Reply to Referee #2

by Johannes Hepp, Michael Zech & co-authors

The manuscript written by Hepp and colleagues presents results of laboratory experiments where different plant species were grown and hydrogen and oxygen isotope ratios were measured on different organic compounds. The dual isotope approach is a valuable and important step toward better paleoclimate reconstructions, but I wonder how comparable these two compounds are. There are differences in the ways these two compounds are synthesized and I think a more in depth discussion of these mechanisms is necessary in order to confidently use them, especially for paleoenvironmental reconstructions.

→ We are very grateful to anonymous Referee #2 for her/his judgement that our coupled $\delta^2H_{n\text{-alkane}}$- $\delta^{18}O_{sugar}$ paleohygrometer approach is a valuable and important step towards better paleoclimate reconstructions. We agree that *n*-alkane and sugar biomarkers are biosynthesized differently. However, the principles are the same for both biomarker classes: they (i) reflect the isotopic composition of precipitation modified by (ii) leaf water enrichment due to evapotranspiration and (iii) biosynthetic fractionation. We feel that a more in depth discussion of further specific and complicating mechanistic details would be beyond the scope of this manuscript. As pointed out by Reviewer#2 (and Reviewer#1), our MS is already rather lengthy and needs refocusing. For further mechanistic details we therefore suggest to refer our readers to the literature.

Overall, the manuscript is rather lengthy and could be made more concise by refocusing the discussion. The discussions about biosynthesis should be revisited and revised, because as written now they are a bit unclear. It might be worth it to discuss biosynthesis and effects that might have on isotopic values first, then move to a discussion about how comparable isotopic values of these two compounds really are. This could be followed by extracellular factors that influence these proxies and the comparison with published data and what this might mean overall. There is also a model presented here, but the results of that model are peppered throughout the discussion which make it difficult to follow. It would be good to make this clear, perhaps by dedicating a section solely to the model-data comparison. Finally, a number of sentences would benefit from restructuring because as written now they are hard to follow. Please pay attention to grammar and appropriate phrasing throughout.

→ Thank you for raising these issues and providing suggestions how to restructure and improve our manuscript. We will do our best to improve clarity during the revision.

Specific comments:

Lines 42-44 : Consider rewording this to: 'can relative humidity be accurately reconstructed from leaf water isotope values'.

→ We will readily change this as suggested.

Line 43: Should be 'enable'

→ Will be changed.

Line 45: robust source water reconstruction?

→ As mentioned in the replies to Referee #1, we will readily clarify the whole manuscript also regarding source water (e.g. plant source water vs. source water for biosynthesis…).

Line 60: it might be better to explain this differently. 'getting worse' sounds very informal.

→ Will be changed.

Line 73: 'with respect to' instead of 'in respect'

→ Will be changed.

Line 80: It would be good to discuss the correlation between d2H and d18O in meteoric waters here.

→ Will be added.

Line 82: Please explain the climate transect. Altitudinal?

→ This information will be added.

Lines 123-124: were these temperature and humidity values for all of the chambers? Please better explain the set up, e.g., two chambers were kept at a temperature of X and humidity of Y. Also, please remove the additional 'and' on line 124.

→ Will be changed.

Line 152: pyrolysis mode

→ Will be changed.

Line 211: 'where' not 'were'

→ Will be changed.

Line 290: weighted mean of C29 and C31?

→ Yes, will be added.

Line 314: why is it better to use the weighted mean instead of the individual d18O for arabinose and xylose?

→ We expect the individual sugars to be more prone to analytical uncertainties. Please compare with alkanes, where often weighted means are used, too.

Line 322: what is the offset?

→ The offset reflects the fractionation between biomarker and leaf water (ε_{bio}). This will be added during the revision.

Line 328: change 'relation' to 'correlation'

→ Will be changed.

Lines 407 – 412 : The way you discuss the biosynthesis here is unclear. It reads like you are saying hydrogen is added to a lipid in the chloroplast and the cytosol and on top of that photosynthesis and the pentose phosphate pathway add other hydrogen. NADPH is reduced by different sources in the chloroplast and the cytosol (see Schmidt et al., 2003). This reduced NADPH is then used in lipid biosynthesis in these separate compartments. Please be careful how you discuss this. Also it should be pentose phosphate 'pathway' not cycle. Furthermore, are you sure the n-alkanes are synthesized in the cytosol and not in the endoplasmic reticulum? The Schmidt et al. (2003) and Cormier et al. (2018) papers both provide excellent explanations of this and effects of biosynthesis on isotopic fractionation of lipids (specifically have a look at figure 5 from Cormier et al., 2018 for the n-alkane synthesis). Finally, on line 408: 'modifying/expanding fatty acids' should be changed to 'elongation of fatty acids'.

→ Thank you for raising this issue and giving these explanations. Will be checked and changed.

Figure 1A: It is difficult to distinguish the different shapes in this figure. It might be helpful to remove the lines from these plots. The colors from xylem water and soil water are very similar. You might consider choosing two colors with more contrast.

→ Will be changed.