Reply to Referee #1

by Johannes Hepp, Michael Zech, Christoph Mayr & co-authors

Dear associate editor, Johannes Hepp and co-authors,

To start with my conclusion, I think this should be published, it is a beautiful dataset and I have the feeling that this dual isotope method for paleo reconstructions will become more and more important. I do have some issues with the manuscript as is. I had difficulties keeping track of the story a little, it is complex material, but I have the idea the authors have added to, at least, my confusion. I will try to explain what I mean. The measured data clearly shows the isotopic link (similarity) between source water, soil water and xylem water and leaf water moving away from the source water. This leaf water is assumed to be the source for biosynthesis, to some extend at least and on top of that there is biological isotope fractionation. The idea of using both isotopes is that hydrogen isotopes are highly affected by biological fractionation and oxygen isotopes not or less. One reason for that, I read would be O exchange between hemicellulose and leaf water. I had hoped oxygen would also be less dependent on leaf water evaporation, which it could be in paleorecords were bulk soils will be used and not only leaves. Which made me wonder how leaf derived sugars compare to stem or whole plant derived sugars? Especially for the paleo applications the authors mention as a reason for simplifying some of the formulas.

→ We are very grateful to anonymous Referee #1 for her/his encouraging words concerning the potential and value our coupled $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ paleohygrometer approach and the here presented dataset. The idea of using both isotopes is the possibility to reconstruct leaf water that is isotopically both ^2H- and ^{18}O-enriched due to primarily RH-dependent transpiration. This leaf water (i) plots along an evaporation line right of the global meteoric water line in a ^2H-^{18}O diagram and (ii) its isotope signal is incorporated in newly biosynthesized molecules with an in approximation constant biosynthetic fractionation factor. The fractionation between leaf water and *n*-alkanes is strongly influenced by the metabolic pathway of the *n*-alkane biosynthesis including direct hydrogen transfers, exchange reactions, NADPH as hydrogen source (Schmidt et al., 2003, also for more details). Similarly, the oxygen isotopic composition of leaf sugars (sucrose, hemicellulose and cellulose) is strongly influenced by O exchange processes, which cause more positive $\delta^{18}O$ values of sugars compared to leaf water.

We fully agree with Reviewer#1 that O-exchange and thus 'signal damping' is an important issue especially in stem, trunk and root tissues. While these tissues hardly produce *n*-alkanes, (hemi-)celluloses and sugars extracted from such tissues do not show the full leaf water enrichment signal because a partial oxygen exchange with non-enriched stem water (e.g. Zech et al., 2014). We also fully agree with Reviewer'1 that this has to be kept in mind for paleo applications Importantly, this holds also true for the sugars of grasses, which do not record the full leaf water enrichment (e.g. Helliker and Ehleringer, 2002). In the case of grasses, this 'signal damping' affects *n*-alkanes, too. However, such uncertainties can be included in the RH reconstruction via assumptions and sensitivity analysis of the used model, thus allowing quantifying uncertainties of reconstructed RH records as shown e.g. in Hepp et al. (2019).

We will readily and thoroughly check our manuscript during revision in order to avoid possible confusions and to be as clear with our statements as possible.

I have noticed that there is measured leaf water, but also sometimes leaf water isotope values reconstructed based on measured n-alkanes, especially in the comparisons with literature data. On top of that there is evaporative site water isotopic composition especially for H isotopes. At least that was my impression. There is also biosynthetic water that might be different, or water at the site of biosynthesis, again especially for hydrogen. Cytosol versus chloroplast. The role of NADPH is also mentioned. This helps explain the variability, but a lot falls under biosynthetic fractionation, so it explains variability in fractionation. It makes sense all these different versions of water and their impact, but the way the manuscript is organized now I find it extremely confusing. I would suggest a discussion along the main lines ending with some possible explanations for the "scatter". One of the reasons I ask this is because apparently this is also going on for oxygen, leaf water isn't leaf water for oxygens isotopes either. There are isotopic and sucrose synthesis gradients that have to be taken into account. Again, it makes sense, but it is very confusing. And what does this mean for paleo applications? Where are the sugars coming from in paleosols? How is that related to this study? In general, what would be the effect of the oxygen exchange mentioned in the manuscript on paleosamples?

→ For hydrogen, as mentioned in the reply above, we will add more clarity to the discussion especially about the fractionation process during the biosynthesis, which cause the scatter.

We will readily reorganize the whole discussion chapter as suggested in order to make it easier to follow. For oxygen, we will also carefully check and simplify our manuscript where possible in order to avoid confusions.

Readily we will address more explicitly the relevant issues for paleo applications during revision as requested by Reviewer#1.

The authors mention that incubation 4 and 8 are the same, or the climate rooms are, the results are not? Any ideas what might be the reason? Some of the biosynthetic or synthesis water issues mentioned above? Or something different? Already the measured leaf water isotopes are different especially for Eucalyptus.

→ Thank you for raising this issue. Indeed, the climate conditions for experiment 4 and 8 were the same. The experimental run was run twice because only four chambers were available at the same time and replication was needed. Reviewing the data of figure 1 we observe differences between experiment 4 and 8 for *Eucalyptus globulus* which is mainly related to different sampling daytime of leaves between the two experiments. As a diurnal course of light, relative humidity and temperature was simulated in each chamber, steady-state conditions prevailed only during 11 am and 4 pm. In experiments 1-4 the leaf samples were sampled due to time restrictions after that daytime explaining the deviations between experiment 4 and 8 in leaf water isotope composition only. We will explain this in detail in the revised version. This, however, did not influence isotope values of tissue samples which represent the integrated

signal over the entire growing period and does not explain the observed differences in $\delta^2H_{n\text{-alkane}}$. It became also obvious that the $\delta^2H_{n\text{-alkane}}$ values show this difference for all plant types whereas $\delta^{18}O_{sugar}$, $\delta^{18}O_{leaf\text{-}water}$ do not show this as well as $\delta^2H_{leaf\text{-}water}$ for *Vicia faba* and *Brassica oleracea*. The differences in $\delta^2H_{n\text{-alkane}}$ are most likely explainable via the fractionation occurring during biosynthesis of *n*-alkanes, which is (as stated in the first reply) depending not only on leaf water also on plant physiological factors (e.g. water pressure deficit between air and leaf, transpiration rate, assimilation rate, from Schmidt et al., 2003).

We will add this explanation in the reorganized discussion chapter when discussing the scatter in $\delta^2H_{n\text{-alkane}}$ vs. $\delta^2H_{leaf\text{-}water}$.

The authors calculate deuterium excess, I have been told that in highly evaporative systems also the slope of the meteoric waterline could be lower than 8. Could that be applicable to these kinds of systems as well?

→ Yes, we fully agree. Meteoric water lines different from the GMWL (and thus variability of d-excess of precipitation) will certainly affect reconstructions based on our coupled $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ paleohygrometer approach. In the experiment, however, such ambient conditions did not prevail. Both the tank water used for irrigation and the air humidity were regularly sampled and plot on the GMWL. The chambers had a high fresh air supply rate (750 $m^3\ h^{-1}$), so an evaporative isotope enrichment in the chamber was avoided. Only a slight heavy isotope enrichment in the soil water was observed. Thus, the excess variability primarily is the result of leaf water isotopic enrichment.

Specific comments:

It would be great if the symbols in figure 1 could be a bit larger, the difference between squares and triangles is almost in visible. Would it be possible to indicate the T and RH of the different chambers at least in the figure legend, but if possible, in the figure itself?

→ Will readily be changed.

In figure 2 it would be nice if d was defined in the legend. The per mil in the introduction is fine, the other two I would replace with ‰

→ Will readily be changed.

Line 226: n-alkane and sugar biomarkers

→ Will readily be changed.

Line 246: from the latter

→ Will readily be changed.

Line 247: ones or values, not once.

→ Will readily be changed.

Line 278: all three plant spp.

→ Will readily be changed.

Line 415: This could point.... I have a hard time connecting "this" to the previous sentences. I got lost here a little.

→ Will readily be changed during the reorganization of the discussion.

Line472: despite without?

→ We will delete "despite".

Line 472/473: bulk leaf is less enriched than the leaf water at the evaporative sites. Confusing. Bulk leaf water? The measured leaf water is bulk leaf water I assume? Why not use "leaf water" for this and call the other water, "water at the evaporative sites within the leaf" or something similar.

→ Yes, the measured leaf water is bulk leaf water. Will readily be changed during revision in the whole manuscript.

Line 506: This is a very weird way to refer to a figure. You made the figure, it is not measured or observed and therefore a piece of evidence in your reasoning. The sentence starting at therefore till LEL's) can be deleted, I think.

→ Will readily be changed.

Line 550: Or the fact that bulk leaf water as measured does not capture the variability of water within the leaf and potentially important for biosynthesis. At least that has been mentioned several times already. I do think fractionation and variability therein is important, but the authors discussed these different leaf water to extensive to not mention here.

→ Will readily be changed.

Line 553: introduces

→ Will readily be changed.

Literature

Helliker, B. R. and Ehleringer, J. R.: Grass blades as tree rings: environmentally induced changes in the oxygen isotope ratio of cellulose along the length of grass blades, New Phytologist, 155, 417–424, 2002.

Hepp, J., Wüthrich, L., Bromm, T., Bliedtner, M., Schäfer, I. K., Glaser, B., Rozanski, K., Sirocko, F., Zech, R. and Zech, M.: How dry was the Younger Dryas? Evidence from a coupled $\delta^{2}H$–$\delta^{18}O$ biomarker paleohygrometer applied to the Gemündener Maar sediments, Western Eifel, Germany, Climate of the Past, 15, 713–733, doi:10.5194/cp-15-713-2019, 2019.

Schmidt, H.-L., Werner, R. A. and Eisenreich, W.: Systematics of ^{2}H patterns in natural

compounds and its importance for the elucidation of biosynthetic pathways, Phytochemistry Reviews, 2(1–2), 61–85, doi:10.1023/B:PHYT.0000004185.92648.ae, 2003.

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