Reply to Referee #2

by Johannes Hepp, Michael and Roland Zech & co-authors

GENERAL:

The topic of the manuscript is interesting and important as it deals with the evaluation of highly promising proxies used to reconstruct past environmental conditions. While the data produced are rare and are certainly worth publishing, the manuscript has severe flaws that prevent, in my opinion, its publication in this form.

→ While we are grateful to Referee #2 for her/his constructive suggestions helping to improve our manuscript, the two raised ‘major problems’ are certainly no ‘severe flaws’ preventing publication (see our replies below). We therefore see no justification for rejection of our manuscript based on the review provided by Referee #2.

MAJOR PROBLEMS:

A) While reading the manuscript, the connection between GDGT and the plant proxies (i.e. n-alkanes and hemicellulose) is not clear and seems disconnected as if from two separate manuscripts. Moreover, in the section 3.1 of the discussion, the GDGT data are presented in a way leading the readers to believe that these molecules are produced by plants.

→ We are very surprised that Referee #2 considers the GDGT and the n-alkane/sugar biomarker approach as disconnected. We disagree. Both approaches are based on biomarkers/molecular proxies and are used for paleoclimate reconstructions. We clearly state and explain in the introduction and method sections how the applied biomarkers (GDGT’s as well as n-alkanes and sugars) are produced, how calculations are done and how the proxies can be interpreted. Please note that there are plenty of studies in the literature presenting both GDGT and δ^2Hn-alkane results in one publication → certainly no major problem/severe flaw.

B) The other major point is that the authors suggest that it is “often” not feasible to disentangle between the evapotranspirative enrichment from the precipitation signal, but there is at least another well-established method to do so and published in Climate of the Past (see recent Sachse’s group publications, e.g. A dual-biomarker approach for quantification of changes in relative humidity from sedimentary lipid D/H ratios, Climate of the Past, 2017). While this method should at least be mentioned, I also believe the method should be compared to help the readers understand the full set of tools available to study that issue. These two methods are very likely to be highly complementary.

→ Please note that the ‘dual biomarker approach’ of Rach et al. (2017, CP) is not applicable to terrestrial (soil) samples/archives, but only to lacustrine settings. A comparison with our ‘coupled δ^2Hn-alkane-δ^{18}O_{sugar} biomarker approach’ is therefore neither possible nor reasonably within our European climate transect study → certainly no major problem/severe flaw.
For a critical evaluation and assessment of both approaches when applied to lacustrine paleoclimate archives, we kindly refer our readers to Hepp et al. (2019, CP) and to our ‘Reply to D. Sachse and F. Schenk (SC4: Data analysis and paleoclimatic context)’ available online via https://www.climPast-discuss.net/cp-2018-114/cp-2018-114-AC6-supplement.pdf.

Accordingly, the major shortcomings/uncertainties of the ‘dual biomarker approach’ of Rach et al. (2017) are (i) biosynthetic fraction, (ii) the assumption that paleo-lake water is not affected by evaporative enrichment and (iii) the assumption that the alkane nC23 in lacustrine sediments is of aquatic origin. At least for Central European case studies, the latter assumption is certainly not valid, because birch produces considerable amounts of mid-chain n-alkanes such as nC23. This is acknowledged e.g. by Aichner et al. (2018, CP) concluding for a palaeo lake from Poland that “...mid-chain compounds, which are often interpreted as of aquatic origin, are here rather a mixture of aquatic and terrestrial sources, with high proportional input of the latter during certain time periods.”

This short excursion highlights the need for alternative approaches and justifies the testing/evaluation of our ‘coupled δ2Hn-alkane-δ18Osugar biomarker approach’ as it was done by Tuthorn et al. (2015, BG) for an Argentinian climate transect and as is done in the here presented European climate transect study.

SPECIFICS:

Line 298 to 303: This section is not clear due to some typos or mistakes, please reformulate.

→ Changed.

Line 389 to 407: While the difference of ebio is reported at the end of the section (around line 477 to 487), the possibility that a variable ebio could explain the different signals in different types of vegetation, beside the damping effect, is evacuated of the discussion. This should at least be discussed.

→ Changed.

Line 432: Is that referring to simply using isotope values of a single compound? What is that hitherto method (reference missing)? I believe this brings us back to the problem B. The results would gain a lot to be compared with the updated tool box of proxies.

→ The sentence was slightly changed. See also our reply to ‘major problem B’.

Line 444 to 458: The argumentation is not clear/convincing, please reformulate.

→ We deleted the respective sentence from the revised version of the manuscript.

Line 483-484: The idea of a variable ebio is well expressed in general, but references to some recent works is missing that shows even greater variability in n-alkane dD values under different metabolisms (e.g. Cormier et al, 2018 – New Phytologist, Tipple & Ehleringer 2018 – Oecologia, Cormier et al, 2019 – Oecologia)

→ Please note that we already included Cormier et al. (2018) in the actual version of the manuscript and that the fact is mentioned that εbio can range even larger when also the metabolic status of the plants is considered. However, we changed the respective sentence to:
“The wide range in biosynthetic \( ^2 \)H fractionation factors, which can be even larger, is therefore also related to the carbon and energy metabolism state of plants (Cormier et al., 2018).”

*Line 490 to 494: Please reformulate, this section is not clear.*

→ We changed the quoting of Fig. 10B.

*Line 550: If the author are really considering a variable \( \epsilon_{\text{bio}} \), the damping effect can only potentially explain the different signals observed in different types of vegetation. Again, \( \epsilon_{\text{bio}} \) should be part of the points because standing alone, they can induce confusion even if mentioned afterward.*

→ You are right. Gao et al. (2014) and Liu et al. (2016) showed that the \( \epsilon_{\text{bio}} \) of monocot plants could larger than those of dicots. This would therefore course a more negative apparent fractionation factor for grasses compared to trees. We observe that the apparent fractionation is indeed more negative for the grass sites compared to the forest sites. We will included a discussion about the indistinguishable effects of “signal damping” vs. variable \( \epsilon_{\text{bio}} \) along with vegetation types in the respective parts of the manuscript.

**References**


Tuthorn, M., Zech, R., Ruppenthal, M., Oelmann, Y., Kahmen, A., del Valle, H. F., Eglinton, T.,