Interactive comment on “Validation of a coupled $\delta^{2}H_{r-alkane}$-$\delta^{18}O_{sugar}$ paleohygrometer approach based on a climate chamber experiment” by Johannes Hepp et al.

Anonymous Referee #2

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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.

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.

Specific comments:

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

Line 43: Should be ‘enable’

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

Line 73: ‘with respect to’ instead of ‘in respect’

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

Line 82: Please explain the climate transect. Altitudinal?

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.
Line 152: pyrolysis mode
Line 211: ‘where’ not ‘were’
Line 290: weighted mean of C29 and C31?
Line 314: why is it better to use the weighted mean instead of the individual d18O for arabinose and xylose?
Line 322: what is the offset?
Line 328: change ‘relation’ to ‘correlation’

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’.

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.