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Comment on hess-2022-178

Anonymous Referee #1

Referee comment on "Technical note: On uncertainties in plant water isotopic composition following extraction by cryogenic vacuum distillation" by Haoyu Diao et al., Hydrol. Earth Syst. Sci. Discuss., https://doi.org/10.5194/hess-2022-178-RC1, 2022

General comment/Overview

The manuscript by Diao et al. investigates different fractionation processes caused by H-exchange with organic material, sublimation and evaporation processes during cryogenic vacuum distillation of plant samples. The manuscript is certainly of high interest to scientists working with CVD and provides valuable insights into different fractionation processes. However, I feel that the study has certain shortcomings, which prevent the certainty with which some conclusions were made. I also feel that the description of the experiments + results and discussion could be improved to enhance the clarity of the manuscript. Please find my major and minor comments below.

Specific comments

Major

L. 53-58: The authors conclude that there are two critical H-exchange steps: the first one being during rehydration, the second one being during CVD. Yet, according to the authors only the second one is relevant/"of interest" (L. 56), because in a natural set-up, samples are not rehydrated. However, if you think about that plants are also de- and rehydrating naturally, e.g. over the course of a season, this step and a possible H-exchange might also occur under natural conditions and should be taken into account, when comparing source water with plant water. The question is of course, if this exchange is negligible under natural conditions under high plant water fluxes.

Drying procedure of sample material: why did you dry your material only at 60°C and for 24h? I know for organic materials it is common to use 60-70°C. However, I feel that for

such an experiment a completely dry sample (105°C) would have been of immense importance. At least it should have been dried for 48h. This has to be addressed somehow and could potentially compromise your results. Same issue in L. 128.

Over-/undersaturation of sample material: So if some water amounts were not able to fully saturate the material and others oversaturated the material, I would be really careful with the conclusion drawn from this experiment, as it does not reflect "real" plant samples. If relative water content is not an issue, why did you not choose different weights of your samples material to avoid over-/undersaturation?

In general, there are a lot of assumptions in the material and methods sections, such as "Thus, by the end of the rehydration, the isotope ratios of water in the small stem segments are assumed equal to the isotope ratio of the reference water after rehydration (δref after rehyd) and not to be equal to the original reference water (δref)." (L. 137-139). Can you be sure about this? This should be considered in the discussion.

Experiment 3: I am afraid I don't really get the whole experiment. From what I understand you were only interested in the effects after the water has been extracted from the sample, thus the freezing in liquid nitrogen. But why do you then write "before the extraction started"? I guess you left the water in the liquid nitrogen for a certain amount to simulate an extraction? Why not also freeze the reference water at -20°C and extract it the way as in experiments 1 and 2? This would also allow a statement of the effect of heating the water and potential evaporation effects before freezing the water. There is certainly some clarification needed for this experiment.

As four experiments were conducted, it is sometimes really confusing for the reader to follow the argumentation, as you always have to go back to the methods to see, which experiment exactly the authors are talking about. Potentially, Fig. S1 could be moved into the manuscript, but in a clearer manner with a clearer description.

Figure 1-3: There are some undiscussed effects in Figure 1 and 2:

Fig. 1: why do powder materials drop below the reference line at high water amounts? This remains unexplained

Fig. 2: for H, your dH is negative for water samples larger than 400 microl, for 18O, they are still clearly above the reference line. This should certainly be discussed.

Fig. 3: for H in your sublimation and evaporation test, dH is steadily decreasing with absolute water amount. You argue that this is analytical uncertainty, however it is a quite

clear pattern, which remains undiscussed. Also, the fit for dH in the sublimation experiment is somewhat off and not well representative of the data.

Recommendation to extract more than 600 microl:

From the results, I cannot clearly see, why the authors recommend at least 600 microl of water for extraction. In Figure 2 you clearly see that at 600 microl there is still quite a substantial offset for 2H and 18O, in Fig. 3 this is also the case for the evaporation experiment and 2H. In Fig. 4 there is a negative offset at 600 microl for 18O. This should be discussed in more detail.

Plant material: almost all experiments were only conducted on *Larix decidua* This should be included in the discussion, as the results could be completely different for other woody species and especially for herbaceous plants.

Minor

L. 16: Would be good to mention what the tested organic materials exactly were. "organic materials" could be anything

L.32-35: This is certainly questionable these days. I would be more careful with this statement

L. 35: replace faithfully. Do you mean actually?

L. 47-49: This sentence is hard to read, please consider rephrasing it.

L. 53: delete "exactly"

L. 56: Consider deleting the first "the H-exchange of interest".

"Only the latter is of interest, because…"

L. 67-69: please consider rephrasing this sentence, hard to read

L. 80: check for consistency of "vapour" or "vapor" in the whole manuscript (e.g. L. 71)

L. 100: please give the exact values for 2H and 18O

L. 103-110: why did you choose *Larix decidua*? Your conclusions could only be valid for this one species due to wood anatomy, etc.

L. 117: I think you should justify why you used exactly 200 mg

L. 136-137: This is something you suspect, but do not know for sure

L. 157: 2h is kind of an unrealistic timeframe for CVD, but I guess for investigating the evaporation effect it is okay

L. 162-171: from the description it is not clear why you needed this experiment

L. 173: Did you use any material to cover your samples, e.g. glass wool, to avoid particles to be drawn into your vacuum pump?

L. 205: should be Fig 1a & b

L. 220-221: "However, if this was true"

Fig. 1: reference line for d18O is missing

L. 234: causes?

L. 243-244: I don't fully understand how you can exclude that there is a dynamic

exchange during the extraction. You do not know for sure, what H is bound in your samples, although you allowed it to incubate with water of known isotopic composition. Also, the conditions during extraction are also different. I would be careful with such conclusions.

L. 250: The trend for 18O is very similar to the one for 2H. If you remove the outlier at d18O = 16 and RWC = 36, this will also be a linear trend. However, the relationship is still weak ($R^2 = 0.28$) and your data points are strongly scattered

L. 254: Here I disagree. You should use a linear mixed effect model and treat stem segment size as random effect, if stem segment size is not an explanatory variable.

L. 273: what experiments? I fear at this point the reader has already forgotten, which one experiment 3 was

L. 276: suggest? Suppose? Instead of think

L. 301-303: I think this is true for all your experiments, especially because they were mainly conducted on *Larix*

L. 317: I think -7 and +10 ‰ are quite substantial variations

L. 321: please elaborate further on why the pattern was evident for 18O, but not 2H

L. 322-327: this paragraph is controversial and confusing. You kind of invalidate your own results.

L. 328-331: You could also use a different argumentation: the tap water and twig water is closer to the laboratory water vapour than the isotopically depleted water you used and thus, in these conditions of your laboratory, the initial signature of your extracted water determines the magnitude of isotopic fractionation

L. 354: …but there might be a significant correlation in the range of 45 – 53%, as you only have two samples at 57%. Please check this. This could change your conclusion.

Fig. 4: please write *Larix decidua* in italic

L. 350: check the references in the brackets, there seem to be a few extra brackets.

Fig. S1 is lacking a clearer description of the experiments