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Reply on RC2

Haoyu Diao et al.

Author 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-AC2>, 2022

General comments

In the manuscript 'On uncertainties in plant water isotopic composition following extraction by cryogenic vacuum distillation' by Haoyu Diao et al. the authors investigated the biases caused by CVD extraction of plant samples. In different experiments they tested the influence of H-exchange effects, absolute water amount, and evaporation and sublimation enrichments on the isotopic composition (d18-O, d2-H) of the extracted water. The manuscript is well structured and nicely written. The topic fits well to the scope of the journal and appears to be of interest for isotope hydrologists. However, the discussion could be more comprehensive to make things clearer.

Most of my comments and remarks match those of Referee 1, who raised many important points already.

Response: Thank you for your comments. We have revised the manuscript according to your and the comments of Reviewer 1, particularly the discussion, as suggested. Please find the point-to-point response below.

Specific comments

(1) L. 128 ff: I suspect that drying at 60°C for 24h might eventually be too low and/ or too short. Was this procedure tested in advance? Did you check e.g. by weighing the samples after another 24h whether there was still some weight loss or not? Additionally, the 'appropriate' procedure could also be different for the twigs and the small segments. If your stem segments were not completely dry, the residual water will of course be strongly enriched, which will affect your results.

Response: Thank you for pointing this out. As explained in the response to reviewer 1, in our study, the drying procedure of sample material was only for experiment 1. Because the dried samples in the experiment 2 were rehydrated with excess of water for isotopic equilibration and no dried materials were used in the experiment 3 and 4.

For the stem and twig pieces, the original materials were first dried at 60°C for 24 h in order to cut them into pieces and then for weighing. Then, 200 mg of pieces were weighed in Exetainers and dried again at 60°C for 24 h. In total, the sample were actually dried for 48 h as suggested by the reviewer. For the powdered materials, they have already been

dried and stored in a dry environment prior to the experiment. Those samples were used as isotope reference standards for $\delta^2\text{H}$ analysis in our laboratory (Schuler et al. 2022). So, the powdered materials were further dried once in the Exetainers at 60°C for 24 h. We added some sentences to clarified this. See lines: 106–107; 112; 118; 122–126.

We also would like to note that it is very difficult to have completely dry samples, even if at 105°C for 48 h. That’s because moisture in the air will be absorbed within seconds by the dry samples when the samples are taken out from the oven and when opening the cap for injecting the reference water.

To address potential problems regarding our sample drying procedure, we conducted a test using ca. 200 mg stem pieces, stem powder and caffeine at two different drying procedures (60°C, 48 h; 105°C, 48 h). The samples were weighed every 12 h. After 48 h, we opened the cap of Exetainer for 5 s in the lab to simulate the procedure of injecting the reference water. Then the cap closed and samples were weighted again. We added a description of this new test at lines: 132–139. The results are shown in the table below.

Material	T (°C)	The percentage of moisture removed (%)	Water vapour absorbed in 5s (%)
12h	24h	36h	48h
Twig piece	60	5.55±0.06 a	5.60±0.05 b
105	8.68±0.21 a	8.89±0.12 a	8.91±0.16 a
Stem powder	60	5.05±0.04 a	5.21±0.04 b
105	8.28±0.19 a	8.32±0.25 a	8.28±0.18 a

Caffeine	60	0.44±0.03 a	0.41±0.09 a
105	1.50±0.15 a	1.59±0.11 a	1.62±0.09 a

Note: Different letters in each row indicate a statistically significance difference at $p < 0.05$ (T-test)

The results show that all the tested materials were dried to a constant weight after 36 h and 12 h at 60°C and 105°C, respectively. However, more moisture was removed at 105°C, especially for twig pieces and stem powder. Noticeably, after opening the cap for 5 s, the samples which were dried at 105°C absorbed more lab water vapour than the samples which were dried at 60°C. These results suggest that a complete drying is very difficult, regardless of whether the samples were dried at 60°C or 105°C. However, the difference of 1-2 % in removed moisture after reabsorption between 60°C and 105°C should not affect the isotopic results of our study, because strongly depleted reference water was used to amplify the differences. It should also be noted, that the higher uncertainty at lower amounts of water is observed across all our experiments, again showing that the remaining moisture in the dried material is not a major driver of our overall results. We have added this table to the supplement, see the new Table S2. Corresponding texts were added at lines: 132–139; 255–264.

(2) I'm not surprised that the differences in the isotope data are dependent on AWA rather than on RWA (e.g. L. 224, L.252). Possible reasons could be

- very small water droplets are exposed to ambient air during collection (L. 180-181)
- for small samples the sample volume might be too small relative to the size of the extraction line i.e. the volume which is filled with water vapour. Can you estimate the size (volume) of your system?

and should be considered in the discussion.

Response: We agree with you regarding the two possible reasons of the observed dependence on AWA rather than on RWC. We discussed these two points in the second paragraph of section 3.3. See "... a bi-directional exchange between water droplets and ... water vapour of the laboratory ..." and "... the smaller the water droplets, the larger the ratio of water vapour volume to water droplets volume in the water collection tube ..." (Line 343–345; 349–350).

As suggested, we estimated the volume of the whole system (including all tubes and lines) to about 3500 cm³. We suppose that the two processes mentioned above could mostly occur in the U-shaped water collection tube (Figure S1) after it is detached from the extraction line. Therefore, we added the volume of the U-shaped water collection tube (78 ml) to the description of Figure S1, illustrating the CVD-extraction line. We have also

mentioned that the system volume may have an effect on the isotope fractionation during CVD extraction at the end of section 3.3 (Line 362–364).

(3) L. 322: You assume that the effect of small amounts is different, depending on the isotope ratio? At least for isotope values in the range of your study it should not be in a measurable range.

Response: Sorry for causing this misunderstanding. We were trying to say that the dampened pattern obtained from the tap water extraction compared to the reference water extraction was not because they had different initial isotope composition (this paragraph), but more likely because of the vapour exchange with the lab air (the next paragraph). The first sentence of this paragraph made this paragraph confusing, but we were not intending to invalidate our own results. Therefore, we have revised this paragraph to improve its clearness and the connection with the next paragraph. See lines: 385–405.

(4) Figure 4: How can the huge differences in SD between D18-O and D2-H (Fig. 4a and c) be explained?

Response: We have checked the accuracy and precision of the isotope analyses for all experiments and found that the precision of quality control standard of the batch with the extracted tap water (previous Fig. 4a, blue dots) was low, explaining these substantial variations. Therefore, we repeated the “tap water” experiment and the analysis of the samples. The results are now given in the new Fig. 5, showing that $\Delta^2\text{H}$ of pure tap water also followed the inversely proportional pattern as found in other experiments.

Technical corrections

(1) Throughout the manuscript: isotope fractionation should almost everywhere be singular not plural, please check.

Response: Done.

(2) L. 71: ‘liquid-vapour’ instead of ‘liquid-vapor’

Response: Done.

(3) L. 89: and L. 263: ‘induced’ instead of ‘introduced’?

Response: Done. We replaced “introduced” by “induced.”

(4) L. 310/ 311: ‘...allowed for investigating...’ instead of ‘...allowed us to investigate...’

Response: Done.

(5) L. 340: ...mean values \pm 1 SD like in Fig. 3?

Response: Yes. Done.

(6) Figure S1: Typo in legend of Experiment 2 ‘Abosolute water amount’

Response: Done. This typo has been fixed.