling redox conditions and determining $^{13}$C-CH$_4$ and $^{13}$C-CO$_2$ values, even within Sphagnum species. Differences in the amount of CH$_4$ beneath hydrophyte habitats and their life forms can also affect the CH$_4$

Table 4. The Slope at Each Sampling Site for the Crossplots of $^{13}$C-CH$_4$

${^{13}}C$-CO$_2$ Between 10 and 40 cm Depths (Figure 9c)

<table>
<thead>
<tr>
<th>Site</th>
<th>Slope of $^{13}$C-CH$_4$ Versus $^{13}$C-CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cuspidatum</td>
<td>-1.10</td>
</tr>
<tr>
<td>R. fauriei</td>
<td>-0.27</td>
</tr>
<tr>
<td>P. australis</td>
<td>1.08</td>
</tr>
<tr>
<td>M. trifoliata</td>
<td>-0.16</td>
</tr>
<tr>
<td>S. palustre</td>
<td>-0.92</td>
</tr>
</tbody>
</table>

and $^{13}$C-CH$_4$ was slightly more positive at the 10 than at the 40 cm depth. This suggests that the effect of CH$_4$ transport and CH$_4$ oxidation in the rhizosphere could not have been detected from the pore water at 10 cm depth in the M. trifoliata site, probably because of much shallower root depth (approximately 5 cm) compared with R. fauriei. Both CH$_4$ concentration and $^{13}$C-CH$_4$ values were similar at both the 10 and 40 cm depths at the P. australis (reed) sites. This is also in agreement with Chanton [2005], who showed little difference in $^{13}$C-CH$_4$ in sediment CH$_4$ and CH$_4$ emitted during the daytime for convective plants (e.g., Typha and Phragmites). They suggested that the convective through-flow system under daylight does not result in molecular-weight-dependent fractionation of CH$_4$. The fact that rhizomes of reeds can grow below 40 cm in depth also contributes to the homogenous distribution of CH$_4$ and $^{13}$C-CH$_4$ down to the depth of 40 cm. From these results, our isotopic data also reflect the habitat-specific CH$_4$ emission mechanisms at our site.

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emission intensity. Hornbrook and Bowes [2007] and Hornbrook [2009] measured the $\delta^{13}$C-CH$_4$ of both pore water and CH$_4$ flux from two ombrogenous and two minerotrophic peatlands, focusing on the trophic status, which impacts CH$_4$ production and emission via control of vegetation assemblages. They reported that the $\delta^{13}$C-CH$_4$ of both pore water and CH$_4$ flux was more negative in ombrogenous bogs than in minerotrophic peatlands. Taking into consideration the variety of hydrophytes growing in peatlands and their transitions can provide useful information for a better understanding of CH$_4$ dynamics and its emission from peat bogs. Although we did not show the isotopic data for the CH$_4$ flux in this paper, our results implied that considerable difference can be shown in $\delta^{13}$C-CH$_4$ values in emitted CH$_4$ from the different type of hydrophytes within a small floating peat bog.

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δ13CH4 VARIATIONS IN HYDROPHYTE SPECIES

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