

LETTER TO THE EDITOR

Dear Dr. van der Meer,

Thank you for your comments and for your continued interest in our manuscript. We have modified the manuscript to address the suggestions of the referees. We believe this has improved the clarity and presentation of the manuscript and we hope that these changes align with your expectations. After consideration we included the new table on summary statistics as supplemental material as much of the statistics already existed in the manuscript (mostly in figures). Statistics on height increment and bulk density have been added in the text.

As you suggest, the $\delta^{13}\text{C}$ story is complex as evidenced by the modest relationships exhibited with environmental factors. *Sphagnum* mosses have no known CO_2 concentrating or transport mechanisms. Studies of physiology (i.e., real-time ^{13}C discrimination during photosynthesis), controlled growth under different environmental conditions, and field values at different water availabilities have confirmed the importance of extra-cellular water as a major determinant of $^{13}\text{C}/^{12}\text{C}$ fractionation. Indeed, the effect of external water was strong enough to overwhelm variation in the structure and placement of photosynthetic cells in a comparison of two *Sphagnum* species (Rice and Giles 1996, PC&E 19:118). The role of diffusion through the liquid phase and its importance as a limit to carbon uptake in bryophytes has been recently reviewed by Hanson et al. (2014, Ch. 6 in Hanson and Rice [eds.] Photosynthesis in Bryophytes and Early Land Plants, Springer).

The results of the present manuscript support that this process remains important when considering even broad geographic regions and within two species. However, there remains significant variation in $\delta^{13}\text{C}$ unexplained by either height above water table or productivity. In section 4.1, we discuss underlying causes that may contribute to that variation. It should be noted that the two species studied were rarely found submerged and differences are not likely due to what is dissolved within pore water in peat—either respired

CO₂ or other organic compounds that may be taken up. However, respired carbon in peatlands may alter both the isotopic composition and the concentration of CO₂ in the atmosphere near the surface where it can be refixed (see Turetsky and Weider 1999, *Ecoscience* 6:587, a reference we have added). We have clarified this in the first paragraph of section 4.1 where we discuss it. Although this might contribute to the variation between sites, it is unlikely that this caused the within site variation, which was higher for both species. Unfortunately, we are not aware of studies that have explored within site variation in refixed CO₂ to allow us to elaborate on its possible effects.

We appreciate your input and welcome any additional suggestions you may have. Thank you for your time.

Gustaf Granath and co-authors

RESPONSE TO REVIEW COMMENTS

These are in general the responses posted online, but with a few clarifications on the made changes.

REVIEWER #1

J. Loisel

General comments: The manuscript by Granath et al presents a large northern dataset of d13C and d18O from Sphagnum magellanicum and Sphagnum fuscum tissues. Results show promise for d18O from plant tissue as a proxy for d18O from precipitation; the relationship between tissue and source water could be used broadly to reconstruct changes in precipitation from peat core records. The relationship between d13C from plant tissue and environmental conditions was shown to be a little more complicated to interpret because of species-specific differences and confounding factors (water table and NPP primarily). The dataset presented here is coherent and spans a broad range of climatic conditions. To my

knowledge, the statistical tests performed are adequate and provide honest/reliable results. In general, this is a much needed review of what is known (and what remains unclear) about the relationships between environmental conditions and the stable isotope signature of Sphagnum tissues. This synthesis might help us better understand Sphagnum physiology and its adaptation to local conditions. Also, the text reads well and should be well received by the BG audience and particularly by the terrestrial ecosystem ecology and paleoclimatology communities. I recommend publication of this manuscript pending that the following specific and technical comments be considered in the final article:

Specific comments:

(1) the use of Sphagnum CAPITULUM for the analysis – we know that many other authors have used stems OR leaves in the past and that these 2 types of tissues have different d13C values (see work by Loader for a discussion on the offset); also, there might be translocation from the apex down to the stems and leaves (see work by Bragazza) – I wonder what a difference it makes to analyze the capitulum vs. the top part of the stem. Could this partly explain the relatively wide spread of data you obtained with d13C?

RESPONSE: We used branches in the capitula for our comparisons because we were interested in matching isotope values with environmental conditions in the present growing season. Given our broad sampling including sites that could experience slow growth due to cold temperatures or water stress, we opted to measure tissue that most likely would reflect recent conditions. Loader et al. (2016; J Quat Res 31:426) also used capitula in a study comparing microclimates and Sphagnum isotope values, presumably for the same reason although not explicitly stated. We prepared samples from 10 capitula from each plot. Loader et al. (2016; above) found a 1.7 per mil range in d13C among 102 Sphagnum capitula growing within a 20 cm² area. We believe our sampling reflects this naturally occurring variation and contributed to the spread of d13C values.

Loader et al. (2007; *The Holocene* 17:403) show that the carbon isotopic values in branch types (hanging vs pendant branches) differ consistently, but that the difference is small (0.26 per mil). However, there is a much greater difference between these and stems (>1 per mil). Moschen et al. (2009; *Chemical Geology* 259:262) found a similar offset.

In summary, for absolute isotopic values and its variation it matters what part of the plant is analysed. However, relationships should remain the same. We added some text to inform the reader that they should be aware of this when interpreting/applying these relationships.

(2) water table measurements at the END of the growing season: I wonder if part of the somewhat weak relationship between $d^{13}C$ and HWT could be caused by a contracted spread of HWT? Assuming that the dry end of the microhabitat remains dry throughout the growing season, but that the wetter microhabitats tend to dry out over time, it is possible that measuring HWT at the end of summer does not provide an accurate picture. If photosynthesis was to preferentially occur early during the growing season (i.e., under wetter conditions) and then stop, the pattern you observe could be in part explained by a sampling bias.

RESPONSE: Our measurements of HWT is a snapshot of the $d^{13}C$ -HWT relationship and may indeed have been tighter if we had measured continuous HWT. This shortcoming is highlighted in the manuscript (second sentence section 4.1). Unfortunately, collection of continuous HWT data was not logistically possible but we argue that HWT in the end of the season is a good proxy for relative HWT differences among locations. Growth mainly occurs in late summer/fall in temperate and boreal regions and therefore HWT at the the end of the season is assumed to be a better proxy of relative HWT during growth than spring HWT. We did measure HWT in the spring as well and found spring HWT and fall HWT to be strongly correlated ($r=0.74$, this number has now been added).

(3) I'd like a precision on the bulk density measurements: it is said that the top 30mm of the stem were used to calculate BD; how was volume determined?

RESPONSE: Also requested by reviewer #3. A known area was cut out carefully to avoid compaction (diameter=10cm). Stems within this area were then trimmed to 30 mm by cutting off the capitula and the lower part. This has now been clarified in the method section.

(4) Figure 2: A discussion on regional differences that you found across your dataset would be useful. For example, are there areas where d13C and HWT was more strongly correlated than others? what about d13C and NPP? Or was NPP more strongly correlated for wetter samples? Same goes with d18O and P, as well as d18O and Evaporation: is there anything else that could be learned from within your dataset?

RESPONSE: These are all good suggestions for further exploratory analyses and also put forward by reviewer #3. It is, however, important to point out that such analyses are of exploratory character as we may find spurious relationships when we perform subset analyses with small sample sizes. The NPP x HWT interaction was actually tested but we missed to give this result (in the text it says "we removed negligible interactions"). This has been fixed (see Table heading). In the revised version we discuss areas/data points that do not fit the overall patterns.

(5) Figure 3: i'm curious to know more about the d18O values between -20 and -15 permille; they are almost all poorly predicted by your linear model. Where do they come from? what might explain their 'unusual' signature?

RESPONSE: *Samples with these values occur in continental interiors both in Canada (Northwest Territories, NWT) and in West Siberia. As the d18O model suggests, these sites are expected to experience precipitation with low d18O values. In fact, the linear relationship looks pretty robust along the d18O values. One value, from NWT, does have a much lower value than predicted but we have no idea why except that the 18Oprecip model is less accurate in this region. We added some of these details in the revised version.*

Technical corrections:

line 204: add a space between and_are

line 251: we first built (change the build for built) ... and WERE identified (change ARE for WERE). Everything else in here uses past tense

line 286-287: "S. magellanicum..." should follow the previous sentence; there is currently a 'line jump'.

RESPONSE: Thank you for pointing out these correction.

REVIEWER #3

Anonymous

This paper addresses important questions about how the isotope composition of Sphagnum is controlled by environmental conditions: which is key to using peat bogs as palaeoenvironmental archives. The authors have chosen two cosmopolitan species, which allows the important subject of species specificity of signals to be addressed, and have a good distribution of sample locations from around the Holarctic, the regions where using Sphagnum as a paleoclimate archive is potentially feasible. The differential sensitivity of the two Sphagnum species to the environmental variables is an important result, even when growing in close proximity. The relationship ^{18}O and the annual precipitation is interesting and an important result that has wide ranging relevance. The relationships with ^{13}C are much more complicated! I think that to make the most of the data set, the results section needs to be expanded, with more description of the raw data, which will give the reader a better understanding of the data. The impact of the many environmental variables is very complex, hence several of the relationships have very low explanatory power: there needs to be more critical analysis of the statistics teasing out those that have clear biological relevance. One aspect on which there is no comment is any regional variation in values / relationships, which would be interesting.

RESPONSE: Also reviewer #1 pointed out the need for a more detailed result section and further analyses on regional differences. We agree that this is a useful addition and the revised version we include an overview of the variables (means, SDs, ranges) and comments on data points that diverge from the overall trends (site location). Summary statistics were added as a

supplementary table (Table S2) and in the text for height increment and bulk density.

Regarding the statistical analyses: We think the reviewer refer to the $\delta^{13}\text{C}$ results, and the effect of NPP, ET and temperature that are discussed although their R^2 -values are rather low. We believe that NPP result is still relevant as this is an expected relationship with clear theoretical basis. Our discussion regarding ET and temperature are, however, less relevant as the explanatory power was very low and the underlying mechanism not as clear. Thus, in the revised version some parts have been deleted and we only briefly discuss these variables.

Specific comments:

Line 72: I think it would be better to replace “elements” with “compounds”, as although it is isotopic composition of C and O being analysed, they are not abstracted from the atmosphere in their elemental form, and are analysed within compounds.

RESPONSE: This sentence is changed to “..... depend on nutrients, water and CO_2 uptake from the atmosphere.”

Line 73: compositions (rather than composition)

RESPONSE: OK.

Line 74: Can be difficult to determine if the material is dead, some may spontaneously start to regrow if exposed to light.

RESPONSE: Correct. ‘dead’ has been removed from the sentence.

Line 83: Holarctic spelling

RESPONSE: Will be fixed.

Line 83: Were the differences significant in $\delta^{13}\text{C}$ between species

RESPONSE: Yes, but we prefer to avoid P -values in the abstract.

Line 85: Where R2 is only 6%, I'm doubtful of its importance as a significant predictor: I think this needs expansion and may be easier to leave out of the abstract

RESPONSE: As the relationship between $\delta^{13}C$ and NPP was a part of our aims, we would like to include this result in the abstract. We added a few words about the poor strength of this relationship on L87.

Line 90: Expand HWT and NPP at first use Introduction

RESPONSE: We assume the reviewer means 'Abstract' here (L90 is in the Abstract section). Regardless, HWT and NPP are written out at first use, both in the Abstract and the Introduction.

Line 97: forcings (rather than forcing), responses (rather than response)

RESPONSE: OK.

Line 101: replace "is" with "are"

RESPONSE: OK.

Line 106-113: This paragraph is a bit unclear. It is the isotopic composition of the CO_2 that is in the chloroplast, rather than purely its concentration that is important for the extent of carbon isotope composition. Thus, if the rate of diffusion is slow, and assimilation continues by the moss, the carbon concentration will decrease, but what is more important is that the proportion of $^{13}CO_2$ will increase and consequently discrimination against $^{13}CO_2$ will decrease.

RESPONSE: Yes, this is what we meant and it is mentioned in the following sentences. It has been clarified in the revised version.

Line 113: remove "consequently".

RESPONSE: We kept this.

Line 113: Respiratory CO_2 can be fixed when the mosses are not submerged: particularly close to the ground the isotopic composition of the source CO_2

may vary in space and time depending on the extent of mixing between any respired CO₂ at the bog surface, and the well mixed atmosphere above.

RESPONSE: This is correct. This potential mechanism has been included in the revised version with references (eg Limpens et al. Journal of Vegetation Science 19(6):841-848. 2008, <https://doi.org/10.3170/2008-8-18456>)

Line 147: CO₂: subscript rather than superscript

RESPONSE: OK.

Line 155: compositions (rather than composition) Methods

RESPONSE: OK.

Line 187: How was the end of the growing season identified?

RESPONSE: The end of the growing season was defined as “when there is risk of snowfall or frost to occur”. Of course, some sites are remotely located and it is hard for a researcher to time this. Hence, growth measurements may stop before the “true” end of the growing season. However, this last period likely has negligible growth. We describe this in the revised version.

Table S1: mark which / both species were collected from each site

RESPONSE: An additional column indicating the species sampled at each site has been added to Table S1.

Line 204: space between and and are

RESPONSE: OK.

Line 215: However, cellulose may be more applicable for a comparison to palaeo studies, in which case the differential breakdown of different components means analysing a single component can increase the accuracy. Furthermore, may be a significant contributor to species specific differences. Furthermore, whilst trying to pin down influencing factors which previous studies have shown to be very complex, whilst there is a strong relationship between the composition of organic matter and cellulose, particularly for 18 O, 30-50% variation in cellulose-OM relationship is not explained using OM, and

the mean annual modelled water leaves 24% to be explained...cellulose maybe could have been measured and precip collected to facilitate explanations

RESPONSE: We agree that cellulose extraction would have improved our ability to develop quantitative isotope-environment transfer functions that would have facilitated the connection with paleo studies. Unfortunately, this was not feasible for the present study. We believe the value of our study arises from the broad geographic sampling linking contemporary isotope signatures to environmental conditions, which is adequately addressed using isotopes derived from organic matter. In addition, given the high number of research participants, many of whom visited sites only at the start and the end of the growing season, we were unable to perform the regular rainfall collection necessary to determine annual average $d^{18}O$ in precipitation. Instead we relied on modelled data, which has shown to be very accurate and has the benefit that it is easy to use our results. These arguments and explanations has been incorporated in the Method section.

Line 234: when were the HWT measurements made? Depending when most of the growth occurred, this could have a significant impact on both isotope relationships.

RESPONSE: Also commented on by reviewer #1. We here repeat the same response.

Our measurements of HWT is a snapshot and the $d^{13}C$ -HWT relationship may have been tighter with continuous HWT data. This is also pointed out in the manuscript (second sentence section 4.1). Now continuous HWT data was not logistically possible but we argue that HWT in the end of the season is a good proxy for relative HWT differences among locations. Growth mainly occur in late summer/fall in temperate and boreal regions and therefore HWT at the the end of the season is assumed to be a better proxy of relative HWT during growth than spring HWT. We did measure HWT in the spring as well and spring HWT and fall HWT was strongly correlated ($r=0.74$, this is now in the manuscript).

Line 240: how long a period were the pins in place? The calculations for NPP need more detail both for the amount of vertical growth, and the bulk density measurements as that can be very difficult to do accurately on loose sphagnum

*RESPONSE: Growing season (the time wires were in the field) varies among sites. Bulk density can be hard to estimate accurately but it is easier to get precise values for denser species like *S.fuscum* and *S.magellanicum* as they grow in slightly drier habitats. We will add information about mean and variation in height growth and bulk density (Result section).*

Line 251: built rather than build

RESPONSE: OK.

Results: Need more details in the results section – the results need to be described at the beginning. What are the ranges of the raw data for the isotope values, what are the growth rates, bulk densities, water table depths etc.

RESPONSE: We have added a table (Table S2) showing the means, SDs and ranges.

Line 272: Table 1 is unclear: need means of both ^{13}C and ^{18}O values rather than just the variation. Add per mille sign to SD values. Unclear what the proportion of variance is referring to: is this the variance explained by the mixed effect model?

RESPONSE: Table 1 shows the variation and how it is partitioned between with-site and between-site. One of the aims of the study was to investigate where most variation in isotopic variation can be found in Sphagnum. Hence, there is no need showing the means in Table 1, and similar information is given in figure 2-3. However, the table caption was brief and we added information on what it actually shows (eg the definition of proportion variance: that it is the proportion of total variance).

Line 268-272: Are the relationships between $\delta^{13}\text{C}$ and HWT significant?

RESPONSE: Yes, and this information is given in Table 2.

Fig 2: How many samples per site into each line? If its only two per site ($N_{\text{site}} = 80$, $N = c. 160$), is that enough info for a valid calculation: I'm not convinced the site lines are meaningful. Plot confidence interval on pooled regression lines. The individual site lines make it hard to see the overall averages.

RESPONSE: Number samples per site varies, but is mostly two. Site is a random factor and lines show the estimated response per site. The benefit of showing individual lines is that the reader can evaluate if within-site trends follow the between site trends. Here they do so, but it does not have to be the case (think Simpson's paradox). Therefore we think it is a more honest illustration of the analyses (and data) to plot the individual lines. Confidence intervals (CIs) depict another sort of variation that can be found in Table 2 (SEs of regression coefficients). With the population level lines being close to each other, CIs for each species may be hard to distinguish for the reader. To illustrate CIs, it is probably necessary to split the figure into two panels, but then the species-specific responses may be less obvious.

We agree that the average lines are hard to see because points are plotted on top of them. We have included a clearer version of this graph.

See also next comment.

Fig 3: Plot confidence interval on regression lines.

RESPONSE: Similar to Figure 2, the two lines are rather close to each other and the confidence intervals (CIs) will be hard to distinguish. Unless we split the figure into two panels, such CIs may not be very informative for the reader. Details on the regression lines (SEs) can be found in Table 2 for the readers that want such details.

All together, we are not convinced that adding CIs will significantly improve our figures. At the same time, we don't have particularly strong opinions and if the editor prefers CIs we are open to change the figures accordingly.

Discussion

Line 324: This overstates the influence that you measured on $d_{13}C$, especially of ET, which had “weak evidence” for *S. magellanicum*.

RESPONSE: We agree that this was not correctly worded. The evidence for ET (and temperature) was in general weak with low R2s and we have shortened this in the new version.

Line 335 “influenced by many unknown factors”: could this be expanded and made a little more specific?

RESPONSE: Good point. We have clarified this and we briefly mention the complex interactions among environmental factors that may affect Sphagnum growth across our sites.

Line 345: Do you mean precipitation amount?

RESPONSE: Yes. Now corrected.

Line 373: Sphagnum doesn't actively control the water availability: it is a passive process, influenced by growth form etc. I think that “control” implies that it is an active process.

RESPONSE: Reworded as this is mostly a passive process.

Line 391: May not be generalisable across moss species: sphagnums are generally wet so tightly coupled to the source water, mosses which rapidly hydrate and desiccate repeatedly may be less tightly coupled to the source water and more dependent on evaporative processes.

RESPONSE: Specified that we mean peatland mosses.

Environmental and taxonomic controls of carbon and oxygen stable isotope composition in *Sphagnum* across broad climatic and geographic ranges

5 Gustaf Granath¹, Håkan Rydin¹, Jennifer L. Baltzer², Fia Bengtsson¹, Nicholas Boncek³, Luca
Bragazza^{4,5,6}, Zhao-Jun Bu^{7,8}, Simon J. M. Caporn⁹, Ellen Dorrepaal¹⁰, Olga Galanina^{11,40}, Mariusz
Gałka¹², Anna Ganeva¹³, David P. Gillikin¹⁴, Irina Goia¹⁵, Nadezhda Goncharova¹⁶, Michal Hájek¹⁷,
Akira Haraguchi¹⁸, Lorna I. Harris¹⁹, Elyn Humphreys²⁰, Martin Jiroušek^{21, 22}, Katarzyna Kajukalo¹²,
Edgar Karofeld²³, Natalia G. Koronatova²⁴, Natalia P. Kosykh²⁴, Mariusz Lamentowicz¹², Elena
Lapshina²⁵, Juul Limpens²⁶, Maiju Linkosalmi²⁷, Jin-Ze Ma^{7,8}, Marguerite Mauritz²⁸, Tariq M. Munir²⁹,
10 ³⁰, Susan Natali³¹, Rayna Natcheva¹³, Maria Noskova[†], Richard J. Payne^{32, 33}, Kyle Pilkington³, Sean
Robinson³⁴, Bjorn J. M. Robroek³⁵, Line Rochefort³⁶, David Singer^{37,41}, Hans K. Stenøien³⁸, Eeva-Stiina
Tuittila³⁹, Kai Vellak²³, Anouk Verheyden¹⁴, James Michael Waddington¹⁹, Steven K. Rice³

¹Department Ecology and Genetics, Uppsala University, Norbyvägen 18D, Uppsala, Sweden

²Biology Department, Wilfrid Laurier University, 75 University Ave. W., Waterloo, ON, N2L 3C5, Canada

15 ³Department of Biological Sciences, Union College, Schenectady, NY, US

⁴Department of Life Science and Biotechnologies, University of Ferrara, Corso Ercole I d'Este 32, I-44121 Ferrara, Italy

⁵Swiss Federal Institute for Forest, Snow and Landscape Research, WSL Site Lausanne, Station 2, 1015 Lausanne, Switzerland

20 ⁶Ecole Polytechnique Fédérale de Lausanne EPFL, School of Architecture, Civil and Environmental Engineering ENAC, Laboratory of ecological systems ECOS, Station 2, 1015 Lausanne, Switzerland

⁷Institute for Peat and Mire Research, Northeast Normal University, State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, 5268 Renmin St., Changchun 130024, China

⁸Jilin Provincial Key Laboratory for Wetland Ecological Processes and Environmental Change in the Changbai Mountains, 5268 Renmin St., Changchun 130024, China

25 ⁹School of Science and the Environment, Division of Biology and Conservation Ecology, Manchester Metropolitan University, Manchester, M1 5GD, UK

¹⁰Climate Impacts Research Centre, Dept. of Ecology and Environmental Science, Umeå University, 98107 Abisko, Sweden

¹¹Institute of Earth Sciences, St. Petersburg State University, Universitetskaya nab., 7-9, Russia, 199034, St.Petersburg, Russia

30 ¹²Department of Biogeography and Paleoecology, Adam Mickiewicz University in Poznan, Bogumiła Krygowskiego 10, 61-680 Poznan, Polen

¹³Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Yurii Gagarin Str., 1113 Sofia, Bulgaria

¹⁴Department of Geology, Union College, Schenectady, NY, USA

35 ¹⁵Babeş-Bolyai University, Faculty of Biology and Geology, Department of Taxonomy and Ecology, 42 Republicii Street, RO-400015, Cluj Napoca, Romania

¹⁶Institute of Biology of Komi Scientific Centre of the Ural Branch of the Russian Academy of Science, Russia

¹⁷Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlarska 2, CZ-61137, Brno, Czech Republic

40 ¹⁸Department of Biology, The University of Kitakyushu, Kitakyushu 8080135, Japan

¹⁹School of Geography and Earth Sciences, McMaster University, 1280 Main Street West, Hamilton, ON, L8S 4K1, Canada

²⁰Department of Geography and Environmental Studies, Carleton University, Ottawa, Canada

- 21Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlarska 2, CZ-61137, Brno, Czech Republic
- 45 22Department of Plant Biology, Faculty of AgriSciences, Mendel University in Brno, Zemedelska 1, CZ-61300, Brno, Czech Republic
- 23University of Tartu, Institute of Ecology and Earth Sciences, Lai st 40, Tartu 51005, Estonia
- 24Laboratory of Biogeocenology, Institute of Soil Science and Agrochemistry, Siberian Branch of Russian Academy of Sciences, Ak. Lavrent'ev ave., 8/2, Novosibirsk, 630090, Russia
- 50 25Yugra State University, Chekhova str, 16, Khanty-Mansiysk, 628012, Russia
- 26Plant Ecology and Nature conservation group, Wageningen University, Droevendaalse steeg 3a, 6708 PD Wageningen, the Netherlands
- 27Finnish Meteorological Institute, Erik Palménin aukio 1, FI-00560 Helsinki, Finland
- 28Center for Ecosystem Science and Society (EcoSS), Department of Biological Sciences, Northern Arizona University, POBox 5620, Flagstaff, AZ 86011, USA
- 55 29Department of Geography, University of Calgary, 2500 University Dr. NW, Calgary, AB, T2N 1N4, Canada
- 30Department of Geology, St. Mary's University, Calgary, AB, T2X 1Z4, Canada
- 31Woods Hole Research Center, 149 Woods Hole Road, Falmouth MA 02540, USA
- 32Environment, University of York, York YO105DD, UK
- 60 33Penza State University, Krasnaya str., 40, 440026 Penza, Russia
- 34Department of Biology, SUNY-Oneonta, Oneonta, NY, USA
- 35Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK
- 36Department of Plant Science and Center for Northern Studies, Laval University, QC, Canada
- 37Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, CH-2000 Neuchâtel, Switzerland
- 65 38NTNU University Museum, Norwegian University of Science and Technology, Erling Skakkes gate 47, N-7491 Trondheim, Norway
- 39School of Forest Sciences, University of Eastern Finland, B.O. Box 111, FIN-80110 Joensuu, Finland
- 40Komarov Botanical Institute Russian Academy of Sciences, Professor Popov st. 2, 197376, St.Petersburg, Russia
- 70 41Department of Zoology, Institute of Biosciences, University of São Paulo, 05508-090, Brazil
- †deceased, 27 August 2017

Correspondence to: Gustaf Granath (gustaf.granath@gmail.com)

Abstract. Rain-fed peatlands are dominated by peat mosses (*Sphagnum* sp.), which for their growth depend on elements/nutrients, water and CO₂ uptake from the atmosphere. As the isotopic compositions of carbon (^{12,13}C) and oxygen (^{16,18}O) of these *Sphagnum* mosses are affected by environmental conditions, the ~~dead~~ *Sphagnum* tissue accumulated in peat constitutes a potential long-term archive that can be used for climate reconstruction. However, there is a lack of adequate understanding of how isotope values are influenced by environmental conditions, which restricts their current use as environmental and palaeoenvironmental indicators. Here we tested (i) to what extent C and O isotopic variation in living tissue of *Sphagnum* is species-specific and associated with local hydrological gradients, climatic gradients (evapotranspiration, temperature, precipitation), and elevation; (ii) if the C isotopic signature can be a proxy for net primary productivity (NPP) of *Sphagnum*; and (iii) to what extent *Sphagnum* tissue δ¹⁸O tracks the δ¹⁸O isotope signature of precipitation. In total, we analysed 337 samples from 93 sites across North America and Eurasia using two important peat-forming *Sphagnum* species (*S. magellanicum*, *S. fuscum*) common to the Holarctic realm. There were differences in δ¹³C values between species. For *S. magellanicum* δ¹³C decreased with increasing height above the water table (HWT, R²=17%)

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and was positively correlated to productivity ($R^2=7\%$). Together these two variables explained 46% of the between-site variation in $\delta^{13}\text{C}$ values. For *S. fuscum*, productivity was the only significant predictor of $\delta^{13}\text{C}$ but had low explanatory power (total $R^2=6\%$). For $\delta^{18}\text{O}$ values, ca. approximately 90% of the variation was found between sites. Globally-modelled annual $\delta^{18}\text{O}$ values in precipitation explained 69% of the between-site variation in tissue $\delta^{18}\text{O}$. *S. magellanicum* showed lower $\delta^{18}\text{O}$ enrichment than *S. fuscum* (-0.83‰ lower). Elevation and climatic variables were weak predictors of tissue $\delta^{18}\text{O}$ values after controlling for $\delta^{18}\text{O}$ values of the precipitation. To summarise, our study provides evidence for **(a)** good predictability of tissue $\delta^{18}\text{O}$ values from modelled annual $\delta^{18}\text{O}$ values in precipitation, and **(b)** the possibility to relate tissue $\delta^{13}\text{C}$ values to HWT and NPP, but this appears to be species-dependent. These results suggest that isotope composition can be used at a large scale for climatic reconstructions but that such models should be species-specific.

95 **1 introduction**

Peatlands in temperate, boreal and arctic regions form large reservoirs of carbon, which are vulnerable to release under expected changes in global climate and land management (Rydin and Jeglum 2013, Loisel et al. 2014). Because peat decomposes slowly and gradually accumulates, it preserves information on past peatland ecosystem dynamics and responses to allogenic and autogenic forcings. Palaeoenvironmental studies of peat may, therefore, help anticipate future responses of these globally important ecosystems to climate change (Loader et al. 2016). Past climate and local hydrology can be estimated using a variety of biotic and biogeochemical proxies, including the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of organic material. (e.g. van der Knaap 2011, Royles et al. 2016, Lamentowicz et al. 2015). However, the environmental (e.g. climate) and biotic (e.g. species identity) controls of isotope differentiation in peatland-dwelling plants are still poorly understood; and current assumptions regarding these controlling factors are yet to be tested at larger spatial scales.

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Sphagnum mosses are the most dominant peat-forming plant group in acidic peatlands. The composition of stable isotopes of carbon and oxygen in their tissues is affected by different environmental conditions, operating through their impact on fractionation processes. When not submerged, carbon isotope signals in bulk tissues or components such as cellulose depend mainly on the concentration and isotopic composition of $[\text{CO}_2]$ in the chloroplast ($[\text{CO}_2]_c$), which alters isotope discrimination during biochemical fixation of CO_2 ~~and by fractionation caused by diffusion to the chloroplast~~ (Farquhar et al. 1989, O'Leary 1988). In mosses, the CO_2 concentration in the chloroplast, $[\text{CO}_2]_c$ ~~is has been shown to be~~ determined by temperature, light availability, CO_2 partial pressure and, most importantly, plant water status (Finsinger et al. 2013, van der Knaap et al. 2011, Ménot and Burns 2001, Ménot-Combes et al. 2004, Royles et al. 2014, Skrzypek et al. 2007a, Kaislahti Tillman et al. 2013). When wet, external water films on leaf surfaces impede diffusion and $[\text{CO}_2]_c$ is lowered (Rice and Giles 1996, Rice 2000, Williams and Flanagan 1996); consequently, the proportion of fixed ^{13}C increases due to internal drawdown of the preferred isotope ^{12}C . When submerged, assimilation of respired or methane-derived CO_2 can alter $[\text{CO}_2]$ and also the C -isotopic composition of C in *Sphagnum* (Raghoebarsing et al. 2005). Even when not submerged, respiratory carbon can be refixed by

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Sphagnum (Turetsky and Wieder 1999, Limpens et al. 2008). Given that respired CO₂ is isotopically lighter than that in the atmosphere, it may also contribute to variation in tissue isotope values. Despite many detailed studies, there remains

120 uncertainty about how the multiple controls on ¹³C isotope values combine to determine isotopic composition, and how universal the proposed mechanisms are on a global scale. This uncertainty currently restricts the utility of C isotope signals as a palaeoclimatic/palaeoenvironmental indicator in peatlands (Loader et al. 2016).

Oxygen isotope values in moss tissues depend on the isotopic composition of the water sources, enrichment associated with
125 evaporation from the moss surface and biochemical fractionation (Dawson et al. 2002). Once on the plant, ¹⁸O present in water equilibrates with that in atmospheric CO₂ prior to fixation as well as being incorporated directly during hydrolysis reactions, especially during the initial stages of carbon fixation (Gessler et al. 2014, Sternberg et al. 2006). Hence, variation in tissue oxygen isotopes reflect environmental conditions that control source water (rainfall, snowfall, groundwater) as well as fractionation caused by evaporation prior to fixation which is controlled by micrometeorological conditions (mainly
130 temperature, relative humidity and incident energy) (Daley et al. 2010, Moschen et al. 2009, Royles et al. 2013, Kaislahti Tillman et al. 2010). Oxygen isotope composition has, therefore, been used to reconstruct climatic conditions and to infer the dominant water source in peatlands (Aravena and Warner 1992, Ellis and Rochefort 2006, van der Knaap et al. 2011). Ongoing measurements of oxygen isotopes in precipitation across the globe (Bowen 2010, IAEA/WMO 2015) have generated models that predict spatial patterns in oxygen isotope composition of precipitation based on temperature,
135 elevation, atmospheric residence time and circulation patterns (e.g. Bowen 2010). Once isotopic composition of the source water is accounted for, variation in moss tissue isotopic values should be largely determined by fractionation that accompanies evaporation from the surface of plants. How well oxygen isotopes in *Sphagnum* tissues reflect atmospheric water or plant surface water depend on local weather conditions such as precipitation, air temperature and humidity. For example, Bilali et al. (2013) suggest that oxygen isotopes in *Sphagnum* mosses from maritime bogs will track variation in
140 precipitation patterns whereas isotopic values in continental habitats will be more dependent on summer temperature, as temperature and humidity are more variable in those regions. At local scales, oxygen isotope values also vary as a function of temperature and humidity. Aravena and Warner (1992) found differences that correspond with changes in microtopography. Elevated microsites (hummocks) were enriched in ¹⁸O, which they ascribed to higher evaporation compared to that of neighbouring wet depressions (hollows). However, as with ¹³C, there remains uncertainty in how ¹⁸O
145 signatures relate to environmental factors and species identity, and to what extent global ¹⁸O patterns in precipitation dominate over local processes.

Stable isotopes can also serve as indicators of primary productivity (NPP) (Rice and Giles 1996, Williams and Flanagan 1996, Rice 2000). However, few studies have explored these relationships in the field. In a multispecies comparison of peat
150 mosses, Rice (2000) found that plants with higher relative growth rates had lower discrimination against ¹³C and therefore were more enriched in ¹³C. This was attributed to the local environment, with fast growing plants of wetter microhabitats

having thicker water films that inhibits CO₂ diffusion into the plant, and to species-specific differences in maximum rates of photosynthesis. Both factors would reduce internal [CO₂] and thereby lower discrimination. In line with this, a warming experiment by Deane-Coe et al. (2015) reported a positive relationship between moss net primary productivity (NPP) and δ¹³C values for tundra mosses (*Dicranum*, *Pleurozium*, *Sphagnum*). Clearly, carbon isotope values show promise as indicators of peat moss contemporary growth, and potentially as a NPP proxy in paleoecological studies. This could be particularly valuable to differentiate productivity and decomposition controls in long-term carbon accumulation studies. To date, we are not aware of attempts to explore the robustness of these relationships across large spatial scales.

Together, tissue carbon and oxygen isotope compositions are controlled both by environmental factors at micro- and macro-scales, and by species-specific differences that relate to water balance and carbon dynamics in peat mosses. Paleoecological studies rely on such environment—isotope relationships for environmental reconstructions (Ellis and Rochefort 2006, van der Knaap et al. 2011). The underlying mechanisms are, however, rarely fully explored using known environmental gradients (but see Ménot and Burns 2001 for an example), or only tested across narrow bands of environmental variation, often with sets of correlated environmental factors (Loader et al. 2016). Moreover, interactions with biotic factors such as species identity have received little attention despite the large variations in *Sphagnum* species dominance commonly observed down peat cores (e.g. Ménot and Burns 2001). Here we aim to provide a robust, cross-scale evaluation of how environmental factors and species identity influence the C and O isotope compositions of *Sphagnum* using two common and widely distributed peat-forming species (*S. magellanicum* and *S. fuscum*) that are primarily rain-fed. To achieve this, we performed a unprecedented large sampling campaign across the Holarctic realm.

Specifically, we (i) investigated relationships between C and O isotope values and factors known to influence plant water availability (height above the water table - HWT, temperature, evaporation and precipitation) and CO₂ partial pressure (elevation), and tested if their effects were modified by species identity; (ii) tested the prediction that *Sphagnum* tissue δ¹³C values are associated with NPP; (iii) tested if tissue δ¹⁸O in rain-fed Sphagna is predicted by the δ¹⁸O isotope signature in precipitation but modified by negative relationships with precipitation and positive ones with temperature/evaporation. Across these objectives we examined how C and O isotope values varied with scale (within-peatland versus between-peatlands) and to what extent HWT and NPP could explain variation within and between peatlands.

2 Materials and Methods

2.1 Study species and collection sites

Our study focused on two common peat-forming *Sphagnum* species, *S. fuscum* (Schimp.) H. Klinggr. (circumpolar distribution) and *S. magellanicum* Brid. (cosmopolitan distribution). In general, these species are confined to primarily rain-fed peatlands (bogs), and described as hummock (*S. fuscum*) and lawn (*S. magellanicum*) species. However, *S.*

magellanicum is a species with a very broad niche and found in a range of habitats with varying degrees of groundwater influence (Flatberg 2013). These species are easy to identify but recent research has shown that the dark European morph of *S. fuscum* is conspecific to the North American *S. beothuk* (Kyrkjeeide et al. 2015), and *S. magellanicum* has been shown to consist of two genetically diverged morphotypes (Kyrkjeeide et al. 2016). Unpublished genetic data suggest that samples collected in our study consist of both *S. magellanicum* morphs (approximately 50/50) and possibly one or two samples of *S. beothuk* (Pers. comm. N. Yousefi). Hence, we here treat our species as aggregates (i.e. species collectiva), *S.fuscum* coll. and *S. magellanicum* coll..

The two species were sampled across the Holarctic region at a total of 93 sites (Figure 1; Supplemental Table S1) at the end of the growing season. To make comparisons between species and between sites possible, we focused on habitats where both species can be found and have low influence of surrounding groundwater. Thus, we only sampled bogs (including a few poor fens with ombrotrophic character) and open (no tree canopy) habitats. Sampling was conducted mainly during 2013, but a few sites were sampled at a similar time of year in 2014. At each site two patches (minimum 10 m apart) for each species were sampled (except for 11 sites that contained only one patch for one species). At each sampling patch we recorded moss growth, HWT (height above the water table) and GPS coordinates, and collected a moss sample (78 cm² and 5 cm deep) at the end of the growing season (September to November depending on location and generally coincided with when there was a risk of the first snowfall to occur). Moss samples were dried (24 hrs at 60-65 °C) within 72 h, or alternatively immediately frozen and later thawed and dried. The apical part (the capitula, top 1 cm) of the dried plant shoots was used for isotope analysis, while the stem section was used for bulk density estimation to calculate moss NPP.

2.2 Isotope determination

Ten capitula from each patch were selected and finely chopped with a single-edge razor by hand and mixed. Capitula were chosen as they reflect the most recently fixed organic matter and should relate better to recent growing season conditions. In Sphagnum, $\delta^{13}\text{C}$ from the capitulum is similar to that of branches within the top 15 cm plants, but is approximately 1-2 ‰ less negative than stems (Loader et al. 2007). For $\delta^{18}\text{O}$, the offset between branches and stems is around 1 ‰ (Moschen et al. 2009). Standard deviations of repeated samples were 0.6 and 0.7 ‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively. Approximately 0.5 mg dry sample was packed in tin cups for $\delta^{13}\text{C}$ analyses, and ~0.2 mg in silver cups for $\delta^{18}\text{O}$ analyses. Samples were analyzed at Union College (Schenectady, NY, USA) using a Thermo Delta Advantage mass spectrometer in continuous flow mode connected (via a ConFlo IV) to a Costech Elemental Analyzer for $\delta^{13}\text{C}$ analysis or a Thermo TC/EA for $\delta^{18}\text{O}$ analyses. Isotope values are presented as $1000 \times (R_{\text{sample}}/R_{\text{standard}}-1)$, where R_{sample} and R_{standard} are the ratios of heavy to light isotopes (e.g., $^{13}\text{C}/^{12}\text{C}$) and are referenced to VPDB and VSMOW for C and O, respectively. Carbon isotope data were corrected using sucrose (IAEA-CH-6, -10.449‰), acetanilide (in house, -37.07‰), and caffeine (IAEA-600, -27.771‰). Oxygen isotope data were corrected using sucrose (IAEA-CH-6, 36.4‰), cellulose (IAEA-C3, 31.9‰) and caffeine (IAEA-600, -3.5‰) with values from Hunsinger et al. (2010). Oxygen isotope standardization was further checked with the whole wood

standards USGS54 and USGS56. The combined instrument uncertainty for $\delta^{13}\text{C}$ (VPDB) is $< 0.1\text{‰}$ based on the in-house acetanilide standard and $< 0.5\text{‰}$ for $\delta^{18}\text{O}$ (VSMOW) based on the cellulose standard (IAEA-C3).

220 We performed isotope analyses on whole-plant tissue rather than on cellulose extracts. In living *Sphagnum* samples, there is strong linear relationship between the isotopic composition of these two components for both $\delta^{13}\text{C}$ (R^2 values 0.89-0.96; Kaislahti Tillman et al. 2010, Ménot and Burns 2001, Skrzypek et al. 2007b) and for $\delta^{18}\text{O}$ (R^2 values 0.53-0.69; Kaislahti Tillman et al. 2010, Jones et al. 2014). Focussing on whole-plant tissue allowed us to analyze a higher number of samples for this study, allowing larger numbers of sites and more replication.

225 2.3 Environmental variables

The modelled $\delta^{18}\text{O}$ signal in meteoric water (precipitation) (Bowen and Wilkinson 2002) was obtained from <http://www.waterisotopes.org> as annual and monthly isotope ratio estimates at 10' resolution. These global estimates have shown to be highly accurate ($R^2 = 0.76$ for mean annual $\delta^{18}\text{O}$ in precipitation) and are based on absolute latitude and elevation and account for regional effects on atmospheric circulation patterns (for details see Bowen 2010, IAEA/WMO
230 2015, Bowen 2017). To test which temporal period of $\delta^{18}\text{O}$ values in precipitation showed the highest correlation with tissue $\delta^{18}\text{O}$ values, we calculated annual (Jan-Dec), growing season (May-Oct), winter-spring (Jan-April) mean isotope ratio. We calculated both unweighted means and weighted against precipitation for each month. Monthly precipitation (PRECTOTCORR), land evapotranspiration (EVLAND) and surface air temperature (TLML) for each site and year of sampling (2013 or 2014) were retrieved from the NASA GESDISC data archive, land surface and flux diagnostics products
235 (M2T1NXLND, M2TMNXFLX; resolution longitude 0.667°, latitude 0.5°; Global Modeling and Assimilation Office 2015ab). Total precipitation and evapotranspiration (ET), and mean temperature, from April to October were used as predictors in the statistical models. As ET can be compensated for by precipitation, we used the ET/P quotient as a predictor for the effect of water loss. A high value (>1) indicates a net loss of water to the atmosphere. Site altitude was retrieved from a global database using the R package *elevatr* (ver 0.1-2, Hollister and Shah 2017).

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The distance from the moss surface to the water table (height above the water table, HWT), was measured using water wells (commonly a PVC pipe, 2–5 cm in diameter and slotted or perforated along the sides) with a “plumper” (a cylinder on a string that makes a ‘plump’ sound when it hits the water surface) or a “bubbler” (a narrow tube that makes bubbles when it hits the water surface while the user blows in it). HWT was measured in the spring and in the fall and there was a strong
245 correlation between the two occasions ($r = 0.74$). As growth mainly occurs in late summer/fall in temperate and boreal regions, we used HWT at the the end of the season as the proxy of relative HWT between sites.

2.4 Moss growth

Moss growth (or productivity, NPP) was measured with a modified version of the cranked wire method (see Clymo 1970, and Rydin and Jeglum 2013 for details), with bristles from a paint brush spirally attached to a wire. These ‘brush wires’ were inserted in the moss layer with the end of the wire protruding above the surface. Height increment (i.e. vertical growth) was measured over the growing season as the change in distance (to nearest mm) between the moss layer and the top of the wire. A minimum of three wires were inserted within a 1 x 1 m uniform area (same microhabitat, vegetation and general structure). To determine moss bulk density (kg m^{-3}) we dried (24 hrs at 60-65 °C) the top 30 mm of the stems (area 78 cm²) in our collected core (see Sect. 2.1). Biomass growth on an area basis ($\text{g m}^{-2} \text{year}^{-1}$) was calculated as height increment \times bulk density.

2.5 Statistical analyses

To test and quantify the influence of environmental variables and species identity on isotope composition, we used linear mixed models in R (R core team 2016), employing the R package *lme4* ver 1.1-12 (Bates et al. 2015). Site dependence (i.e. multiple samples from the same site) was accounted for by adding site as a random factor. For tissue $\delta^{13}\text{C}$, we first fitted two separate models to test the independent effects of HWT, NPP and species identity (*S. fuscum* and *S. magellanicum*), and if the HWT or NPP effect varied between species by fitting a species interaction term. To test the explanatory power of environmental variables (ET/P, precipitation, temperature, elevation) we first constructed a base model with HWT and NPP as they were identified as the main predictors in literature. For simplicity we removed negligible interactions from this model. Each environmental variable and their interaction with species was then tested against the base model. For tissue $\delta^{18}\text{O}$, we first explored which temporal period of modelled $\delta^{18}\text{O}_{\text{precip}}$ (annual, growing season, winter-spring) had the highest explanatory power and if the relationship varied between species. The identified best model was then used as base model to separately test each environmental variable (HWT, ET/P, precipitation, temperature) and its interaction with species.

The proportion of variance explained by the predictors was calculated at the site level (Gelman and Hill 2007) or as marginal R^2 (Nakagawa and Schielzeth 2013; R package *piecewiseSEM* ver 1.1-4, Lefcheck 2015). Although our study focused on explained variance by predictors, we also performed statistical tests of predictors and their interactions using type-2 (main effects tested after all the others in the model but without the interaction term) *F*-tests, applying Kenward-Roger adjustments to the degrees of freedom, as implemented in the *car* package (ver. 2.1-3, Fox and Weisberg 2011). Standard model checking was performed (e.g., residual analyses and distribution of random effects), to ensure compliance with model assumptions. Covariances between predictors were small ($r < 0.15$) or moderate ($r = 0.40-0.50$ between ET/P, precipitation and temperature) and this multicollinearity had minor impact on model estimates.

3 Results

The geographically broad data collection resulted in large variation of isotope values and explanatory variables (Table S2). Due to uncertainty in height increment measurement we recorded a few negative values resulting in negative NPP. These values were kept in the analyses. Mean and standard deviation (in parenthesis) of height increment (HI, mm) and bulk density (BD, kg m⁻³) were: HI for *S. fuscum* 14.3 (10.1) and *S. magellanicum* 19.5 (14.1), BD for *S. fuscum* 17.8 (9.9) and *S. magellanicum* 10.2 (7.6).

3.1 $\delta^{13}\text{C}$ signal

Variation in *Sphagnum* tissue $\delta^{13}\text{C}$ values was marginally greater within sites than between sites (Table 1). HWT predicted the $\delta^{13}\text{C}$ values, but the relationship differed between the two species (Table 2, Figure 2). Although $\delta^{13}\text{C}$ values decreased with increasing HWT for both species, the slope was less steep for *S. fuscum* and this species had slightly higher $\delta^{13}\text{C}$ values overall. In separate models for the two species, HWT for *S. fuscum* had near zero explanatory power, while for *S. magellanicum* HWT explained 33% of the between-site variation, and 17% of the total variance (i.e., marginal R^2).

Measured $\delta^{13}\text{C}$ values were related to moss productivity (NPP), and $\delta^{13}\text{C}$ values increased by 0.0023‰ (SE: 0.00048) for each mg biomass produced per m². NPP explained 11% of the between-site variation in $\delta^{13}\text{C}$ and 7% of the total variation. HWT and NPP, explained 48% of the between-site variation of $\delta^{13}\text{C}$ in *S. magellanicum*, and 24% of the total variation. Corresponding values for *S. fuscum* were 6% and 7%, respectively. Of the additional environmental variables tested, we found weak evidence that ET/P and temperature were positively correlated with $\delta^{13}\text{C}$, but only for *S. magellanicum* (Table 2).

3.2 $\delta^{18}\text{O}$ signal

Sphagnum tissue $\delta^{18}\text{O}$ values varied more between sites than within sites, and at similar magnitude and proportions for both species (Table 1). Tissue $\delta^{18}\text{O}$ values were predicted by the spatially explicit estimates of $\delta^{18}\text{O}$ values isotope signature in precipitation (Figure 3, Table 3). Annual mean $\delta^{18}\text{O}_{\text{precip}}$ explained 69% of the variation in $\delta^{18}\text{O}_{\text{tissue}}$ between sites. This was similar to mean winter-spring (Jan-Apr) $\delta^{18}\text{O}_{\text{precip}}$ values (75% explained), but higher than growing season (Apr-Sep) $\delta^{18}\text{O}_{\text{precip}}$ (58%). Using precipitation-weighted $\delta^{18}\text{O}_{\text{precip}}$ values resulted in lower percentages of explained variance for all three time periods (R^2_{site} : annual 52%, Jan-Apr 65%, Apr-Sep 52%). *S. magellanicum* had consistently lower $\delta^{18}\text{O}$ values than *S. fuscum* (-0.83‰), but both species had a similar relationship between tissue $\delta^{18}\text{O}$ and $\delta^{18}\text{O}_{\text{precip}}$ (Figure 3, Table 3).

Height above the water table (HWT) at the end of the growing season was, on average, 11 cm lower in *S. magellanicum* patches (= wetter habitat) compared to *S. fuscum* (HWT = 33 cm) patches ($F_{1,224} = 131.9$, $P < 0.0001$). However, we found only very weak support for the hypothesis that HWT predicts tissue $\delta^{18}\text{O}$ values, as HWT explained <1% of the $\delta^{18}\text{O}$

variation (Table 2). There was negligible influence of the additional environmental variables on $\delta^{18}\text{O}$ values (Table 2). ET/P was associated with higher $\delta^{18}\text{O}$ values in *S. magellanicum* and lower in *S. fuscum* (but not different from zero effect), while increasing temperature was weakly associated with overall lower $\delta^{18}\text{O}$ values.

4 Discussion

4.1 Stable carbon isotope discrimination in *Sphagnum*

Our data were consistent with the hypothesis that moss growing closer to the water table (low HWT) has reduced carbon isotope fractionation, leading to greater fixation of $^{13}\text{CO}_2$ and more ^{13}C enriched tissue (Rice and Giles 1996, Williams and Flanagan 1996). Given that the water table position was measured in different places at different times and all are one-time measurements, this result is remarkably robust. For example refixation of ^{12}C enriched substrate-derived CO_2 in living *Sphagna* (Turetsky and Weider 1999, Raghoebarsing et al. 2005) can potentially contribute to within-site variation in $\delta^{13}\text{C}$ as it potentially affects both the ambient concentration of CO_2 as well as its isotopic composition. Interestingly, the strength of this $\delta^{13}\text{C}$ - HWT relationship differed in the two species, with *S. magellanicum* exhibiting a greater reduction in $\delta^{13}\text{C}$ in response to drier conditions (high HWT) than *S. fuscum*. The weaker effect of HWT on $\delta^{13}\text{C}$ values in *S. fuscum* is likely a consequence of limited fluctuation in tissue water content as this species is well known to store abundant water within capillary spaces and resist drying (Rydin 1985), thus maintaining the waterfilm that hampers fractionation. Loader et al. (2016) reported a similar slope estimate for *S. magellanicum* in a single peatland and several studies have confirmed effects of contrasting microtopography (i.e. hummock—hollow differences) using multi-species comparisons (Price et al. 1997, Loisel et al. 2009, Markel et al. 2010). As such, our results suggest that species-specific differences in carbon isotope discrimination in *Sphagnum* are related to water retention capacity and, consequently, become more apparent under drier conditions. This supports the results of previous, smaller-scale studies (Rice 2000).

The influence of species identity on the relationship between $\delta^{13}\text{C}$ values and water table position has important implications for palaeoenvironmental reconstructions based on $\delta^{13}\text{C}$ values. The relationship between $\delta^{13}\text{C}$ and HWT has been used in paleoecological reconstructions of surface wetness (e.g., Loisel et al. 2009). In our dataset the strength of the relationship was weaker than previously reported. For instance, Loader et al. (2016) reported $R^2 = 54\%$ for *S. magellanicum* in a single site. Given the characteristics of our data (large-scale, circumpolar), the explanatory power ($R^2_{\text{marginal}} = 17\%$) can be considered acceptable and comparable to other proxies such as testate amoebae (16% in Loader et al. 2016; Sullivan and Booth 2011). Our results imply that isotopic signals of peatland wetness in hummock-dwelling species (such as *S. fuscum* spp.) may be weaker, or absent, compared to lawn species. It is therefore important that the same species, or species type (e.g., lawn species as they likely have a broad HWT niche), are used if $\delta^{13}\text{C}$ values are employed as a proxy to infer changes in HWT.

340 We also identified evidence that evapotranspiration (ET), and productivity (NPP) modify $\delta^{13}\text{C}$ values; although the effect of ET was weak and restricted to *S. magellanicum*. We expected a stronger relationship as ET and temperature control $\delta^{13}\text{C}$ by increasing water loss at the moss surface and reducing the diffusive resistance (i.e., reducing CO_2 limitation), which enables discrimination against ^{13}C (Williams and Flanagan 1996). ~~This mechanism requires the moss surface to partially dry out under high evaporative demand, which only occurs in hollow lawn species and not, or to a much lesser extent, in~~
345 ~~hummock species due to high water retention, strong capillarity forces, and reduced boundary layer conductance. This can explain the stronger effect of ET/P and temperature (i.e. net water loss) on $\delta^{13}\text{C}$ in *S. magellanicum*.~~ NPP only explained a small proportion of the variation in $\delta^{13}\text{C}$ values but the relationship was apparent across species. Several studies have proposed the use of $\delta^{13}\text{C}$ values relative abundance to infer *Sphagnum* productivity (e.g., Rice and Giles 1996, Rice 2000, Munir et al. 2017) and our study is the first to test this at the pan-Holarctic scale. Deane-Coe et al. (2015) investigated $\delta^{13}\text{C}$
350 values across moss species (including *Sphagnum*) and years at one site and found a weak relationship between productivity and $\delta^{13}\text{C}$ values ($R^2=0.10$ and 0.31 , respectively). Similarly, Rice (2000) reported that relative growth rate explained about 25% of the variation in ^{13}C discrimination. We did not find as strong a relationship ($R^2 < 0.12$), but our study was geographically broader and less controlled; and thereby consequently, our results were likely influenced by more complex interactions among environmental factors that affect *Sphagnum* growth across our sites many unknown factors. Nevertheless,
355 our results indicate independent effects of evaporation and productivity on $\delta^{13}\text{C}$ values. The lack of a strong NPP pattern somewhat limits the ability to infer productivity of *Sphagnum* in paleoecological studies.

4.2 Global patterns of $\delta^{18}\text{O}$ values in *Sphagnum*

Modelled $\delta^{18}\text{O}$ values in precipitation (Bowen 2010) explained much of the variation in $\delta^{18}\text{O}_{\text{tissue}}$ values between sites ($R^2=68\%$ for annual mean $\delta^{18}\text{O}_{\text{precip}}$). The percent variance explained was even higher if the spring period for modelled
360 $^{18}\text{O}_{\text{precip}}$ was used, but lower for the growing season average. This result does not necessarily mean that spring season water was utilised by the plants during the growing season. Between-site variation in $^{18}\text{O}_{\text{precip}}$ values are much larger in the winter (Figure S1, see end of document), more effectively discriminating maritime and continental regions (Bowen 2010). The better fit may simply be an effect of a more distinct separation of $^{18}\text{O}_{\text{precip}}$ in the winter data. Although the $\delta^{18}\text{O}_{\text{tissue}} - ^{18}\text{O}_{\text{precip}}$ relationship presented here is robust, a few $\delta^{18}\text{O}$ values are less well-predicted by the regression model and they originate
365 from Northwest Territories (Canada) and West Siberia (Russia). Likely, this suggests that the $^{18}\text{O}_{\text{precip}}$ model is less accurate in these interior regions with fewer collection stations.

In contrast, the data did not support a negative correlation between precipitation amount and $\delta^{18}\text{O}_{\text{tissue}}$ values; and $\delta^{18}\text{O}_{\text{tissue}}$ values were only weakly affected by predictors associated with water loss (ET/P and/or temperature) and species identity.
370 The indication of ^{18}O enrichment in *S. magellanicum* due to ET/P was expected as the lighter isotope ^{16}O needs less energy to vaporize. However, the opposite trend was suggested for *S. fuscum*, and surprisingly, higher surface temperatures decreased ^{18}O enrichment. Hence we conclude that climatic variables associated with water loss were weak predictors after controlling

for $\delta^{18}\text{O}_{\text{precip}}$ values. This result may not be too unexpected as laboratory experiments have so far failed to relate ^{18}O enrichment in *Sphagnum* to differences in evaporation rates (Brader et al. 2010).

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There have been few regional studies on moss $\delta^{18}\text{O}_{\text{tissue}}$ values that span gradients of $\delta^{18}\text{O}_{\text{precip}}$ values (Royles et al. 2016, Skrzypek et al. 2010) and most interpretations of moss $\delta^{18}\text{O}_{\text{tissue}}$ - climate relationships come from peat core studies (e.g. van der Knaap et al. 2011). In antarctic non-*Sphagnum* peat banks variation in $\delta^{18}\text{O}_{\text{cellulose}}$ values tracked $\delta^{18}\text{O}$ values in moss water across a latitudinal gradient (61°S-65°S) despite a lack of difference in $\delta^{18}\text{O}_{\text{precip}}$. This result led Royles et al. (2016) to suggest that moss water and tissue $\delta^{18}\text{O}$ values are better temporal integrators of source water than point rainfall measurements. The authors interpreted site-to-site differences as relating to differential evaporative enrichment and other physio-chemical factors that affect ^{18}O exchange, fixation and biochemical synthesis. Similar patterns may also occur along elevational gradients as $\delta^{18}\text{O}_{\text{tissue}}$ values are consistent with expected isotopically heavier source water at high elevations controlling tissue signals, but with small sample sizes (n=7) patterns remain unclear (Skrzypek et al. 2010). The present study provides a much greater range of geographical and environmental variation, and arrives at similar conclusions — $\delta^{18}\text{O}_{\text{tissue}}$ values in *Sphagnum* strongly track source water.

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Interestingly, the relationship between $\delta^{18}\text{O}_{\text{tissue}}$ and $\delta^{18}\text{O}_{\text{precip}}$ values detected here is very similar to that proposed some time ago by Epstein et al. (1977); $\delta^{18}\text{O}_{\text{cellulose}} = 27.33 + 0.33 \times \delta^{18}\text{O}_{\text{precip}}$ [note that Jones et al. 2014 show high correspondence between $\delta^{18}\text{O}_{\text{cellulose}}$ and $\delta^{18}\text{O}_{\text{tissue}}$ values]). However, our data suggest a slightly steeper slope and lower intercept, particularly for *S. magellanicum*. The species effect on $\delta^{18}\text{O}$ suggests a difference in the degree of evaporation from the plant surface prior to uptake of water. The lower $\delta^{18}\text{O}$ values for *S. magellanicum* compared to *S. fuscum* (-0.83‰); is comparable to the results from bogs in Canada for the same species (-2.2‰, Aravena and Warner 1992) and between a hollow and a hummock species in The Netherlands (-2‰, Brenninkmeuer et al. 1982). This suggests that the absorbed water in this *S. magellanicum* was subject to less evaporation. In *Sphagnum* plants, control the water available on their surface water is largely affected by capillarity, water storage and reducing conductance with compact morphology. Plant traits that enhance these functions are more pronounced in species and individuals found at high HWT as these characteristics maintain high tissue water content (Hayward and Clymo 1982, Laing et al. 2014, Waddington et al. 2015). Consequently, during droughts, *Sphagnum* species growing close to the water table will dry out quickly as the evaporative demand cannot be balanced, and simultaneously photosynthesis is shut down. *Sphagnum* species higher above the water table wick water from below and store water effectively, thereby remaining photosynthetically active while water is lost due to evaporation. This mechanism would result in ^{18}O enrichment being higher above the water table (Brenninkmeuer 1982, Aravena and Warner 1992), and explains the positive relationship between HWT and $\delta^{18}\text{O}$ in *S. magellanicum* reported by Loader et al. (2016) along a 10 m transect. We found a weak positive relationship of $\delta^{18}\text{O}$ with HWT, which suggests that HWT cannot entirely explain species-specific differences in ^{18}O enrichment. Instead, this can be attributed to lower water retention (i.e. higher evaporation at the same water deficit) in *S. magellanicum* compared to *S. fuscum* (Clymo 1973, McCarter and Price 2014). Although species

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differences in ^{18}O have been reported (Aravena and Warner 1992, Zanazzi and Mora 2005, Bilali et al. 2013), our study suggests that the species-specific $\delta^{18}\text{O}$ signals may not simply be a consequence of growing at different HWT but can rather reflect distinct water retention capacity in these species.

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The strong influence of $\delta^{18}\text{O}_{\text{precip}}$ values and, to a much lesser extent, environmental variables related to water loss, combined with a relatively small within-site variation in $\delta^{18}\text{O}_{\text{tissue}}$ values, suggest that macroclimatic drivers, such as precipitation inputs, largely determine the $\delta^{18}\text{O}$ value of peatland moss tissue. These results are promising for the use of oxygen isotopes in large-scale paleoecological reconstructions from peat cores (Ellis and Rochefort 2006, Chambers et al. 2012, Daley et al. 2010), although a better understanding of O isotope fractionation within tissue components and their decay relationships would improve their utility. Moreover, the simple relationships presented here can potentially be utilised to trace changes in $\delta^{18}\text{O}_{\text{precip}}$ values that mirrors climate variability.

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5 Conclusions

Our study provides new insights into large-scale variation in *Sphagnum* tissue isotopic signature and suggests that isotopic composition can be used for climatic reconstructions. We show a close link between precipitation and tissue $\delta^{18}\text{O}$ values and conclude that variation in $\delta^{18}\text{O}$ values are mainly driven by the macroclimate, but species differences exist. In contrast, $\delta^{13}\text{C}$ values were strongly related to local microtopography while the influence of macroclimate was negligible but also weakly related to macroclimate. As suggested in earlier studies, $\delta^{13}\text{C}$ values were also weakly associated with NPP. These conclusions were most strongly supported for the cosmopolitan *S. magellanicum* complex and species identity should be accounted for in future carbon isotope studies to avoid spurious conclusions.

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6 Data and code availability

Data and R-script to reproduce results are available for review at <https://github.com/ggranath/isotopeSphagnum>. Upon acceptance these files will be moved to a permanent public repository.

7 Supplementary material

430 | Figure S1, ~~and~~ Table S1 and Table S2.

8 Author contribution

SKR, GG, HR initiated the study and formulated the research objectives. All authors were involved in data collection and SKR, NB, KP, AV, DPG performed the isotope analyses. GG performed the statistical analyses and wrote the first draft with input from SKR and HR. All authors read and commented on the manuscript and approved the final version.

435 9 Competing interests

The authors declare that they have no conflict of interest.

10 Acknowledgement

To the memory of coauthor and *Sphagnum* enthusiast Maria Noskova, who passed away, tragically, before this paper was finished. We thank [Union College and](#) the U.S. National Science Foundation for providing funding for Union isotope ratio
440 mass spectrometer and peripherals (NSF-MRI #1229258) and Sarah Katz for laboratory assistance. Data collection was supported by the Russian Science Foundation, grant 14-14-00891 and by the Russian Foundation for Basic Research according to the research projects No 14-05-00775 and No 15-44-00091, University of Ferrara (FAR 2013 and 2014), the Polish National Centre for Research and Development within the Polish-Norwegian Research Programme within the project WETMAN (Central European Wetland Ecosystem Feedbacks to Changing Climate Field Scale Manipulation, Project ID:
445 203258), [and the National Science Centre, Poland ID: 2015/17/B/ST10/01656](#), institutional research funds from the Estonian Ministry of Education and Research (grant IUT34-7), the Natural Sciences and Engineering Research Council of Canada Discovery Grants program awarded to JLB, an NSERC Strategic Grant, and with generous support awarded to L.I.H from the W. Garfield Weston Foundation Fellowship for Northern Conservation, administered by Wildlife Conservation Society (WCS) Canada, and National Science Foundation (NSF-1312402) to SMN. We acknowledge the Adirondack and Maine
450 offices of The Nature Conservancy, the Autonomous Province of Bolzano (Italy), Staatsbosbeheer and Landschap Overijssel (the Netherlands), the Greenwoods Conservancy, NY and the University of Orono, ME for access to field sites.

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	Species	N _{site}	N _{obs}	Within-site		Between-site	
				Std. Dev.	Proportion variance (%)	Std. Dev.	Proportion variance (%)
δ ¹³ C	<i>S. fuscum</i>	80	169	0.9	56	0.8	44
	<i>S. magellanicum</i>	83	168	1	51	0.98	49
δ ¹⁸ O	<i>S. fuscum</i>	80	168	0.7	13	1.83	87
	<i>S. magellanicum</i>	83	167	0.67	10	2	90

Table 1. Sample sizes, standard variation and overall partitioning of measured variation for each species and response (δ¹³C and δ¹⁸O). N_{site} is the number of sites and N_{obs} the total sample size. Standard deviation of the responses is given for within and between sites, together with the proportion of total variance measured between sites and within sites.

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variable	effect	F	DF	P	N	R2site	R2marginal
HWT		26.8	1, 67	<0.001	311		
species	-0.88+0.13*	43	1, 274	<0.001			
HWT*species	S.fus: -0.021+-0.008 S.mag: -0.045+-0.008	6	1, 235	0.01		0.32	0.18
HWT [<i>S.magellanicum</i>]	-0.04+-0.008	26	1, 134	<0.001	158	0.33	0.17
NPP	0.0023+-0.0005	23.1	1, 309	<0.001	318	0.12	0.07
species	-0.38+-0.12	9.4	1, 266	<0.01		0.01	0.01
NPP*species		0.7	1, 290	0.41			
HWT		25.5	1, 246	<0.001	295		
species	-0.62+-0.13*	22.1	1, 269	<0.001			
NPP	0.0022+-0.0005	22.4	1, 281	<0.001		0.11	0.07
HWT*species	S.fus: -0.012+-0.007 S.mag: -0.042+-0.007	10.6	1, 267	<0.01		0.2	0.12
elevation	0.00035+-0.0002	2.7	1, 81	0.11	295	0.03	0.01
elevation*species		0.5	1, 233	0.47			
ET/P		0.2	1, 90	0.66	295		
(ET/P)*species	S.fus: -0.33+-0.40 S.mag: 0.78+-0.44	5	1, 266	0.03		-0.02	0.01
P	-0.00013+-0.0006	0	1, 80	0.83	295	-0.01	0
P*species		1.2	1, 248	0.27			
T		0	1, 91	0.97	295		
T*species	S.fus: -0.051+-0.034 S.mag: 0.087+-0.041	9.7	1, 273	<0.01		-0.05	0.02

490 *The effect of *S. magellanicum* compared to *S.fuscum* at HWT 28 cm.

495 **Table 2. Results from linear mixed-models for $\delta^{13}\text{C}$ values. Statistical tests are based on type-2 *F*-test using Kenward-Roger adjusted degrees of freedom. The second model only included *S. magellanicum*. Elevation [m asl] and the three climatic variables (growing season sums and means: ET/P, P [mm], temp [°C]) were tested one by one in the model including HWT [Height above the Water Table, cm], species and NPP [$\text{mg m}^{-2} \text{year}^{-1}$]. **For simplicity, the negligible HWT \times NPP term was dropped from this model (P = 0.36).** Estimated effects (+/- SEs) are only given for main effects if interactions were considered negligible. These effects are slopes for continuous variables (all variables except species) and for species (categorical) the difference between *S. magellanicum* and *S. fuscum* (i.e. *S. fuscum* being the reference level). In the presence of an interaction between HWT and species, the species effect was estimated at mean HWT. R^2_{site} = explained between-site variance, R^2_{marginal} = explained total variance.**

variable	effect	F	DF	P	N	R2site	R2marginal
annual precip d18O	0.43+-0.035	148.4	1, 95	<0.001	335	0.69	0.5
species	-0.83+-0.083	101.3	1, 250	<0.001			0.05
annual precip d18O*species		1.9	1, 261	0.16			
Apr-Sep precip d18O	0.49+-0.049	100.5	1, 94	<0.001	335	0.58	0.42
species	-0.83+-0.083	99.4	1, 249	<0.001			0.05
Apr-Sep precip d18O*species		1.4	1, 256	0.24			
Jan-Apr precip d18O	0.37+-0.027	187.2	1, 96	<0.001	335	0.75	0.55
species	-0.84+-0.083	102.3	1, 252	<0.001			0.05
Jan-Apr precip d18O*species		2.3	1, 265	0.13			
annual precip d18O	0.41+-0.038	111.9	1, 88	<0.001	310	0.64	0.46
HWT	0.015+-0.005	10.4	1, 288	<0.01		0	0.01
ET/P		0.1	1, 99	0.81	335		
(ET/P)*species	S.fus: -0.39+-0.48 S.mag: 0.28+-0.50	3.5	1, 266	0.06		0	0
P	-0.0005+-0.0008	0.4	1, 99	0.54	335	0	0
P*species		0.8	1, 257	0.37			
T	-0.14+-0.051	7.4	1, 96	0.01	335	0.02	0.02
T*species		1.6	1, 274	0.21			

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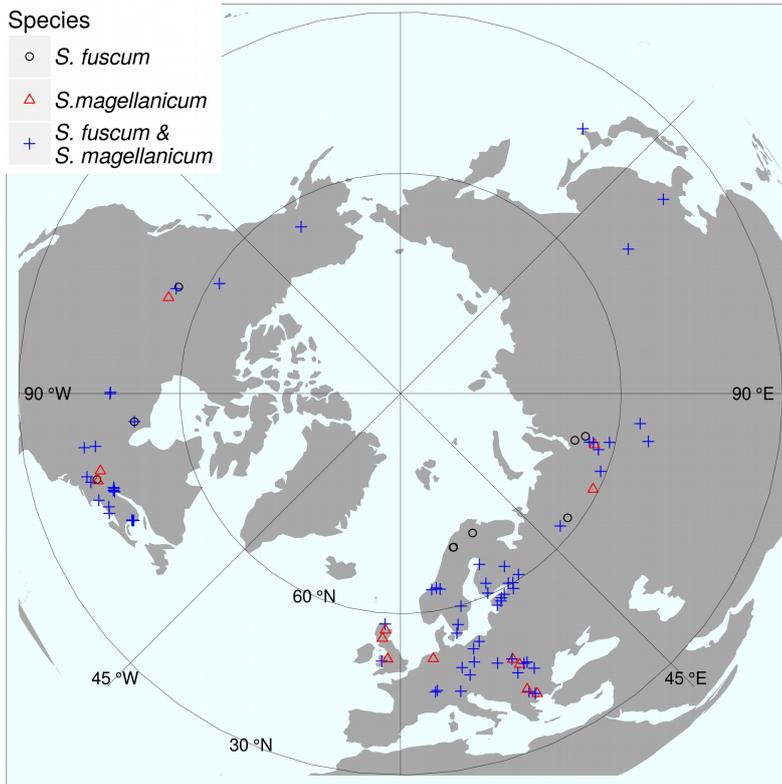
Table 3. Results from linear mixed-models for $\delta^{18}\text{O}$ values. Statistical tests are based on type-2 *F*-test using Kenward-Roger adjusted degrees of freedom. Three time periods for modelled $\delta^{18}\text{O}$ values (‰) in precipitation were tested individually: annual mean, growing season (Apr-Sep) and spring (Jan-Apr). The three climatic variables (growing season sums and mean: ET/P, P [mm], temp [°C]) were tested one by one in a model including HWT [cm] and mean annual $\delta^{18}\text{O}$ values). Estimated effects (+/- SEs) are only given for main effects if interactions were considered of negligible. These effects are slopes for continuous variables (all variables except species) and for species (categorical) the difference between *S. magellanicum* and *S. fuscum* (i.e. *S. fuscum* being the reference level). R^2_{site} = explained between-site variance, R^2_{marginal} = explained total variance.

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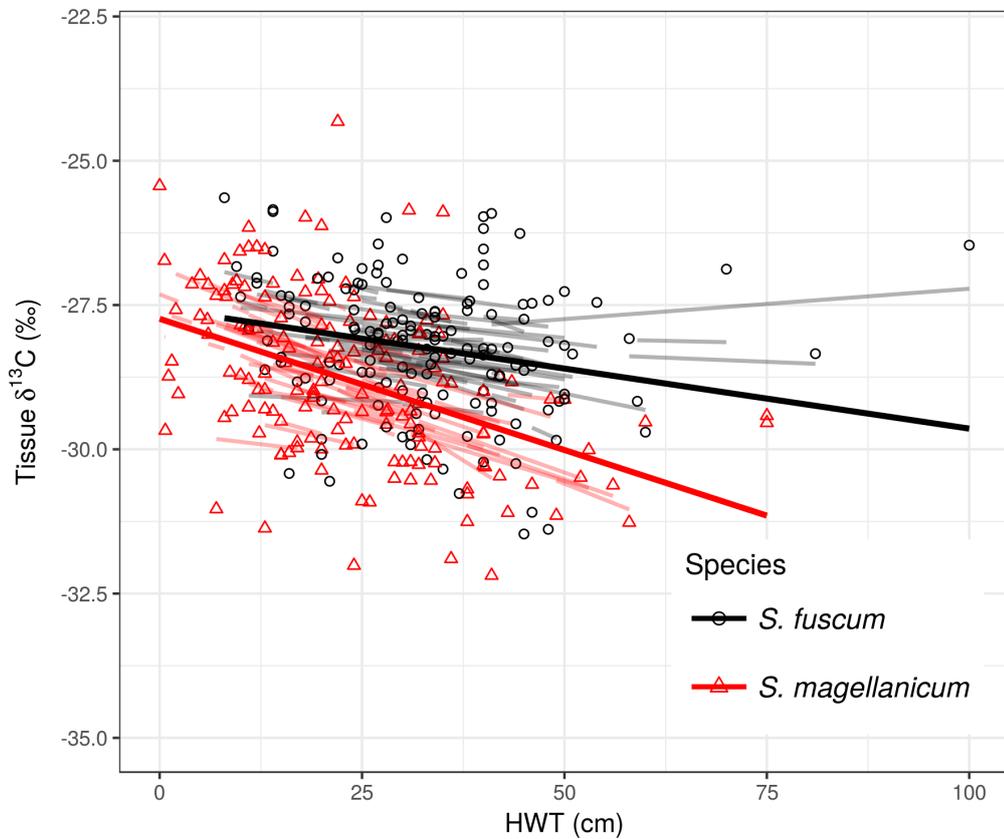


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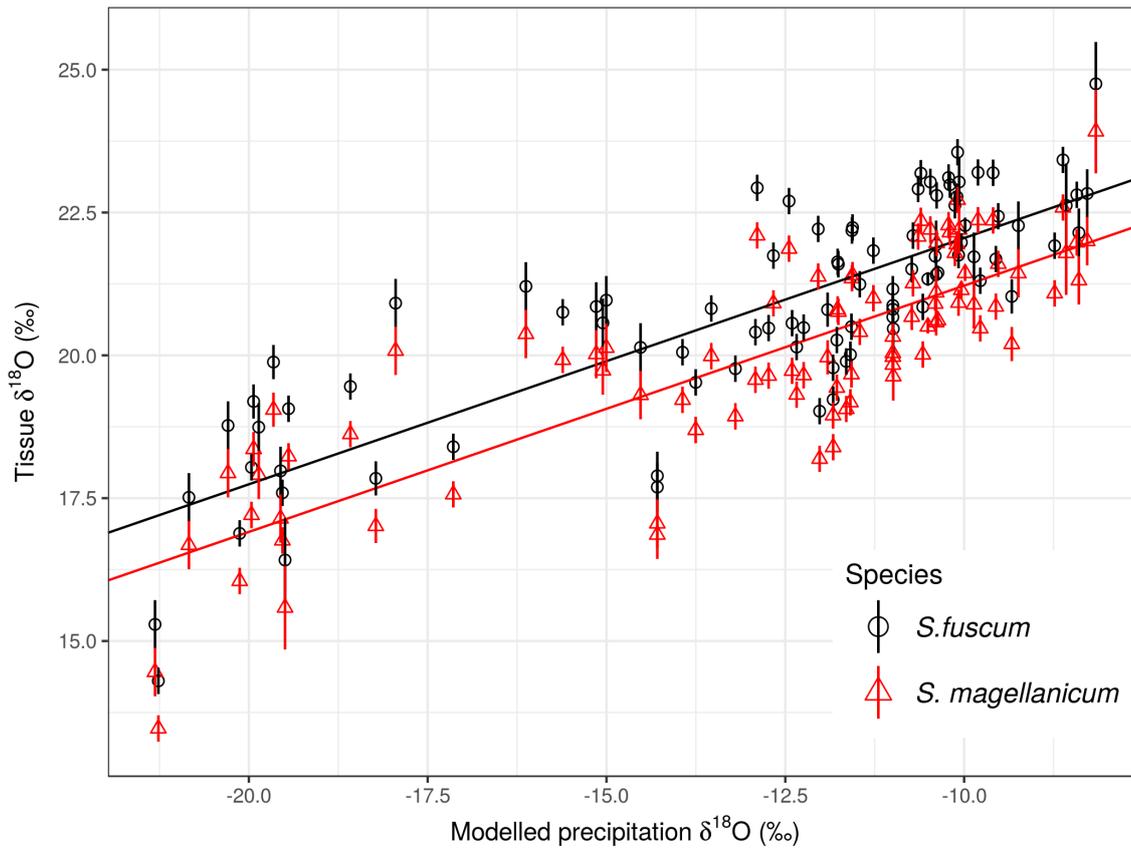
Figure 1. Map illustrating sample sites for the investigated species. At some sites only one of the two *Sphagnum* species was sampled, indicated by red triangles or black circles, otherwise sites contained both species (blue crosses). The map is centered on the North Pole and has an orthographic projection. Geographical ranges: latitude 41.6N-69.1N, elevation 2 - 1 829 m asl. See Table S1 for details.

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545 **Figure 2.** Relationship between height above the water table (HWT, measured at the end of the growing season) and $\delta^{13}\text{C}$ values in two *Sphagnum* species sampled across the Holarctic realm. Pale lines represent relationships for individual sites, while thicker lines show the pooled regression line in a mixed-effect model. Equations: *S. fuscum*, $\delta^{13}\text{C} = -27.56 - 0.021 \times \text{HWT}$; *S. magellanicum*, $\delta^{13}\text{C} = -27.74 - 0.045 \times \text{HWT}$. $N_{\text{site}} = 83$, $N_{\text{total}} = 311$. See also Table 1.



560 **Figure 3.** Association between modelled annual mean $\delta^{18}\text{O}_{\text{precip}}$ values and $\delta^{18}\text{O}$ values in two *Sphagnum* species. Data show predicted site means (BLUPs) and error bars represent the approximate 95% confidence intervals ($2 \times \text{SE}$). Regression lines with different intercepts ($P < 0.001$, Table 2) illustrate the relationship between modeled $\delta^{18}\text{O}_{\text{precip}}$ and *Sphagnum* $\delta^{18}\text{O}$. Equations: *S. fuscum*, $26.36 + 0.43 \times \delta^{18}\text{O}_{\text{precip}}$ ($n = 1-2$, $N_{\text{site}} = 80$); *S. magellanicum*, $25.53 + 0.43 \times \delta^{18}\text{O}_{\text{precip}}$ ($n = 1-2$, $N_{\text{site}} = 83$).