Reply to Referee #2

by Johannes Hepp, Michael Zech & co-authors

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 sugard18O data for reconstructing paleo-humidity. This is a very detailed and well-describedstudy that is likely to attract attention from biogeochemists, paleoecologists and pale-oclimatologists who use biomarker isotopes for studying climate change in the past.

→ We are very grateful to anonymous Referee #2 for her/his encouraging words concerning the attraction of our study and manuscript for different biogeoscientifically working communities.

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?

→ Thank you for raising that issue about the paleo application of the here validated coupled $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ paleohygrometer approach. Indeed, there are already some first paleo applications of this approach (Hepp et al., 2017, 2019; Zech et al., 2013) as well as first climate transect validation studies (Hepp et al., 2020; Tuthorn et al., 2015) published. Similarly, the stability of the biomarker isotope signals during degradation was studied amongst others by our working group (Zech et al., 2011, 2012).

In brief, *n*-alkanes and sugars can be extracted compound specifically investigated from plants, soils and a wide range of different sediments; so no intact plant tissues of fossilized leaves are necessary. When using lake sediments a thorough terrestrial versus aquatic source identification of the biomarkers should be made in order to know whether leaf water or lake water are reconstructed. We will gladly include during the revision the existing paleo applications and the paleo applicability in general.

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?

→ The three species were primarily chosen because they are very different. This allows best to check for species-dependencies. An additional criterion was the resilience of the taxa to the climatic conditions and sufficient growth during the experiments. Differences between the taxa might be explained by different leaf sizes and geometries, possibly affecting, e.g., the Peclet effect. But please note that the differences in biosynthetic fractionation shown in Fig. 4 are statistically not significant. We do not know whether this is caused by the rather low sample number of our study (n = 24) or not. Still, the yielded mean biosynthetic fractionation factors over all species are -156‰ and +27‰ for δ^2H of *n*-alkanes and $\delta^{18}O$ of sugars, respectively. This is well in agreement with data from the literature (usually -160‰ and +27‰, respectively). Hence, for paleo applications it seems well justified to assume in approximation constant biosynthetic fractionation factors (at least for oxygen) when reconstructing leaf water isotopic composition from *n*-alkane and sugar biomarkers. As stated also in our reply to Reviewer#1, we will gladly address this issue and other relevant issues for paleo applications more explicitly during revision.

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.

→ During revision we will ensure a good scale for all figures and reorganize especially figure 1.

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.

→ Will readily be changed.

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.

→ Will readily be changed.

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.

→ During revision we will ensure a good scale for all figures and reorganize especially figure 6. We will add a line for the tank water instead of a boxplot with one data point.

Literature

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