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

Anonymous Referee #2

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Hepp et al. describe a project that focuses on investigating the role of environmental conditions on stable isotopic composition of modern plant biomarkers. The authors grew three higher plant species in growth chambers under controlled conditions (varied temperature and relative humidity) and then measured the d2H values of leaf-wax n-alkanes and the d18O values of hemicellulose sugars. These data were supplemented by isotope data (oxygen and hydrogen) for soil, xylem and leaf waters. The goal of the project was to investigate the usefulness of integrating the n-alkane d2H and sugar d18O data for reconstructing paleo-humidity. This is a very detailed and well-described study that is likely to attract attention from biogeochemists, paleoecologists and paleoclimatologists who use biomarker isotopes for studying climate change in the past.

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The manuscript fits the scope of Biogeosciences quite well, however, there are several issues I would like to be resolved before this work is published. For this manuscript to be attractive to the readers of this journal, the authors need to have a more detailed discussion of what the implications of their findings are and, more importantly, how the information generated in this project could be used in the paleo context.

First: the applicability of this approach to paleo records The authors do a very thorough job providing a theoretical rationale for their approach and demonstrating that the biomarker data they've obtained can be used to reconstruct the isotopic composition of leaf water. Moreover, the dual biomarker approach could work well for estimating paleo relative humidity. However, it is not clear how exactly this approach would work with sedimentary n-alkanes and sugars, provided the latter survive the fossilisation process. What type of sedimentary material would be needed with this approach, i.e. would it be intact plant fossils or would this work on dispersed particular plant matter as well?

Second: the choice of plants The authors have used three very different – morphologically and physiologically speaking – higher plants without really explaining why they chose these particular plants. What guided their choice and why do they think the isotope data they've generated (and the fractionation factors they've calculated for these species) are representative of how the dual d2H – d18O approach works in general in higher plants. The species-specific hydrogen isotope fractionation between leaf water and biomarkers shown in Fig. 4 indicates that there are considerable differences among the three species. At the same time, the authors state on lines 99-100 "In case the biosynthetic fractionation is known and constant, there is a great potential to derive RHair from coupling d2Hn-alkane with d18Osugar values." But for paleo samples, we wouldn't really know what the fractionation was. How would we deal with this issue when looking at a paleo record?

Several other suggestions regarding the quality of figures:

Figure 1. This figure is very busy. It needs to be split into 3 figures (climate chamber

conditions, d2H of waters and n-alkanes, and d18O of waters and sugars). At this scale, it is difficult to see what is going on.

Figure 2. The font in the legend is too small. I expect that I won't be legible when transferred to a published figure. Also, when showing biomarkers, it would be useful to identify the three different plants used for this study, like is done in Figure 3.

Figure 3. The choice of symbol colours when showing different plants could be improved. At this scale, it is difficult to see the difference between purple and black. The symbols themselves could also be made larger.

Figure 6. The same issue as mentioned for Figure 1, i.e., the figure should be split into two figures to make the text more legible. Also, does it make sense to show tank water data as a "box"? It would look better if plotted as a single data point.

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