RESEARCH ARTICLE

Key Points:
- D/H and $^{13}C/^{12}C$ ratios of leaf waxes constrain proportion recycled methane in *Sphagnum*
- High water table supports increased *Sphagnum* methane recycling
- Methane transport through sedge aerenchyma inhibits methane recycling

Supporting Information:
- Appendix Table 1
- Readme
- R script

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A novel framework for quantifying past methane recycling by *Sphagnum*-methanotroph symbiosis using carbon and hydrogen isotope ratios of leaf wax biomarkers

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Abstract The concentration of atmospheric methane is strongly linked to variations in Earth's climate. Currently, we can directly reconstruct the total atmospheric concentration of methane, but not individual terms of the methane cycle. Northern wetlands, dominated by *Sphagnum*, are an important contributor of atmospheric methane, and we seek to understand the methane cycle in these systems. We present a novel method for quantifying the proportion of carbon *Sphagnum* assimilates from its methanotrophic symbionts using stable isotope ratios of leaf-wax biomarkers. Carbon isotope ratios of *Sphagnum* compounds are determined by two competing influences, water content and the isotope ratio of source carbon. We disentangled these effects using a combined hydrogen and carbon isotope approach. We constrained *Sphagnum* water content using the contrast between the hydrogen isotope ratios of *Sphagnum* and vascular plant biomarkers. We then used *Sphagnum* water content to calculate the carbon isotope ratio of *Sphagnum*'s carbon pool. Using a mass balance equation, we calculated the proportion of recycled methane contributed to the *Sphagnum* carbon pool, "PRM." We quantified PRM in peat monoliths from three microhabitats in the Mer Bleue peatland complex. Modern studies have shown that water table depth and vegetation have strong influences on the peatland methane cycle on instrumental time scales. With this new approach, $\delta^{13}C$ of *Sphagnum* compounds are now a useful tool for investigating the relationships among hydrology, vegetation, and methanotrophy in *Sphagnum* peatlands over the time scales of entire peatland sediment records, vital to our understanding of the global carbon cycle through the Late Glacial and Holocene.

1. Introduction

Atmospheric concentrations of methane, an important greenhouse gas, have changed dramatically over the late glacial and Holocene. While these changes in concentration are well constrained by samples from ice cores, the reasons for the observed changes remain in the realm of hypothesis. Several lines of evidence have emerged from studies of methane in ice cores implicating northern peatlands as an important methane source, including higher concentrations of methane in the northern hemisphere relative to the southern hemisphere, indicating a northern source [*Chappellaz et al.*, 1997; *Dallenbach et al.*, 2000], and depleted H and C isotope ratios of atmospheric methane in the Holocene relative to the glacial, indicating a biogenic, wetland source [*Möller et al.*, 2013]. Other studies of peatland sediments have attempted to address the atmospheric methane source question by synthesizing peatland initiation ages or peatland ecological changes though time and comparing these to the changes in methane concentration in ice [*Macdonald et al.*, 2006; *Korhola et al.*, 2010; *Yu et al.*, 2013]. While it seems likely that peatland dynamics are responsible for much of the variability of atmospheric methane, all of this evidence is fundamentally circumstantial, and no study has directly measured any part of the peatland methane cycle over the Holocene or late glacial. We present here a novel method for reconstructing one part of the peatland methane cycle—the proportion of methane recycled by methanotroph/*Sphagnum* symbiosis—using the carbon and hydrogen isotope ratios of sedimentary biomarkers in northern peatlands. While it is difficult to directly connect this parameter to methane flux to the atmosphere, it is an important part of the peatland methane cycle that has not previously been quantified.

The methane cycle has been directly measured in modern peatlands, and although emissions from peatlands account for about 10% of atmospheric methane today [*Bridgham et al.*, 2006], it has also been observed that these peatlands do not emit nearly as much methane as is produced at depth [*Hornibrook et al.*, 2009]. This apparent loss of methane has been ascribed to bacterially mediated oxidation in the upper
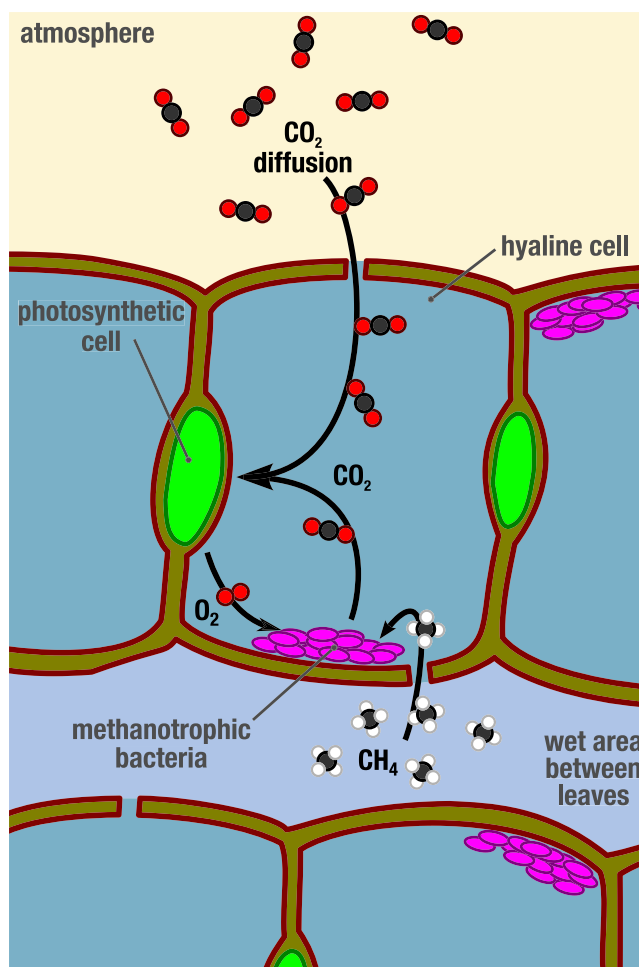
Figure 1. Schematic diagram illustrating the two sources of carbon for *Sphagnum magellanicum* CO_2 from the atmosphere must diffuse through water in the hyaline cells before reaching the photosynthetic cells. Oxygen from *Sphagnum* photosynthesis is used by the methanotrophs to respire methane, and the resulting CO_2 is in turn used by *Sphagnum*.

layers of peat. The bacteria responsible for this consumption of methane have been found to be symbiotic with *Sphagnum*; methanotrophic bacteria reside inside the hyaline (water-holding) cells of *Sphagnum*, which uses the CO_2 produced by methane oxidation for photosynthesis [*Raghoebarsing et al.*, 2005; *Kip et al.*, 2010; *Larmola et al.*, 2010]. The resulting O_2 from *Sphagnum* photosynthesis is used by the methanotrophs to oxidize methane (Figure 1). For modern peatlands, recent investigations have begun to constrain with increasing detail the relationships among vegetation type, hydrology, methane flux, and the activity of *Sphagnum*-methanotroph symbiosis [*Chen et al.*, 2008; *Kip et al.*, 2010]. These studies are limited, however, to the instrumental period. No proxy for any part of the peatland methane cycle currently exists for testing the applicability of uniformitarianism to the relationships we observe in the modern, or for tracking changes in the methane cycle of the past.

The depth of the water table, or, correspondingly, the size of the aerated zone, exerts a strong control on the peatland methane cycle [*Blodau*, 2002; *Roulet et al.*, 2007]. While peatlands are, in general, saturated, anoxic, acidic environments, hydrologic balance does vary, creating conditions that are more or less favorable for methanogenesis or methanotrophy. The surface of a peatland is also heterogeneous, and the typical "hummock and hollow" microtopography produces locations within a peatland where the water table depth can be as different as 60 cm [*Booth*, 2002]. During times or in locations where the water table is high, anoxic conditions are allowed to fully develop and persist in the peat, fostering an active methanogenic community. Methanotrophy is also found to be most active in locations where the water table is close to or at the surface of the peat [*Basiliko et al.*, 2004; *Raghoebarsing et al.*, 2005; *Kip et al.*, 2010; *Larmola et al.*, 2010]. Based on these observations, we expect that *Sphagnum*-methanotroph symbiosis should be most efficient under high water table conditions.

The vegetational composition at the peatland surface also has a strong influence on the methane cycle. Measurements of methane flux from peatlands in North America and Europe have shown that areas with abundant *Sphagnum* have much lower rates of methane flux than areas with abundant sedges (*Cyperaceae*). Sedges have aerechyma, spongy tissue that transports oxygen from the atmosphere to the roots. These structures also allow methane produced in the rooting zone of these plants to escape to the atmosphere before it is oxidized by methanotrophic bacteria [*Segers*, 1998; *Frenzel and Karofeld*, 2000; *Laanbroek*, 2010]. We therefore expect that *Sphagnum* living amongst abundant sedges will have a lower proportion of carbon from recycled methane as *Sphagnum* without nearby sedges.

Promise for a methane cycle paleo-proxy lies in the carbon isotope ratios of *Sphagnum*. Because biogenic methane, such as that produced in peatlands, has a distinctly more negative carbon isotope ratio, the CO_2 that results from the oxidation of that methane also has a distinct carbon isotope ratio. *Sphagnum* using this CO_2 for photosynthesis should have a significantly more negative carbon isotope ratio than a typical C3 plant. Indeed, *Sphagnum* has been found to have a light carbon isotope signature for bulk plant material as well as individual compounds, particularly leaf waxes. Here as in previous work, tricosane is used as an effective biomarker for *Sphagnum* in peatland sediment [*Baas et al.*, 2000; *Nott et al.*, 2000; *Pancost et al.*, 2002; *Xie et al.*, 2004; *Nichols et al.*, 2006; *Bush and McInerney*, 2013]. Confounding this carbon isotope approach, however, is the fact that moisture content strongly affects the carbon isotope ratio of *Sphagnum* in addition to the isotope ratio of the source carbon [*Williams and Flanagan*, 1996]. Here we disentangle these two effects using a combined hydrogen and carbon isotope approach.

We present a method for quantifying past changes in the proportion of carbon in *Sphagnum* coming from recycled methane by independently constraining the water content of *Sphagnum* using a hydrogen isotope method. *Sphagnum* has no vascular system, and thus can only get water by capillary action from the top ∼20 cm of the peatland, which has an isotope ratio strongly affected by evaporation. Vascular plants have roots that can obtain water from below the surface that is not affected by evaporation [*Nichols et al.*, 2010]. Therefore, the contrast in δD between *Sphagnum* and vascular plant biomarkers can be used to estimate evaporative water loss from within and between *Sphagnum* leaves—approximately equivalent to *Sphagnum* water content [*Nichols et al.*, 2010]. *Sphagnum*'s carbon isotope discrimination from CO_2 to biomass is related to water content by an experimentally derived function. Thus, we can use hydrogen isotope ratios of biomarkers to derive the carbon isotope ratio of the carbon dioxide used by *Sphagnum*. We then use a mass balance equation and the distinctive $\delta^{13}C$ of methane derived and atmospheric CO_2 to find the proportion of each used by *Sphagnum*.

We applied our new method to short cores from three different sites within the Mer Bleue peatland complex near Ottawa, ON, Canada. Scores of studies have been performed at this site, including measurements of methane flux [*Moore et al.*, 2011]. We chose a site at a *Sphagnum capillifolium*-dominated lawn with a higher water table (the "Lawn" site), a *Sphagnum magellanicum*-dominated site with a lower water table (the "Hummock" site), and an *Eriophorum vaginatum* and *Sphagnum capillifolium*-dominated site with variable water table dynamics (the "Tussock" site). Each of the three sites is located adjacent to long-term methane flux measurement sites. We chose these sites to be able to test the influence of both water table and vegetation on *Sphagnum*-methanotroph symbiosis.

2. Methods

A peat monolith was sampled from each of the three target locations, the *Sphagnum capillifolium* lawn, the *Sphagnum magellanicum* hummock, and the *Eriophorum vaginatum* tussock. Twenty contiguous, 1 cm thick peat samples were cut from each monolith. Extraction and purification of *n*-alkanes from peat samples was performed according to previously described methods [*Nichols et al.*, 2010]. Briefly, peat samples were freeze-dried, and lipids were extracted with hexane by ultrasonic agitation. Total lipid extracts were separated over a silica gel flash column. *n*-Alkanes elute with hexane and were quantified by gas chromatography-flame ionization detection (GC-FID). Carbon and hydrogen isotope ratios of *n*-alkanes were measured by gas chromatography-continuous flow isotope ratio mass spectrometry. Carbon isotopes were measured at the Woods Hole Oceanographic Institution Organic Mass Spectrometry Facility. Compounds eluting from the GC are oxidized to CO_2 at 1000°C in an alumina oxidation reactor with copper-nickel wire catalyst. Hydrogen isotope measurements were made at the Lamont Doherty Earth Observatory Biogeochemistry Laboratory on a Thermo DeltaV coupled to a Thermo Trace GC through a Thermo IsoLink and ConFlo IV. Eluting compounds were pyrolyzed to hydrogen gas at 1420°C in an empty alumina reactor. Evolved gases are introduced to the IRMS through a continuous flow device, and isotope ratios are calculated using a calibrated laboratory reference gas. Standard deviations of D/H measurements are <2‰ based on long-term repeat analyses of a lipid standard or <1.5‰ based on triplicate analysis of a single sample. H_3^+ factor during analysis of Mer Bleue samples remained consistently at 4.6.

3. Results and Discussion

We measured the stable hydrogen and carbon isotope ratios of tricosane and nonacosane from 20 contiguous 1 cm samples from each monolith. The clear separation between the two compounds in isotope space
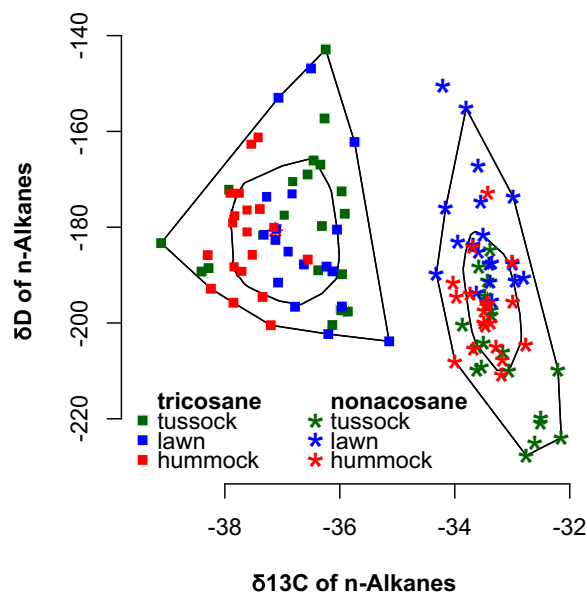
Figure 2. Bagplots of isotope ratio measurements from *n*-alkanes at three sites in Mer Bleue Bog. A bagplot is a two-variable variation of a box-and-whisker plot. The inner polygon (the "bag") for each data group circumscribes the *n*/2 datapoints closest to the two-variable median, while the outer polygon (the "loop") circumscribes all the points that fall within the "fence" which is a polygon three times larger than the "bag." Points outside the "loop" are considered outliers [*Rousseeuw et al.*, 1999].

(Figure 2) reflects their different source plants, reinforcing the utility of tricosane and nonacosane as biomarkers for *Sphagnum* and vascular plants, respectively. Most distinctive is the complete separation on the carbon isotope scale. Tricosane is consistently depleted relative to nonacosane by 3–4‰. This depletion supports the hypothesis that *Sphagnum* carbon isotope ratios are strongly influenced by respired methane.

The interpretation of carbon isotope ratios of *Sphagnum* compounds in peatland surface samples and peat sediments has confounded researchers in the past [e.g., *Xie et al.*, 2004], due to the competing influences of carbon source and moisture-controlled isotope discrimination [*Markel et al.*, 2010]. Although laboratory experiments have shown a clear relationship between *Sphagnum* moisture content and carbon isotope discrimination [*Williams and Flanagan*, 1996], this relationship is much less clear in real peatland environments [*Loisel et al.*, 2009; *Markel et al.*, 2010]. The impact on *Sphagnum* δ^{13}C of CO_2 from methane respired by symbiotic methanotrophic bacteria is, however, clear from some laboratory experiments and field studies [*Nichols et al.*, 2009; *Kip et al.*, 2010]. In order for carbon isotope ratios of *Sphagnum* compounds to be a useful paleoenvironmental tool, we must separate the effects of methanotrophy and water content.

To use our carbon isotope ratio measurements to constrain the contribution of respired methane to *Sphagnum*, we independently constrain the water content of *Sphagnum* using hydrogen isotopes. This method has been described and discussed previously [*Nichols et al.*, 2010]. Because *Sphagnum* has no vascular system for water transport, it accesses water only by capillary action, and therefore uses water only from approximately the upper 20 cm of the peatland. This water is highly susceptible to evaporation, which strongly enriches the hydrogen isotope ratios of *Sphagnum* water. As water evaporates from inside and among the leaves of the *Sphagnum* (i.e., water content decreases), the hyaline cells of the *Sphagnum* and the surface of the peatland itself shrink as water is removed to the atmosphere [*Clymo and Hayward*, 1982; *Kim and Verma*, 1996], providing a progressively more enriched pool of water for *Sphagnum* to use [*Nichols et al.*, 2010]. This evaporation signal is transferred to *Sphagnum* biomarkers (e.g., tricosane). Vascular plants, however, use water found deeper in the acrotelm, which is protected from evaporation by the shrinking peatland surface [*Kim and Verma*, 1996]. Thus, the δD signature of vascular plant biomarkers (e.g., nonacosane) is not affected by what in other terrestrial environments would be called soil water evaporation. With the δD of *Sphagnum* and vascular plant biomarkers, the fraction of water evaporated—essentially the *Sphagnum* water content—can be calculated using a Rayleigh evaporation model (equation (1)). The term, *f*, is the fraction of water remaining in *Sphagnum* after evaporation, or the fractional water content of *Sphagnum*. This value is calculated by comparing the δD of *Sphagnum*'s water with the δD of the water used by vascular plants [*Nichols et al.*, 2010].

$$\frac{\delta D_a - \delta D_s}{\varepsilon_k - \varepsilon^*} = \ln f \qquad (1)$$

$$\varepsilon_{vasc} + \left(\frac{\varepsilon_{vasc} \times \delta D_{C_{29}}}{1000} \right) + \delta D_{C_{29}} = \delta D_a \tag{2}$$

$$\varepsilon_{sph} + \left(\frac{\varepsilon_{sph} \times \delta D_{C_{23}}}{1000} \right) + \delta D_{C_{23}} = \delta D_s \tag{3}$$

where

δD_a = acrotelm water

δD_s = Sphagnum water

ε_k = kinetic fractionation factor

ε^* = equilibrium fractionation factor

$\delta D_{C_{29}}$ = hydrogen isotope ratio of nonacosane

$\delta D_{C_{23}}$ = hydrogen isotope ratio of tricosane

ε_{vasc} = enrichment factor between acrotelm water and nonacosane

ε_{sph} = enrichment factor between Sphagnum water and tricosane

f = fraction of water remaining after evaporation.

Values for kinetic and equilibrium fractionation in equation (1) (ε_k and ε^*, respectively) assume a temperature of 288 K and a relative humidity of 75%. These assumed values are reasonable for any region containing peatlands [*Halsey et al.*, 2000], and do not have a strong effect on the calculated value for ln f [*Nichols et al.*, 2010]. In equation (2), δD_s is calculated using an experimentally derived relationship [*Nichols et al.*, 2010]. δD_a, is derived from the δD of nonacosane using an apparent fractionation for C3 dicotyledons, $-113\permil$ [*Sachse et al.*, 2006; *Smith and Freeman*, 2006] in the hummock and lawn locations and an apparent fractionation for C3 monocotyledons, $-146\permil$ in the tussock core [*Sachse et al.*, 2012]. By using fractionation factors that correspond with the dominant vascular vegetation, we find δD_p values that are consistent among the three locations (supporting information Table A1). Because the age/depth relationship is different for each core, we do not expect perfect depth-for-depth matches, but we do see a close relationship between the tussock and hummock cores (Figure 3).

The apparent fractionation factor between vascular plant leaf wax δD and peatland water δD is perhaps the largest source of uncertainty in our calculations. Much work has been done to understand the controls on this apparent fractionation, and recent publications have cautioned against direct translation of leaf wax δD into precipitation δD because of the impact of leaf water enrichment [*Smith and Freeman*, 2006; *Hou et al.*, 2008; *Kahmen et al.*, 2013a, 2013b]. The characteristics of peatland environments, however, minimize the effects of leaf water evaporative enrichment. Leaf water enrichment strongest in environments with low relative humidity [*Hou et al.*, 2008; *Kahmen et al.*, 2013a]. Peatlands necessarily exist in areas of very high relative humidity, about 70–80% [*Gorham*, 1991]. The average relative humidity for Mer Bleue is well within this range, at about 75%. The influence of leaf water enrichment is also low in riparian areas [*Tipple et al.*, 2013], which are akin to peatlands in that the rooting zone of vascular plants is often saturated. Given the uncertainty in ε_{vasc}, we have attempted to account for all influences by using the empirically derived mean values for this parameter. We also attempt to propagate the uncertainty around these empirical values through our PRM calculation using a method described below. Fortunately, our PRM calculation is flexible. Even as our understanding of ε_{vasc} evolves, our framework for calculating PRM would remain unchanged, and can easily accommodate updated methods for estimating peatland water δD.

Once we have calculated f for each sample, we can then use ln f to calculate the isotope discrimination factor, Δ_f, using an experimentally derived relationship (equation (4)) between *Sphagnum* moisture content and carbon isotope discrimination shown in Figure 4 [*Williams and Flanagan*, 1996].

$$-7.060 \times \ln f + 14.394 = \Delta_f \tag{4}$$

We can then use the $\delta^{13}C$ of tricosane ($\delta^{13}C_{C_{23}}$) and this enrichment factor to reconstruct this carbon isotope for the CO_2 used by *Sphagnum*, $\delta^{13}C_{sph}$ (equation (5)).
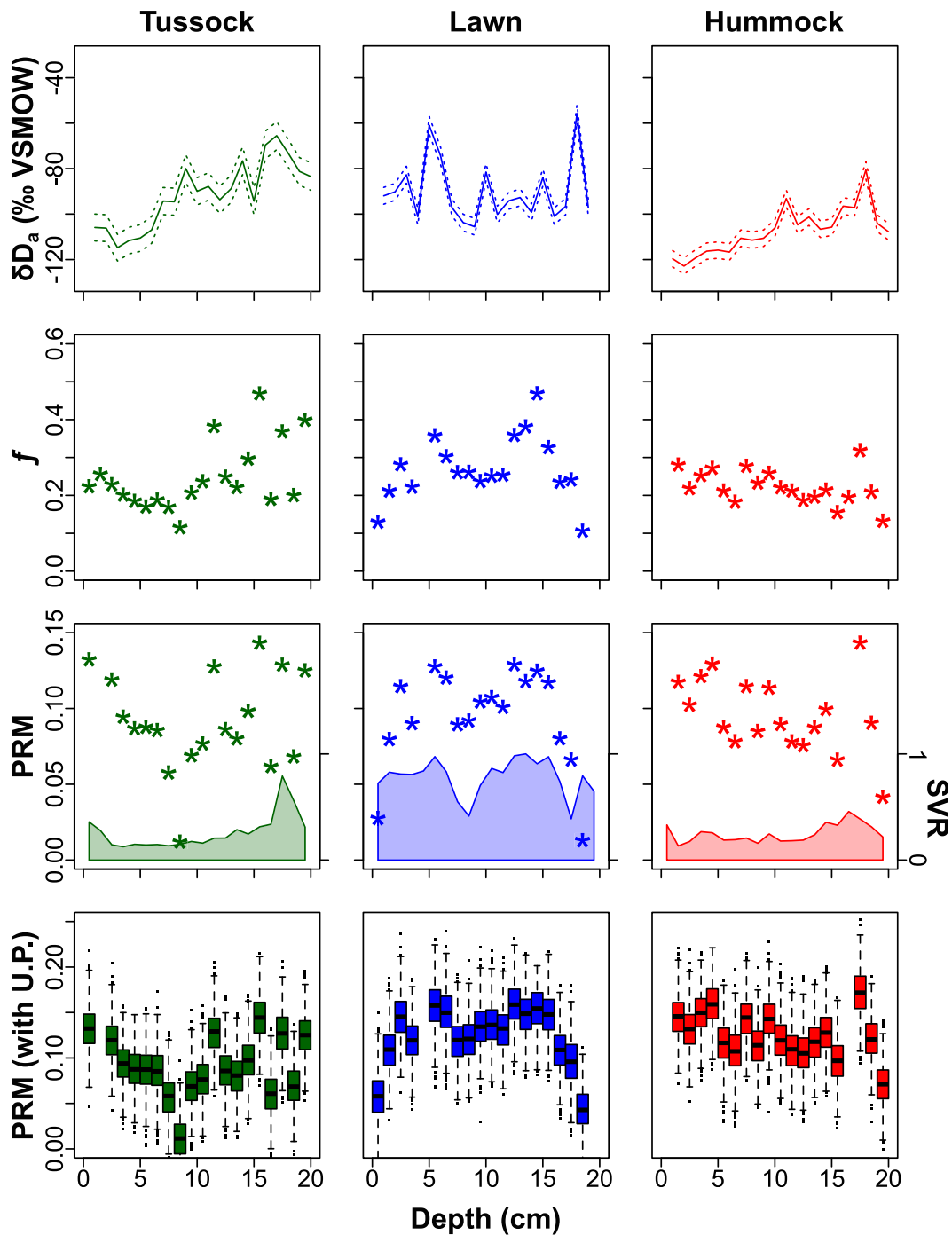
Figure 3. Each column contains data from one of the three monoliths, tussock, lawn, and hummock. (first row) δD of peatland water for each core location with 95% confidence intervals calculated from the *Sachse et al.* [2012] estimations of ε_{vasc}. (second row) Calculated values for f, or the water content of *Sphagnum* based on δD of tricosane and nonacosane. (third row) PRM calculations along with calculations of *Sphagnum*/Vascular Ratio "SVR," which is the proportional contribution of tricosane to the sum of tricosane and nonacosane [*Nichols et al.*, 2006]. (fourth row) PRM with uncertainty propagation: box-and-whisker plots of 1000 instances of PRM calculation, each using different values for ε_{vasc} randomly selected from a normally distributed set of values with a mean and standard deviation equal to that of the plant type as described in the text.

$$\delta^{13}C_{C_{23}} - \Delta_f = \delta^{13}C_{sph} \tag{5}$$

The CO_2 that *Sphagnum* uses, with a carbon isotope value represented by $\delta^{13}C_{sph}$, is a mixture of methane respiration product and atmospheric CO_2. The percent respired-methane-derived C used by *Sphagnum* (PRM) can be calculated using a mass balance equation (equation (6)).
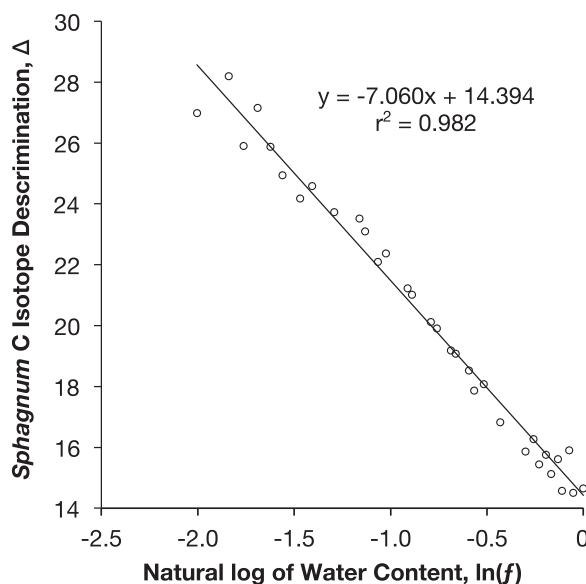
Figure 4. Carbon isotope discrimination as a function of the natural log of fractional water content. These data are redrawn from *Williams and Flannagan* [1996]. The regression is calculated by the least squares method.

$$\frac{\delta^{13}C_{sph} - \delta^{13}C_{atm}}{\delta^{13}C_{CH_4} - \delta^{13}C_{atm}} \times 100\% = PRM$$

(6)

The two end-member mass balance equation (equation (6)) is used to calculate how much carbon in *Sphagnum* comes from methane respired by symbiotic methanotrophs as a percent of the total carbon. The end-members are the measured carbon isotope ratio of methane from this peatland, $-75‰$ [*Roulet et al.*, 2007], and the carbon isotope ratio of the local atmosphere. Rather than using a fixed value for $\delta^{13}C$ of atmospheric CO_2, we reconstructed this value from the $\delta^{13}C$ of nonacosane (equation (7)) using average enrichment values for bulk C3 plants ($-20.1‰$) and for the enrichment between bulk plant material and leaf wax *n*-alkanes ($-6.4‰$) [*Conte et al.*, 2003; *A Hobbie and Werner*, 2004].

$$(\varepsilon_{C3} + \varepsilon_{alk}) + \frac{\left[(\varepsilon_{C3} + \varepsilon_{alk}) \times \delta^{13}C_{C_{29}}\right]}{1000} \delta^{13}C_{C_{29}} = \delta^{13}C_{atm}$$

(7)

By reconstructing this value, rather than using an assumed value based on the whole atmosphere, we account for the potential isotopic effects of the addition of CO_2 from other sources of respiration, thus isolating the contribution of respired methane. This is necessary because the local atmosphere near the surface of peatlands can be depleted in $^{13}CO_2$ relative to the whole atmosphere due to the addition of CO_2 from respiration. The value, $\delta^{13}C_{atm}$ represents the carbon isotope value of CO_2 used by plants without methanotrophic symbionts. Using this approach, we find reconstructed values for atmospheric CO_2 of $-7.05‰ \pm 0.48$. This is consistent with direct atmospheric measurements globally. This consistent value for atmospheric CO_2 also supports the conclusion that respired methane is not an important component of the atmosphere directly above the peatland surface, suggesting that most of the CO_2 from respired methane is reincorporated into *Sphagnum* and not released to the atmosphere.

Because the apparent fractionation factor between water and lipids for vascular plants (ε_{vasc}) is a large source of uncertainty, we propagated this uncertainty through our PRM calculation. For each depth interval in the three cores, we calculated the PRM 1000 times, each time choosing an ε_{vasc} at random from a normally distributed population of values with a mean and standard deviation equal to that reported in the literature for each vegetation type: for the tussock core, we use the value for C3 monocots ($\mu = -146$, $\sigma = 27$) and for the hummock and lawn cores we used values for C3 dicots ($\mu = -113$, $\sigma = 30$) [*Sachse et al.*, 2012]. The results of the PRM calculations with the propagation of uncertainty are displayed in Figure 4.

3.1. Percent Respired Methane at Mer Bleue

We applied our PRM calculation to sediments from the three different locations at Mer Bleue, a *Sphagnum capillifolium*-dominated lawn, the *Sphagnum magellanicum*-dominated hummock, and an *Eriophorum vaginatum* tussock with *Sphagnum capillifolium*. Uptake of respired methane by *Sphagnum* at all three locations was up to 16% (Figure 3). The range of values is reasonable, given the maximum of 20% assimilation of methane-derived carbon by *Sphagnum* samples in vitro [*Raghoebarsing et al.*, 2005].

Respired methane is a greater source of carbon for *Sphagnum* in the Lawn habitat with a higher water table than in Hummock habitat with a lower water table. This is a reasonable result, as laboratory experiments
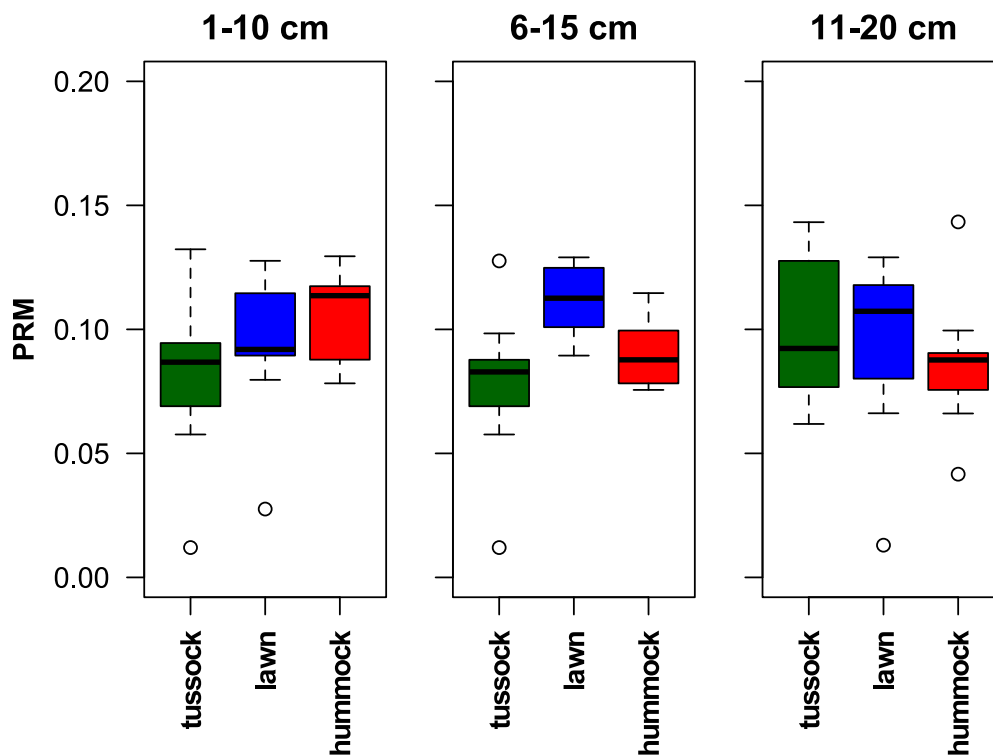
Figure 5. Box-and-whisker plots of PRM values from three sections of each of the cores.

have shown that methane consumption by symbiotic methanotrophs is greater when the *Sphagnum* is wetter [*Raghoebarsing et al.*, 2005] and that the potential for methane consumption is greater in *Sphagnum* found in peatland areas where the water table is higher [*Basiliko et al.*, 2004; *Kip et al.*, 2010]. If water table were the only factor controlling methane consumption, we would expect that the PRM would be lowest at the Hummock site, with the lowest water table. The PRM values at the Hummock site are often, but not consistently lower than at the Lawn site. Additionally, we find that the Tussock site has consistently lower PRM values than the other two sites (Figures 3 and 5).

Methanotrophs living at the Tussock site likely have less access to methane produced in the peatland, contributing to lower PRM values at this site. Sedges, including *Eriophorum vaginatum* have aerenchyma, structures which allow air from the atmosphere to oxygenate the plant's roots. These structures also allow methane to escape the peatland into the atmosphere, making it unavailable to methanotrophs in *Sphagnum*. Direct measurements of methane flux at the three sites show the presence of *Eriophorum* results in a threefold to fourfold increase in flux when water table is constant [*Moore et al.*, 2011]. Aerenchymous plants are thus a likely cause for reduced PRM values at the Tussock site. PRM values at the tussock site are not always low, however, especially in the 11–20th centimeters of the monolith (Figure 5). These samples could correspond to times when sedge stomata were, on average, closed for more time, inhibiting the flow of methane through the aerenchyma, causing the Tussock site to behave more like the Lawn site. Alternatively, there may have been fewer sedges at the site during the time represented by these samples. Indeed the relative amount of tricosane is higher in these samples than in the upper part of the monolith (Figure 3) suggesting that more *Sphagnum* was present at that time.

4. Conclusions

We find that the confounding effects of water content and carbon source on carbon isotope ratios of *Sphagnum* compounds can be disentangled by our combined hydrogen and carbon isotope approach. Using this new method, we are able to quantify the proportion of carbon from methane assimilated by

Sphagnum, and reconstruct changes in this important carbon cycle parameter in the past. We find, as before, that water table depth and vegetation have strong influences on methane recycling by *Sphagnum*'s methanotrophic symbionts. This new method, however, allows us to quantitatively reconstruct past variability in this symbiosis. Thus, it is possible to test the relationships among hydrology, vegetation, and methanotrophy in *Sphagnum* peatlands over Holocene time scales. It is now possible to reconstruct paleohydrology, vegetation change, and methane recycling using the same samples. Such reconstructions are vital to the understanding of the methane cycle in northern peatlands. While the PRM calculation is an important step forward in our understanding of the complicated nature of *Sphagnum* δ^{13}C measurements, further investigation is needed to connect PRM to concentrations of methane in the atmosphere.

References

Baas, M., R. Pancost, B. van Geel, and J. Damste (2000), A comparative study of lipids in Sphagnum species, *Org. Geochem.*, *31*(6), 535–541.

Basiliko, N., R. Knowles, and T. Moore (2004), Roles of moss species and habitat in methane consumption potential in a northern peatland, *Wetlands*, *24*(1), 178–185.

Blodau, C. (2002), Carbon cycling in peatlands—A review of processes and controls, *Environ. Rev.*, *10*(2), 111–134.

Booth, R. K. (2002), Testate amoebae as paleoindicators of surface-moisture changes on Michigan peatlands: Modern ecology and hydrological calibration, *J. Paleolimnol.*, *28*(3), 329–348.

Bridgham, S., J. Patrick Megonigal, J. Keller, N. Bliss, and C. Trettin (2006), The carbon balance of North American wetlands, *Wetlands*, *26*(4), 889–916.

Bush, R. T., and F. A. McInerney (2013), Leaf wax n-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy, *Geochim. Cosmochim. Acta*, *117*, 161–179, doi:10.1016/j.gca.2013.04.016.

Chappellaz, J., T. Blunier, S. Kints, A. Dällenbach, J.-M. Barnola, J. Schwander, D. Raynaud, and B. Stauffer (1997), Changes in the atmospheric CH_4 gradient between Greenland and Antarctica during the Holocene, *J. Geophys. Res.*, *102*(D13), 15,987–15,997.

Chen, Y., M. Dumont, N. McNamara, and P. Chamberlain (2008), Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PLFA analyses, *Environ. Microbiol.*, *10*(2), 446–459.

Clymo, R. S., and P. M. Hayward (1982), *The Ecology of Sphagnum*, pp. 229–289, Springer, Dordrecht, Netherlands.

Conte, M., J. Weber, P. Carlson, and L. Flanagan (2003), Molecular and carbon isotopic composition of leaf wax in vegetation and aerosols in a northern prairie ecosystem, *Oecologia*, *135*(1), 67–77.

Dallenbach, A., T. Blunier, J. Fluckiger, B. Stauffer, J. Chappellaz, and D. Raynaud (2000), Changes in the atmospheric CH_4 gradient between Greenland and Antarctica during the Last Glacial and the transition to the Holocene, *Geophys. Res. Lett.*, *27*(7), 1005–1008.

Frenzel, P., and E. Karofeld (2000), CH_4 emission from a hollow-ridge complex in a raised bog: The role of CH_4 production and oxidation, *Biogeochemistry*, *51*(1), 91–112, doi:10.1023/A:1006351118347.

Gorham, E. (1991), Northern peatlands: Role in the carbon cycle and probable responses to climatic warming, *Ecol. Appl.*, *1*(2), 182–195, doi:10.2307/1941811.

Halsey, L., D. Vitt, and L. Gignac (2000), Sphagnum-dominated peatlands in North America since the last glacial maximum: Their occurrence and extent, *Bryologist*, *103*(2), 334–352.

Hobbie, E. A., and R. A. Werner (2004), Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: A review and synthesis, *New Phytol.*, *161*(2), 371–385, doi:10.1111/j.1469-8137.2004.00970.x.

Hornibrook, E., H. L. Bowes, A. Culbert, and A. V. Gallego-Sala (2009), Methanotrophy potential versus methane supply by pore water diffusion in peatlands, *Biogeosciences*, *6*, 1491–1504.

Hou, J., W. D'Andrea, and Y. Huang (2008), Can sedimentary leaf waxes record D/H ratios of continental precipitation? Field, model, and experimental assessments, *Geochim. Cosmochim. Acta*, *72*(14), 3503–3517.

Kahmen, A., B. Hoffmann, E. Schefuß, S. K. Arndt, L. A. Cernusak, J. B. West, and D. Sachse (2013a), Leaf water deuterium enrichment shapes leaf wax n-alkane Î'D values of angiosperm plants II: Observational evidence and global implications, *Geochim. Cosmochim. Acta*, *111*, 50–63, doi:10.1016/j.gca.2012.09.004.

Kahmen, A., E. Schefuß, and D. Sachse (2013b), Leaf water deuterium enrichment shapes leaf wax n-alkane Î'D values of angiosperm plants I: Experimental evidence and mechanistic insights, *Geochim. Cosmochim. Acta*, *111*, 39–49, doi:10.1016/j.gca.2012.09.003.

Kim, J., and S. Verma (1996), Surface exchange of water vapour between an open Sphagnum fen and the atmosphere, *Boundary Layer Meteorol.*, *79*(3), 243–264.

Kip, N., J. F. V. Winden, Y. Pan, L. Bodrossy, G.-J. Reichart, A. J. P. Smolders, M. S. M. Jetten, J. S. Sinninghe Damsté, and H. J. M. Op Den Camp (2010), Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems, *Nat. Geosci.*, *3*(9), 617–621, doi:10.1038/ngeo939.

Korhola, A., M. Ruppel, H. SeppA, M. Väliranta, T. Virtanen, and J. WeckstrOm (2010), The importance of northern peatland expansion to the late-Holocene rise of atmospheric methane, *Quat. Sci. Rev.*, *29*(5), 611–617, doi:10.1016/j.quascirev.2009.12.010.

Laanbroek, H. J. (2010), Methane emission from natural wetlands: Interplay between emergent macrophytes and soil microbial processes. A mini-review, *Ann. Bot.*, *105*(1), 141–153.

Larmola, T., E.-S. Tuittila, M. Tiirola, H. Nykänen, P. J. Martikainen, K. Yrjälä, T. Tuomivirta, and H. Fritze (2010), The role of Sphagnum mosses in the methane cycling of a boreal mire, *Ecology*, *91*(8), 2356–2365, doi:10.1890/09-1343.1.

Loisel, J., M. Garneau, and J. Hélie (2009), Modern *Sphagnum* δ^{13}C signatures follow a surface moisture gradient in two boreal peat bogs, James Bay lowlands, Quebec, *J. Quat. Sci.*, *24*(3), 209–214.

Macdonald, G. M., D. W. Beilman, K. V. Kremenetski, Y. Sheng, L. C. Smith, and A. A. Velichko (2006), Rapid early development of circumarctic peatlands and atmospheric CH_4 and CO_2 variations, *Science*, *314*(5797), 285–288, doi:10.1126/science.1131722.

Markel, E. R., R. K. Booth, and Y. Qin (2010), Testate amoebae and δ^{13}C of *Sphagnum* as surface-moisture proxies in Alaskan peatlands, *Holocene*, *20*(3), 1–13, doi:10.1177/0959683609354303.

Möller, L., T. Sowers, M. Bock, R. Spahni, and M. Behrens (2013), Independent variations of CH_4 emissions and isotopic composition over the past 160,000 years, *Nat. Geosci.*, *6*, 885–890, doi:10.1038/ngeo1922.

Moore, T. R., A. Young, J. L. Bubier, E. R. Humphreys, P. M. Lafleur, and N. T. Roulet (2011), A multi-year record of methane flux at the Mer Bleue Bog, Southern Canada, *Ecosystems*, *14*(4), 646–657, doi:10.1007/s10021-011-9435-9.

Nichols, J. E., R. K. Booth, S. Jackson, E. Pendall, and Y. Huang (2006), Paleohydrologic reconstruction based on n-alkane distributions in ombrotrophic peat, *Org. Geochem.*, *37*(11), 1505–1513, doi:10.1016/j.orggeochem.2006.06.020.

Nichols, J. E., M. Walcott, R. Bradley, J. Pilcher, and Y. Huang (2009), Quantitative assessment of precipitation seasonality and summer surface wetness using ombrotrophic sediments from an Arctic Norwegian peatland, *Quat. Res.*, *72*(3), 443–451, doi:10.1016/j.yqres.2009.07.007.

Nichols, J. E., R. K. Booth, S. T. Jackson, E. G. Pendall, and Y. Huang (2010), Differential hydrogen isotopic ratios of Sphagnum and vascular plant biomarkers in ombrotrophic peatlands as a quantitative proxy for precipitation—evaporation balance, *Geochim. Cosmochim. Acta*, *74*(4), 1407–1416, doi:10.1016/j.gca.2009.11.012.

Nott, C., S. Xie, L. Avsejs, D. Maddy, F. M. Chambers, and R. P. Evershed (2000), n-Alkane distributions in ombrotrophic mires as indicators of vegetation change related to climatic variation, *Org. Geochem.*, *31*(2–3), 231–235.

Pancost, R., M. Baas, B. van Geel, and J. Damste (2002), Biomarkers as proxies for plant inputs to peats: An example from a sub-boreal ombrotrophic bog, *Org. Geochem.*, *33*(7), 675–690.

Raghoebarsing, A. A., et al. (2005), Methanotrophic symbionts provide carbon for photosynthesis in peat bogs, *Nature*, *436*(7054), 1153–1156, doi:10.1038/nature03802.

Roulet, N., P. Lafleur, P. Richard, and T. Moore (2007), Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland, *Global Change Biol.*, *13*, 397–411.

Rousseeuw, P. J., I. Ruts, and J. W. Tukey (1999), The bagplot: A bivariate boxplot, *Am. Stat.*, *53*(4), 382–387, doi:10.1080/00031305.1999.10474494.

Sachse, D., J. Radke, and G. Gleixner (2006), δD values of individual n-alkanes from terrestrial plants along a climatic gradient—Implications for the sedimentary biomarker record, *Org. Geochem.*, *37*(4), 469–483, doi:10.1016/j.orggeochem.2005.12.003.

Sachse, D., et al. (2012), Molecular paleohydrology: Interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms, *Annu. Rev. Earth Planet. Sci.*, *40*(1), 221–249, doi:10.1146/annurev-earth-042711–105535.

Segers, R. (1998), Methane production and methane consumption: A review of processes underlying wetland methane fluxes, *Biogeochemistry*, *41*(1), 23–51.

Smith, F., and K. Freeman (2006), Influence of physiology and climate on δD of leaf wax n-alkanes from C_3 and C_4 grasses, *Geochim. Cosmochim. Acta*, *70*, 1172–1187.

Tipple, B. J., M. A. Berke, and C. E. Doman (2013), Leaf-wax n-alkanes record the plant–water environment at leaf flush, *Proc. Natl. Acad. Sci. U. S. A.*, *110*(7), 2659–2664.

Williams, T., and L. Flanagan (1996), Effect of changes in water content on photosynthesis, transpiration and discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$ in *Pleurozium* and *Sphagnum*, *Oecologia*, *108*(1), 38–46.

Xie, S., C. Nott, L. Avsejs, D. Maddy, and F. M. Chambers (2004), Molecular and isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction, *Geochim. Cosmochim. Acta*, *68*, 2849–2862.

Yu, Z., J. Loisel, M. R. Turetsky, and S. Cai (2013), Evidence for elevated emissions from high-latitude wetlands contributing to high atmospheric CH_4 concentration in the early Holocene, *Global Biogchem. Cycles*, *27*, 131–140, doi:10.1002/gbc.20025.