

# ***Interactive comment on “Environmental and taxonomic controls of carbon and oxygen stable isotope composition in *Sphagnum* across broad climatic and geographic ranges” by Gustaf Granath et al.***

**Gustaf Granath et al.**

gustaf.granath@gmail.com

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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

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an important result, even when growing in close proximity. The relationship 18O and the annual precipitation is interesting and an important result that has wide ranging relevance. The relationships with 13C 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 will include an overview of the variables (means, ranges) and comments on data points that diverge from the overall trends (site location). Regarding the statistical analyses: We think the reviewer refer to the d13C results, and the effect of NPP, ET and temperature that are discussed although their R2-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 we will 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 will be changed to “..... depend on nutrients, water and CO2 uptake from the atmosphere.”*

Line 73: compositions (rather than composition)

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*RESPONSE: OK.*

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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' will be removed from the sentence.*

Line 83: Holarctic spelling

*RESPONSE: Will be fixed.*

Line 83: Were the differences significant in d13C between species

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

Line 85: Where R2 is only 6

*RESPONSE: As the relationship between d13C and NPP was a part of our aims, we would like to include this result in the abstract. We will add a few words about the poor strength of this relationship on L90 (current version).*

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 Co2 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,

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and assimilation continues by the moss, the carbon concentration will decrease, but what is more important is that the proportion of  $^{13}\text{CO}_2$  will increase and consequently discrimination against  $^{13}\text{CO}_2$  will decrease.

*RESPONSE: Yes, this is what we meant and it is mentioned in the following sentences. This will be clarified in the revised version.*

Line 113: remove “consequently”.

*RESPONSE: OK.*

Line 113: Respiratory  $\text{CO}_2$  can be fixed when the mosses are not submerged: particularly close to the ground the isotopic composition of the source  $\text{CO}_2$  may vary in space and time depending on the extent of mixing between any respired  $\text{CO}_2$  at the bog surface, and the well mixed atmosphere above.

*RESPONSE: This is correct. This potential mechanism will be included in the next 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:  $\text{CO}_2$ : 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/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 will describe this in the revised version.*

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

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*RESPONSE: An additional column indicating the species sampled at each site will be 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 palao 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

*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 will be 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.

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*RESPONSE: Also commented on by reviewer 1. We here repeat the same response. Our measurements of HWT is a snapshot and the d13C-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 number is not in the current manuscript but will be added).*

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.*

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 will add a table showing the means, SDs and ranges.*

Line 272: Table 1 is unclear: need means of both 13C and 18O values rather than just the variation. Add per mille sign to SD values. Unclear what the proportion of variance

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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 will add information 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 d13C 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 ( $N_{site} = 80$ ,  $N=c. 160$ ), 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 will prepare a clearer version of this graph.*

*See also next comment.*

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Fig 3: Plot confidence interval on regression lines.

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*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 d13C, 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 will shorten 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 will clarify this and 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. Will be 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: Will be reworded as this is mostly a passive process.*

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Line 391: May not be generalisable across moss species: sphagnum 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.

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*RESPONSE: We will specify that we mean peatland mosses.*

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