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Influence of cellulose oxygen isotope variability in sub-fossil *Sphagnum* and plant macrofossil components on the reliability of paleoclimate records at the Mer Bleue Bog, Ottawa, Ontario, Canada

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ABSTRACT

This study provides a new understanding of cellulose oxygen ($\delta^{18}O_{cel}$) isotopic variability in various plant macrofossils, particularly *Sphagnum*, derived from an ~9200 year succession of Holocene peat in Mer Bleue Bog, Ottawa, Ontario, Canada. The variation of $\delta^{18}O_{cel}$ isotopic composition, commonly used as a proxy of paleotemperature and paleoprecipitation, was compared between (1) *Sphagnum* and other plant macrofossils down core and (2) *Sphagnum* species within and between samples.

The most common *Sphagnum* species encountered was *Sphagnum magellanicum* von Bridel, 1798 with lesser amounts of *Sphagnum capillifolium* (Breutel) Steudel, 1824, *Sphagnum fuscum* Klinggräff, 1872 and *Sphagnum angustifolium* Jensen, 1896. There is a statistically significant offset in $\delta^{18}O_{cel}$ isotopic values (mean offset: 1.6‰, standard deviation = 3.2‰, $n = 19$) obtained from *Sphagnum* in comparison with values obtained from other plant macrofossils, particularly rhizomes. The $\delta^{18}O_{cel}$ isotopic offset (mean offset: 0.1‰, standard deviation = 1.0‰, $n = 11$) between *Sphagnum* specimens from the same core horizons, irrespective of the species analyzed, was statistically insignificant at >95%.

These results indicate that $\delta^{18}O_{cel}$ isotopic analysis of bulk peat material with high percentage of rhizome and other vascular plants could result in erroneous paleoclimate reconstructions.

1. Introduction

Proxy-derived reconstructions of past climates result in a greater understanding of the range of natural variability within present day climatic zones and permit the development of better informed predictions of potential future climatic trends. Analyses of peat deposits are particularly useful in this context as peat deposits have long been known to be an ideal archive of paleoclimatic variation (e.g., Brenninkmeijer et al., 1982; Francey and Farquhar, 1982; O'Leary et al., 1986; Aucour et al., 1996; Anderson et al., 1998).

Peat organic matter (i.e., bulk peat or bulk *Sphagnum*) is comprised primarily of cellulose (15–50%), hemicellulose (10–40%), lignin (5–30%), proteins (2–15%) and various lipids (Haider, 1996). Cellulose from these bog environments is commonly used as a paleoclimate proxy because it is amongst the most isotopically stable organic materials known, even under conditions of partial decomposition (Epstein et al., 1976, 1977; Feng et al., 1993; Jędrysek and Skrzypek, 2005).

Several studies carried out on peat bog deposits have shown a strong relationship between plant cellulose isotopic signatures and temperature and/or humidity (e.g., DeNiro and Epstein, 1979, 1981; Edwards et al., 1985; Sternberg et al., 1986; Sukumar et al., 1993; White et al., 1994a,b). The uppermost living part of peat bogs (acrotelms) in ombrotrophic bog settings have $\delta^{18}O_{cel}$ isotope signatures consistent with the interpretation that the source water utilized by plants during cellulose synthesis records the isotopic signature of meteoric water (Dansgaard, 1964; Rozanski et al., 1993; Daley et al., 2009).

As the $\delta^{18}O$ of precipitation at mid- to high latitudes covaries with local air temperature in continental settings (Dansgaard, 1964; Rozanski et al., 1993; Fricke and O'Neil, 1999) several studies have concluded that analysis of $\delta^{18}O_{cel}$ from *Sphagnum* provides one of the most sensitive and reliable proxies for paleotemperature reconstruction in ombrotrophic bog sections (Barbour et al., 2001; Taylor, 2008; Daley et al., 2009, 2010). *Sphagnum*, with its lack of roots, functioning guard cells, vascular tissues and the simplicity by which it incorporates meteoric water into cellulose, is characterized by growth that can be directly linked to the isotopic composition of growing season precipitation. As a result, no significant change in isotopic signature occurs between source water and its accumulation in *Sphagnum* (Ménot-Combes et al., 2002; Zanazzi and Mora, 2005; Daley et al., 2009). However, an enrichment factor of +27‰ associated with cellulose biosynthesis was determined experimentally (Aucour et al., 1996; Ménot-Combes et al., 2002; Zanazzi and Mora, 2005; Daley et al., 2009; Tillman et al., 2010; Skrzypek et al., 2011).

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Paleotemperature reconstructions based on bulk peat have also been carried out (Hong et al., 2000, 2001). However, concerns have been raised that $\delta^{18}O$ signatures obtained from bulk peat, which contains a mixture of cellulose from both vascular and non-vascular plant and other organic material derived from partly decayed plant species, might be significantly different from the $\delta^{18}O_{cel}$ signatures obtained from *Sphagnum* cellulose alone. This is because only the cellulose would remain largely unaltered by the effects of burial, humification and diagenesis over time (Ménot and Burns, 2001; Pancost et al., 2003). The present study investigates $\delta^{18}O_{cel}$ variation for the last ~9200 years. Thus, it is important to take into account the effect of burial and humification on peat material and on $\delta^{18}O$ in particular over several millennia (Ménot and Burns, 2001). It has been shown that long term burial and humification removes the more labile organic fractions susceptible to decomposition, which could change the $\delta^{18}O_{cel}$ values (Ménot and Burns, 2001). In contrast, cellulose is one of the most isotopically stable organic materials known, withstanding even conditions of partial decomposition (Epstein et al., 1976, 1977; Feng et al., 1993; Jędrysek and Skrzypek, 2005). In addition, analyzing a single plant component also reduces variability in isotopic signatures because different plant components, such as vascular and non-vascular plants, have different isotope signatures as a result of their distinct biosynthetic pathways. Consequently, many studies analyzed plant cellulose, rather than cellulose derived from bulk organic material (Leavitt and Danzer, 1993).

A number of studies have investigated the relationship between climate parameters and modern *Sphagnum* cellulose stable isotopic signatures (Ménot and Burns, 2001; Ménot-Combes et al., 2002; Zanazzi and Mora, 2005; Loader et al., 2007; Skrzypek et al., 2007a,b; Moschen et al., 2009). However, *Sphagnum* $\delta^{18}O_{cel}$ has not yet been precisely calibrated to temperature (e.g., Epstein et al., 1976; Feng et al., 1993; Jędrysek and Skrzypek, 2005). This uncertainty is due to the dependence of $\delta^{18}O$ of bog surface water on a combination of several environmental factors. Studies using statistical techniques have reported various relationships between plant $\delta^{18}O_{cel}$ values, humidity, air temperature and precipitation amount (Gray and Thompson, 1976; Burk and Stuiver, 1981), thus complicating the use of $\delta^{18}O_{cel}$ as paleotemperature proxy. For instance Kahmen et al. (2011) combined empirical data analyses with mechanistic model simulations and determined that both air temperature and humidity equally influence $\delta^{18}O_{cel}$ values and that $\delta^{18}O$ values in plant cellulose can be used as a proxy for VPD (leaf to air vapor pressure difference) in tropical ecosystems.

More recently Moschen et al. (2009) investigated the potential use of *Sphagnum* $\delta^{18}O_{cel}$ as a paleoclimate proxy. Cellulose was analyzed from *Sphagnum* branches and stem sections from a 4000 year old peat obtained from the Westeifel volcanic field in Germany. This study demonstrated a significant isotopic offset between *Sphagnum* branch and stem sections (1.5‰ for carbon and 0.9‰ for oxygen). The stable carbon isotopic offset between branch and stem sections decreased with increasing age of the plant material whereas the oxygen isotopic offset was less pronounced but remained consistent in time. However the research of Moschen et al. (2009) as well as the studies outlined did not include a quantitative assessment of the relationship between the variation in plant $\delta^{18}O_{cel}$ and individual plant macrofossils with depth and whether there is an offset between stable isotope values among the various *Sphagnum* species.

In order to address this gap considerable effort has been expended in this study to ensure that plant macrofossil species were isolated and properly identified, particularly as the research involves comparisons between *Sphagnum* and other plant macrofossils, as well as among different *Sphagnum* species within and between samples.

The purpose of this study is (1) to use an improved sampling strategy to provide a new understanding of $\delta^{18}O_{cel}$ variability in peat plant macrofossils in general and in *Sphagnum* in particular in a 6 m peat profile deposited over several thousand years and (2) to quantify the potential of stable isotope analyses of selected peat constituents such as cellulose in paleoclimate research. The inter- and intra-plant $\delta^{18}O_{cel}$ variability is investigated to determine if there is a significant difference in $\delta^{18}O_{cel}$ values between (1) *Sphagnum* and the other plant macrofossils down core and (2) *Sphagnum* species within and between samples.

2. Geographic and geological setting

Mer Bleue Bog is a designated Provincial Conservation Area located in the eastern portion of the National Capital Region of Canada within the city limits of Ottawa, Ontario (45.41°N latitude, 75.48°W longitude, 69 m above mean sea level). The east–west oriented and oval shaped bog encompasses ~28 km^2 and is comprised of three separate lobes (Fig. 1). Deglaciation in the area of Mer Bleue Bog occurred ~13,200 years ago. This part of the lower Ottawa River lowlands was then inundated by post-glacial Lake Iroquois and subsequently by the Champlain Sea marine incursion, which resulted in the deposition of laminated silt and clay over sandy, silty gravel and limestone (Anderson, 1988; Roulet et al., 2007). An extensive postglacial channel system was carved through the area as a result of fluvial outbursts of the Ottawa River during the establishment of the early upper Great Lakes between 12,000 and 9500 cal yBP. Isostatic rebound resulted in a replacement of marine conditions by fresh water in the basin by ~10,600 years ago (Lampsilis Lake phase; Elson, 1969). The present day Mer Bleue peatland lies within an abandoned postglacial channel of the Ottawa River that was eroded into the floor of the Champlain Sea basin. The peatland formed over the past 8400 years, initially as a fen and transitioning to a bog by ca. 7100–6800 cal yBP (Auer, 1930; Mott and Camfield, 1969; Roulet et al., 2007). The modern Mer Bleue Bog is characterized by peat depths varying from 6 m near the center to 0.3 m at the margins (Joyal, 1970; Roulet et al., 2007).

Mer Bleue Bog is a rare domed ombrotrophic bog, dominated by *Sphagnum*, where all nutrients and the water supply derive strictly from precipitation rather than from ground water or river runoff. The overall bog surface has a hummock–hollow microtopography with both hummocks and hollows being primarily covered by *Sphagnum* mosses (Roulet et al., 2007). During the winter season, Mer Bleue Bog is completely frozen and often completely covered by snow, although the frost line only extends down a few cm below the surface. During the spring to fall growing season, fast growing *Sphagnum* dominates the central part of the bog together with cotton grasses, and minor occurrences of cranberries and blueberries. The edge of the bog is also dominated by *Sphagnum* and other plants such as pitcher plants, wild orchids and sundews. Areas of fen remain around the margins of the Mer Bleue Bog and are dominated by aquatic plants including *Potamogeton* ssp. (pond weed), *Nuphar* ssp. (yellow water lily), *Nymphea* ssp. (white water lily), *Typha* spp. (cattail), *Scirpus* spp. (bulrush), and *Ranunculus* ssp. (buttercup).

Hummock-forming *Sphagnum* species are mostly *Sphagnum fuscum* Klinggräff, 1872 and *Sphagnum capillifolium* (Breutel) Steudel, 1780. *S. fuscum* generally occupies the driest area of the bog and occurs on the top of large hummocks. In the hollows *Sphagnum angustifolium* Jensen, 1890 and *Sphagnum magellanicum* von Bridel, 1798 are most common. *S. magellanicum* is the dominant *Sphagnum* species in Mer Bleue Bog. *S. angustifolium* can grow submerged.

It is often difficult to differentiate *Sphagnum* species, particularly down core. However, there are characteristic features that may make it possible to identify individual species. *S. magellanicum* is characterized by a very stiff stem, many large fascicles, a prominent capitulum and is deep red in color. *S. capillifolium* is a smaller plant with tightly packed fascicles. It has a very large capitulum and is bright red in color. *S. fuscum* is small, with a flat capitulum, and is

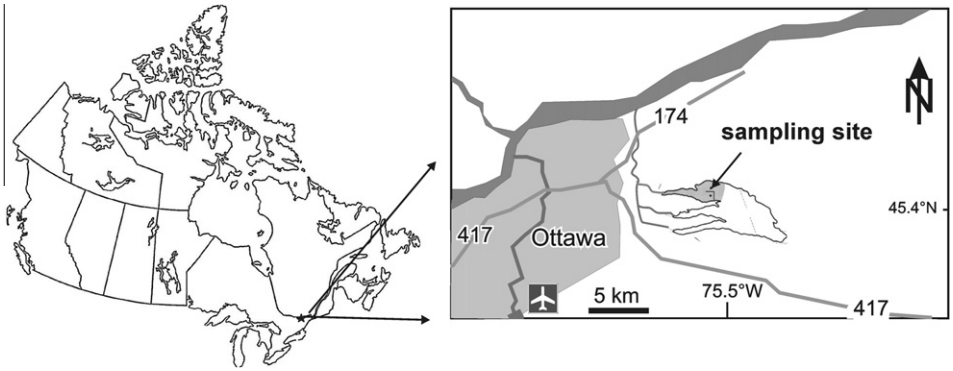

Fig. 1. Location map of the Mer Bleue Bog, Ottawa, Ontario in eastern Canada. Asterisk marks the sampling site at the northwestern arm of Mer Bleue.

dark brown in color. *S. angustifolium* is small, often more compact than the other species and is characterized by a greenish color.

3. Field sampling and material collection

Cores used in the present study were collected in March 2008 using a Russian Auger corer from close to the center of Mer Bleue Bog at N45°24.653′, W75°31.064′ adjacent to the coring location of Roulet et al. (2007). After clearing 70 cm of snow cover, cores were collected very near each other at the top of a very low relief hummock in a lawn-like area to minimize complications arising from marginal variation in the water table. Coring protocol when using a Russian Auger in bog settings stipulates that each core be comprised of offsets collected from two different holes to ensure

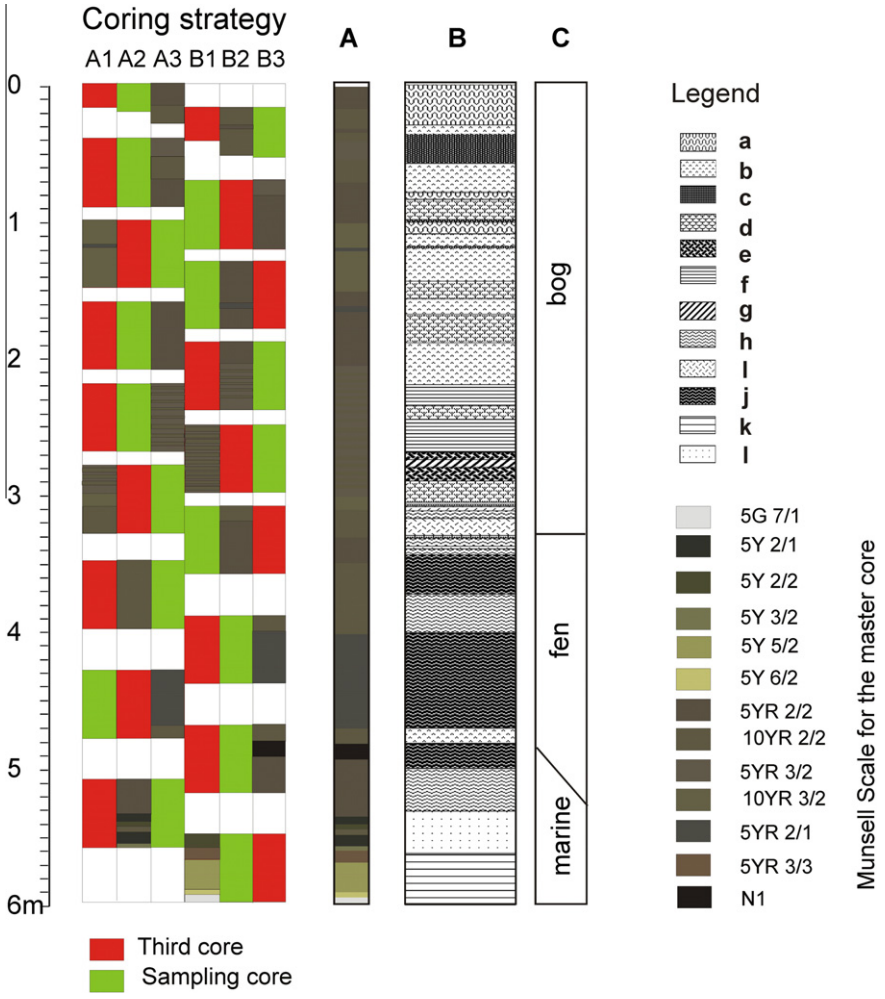

Fig. 2. Mer Bleue coring strategy and sedimentology: Best preserved core used for non-destructive lithological description and archiving (''master core''), sampling core used for geochemical analyses. (A) Rock color code following Munsell Chart (Munsell, 1975), (B) Lithology, lithotypes: a: Coarse grained *Sphagnum* dominated peat facies, b: *Sphagnum* dominant peat facies, c: Charcoal rich peat facies, d: Rhizome dominant peat facies, e; Rhizome dominant peat facies with dark rootlets of pteridophytae, f: Alternation of *Sphagnum* and rhizome dominant peat facies; g: *Sphagnum*-dominant peat facies with dark rootlets of pteridophytae, h: Rhizome dominant peat facies with dark rootlets of pteridophytae and reddish brown leaves, i: *Sphagnum* dominant peat facies with dark rootlets of pteridophytae and reddish brown leaves, j: Dark rootlets of pteridophytae and reddish brown leaves dominant peat facies, k: Marine clay dominant facies, l: Tissue remains of herbacea dominant peat-clay mixed facies. (C) Depositional environments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

complete recovery of the section (e.g., Jowsey, 1966). To meet the sampling requirements of this research triplicate cores were collected, which required extraction of core intervals from six closely spaced holes. The Russian corer permitted the retrieval of cores 50 cm long by 5.5 cm diameter. Core overlap was 20 cm through the uppermost 3.5 m of the core and 10 cm through the lower 2.5 m of the core for a complete recovery of ~6 m of sediment, terminating in the uppermost few cm of the underlying Champlain Sea marine clay deposits (Fig. 2). Retrieved cores were carefully covered in plastic wrap, secured in labeled plastic half-tubes and stored at 4 °C in a core storage facility at Carleton University. The cores were subsequently logged, photographed and X-rayed to identify any sedimentary structures. High resolution subsampling (i.e., 1 cm thick slices) was then carried out at 2 cm intervals in preparation for microscopy and geochemical analyses.

4. Methods

4.1. Plant macrofossil separation

Peat samples were gently heated in a 5% KOH solution for ~30 min to dissolve humic and fulvic acids. The samples were then gently disaggregated on a 125 μm sieve using deionized water. Isolated plant remains on the sieve were transferred to vials and kept immersed in distilled water to avoid damage and disintegration resulting from desiccation. The suspended plant macrofossil remains were examined using an Olympus SZH-1 stereo microscope and identified using several illustrated moss identification guides (Grosse-Brauckmann, 1972, 1974; Lévesque et al., 1988; Smith, 2004).

4.2. Cellulose oxygen isotope analytical technique

For $\delta^{18}O_{cel}$ analyses, plant macrofossils including separated stem sections of *Sphagnum* were hand picked from a Petri dish under a binocular microscope and placed in porcelain crucibles. Where possible, pairs of samples from different *Sphagnum* species from within the same samples were selected. The picked samples were placed in porcelain crucibles and dried in an oven at ~50 °C for 24 h. The samples were then powdered, weighed, labeled, placed in small plastic vials and sent to the University of Saskatchewan for $\delta^{18}O_{cel}$ analysis.

Cellulose extraction methods include modifications of the Jayme–Wise and diglyme-HCl methods (e.g., Cullen and MacFarlane, 2005). The diglyme-HCl method is easier to use but leaves a small lignin residue in the crude cellulose that may affect stable isotope values, whereas α-cellulose produced by the Jayme–Wise method is relatively pure (Cullen and MacFarlane, 2005).

Samples were analyzed at the University of Saskatchewan isotope laboratories using a Thermo Finnigan TC/EA coupled to a Conflo III and Delta Plus XL mass spectrometer. Prior to analysis cellulose samples were heated at 60 °C in a vacuum oven for 2 h to drive off any remaining moisture, then immediately transferred and flushed in a zero blank autosampler. Samples were then dropped under a helium atmosphere into a carbon furnace and pyrolyzed at 1450 °C to form hydrogen and/or carbon monoxide gases. These gases were then carried in a helium stream to a GC column held at 100 °C to separate the gases before being diluted in the Conflo III and passed to the mass spectrometer for analysis. Isotope ratios were blank corrected and reported in per mil notation relative to the VSMOW-VSLAP scale. In-house oxygen standards were calibrated against international standards USGS-34 ($\delta^{18}O = -27.9‰$ VSMOW) and USGS-35 ($\delta^{18}O = 57.5‰$ VSMOW). An intermediate international standard, IAEA-NO3, gave the result $\delta^{18}O = 25.53 \pm 0.27‰$ VSMOW ($n = 23$) during calibration of the in-house standards compared to the accepted value of $\delta^{18}O = 25.6 \pm 0.4‰$ VSMOW. Two in-house standards were subsequently used to set up a calibration line and a third was used to monitor the accuracy of data obtained. The accuracy of $\delta^{18}O$ data was ±0.11‰ ($n = 25$). The %O measurements had an accuracy of ±0.5%. The actual sample errors may be greater than these due to heterogeneity and more accurate data may be obtained through analytical repetition.

4.3. Analysis of variance (ANOVA) method

Single factor ANOVA (e.g., Davis, 2002) was applied to test the null hypothesis (H_0) that $\delta^{18}O_{cel}$ variance between groups of plants is insignificant at confidence level $\alpha = 0.05$, which equals 95% confidence level as compared to the variability within the specific plant groups through depth.

Single factor ANOVA uses only one factor in k levels $i = 1, 2, \ldots, k$. Each level i may have different numbers of observations (=repetitions) n_i. The total number of observations N is defined by:

$$N = \sum_{i=1}^{k} \sum_{j=1}^{n_i} \quad (1)$$

The model for the independent variable to be tested is defined by: $x_{ij} = \mu + \alpha_i + \varepsilon_{ji}$ where μ is the mean of all observations, α is the effect of known factor(s) (i.e., =the variability between each level of factor) and ε is the random error. The source of variation is represented by the sum of squares (SS) where SST (total) = SSG (between levels) + SSE (within levels).

$$\mathrm{SST} = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (x_{ij} - \bar{x})^2 = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (x_{ij})^2 - N\bar{x}^2 \quad (2)$$

$$\mathrm{SSG} = \mathrm{SS}_{\alpha} = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (\bar{x}_i - \bar{x})^2 = \sum_{i=1}^{k} n_i (\bar{x}_i - \bar{x})^2 \quad (3)$$

$$\mathrm{SSE} = \sum_{i=1}^{k} \sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)^2 \quad (4)$$

where $\bar{x}_i$ refers to the mean values for the observations for each level i.

Then, the means of squares (MS) are calculated by $\mathrm{MS}_{total} = \mathrm{SS}_{total}/\mathrm{DF}_{total}$ with MSG (between levels) = SSG/DFG, MSE (within levels) = SSE/DFE with DF referring to "degrees of freedom". Finally, the F-test can be used to determine the equality of variances and the F_{crit} (F critical) is provided by probability distribution called the F-distribution (e.g., Davis, 2002). The test value F is calculated by $F_{calc} = \mathrm{MSG/MSE}$. If $F_{calc} \geqslant F_{crit}$ than H_0 is rejected at the confidence level = 100% $(1 - p)$. The p value (P) is defined as the smallest level of significance at which the null hypothesis (H_0) would be rejected for specific test (e.g., Davis, 2002).

The null hypotheses (H_0) were tested to determine that all means of $\delta^{18}O_{cel}$ values being compared between different plant macrofossils were statistically the same.

5. Results

5.1. Core sedimentology

The Mer Bleue Bog sequence consists predominantly of well humified herbaceous peat, with abundant remains of *Sphagnum* mosses as well as the remains of monocotyledon rhizomes. The degree of decomposition within the Mer Bleue core sequence was estimated in the laboratory by evaluating the change in peat color and compaction degree with the naked eye in conjunction with the

Munsell soil color chart (Munsell, 1975). As would be expected the degree of decomposition of the peat sediments increased with depth (Fig. 2).

Recovered core material was primarily comprised of relatively fresh and uncompacted *Sphagnum* material within the top 25 cm of the core, which consisted of poorly decayed and weakly compacted *Sphagnum* dominated plant material in the oxic zone (acrotelm). Below 25 cm depth the peat became anaerobic (catotelm) until a depth of 73.5 cm, where the peat was fully compacted.

The interval from 73.5–320 cm consisted of compacted and decomposed peat sediments, primarily *Sphagnum* mosses. Throughout the upper 320 cm, *Sphagnum* was the dominant plant macrofossil making up ~80% of the core. *Sphagnum* species observed through this interval include *S. fuscum*, *S. magellanicum*, *S. capillifolium*, *S. angustifolium* and minor occurrence of *Sphagnum papillosum* Lindberg, 1872. Fen deposition transitioned to bog deposition over a short depth interval (~330–320 cm). Peat formed under fen conditions characterized the core from ~330–500 cm. Peat sediments deposited below 330 cm were decomposed and less compacted than the peat higher in the section and was comprised of *Sphagnum*, rhizomes, wood fragments, roots, networks of pteridophytae, seeds, charcoal, unknown reddish-brown leaves and other minor plant macrofossil components. The lowermost meter of the core (~500–600 cm) was characterized by several alternating bands of fine peat and marine clay, with a fen-marine clay transitional zone being found between ~500–590 cm and typical marine clays being found from ~590–600 cm.

The plant macrofossil assemblages observed in the Mer Bleue Bog cores were characterized by considerable variation with depth and permitted recognition of 12 distinct macrofossil biofacies (Fig. 2):

1. Coarse grained, *Sphagnum* dominated facies (up to 90%);
2. *Sphagnum* dominant facies (up to 80%) with less than 20% wood fragments, charcoal, rootlets and rhizome;
3. Charcoal rich facies (up to 4%);
4. Rhizome dominant facies (up to 90%);
5. Rhizome dominant facies with dark rootlets and root networks of pteridophytae;
6. Alternating bands of *Sphagnum* rich layers and rhizome rich layers;
7. *Sphagnum* dominant facies with root networks of pteridophytae;
8. Rhizome dominant facies (<70%) with up to 20% root networks of pteridophytae and unidentified reddish-brown leaves, minor to rare *Sphagnum* (5%), and roots + wood fragments (all about 15%);
9. *Sphagnum* dominant facies (up to 70%) with up to 20% root network of pteridophytae and unidentified reddish-brown leaves, and minor to rare rhizome + roots + wood fragments (all about 15%);
10. Root networks of pteridophytae (>30%) and unknown reddish-brown leaves;
11. Marine clay dominant facies (more than 60%) with minor tissue remains of herbacae and no *Sphagnum*;
12. Tissue remains of herbacea dominant facies (more than 60%) with marine clay (less than 40%), minor rootlets and no *Sphagnum*.

5.2. Cellulose oxygen isotope composition

Cellulose $\delta^{18}O$ signature determination was based mainly on analysis of *Sphagnum* macrofossils. Other plant macrofossil $\delta^{18}O_{cel}$ were analyzed for comparison with the *Sphagnum* $\delta^{18}O_{cel}$ results from the same horizons and on their own in some sections of the core where *Sphagnum* was absent (Fig. 3; Table 1).

The results show that *Sphagnum* $\delta^{18}O_{cel}$ values range from ~14‰ to 25‰, with most samples falling in a narrower band decreasing from 19‰ in surface peat sediments to 16‰ deeper in the core (Fig. 3). The values obtained from the plant macrofossil species had $\delta^{18}O_{cel}$ values ranging from ~7‰ to 26‰, but were generally characterized by lower $\delta^{18}O_{cel}$ values than obtained for *Sphagnum* at the same core intervals. The plant oxygen percentage (O%) ranged from ~20% to 50% and showed similar trends and differences between *Sphagnum* and other plant macrofossils, with the exception of rhizome values that are up to 10% below those of *Sphagnum* cellulose (Fig. 3).

5.3. Results of analysis of variance (ANOVA)

Paired $\delta^{18}O_{cel}$ analyses were obtained from coexisting rhizomes and *Sphagnum* species (*S. fuscum*) in 11 samples at several core depths. Nineteen additional $\delta^{18}O_{cel}$ paired analyses were obtained from rhizomes coexisting with other *Sphagnum* species. Paired $\delta^{18}O_{cel}$ analyses were also obtained from five samples with coexisting *S. capillifolium* and *S. fuscum* and six samples with coexisting *S. magillanicum* with *S. fuscum*. These sample pairs formed the data set for statistical evaluation.

Results of ANOVA (Table 2) indicate that the different *Sphagnum* species have statistically similar $\delta^{18}O_{cel}$ values where they coexist. The average difference was 0.18‰ between *S. capillifolium* and *S. fuscum* and 0.06‰ between *S. magellanicum* and *S. fuscum.* In contrast the $\delta^{18}O_{cel}$ values obtained for rhizomes were characterized by average values of ~16.7‰, more than 1.5‰ below the average *Sphagnum* sp. values (~18.3‰; Table 2).

To put these results in context, the variability between rhizome and *Sphagnum* $\delta^{18}O_{cel}$ results within samples (i.e., at the same sample depths) was more than three times higher than the observed variability between samples (see F_{calc}, Table 2). This variability between rhizome and *Sphagnum* $\delta^{18}O_{cel}$ is slightly below the 95% confidence level which has been tested for. Thus, at the 95% confidence level, the null hypothesis that $\delta^{18}O_{cel}$ variability between rhizome and *Sphagnum* is statistically insignificant cannot be rejected. The *p*-value of 0.08 suggests however that the null hypothesis would be rejected based on 100% $(1 - p) = 100\%$ (1–0.08) = 92% confidence level (Table 2).

For the *Sphagnum* pairs $F_{calc} \ll F_{crit}$, so the null hypothesis cannot be rejected. A linear correlation test was applied to determine whether the $\delta^{18}O_{cel}$ signature obtained from the rhizomes displayed the same variability as *Sphagnum* samples with depth to determine whether rhizomes could be used if a static offset was applied. The linear correlation between pairs of rhizomes and *Sphagnum* was insignificant ($R^2 = 0.006$, Fig. 4), indicating in addition to the ANOVA results that rhizome $\delta^{18}O_{cel}$ data cannot be mixed with *Sphagnum* for $\delta^{18}O_{cel}$ analysis based temperature reconstruction. In contrast, the coefficient of determination $R^2 = 0.51$ between 6 pairs of *S. magellanicum* with *S. fuscum* $\delta^{18}O_{cel}$ from the same depth is significant at around 90% (Fig. 4). As indicated by ANOVA results (Table 2), the average $\delta^{18}O_{cel}$ difference among different *Sphagnum* species from the same depth is <0.2‰ which is small compared to the ~6‰ range in *Sphagnum* $\delta^{18}O_{cel}$ values through the core (see Fig. 2).

Thus, cellulose $\delta^{18}O$ values obtained from both *Sphagnum* species could be used interchangeably for temperature reconstruction. As an additional test, $\delta^{18}O_{cel}$ analyses were carried out on sub-samples of *S. magellanicum*, *S. capillifolium* and rhizomes from samples at 58 cm, 202 cm and 242 cm core depths, respectively (Table 1).

Analysis results show only a small offset (19.72 – 19.36‰ = 0.36‰) between the *S. magellanicum* duplicates, and (17.71 – 17.09‰ = 0.62‰) between the *S. capillifolium* duplicates whereas the difference between the two rhizome sub-samples was four times larger (18.18 – 16.77‰ = 1.41‰). The standard

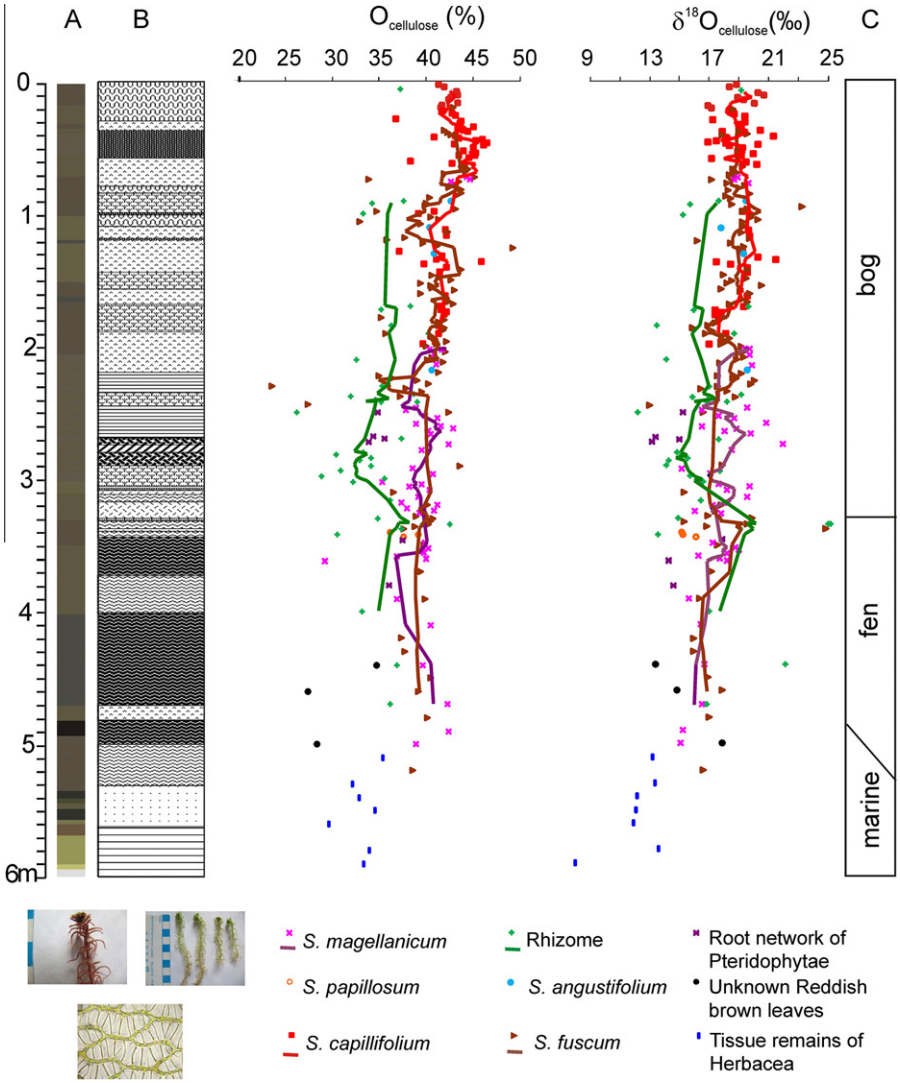

Fig. 3. Oxygen concentration and oxygen isotope data of cellulose from different plant matter of the Mer Bleue Bog core with five point running average for *S. fuscum*, *S. capilliforum*, *S. magillanicum* and rhizome. For details on lithology and peat color see Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

deviation (σ) of the offset between all *Sphagnum*–*Sphagnum* couplets ($n = 11$) is 1‰, compared to all *Sphagnum*–rhizome couplets ($n = 19$) with $\sigma = 3.2$‰. Analytical results from the two samples pairs also indicated that root networks of pteridophytae are characterized by significantly lower $\delta^{18}O_{cel}$ values than the *Sphagnum* samples (samples #125, #181, see Table 1).

Similarly, three sample pairs of *Sphagnum* and unknown reddish-brown leaves (samples #220, #230 and #250) also showed large and highly varying cellulose oxygen isotope value differences ranging from +3‰ to −2‰ (see Table 1). *Sphagnum* and tissue remains of herbacea were never found together in the same samples. Thus offsets and variability between these two groups could not be evaluated.

6. Discussion

The comparative approach taken in this study, where the $\delta^{18}O_{cel}$ values for a variety of individual *Sphagnum* species and other plant macrofossils from the same core horizons were compared against each other, indicates that the use of bulk peat cellulose could lead to erroneous paleotemperature reconstructions. Bulk peat cellulose is composed of a mixture of vascular and non-vascular plant with undetermined percentage of their relative contribution of cellulose mass. For this study, only well preserved *Sphagnum* stems with minor or no significant decay features were picked by microscopic examination. The clear offset of O% and $\delta^{18}O_{cel}$ between *Sphagnum* and the marine matter (Fig. 3) also shows that no sample homogenization occurred associated with mixing of different plant material. Our $\delta^{18}O_{cel}$ results have shown that there is a significant offset between results obtained for non-vascular *Sphagnum* species and vascular plants such as rhizomes and other plant macrofossils (Fig. 4). This finding indicates that the mixing of vascular and non-vascular plants during $\delta^{18}O_{cel}$ isotopic analysis in a stratigraphic section could also result in an erroneous paleotemperature reconstruction. The influence of fluctuating ratios between vascular (e.g., non-*Sphagnum*) and non-vascular (e.g., *Sphagnum*) plants on $\delta^{18}O$ values of cellulose ($\delta^{18}O_{bc}$) in bulk peat can be expressed by

$$\delta^{18}O_{bc} = \frac{x\delta^{18}O_{sc} + \delta^{18}O_{nsc}(100\% - x)}{100\%} \quad (5)$$

with $x = 0 \ldots 100\%$, $\delta^{18}O_{sc}$ represents $\delta^{18}O$ of *Sphagnum* cellulose and $\delta^{18}O_{nsc}$ denotes $\delta^{18}O$ of non-*Sphagnum* cellulose. The changes in the uncertainty related to mixing follows the error propagation rules and the 95% confidence intervals ($\pm 2\sigma$) are calculated from for each percentage of mixture. The end members are for pure non-*Sphag-*

Table 1
Oxygen isotope ratio and oxygen concentration of plant cellulose from Mer Bleue Bog, Ottawa, Ontario.

Sample ID	Depth (cm)	% O	δ^{18}O (‰, VSMOW)	Macrofossils Taxa	Sample ID	Depth (cm)	% O	δ^{18}O (‰, VSMOW)	Macrofossils Taxa
1yss	2	41.4	18.81	*S. capillifolium*	98yss	196	41.0	17.74	*S. capillifolium*
2yss	4	42.0	18.32	*S. capillifolium*	99bss	198	41.2	17.80	*S. fuscum*
3r	6	37.3	19.14	Rhizome	99yss	198	39.7	16.94	*S. capillifolium*
4yss	8	43.3	20.22	*S. capillifolium*	100	200	40.0	19.11	*S. fuscum*
5yss	10	43.4	20.64	*S. capillifolium*	l01dbss	202	41.8	19.36	*S. magellanicum*
6yss	12	42.7	18.38	*S. capillifolium*	101 dbss repeat	202	40.5	19.72	*S. magellanicum*
7yss	14	42.4	19.28	*S. capillifolium*	102bss	204	42.1	19.18	*S. fuscum*
8yss	16	43.4	20.05	*S. capillifolium*	103dbss	206	40.9	19.68	*S. magellanicum*
9Jyss	18	41.7	16.91	*S. capillifolium*	104bss	208	40.6	19.26	*S. fuscum*
10yss	20	41.7	17.13	*S. capillifolium*	105bss	210	40.1	18.79	*S. fuscum*
10bss	20	42.9	18.58	*S. fuscum*	105r	210	32.7	14.05	Rhizome
11yss	22	41.9	18.48	*S. capillifolium*	106bss	212	41.9	17.72	*S. fuscum*
12yss	24	42.8	18.64	*S. capillifolium*	107dbss	214	41.1	19.81	*S. magellanicum*
13yss	26	43.2	19.34	*S. capillifolium*	109bss	218	40.7	19.53	*S. angustifolium*
14yss	28	36.8	17.17	*S. capillifolium*	110bss	220	41.4	19.11	*S. fuscum*
15yss	30	43.8	19.36	*S. capillifolium*	111bss	222	35.0	17.49	*S. fuscum*
16yss	32	43.7	18.95	*S. capillifolium*	112bss	224	36.4	18.56	*S. fuscum*
17yss	34	45.1	19.08	*S. capillifolium*	113Lbss	226	39.1	20.00	*S. angustifolium*
Sb1yss	35	43.6	18.99	*S. capillifolium*	114bss	228	40.3	19.74	*S. fuscum*
18yss	36	43.4	20.43	*S. capillifolium*	115bss	230	23.7	17.55	*S. fuscum*
Sb2yss	37	44.0	17.82	*S. capillifolium*	115r	230	35.4	16.95	Rhizome
Sb2bss	37	41.9	18.94	*S. fuscum*	116bss	232	37.9	16.43	*S. fuscum*
19yss	38	44.5	19.32	*S. capillifolium*	117bss	234	39.6	18.16	*S. fuscum*
Sb3bss	39	42.3	19.49	*S. fuscum*	118r	236	38.7	19.14	Rhizome
20bss	40	42.6	17.88	*S. fuscum*	119r	238	32.0	15.86	Rhizome
Sb4yss	41	43.1	21.32	*S. capillifolium*	119bss	238	37.3	18.70	*S. fuscum*
21yss	42	40.8	18.13	*S. capillifolium*	120r	240	34.7	16.88	Rhizome
Sb5yss	43	43.6	18.15	*S. capillifolium*	121r	242	35.3	16.77	Rhizome
22yss	44	46.0	17.41	*S. capillifolium*	121r repeat	242	39.1	18.18	Rhizome
Sb6yss	45	45.4	16.89	*S. capillifolium*	121dbss	242	32.5	13.89	*S. magellanicum*
23yss	46	46.6	18.13	*S. capillifolium*	122DS	244	27.5	12.92	Root networks of Pteridophytae
Sb7yss	47	43.0	20.28	*S. capillifolium*	122dbss	244	37.3	14.92	*S. magellanicum*
24yss	48	46.2	18.36	*S. capillifolium*	123dbss	246	39.0	19.54	*S. magellanicum*
25yss	50	45.1	19.18	*S. capillifolium*	124dbss	248	37.9	16.46	*S. magellanicum*
26yss	52	44.0	18.92	*S. capillifolium*	125r	250	34.9	15.19	Rhizome
27yss	54	45.4	19.97	*S. capillifolium*	125DS	250	26.3	12.11	Root networks of Pteridophytae
27.5yss	55	44.9	19.40	*S. capillifolium*	125bss	250	42.5	16.56	*S. fuscum*
28yss	56	43.6	19.15	*S. capillifolium*	126dbss	252	40.0	17.58	*S. magellanicum*
28.5yss	57	46.6	17.21	*S. capillifolium*	127dbss	254	41.3	18.67	*S. magellanicum*
29yssRE	58	46.2	17.71	*S. capillifolium*	129dbss	258	39.0	20.83	*S. magellanicum*
29yss	58	44.7	17.09	*S. capillifolium*	130dbss	260	41.6	16 47	*S. magellanicum*
29.5yss	59	46.3	18.68	*S. capillifolium*	131dbss	262	42.9	18.82	*S. magellanicum*
30yss	60	38.3	19.47	*S. capillifolium*	132dbss	264	40.2	17.98	*S. magellanicum*
30.5yss	61	47.3	19.43	*S. capillifolium*	133dbss	266	40.5	19.78	*S. magellanicum*
31yss	62	42.8	20.15	*S. capillifolium*	134DS	268	34.5	13.33	Root networks of Pteridophytae
31.5yss	63	45.4	19.06	*S. capillifolium*	135DS	270	35.6	14.95	Root networks of Pteridophytae
32bss	64	44.8	19.23	*S. fuscum*	135r	270	37.4	17.63	Rhizome
32.5yss	65	44.5	17.17	*S. capillifolium*	136DS	272	33.9	13.11	Root networks of Pteridophytae
33bss	66	44.5	18.57	*S. fuscum*	137dbss	274	42.5	21.92	*S. magellanicum*
33.5yss	67	45.9	20.75	*S. capillifolium*	139dbss	278	39.6	18.46	*S. magellanicum*
34bss	68	44.3	18.78	*S. fuscum*	139r	278	lost	lost	Rhizome
34ayss	69	45.5	18.24	*S. capillifolium*	140r	280	33.3	14.80	Rhizome
35bss	70	42.0	18.34	*S. fuscum*	141r	282	30.5	15.08	Rhizome
35ayss	71	45.0	20.17	*S. capillifolium*	142r	284	34.1	15.54	Rhizome
36bss	72	45.3	18.74	*S. fuscum*	143r	286	33.0	14.26	Rhizome
36dbss	72	44 3	18.86	*S. magellanicum*	145r	290	34.2	14.06	Rhizome
36ayss	73	45 9	19.52	*S. capillifolium*	145bss	290	43.7	17.82	*S. fuscum*
37dbss	74	44.7	18.61	*S. magellanicum*	146dbss	292	38.6	15.11	*S. magellanicum*
37bss	74	34.0	17.11	*S. fuscum*	147r	294	30.9	17.71	Rhizome
38dbss	76	42.7	19.57	*S. magellanicum*	148dbss	296	40.8	17.03	*S. magellanicum*
38bss	76	43.5	18.16	*S. fuscum*	149r	298	28.9	15.67	Rhizome
39bss	78	43.1	19.36	*S. fuscum*	150r	300	35.6	16.20	Rhizome
40bss	80	42.5	18.97	*S. fuscum*	151r	302	32.3	16.56	Rhizome
41bss	82	43.8	18.21	*S. fuscum*	151dbss	302	35.3	16.34	*S. magellanicum*
42bss	84	43.7	19.68	*S. fuscum*	152dbss	304	39.5	17.63	*S. magellanicum*
43bss	86	43.3	18.09	*S. fuscum*	153dbss	306	38.3	19.71	*S. magellanicum*
44bss	88	40.4	18.45	*S. fuscum*	154dbss	308	40.5	18.16	*S. magellanicum*
45r	90	37.7	17.60	Rhizome	155dbss	310	36.6	15.27	*S. magellanicum*
45Lbss	90	42.7	19.43	*S. angustifolium*	157dbss	314	39.2	19.54	*S. magellanicum*
46bss	92	40.1	18.49	*S. fuscum*	158r	316	36.2	16.40	Rhizome
46r	92	34.3	15.65	Rhizome	159dbss	318	37.4	18.25	*S. magellanicum*
47bss	94	39.9	23.21	*S. fuscum*	160bss	320	40.2	17.75	*S. fuscum*
48yss	96	43.1	19.56	*S. capillifolium*	160dbss	320	41.3	17.21	*S. magellanicum*

(*continued on next page*)

Table 1 (*continued*)

Sample ID	Depth (cm)	% O	δ^{18}O (‰, VSMOW)	Macrofossils Taxa	Sample ID	Depth (cm)	% O	δ^{18}O (‰, VSMOW)	Macrofossils Taxa
49bss	98	34.8	19.18	*S. fuscurn*	161dbss	322	38.1	17.34	*S. magellanicum*
49yss	98	41.0	19.23	*S. capillifolium*	162dbss	324	40.9	15 97	*S. magellanicum*
50r	100	33.3	15.22	Rhizome	162bss	324	39.6	17.38	*S. fuscum*
50yss	100	39.3	19.73	*S. capillifolium*	163dbss	326	39.4	17.76	*S. magellanicum*
51bss	102	40.5	19.80	*S. fuscum*	164bss	328	39.0	16.89	*S. fuscum*
52bss	104	41.4	20.27	*S. fuscum*	165r	330	35.0	14.89	Rhizome
53dbss	106	33.0	19.21	*S. magellanicum*	165bss	330	40.8	19.89	*S. fuscum*
54dbss	108	39.5	19.22	*S. magellanicum*	166bss	332	40.6	15.26	*S. fuscum*
55Lbss	110	40.5	17.76	*S. angustifolium*	167r	334	42.6	24.96	Rhizome
56yss	112	42.1	19.65	*S. capillifolium*	167r repeat	334	39.8	25.18	Rhizome
57bss	114	42.3	19.12	*S. fuscum*	167bss	334	40.4	19.51	*S. fuscum*
58yss	116	42.2	19.68	*S. capillifolium*	168bss	336	38.9	16.79	*S. fuscum*
59bss	118	37.8	19.70	*S. fuscum*	169r	338	37.4	19.06	Rhizome
60bss	120	35.9	16.20	*S. fuscum*	169bss	338	37.7	24.82	*S. fuscum*
61bss	122	39.8	18.44	*S. fuscum*	170syss	340	36.3	15.13	*S. papillosum*
62bss	124	41.2	19.32	*S. fuscum*	171r	342	30.6	13.53	Rhizome
63bss	126	49.3	19.06	*S. fuscum*	171syss	342	39.3	15.17	*S. papillosum*
64yss	128	37.2	19.23	*S. capillifolium*	172syss	344	37.7	16.08	*S. papillosum*
65Lbss	130	40.9	19.31	*S. angustifolium*	173DS	346	37.5	17.88	Root networks of Pteridophytae
66yss	132	41.7	20.29	*S. capillifolium*	174dbss	348	39.9	17.23	*S. magellanicum*
67yss	134	41.1	21.42	*S. capillifolium*	175bss	350	39.8	17.51	*S. fuscum*
68yss	136	45.9	17.47	*S. capillifolium*	176dbss	352	40.3	18.71	*S. magellanicum*
69yss	138	39.8	18.37	*S. capillifolium*	177dbss	354	39.7	18.99	*S. magellanicum*
70yss	140	42.3	19.49	*S. capillifolium*	178dbss	356	39.8	18.14	*S. magellanicum*
71bss	142	43.7	19.50	*S. fuscum*	179dbss	358	37.0	16.21	*S. magellanicum*
72yss	144	41.5	19.50	*S. capillifolium*	180dbss	360	40.0	17.69	*S. magellanicum*
73bss	146	40.7	17.95	*S. fuscum*	181DS	362	22.1	14.24	Root networks of Pteridophytae
74bss	148	41.4	19.42	*S. fuscum*	181dbss	362	29.3	18.18	*S. magellanicum*
75bss	150	42.6	18.50	*S. fuscum*	185bss	370	39.5	17.08	*S. fuscum*
76bss	152	39.5	18.16	*S. fuscum*	190DS	380	36.1	14.59	Root networks of Pteridophytae
77bss	154	40.5	20.52	*S. fuscum*	195bss	390	39.9	16.33	*S. fuscum*
78bss	156	40.3	17.80	*S. fuscum*	195dbss	390	37.0	15.63	*S. magellanicum*
79bss	158	43.1	19.02	*S. fuscum*	200r	400	33.3	16.97	Rhizome
80bss	160	39.4	19.69	*S. fuscum*	205dbss	410	40.5	16.39	*S. magellanicum*
81yss	162	42.1	19.50	*S. capillifolium*	210bss	420	37.4	15.88	*S. fuscum*
82bss	164	42.2	19.15	*S. fuscum*	215bss	430	37.8	15.91	*S. fuscum*
83bss	166	42.5	19.56	*S. fuscum*	220r	440	36.9	22.08	Rhizome
84bss	168	43.0	18.30	*S. fuscum*	220rbl	440	34 8	13.38	Unknown Reddish-brown leaves
85yss	170	41.5	19.28	*S. capillifolium*	220dbss	440	39.7	16.65	*S. magellanicum*
85r	170	38.4	19.61	Rhizome	225bss	450	40.6	16.85	*S. fuscum*
86r	172	35.3	15.93	Rhizome	230bss	460	39.2	17.85	*S. fuscum*
86yss	172	41.6	17.34	*S. capillifolium*	230rbl	460	27.4	14.77	Unknown Reddish-brown leaves
87yss	174	42.3	17.78	*S. capillifolium*	235dbss	470	42.3	16.49	*S. magellanicum*
88yss	176	42.0	17.37	*S. capillifolium*	235r	470	36.3	16.83	Rhizome
89bss	178	35.3	17.94	*S. fuscum*	240bss	480	40.2	16.99	*S. fuscum*
90bss	180	41.9	17.61	*S. fuscum*	245bss	490	42.5	15.17	*S. fuscum*
91bss	182	41.4	17.36	*S. fuscum*	250rbl	500	28.4	17.88	Unknown Reddish-brown leaves
92bss	184	41.4	16.90	*S. fuscum*	250dbss	500	39.0	15.08	*S. magellanicum*
92r	184	36.4	13.47	Rhizome	255trh	510	35.5	13.19	Tissue remains of Herbacea
93bss	186	42.0	15.86	*S. fuscum*	260bss	520	38.6	16.56	*S. fuscum*
94bss	188	41.9	17.18	*S. fuscum*	265trh	530	32.2	13.37	Tissue remains of Herbacea
95yss	190	41.3	18.09	*S. capillifolium*	270trh	540	33.0	12.15	Tissue remains of Herbacea
95bss	190	35.8	16.74	*S. fuscum*	275trh	550	34.7	12.10	Tissue remains of Herbacea
95r	190	40.2	18.66	Rhizome	280trh	560	29.8	11.94	Tissue remains of Herbacea
96bss	192	40.6	17.82	*S. fuscum*	290trh	580	34.0	13.55	Tissue remains of Herbacea
97bss	194	41.0	18.29	*S. fuscum*	295trh	590	33.5	7.99	Tissue remains of Herbacea
98bss	196	42.2	18.06	*S. fuscum*					

Table 2
ANOVA – Single factor of 5 O data.

Group 1	Group 2	Average	Average	No of pairs	Mean of squares		F_{calc}	*P*-value	F_{crit}
		Group 1	Group 2		Between groups	Within groups			95%
S. capillifolium	*S. fuscum*	17.96	18.14	5	0.08	0.82	0.10	0.76	5.32
S. magellanicum	*S. fuscum*	17.64	17.58	6	0.01	1.67	0.01	0.93	4.96
S. fuscum	Rhizome	18.71	16.36	11	30.27	9.20	3.29	0.08	4.35
Sphagnum sp.	Rhizome	18.26	16.70	19	22.98	7.26	3.17	0.08	4.11

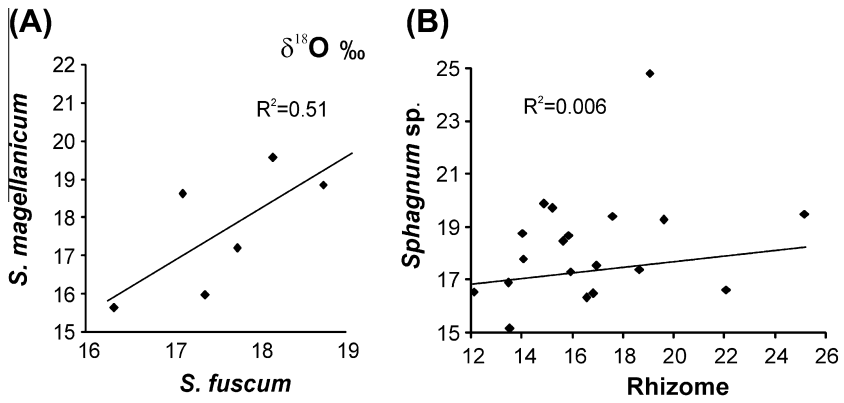

Fig. 4. Correlation of cellulose oxygen isotope data of different plant taxa with coefficient of determination of linear trend line (R^2). (A) *Sphagnum magellanicum* vs. *Sphagnum fuscum* and (B) all lumped *Sphagnum* species vs. rhizome. Black lines in graph area show linear regression lines.

num derived from rhizome 16.7‰ with σ = 1‰ (table 2), and for pure *Sphagnum* mixture 18.26‰ with σ = 0.64‰ (table 2).

A contribution of 20% of non-*Sphagnum* plants decreases the $\delta^{18}O_{cel}$ in bulk peat by 0.3‰ and increases the 95% confidence interval from 2.5‰ to 3‰ (Fig. 5). Thus only bulk peat cellulose with very small amounts of vascular plants may be acceptable for paleotemperature reconstruction. This would allow avoiding the time consuming separation of species for isotope analysis. In the Mer Bleue core, the peat lithology varied strongly ranging from dominantly *Sphagnum* peat to dominantly rhizome bearing peat (see Fig. 2) and the use of $\delta^{18}O_{cel}$ from bulk peat would not be acceptable for paleotemperature reconstruction. Consequently, given the significant differences and inconsistencies characterizing $\delta^{18}O_{cel}$ signatures obtained for rhizome and other non-*Sphagnum* plants, the use of bulk peat cellulose in paleotemperature reconstructions misrepresents the true $\delta^{18}O$ signature of source waters. In contrast, the use of bulk *Sphagnum* will unlikely result in any misrepresentation of the $\delta^{18}O_{cel}$ signature and segregation of *Sphagnum* species prior to isotope analyses is not necessary. Results from bulk peat cellulose would be comprised of mixed sources of $\delta^{18}O_{cel}$ and would thus not be comparable to $\delta^{18}O$ in source water. In the case of *Sphagnum*, $\delta^{18}O_{cel}$ in a single *Sphagnum* species or in a mixture of different *Sphagnum* species will provide much more accurate paleotemperature reconstructions.

Although not dealt with in this research, previous research on $\delta^{13}C_{cel}$ isotopic signatures in various bog plant macrofossils, came to similar conclusions (Ménot and Burns, 2001). In that study the reproducibility and the shift between $\delta^{13}C$ values in the cellulose

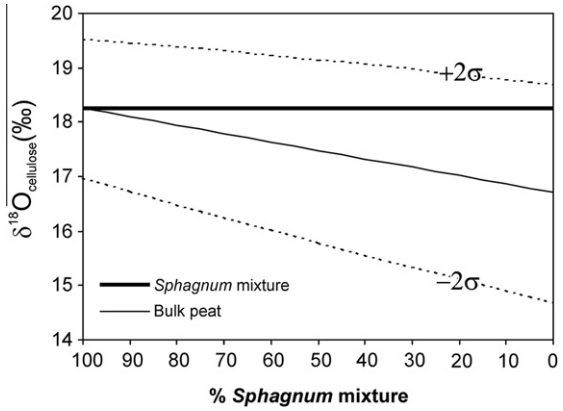

Fig. 5. Effect of mixture of cellulose in bulk peat from vascular (e.g., rhizome) and non-vascular plants (e.g., *Sphagnum*) on oxygen isotope data based on average values from this study (*Sphagnum* = 18.3, rhizome 16.7‰). Black vertical bar: ±1σ = 1‰ determined between *Sphagnum* species in this study.

fraction and whole plant (bulk) in both vascular and non-vascular plants from ombrotrophic peat bogs, along an altitude transect in the Swiss Alps was examined. Ménot and Burns (2001) correlated the difference between bulk and cellulose $\delta^{13}C$ values to the presence in bulk plant material of lipid and lignin fractions, depleted in $\delta^{13}C$ (Park and Epstein, 1960). They suggested that for paleoclimate work it is important not to neglect the effect of burial and humification of peat material on $\delta^{13}C$ values. In their view, these factors (i.e., burial and humification) will remove the more labile organic fractions, which could change the $\delta^{13}C$ values of bulk organic matter even within individual species.

The observations of Ménot and Burns (2001) regarding the applicability of $\delta^{13}C_{cel}$ are in accordance with our conclusions regarding the applicability of $\delta^{18}O_{cel}$ analysis in bog settings. We conclude that the $\delta^{18}O_{cel}$ analysis of individual non-vascular species (i.e., *Sphagnum*) is crucial if reliable isotope-proxy temperature records are to be obtained from peat. Our conclusions thus contrast considerably with those of Skrzypek et al. (2007a) who reported that because plant cellulose is considered the most isotopically stable chemical compound, even under conditions of partial decomposition (Epstein et al., 1976; Feng et al., 1993; Jędrysek and Skrzypek, 2005), the primary $\delta^{18}O_{cel}$ plant composition can be well preserved in bulk organic matter, especially in acid bog or fen conditions (Jędrysek and Skrzypek, 2005). The results reported in the present study indicate that while bulk samples obtained from a bog surface may be internally consistent, the $\delta^{18}O_{cel}$ obtained for non-*Sphagnum* components rapidly diverges down core.

The observed statistically insignificant average $\delta^{18}O_{cel}$ isotopic offset between *Sphagnum* pairs within samples and between species at the same core horizons (Table 2) suggests that down core identification of the exact *Sphagnum* species is not critical. This finding is fortunate as identification of exact *Sphagnum* species can be particularly difficult in old peat material where leaves are mostly detached from their branches and or their stems. The variability of up to ±1.5‰ between different *Sphagnum* species appears high, but this range is in accordance to what has been found in multispecies surveys on monthly scale covering an entire growth season in the British Isles (Daley et al., 2010), with similar insignificant average offset between species.

As the $\delta^{18}O_{cel}$ isotopic ratio remains uniform the mixing of *Sphagnum* species is unlikely to contribute to any erroneous estimates of paleotemperature variation, but due to the $\delta^{18}O_{cel}$ variability it is suggested that repetitive measurements be used to increase the precision in paleotemperature record reconstruction. In addition, multiple measurements over time or depth can be averaged providing reduced stratigraphic resolution (e.g., no interpretation for <20 year fluctuations) but better confidence in the lower frequency (e.g., interpretation of >100 year frequency).

Our findings are also consistent with the results of a recent study that focused on carbon and oxygen isotope ratios in cellulose extracted from *Sphagnum* branches and stem sections sampled from a 4000 year old peat located in the Westeifel volcanic field in Germany (Moschen et al., 2009). Although the Moschen et al. (2009) study did not quantify the offset in $\delta^{18}O_{cel}$ values between different *Sphagnum* species, it did demonstrate that the $\delta^{18}O_{cel}$ offset observed between branches and stem sections obtained from the same *Sphagnum* specimens at various core depths was statistically insignificant. This insignificance is confirmed herein through the $\delta^{18}O_{cel}$ isotopic analysis of *Sphagnum* pairs from different species and indicates that paleotemperature proxy reconstructions utilizing *Sphagnum* will provide reliable results. The variability in O% is predominantly due to often low values in the rhizome cellulose (Fig. 3). In contrast the *Sphagnum* O% values show low variability (Fig. 3) and do not exhibit a typical fan shaped alteration pattern with increasing depth, but, on the contrary, a tight band (e.g., Veizer et al., 1999). This also supports the primary nature of geochemical signatures in *Sphagnum* cellulose.

Based on our observation that *Sphagnum* plants provide a much more reliable $\delta^{18}O_{cel}$ isotopic results than obtained from either rhizomes or vascular plants, we make two specific recommendations regarding $\delta^{18}O_{cel}$ isotopic analysis of peat bog material: (1) either individual *Sphagnum* branches, preferably stems should be analyzed and (2) although it is preferable to restrict analysis to a single *Sphagnum* species, the statistically insignificant offsets observed between species will result in reliable data even if hard to identify stems from different species are analyzed.

7. Conclusions

There is a statistically significant difference in the $\delta^{18}O_{cel}$ isotopic ratios obtained from non-vascular *Sphagnum* and the vascular plant macrofossils typically found in cores. This offset is recorded within and between samples. This result suggests that the use of bulk peat material without consideration of the observed differences between the isotopic composition of *Sphagnum* and the other plant macrofossils could lead to erroneous conclusions concerning the magnitude of paleoclimate variation.

There is a consistent and significant correlation between the $\delta^{18}O_{cel}$ isotopic ratios obtained from different *Sphagnum* species analyzed from the same sample. The observed statistically insignificant offsets between the cellulose oxygen isotopic composition of the different *Sphagnum* species analyzed implies that segregation of these species prior to isotope analyses is not necessary. Since the $\delta^{18}O_{cel}$ ratios observed in *Sphagnum* and rhizomes are statistically uncorrelated, the use of bulk *Sphagnum* will unlikely result in any misrepresentation of the $\delta^{18}O_{cel}$ signature.

Although it is preferable to restrict analysis to single *Sphagnum* species as was the case in the present study, the statistically insignificant offsets observed between species suggest that *Sphagnum* remains could also be used for $\delta^{18}O_{cel}$ based temperature reconstructions.

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