Comment on hess-2022-37

Anonymous Referee #2

The work presented in HESSD by Magh and colleagues describes a new approach to sample xylem water for isotopic analysis in trees. In this technical note, the authors conducted a lab trial and a field trial to show the suitability of the proposed method. The general motivation of the study is to provide a cost-efficient approach to overcome the limitations of tree water sampling in field conditions and the potential bias of cryogenic extraction. This is important as this is a current point of debate in the literature (i.e. cryogenic limitation) and the proposed method is interesting.

We thank the anonymous reviewer for their extensive and insightful review of our manuscript. We hope to be able to address all their comments and questions sufficiently and provide a comprehensive revised manuscript.

While the study has a great design and is innovative, and the method provides reliable results in the lab, my main concern is that the proposed approach does not seem to provide reliable data from trees in the field (Fig. 7). This is a holdback, as this should be reliable if the goal is to obtain stem water and overcome limitations. It would be important to present more data in the field, i.e., more than one day, and possibly, in more than one tree (since the approach is "easy to use and cost-effective"), and different environmental conditions, i.e. wet vs dry.

As we indicated in the original draft this method originates from an extensive data set obtained in the field from daily observations of 120 trees over the course of 2 months (see “previous attempts” section in the original draft). We developed the lab trial to develop a method that would allow us to overcome the limitations of the method used in said field trial and tested the new method extensively in the lab. We were able to prove that it does work within the addressed uncertainty range, and then additionally tested it on one tree in the field on one day. We feel the scope of the study, to introduce a new measurement method that can provide basis to overcome spatial limitation of in-situ measurements has been accounted for with the lab trial. We agree, the method needs further testing and is far from perfect and fully investigated. We would like to share it at this point anyways so the community can benefit from our experience and start testing it now.

We will address your concerns in the revised version of the manuscript to provide the basis for improvements of the method.

The authors show a statistically significant difference between the *in situ*, defined in the paper as the “gold standard”/ “true value” and the VSVS data, even after the correction for storage is applied. This difference is evident even for the zero-day storage (Fig. 7) for d18O. While for d2H, there is no difference with some days (e.g. 1 and 7), it did not show a clear pattern. Thus, the reliability of the data is uncertain because the effect seemed random (e.g., why is 0-day results different from the i*n situ* and not 1-day?). This is difficult to understand with a single trial in the field.

We agree there are limits to the field data we provided. However, as mentioned above, we aimed at providing a new approach the community can further develop and improve. The reason for the field data to not match the in-situ observations can be manifold and we will address some reasonable explanations in the revised manuscript.

Suppose one can sample vapour with higher or lower water content (ppmV), as the authors even experienced (L166-168), and as we know the water content of the wood

changes largely depending on the water status (e.g. high water content in the wood because of well-water conditions or low water content due to water stress), how would that interfere with VSVS and storage time, or even the proposed correction? Lab trials usually show fractionation in vapour samples with low ppm. Since the authors are offering/reporting a new approach, it would be interesting to understand this before we, as a community, start to apply the technique broadly. Would it be beneficial to use larger vials during drier periods? Have the author's tested different volumes of vials for larger vapour storage?

Vapor pressure is related to the curvature of the bounding surface and would therefore only be reduced in either very dry soils or very dry tree tissues (see e.g. (Thomson, William Sir 1871). Those conditions in a tree would most likely lead to irreversible cavities and therefor dying of the tree (see e.g. (N. McDowell et al. 2008; N. G. McDowell et al. 2022). So, we would assume there is no physical basis for dry conditions in viable trees to lead to smaller vapor concentrations. We will address this in the revised discussion.

We have initially tested even smaller vials (20ml) and discarded those in the very beginning as the length of measurement did not allow for a stable 2 min isotope reading. We will add this to the previous attempts section in the revised manuscript. Havranek et al. (2020) did provide a similar system to ours, that they did test in the lab, showing that large bottles (700ml) provide more stable storage and isotope readings. However, their bottles are very large, and custom made (which makes their approach more expensive and less flexible).

Regarding the borehole, have the authors monitored the wounding effect? Similar to sap flow systems, where the wounding effect can influence sap flow rate measurements (e.g. Wiedemann et al., 2016; Peters et al., 2018), one would expect that we observe a similar effect when sampling for precise natural abundance where the boreholes are much larger than the small sap flow needle. Can the authors comment on this and discuss this limitation? For example, if multiple samples need to be collected from a forest in high- temporal resolution, for how long can one rely on the same borehole? Additionally, conifer species tend to produce resin near the wound, this could additionally result in spectral contamination. How could one define if spectral contamination is an issue in this system?

We have monitored the pitch/resin production after drilling and cleared out the resin using Acetone (see line 141) every day. Only when the production ceased did we install the fittings (after 4 days see below). Additionally, we checked whether there was new resin coming in after five weeks before connecting the tree to the VSVS (see also in an answer to your specific comments below), and there was not.

To check for spectral contamination (e.g. associated with organics/Voc's) we regularly checked the methane ("CH4") variable recorded simultaneously on the Picarro during the measurements. We compared it to the values when measuring the standards which should have close to no spectral contamination. We did not observe any differences. Further, VOC pollution is rather associated with e.g. the EQ bag method, where prolonged storage time may facilitate VOC accumulation which then potentially flaws the obtained isotope readings.

Regarding the reliance on one borehole over time we have to refer to the already published studies conducted by (Marshall et al. 2020; Beyer, Kühnhammer, and Dubbert 2020; Kühnhammer et al. 2022). The longest time using the same borehole was studied in Kühnhammer et al. 2021. They did observe reliable results over the course of 2.5 months in a

tropical dry forest. However, the authors also call for a more systematic investigation of how long a borehole can be used. We will add this to the revised discussion.

I think this study is missing a direct comparison with the cryogenic system. In the introduction, the authors refer to the potential bias of the method while this is also the "state-of-the-art extraction process" in this field, so how do the results compare with cryo? Or even the direct-equilibrium bag method?

Cryogenic extraction has often been shown to result in heavily biased data and should therefore no longer be considered the state of the art in our opinion. See for examples (Orlowski, Breuer, and Mcdonnell 2016; Allen and Kirchner 2021; Chen et al. 2021). We will change this "state-of-the-art extraction process" statement in the introduction since cryogenic extraction is no longer that.

We respectfully disagree with the comparison to cryogenic extraction. Our aim was the comparison between in situ and „bottled" vapor to show the potential effect of our storing approach. Whether in-situ is "correct" in terms of representing the stem water is not the scope of this study.

Regarding the comparison to the direct equilibrium bag method: the information we would obtain would be one single observation (most likely from the day installing the borehole) and the xylem composition would for sure have changed by the time the "settling period" of the same was over.

We have tried sampling vapor directly into the bags most commonly used for the bag equilibration method, but handling proved to be much harder than with the vials. We will add this information to the revised manuscript to the section "previous attempts".

Specific comments:

Title: The title mentions soil, but no tests or trials are done for soil in this work.

We will revise the title as also mentioned in the answer to the community comment. It will be more concise.

In line 36, the author refers to "matrix-bound" (assuming soil, as the previously mentioned soil matrix in the above paragraph). Still, in the last lines (L40-42) of this paragraph (L36-42), the authors use references that discuss cryogenic bias that relates to plants (Chen et al., 2020 and Allen and Kirchner, 2021). It would be helpful to be clearer and refer specifically to plant cryogenic bias in the text. Or, if the authors want to refer to cryogenic bias in both soil and plants, it would be helpful to mention it more clearly with appropriate references to both cases.

We will revise this so that the references match the text.

Lines 45- 46. What do the authors mean by altering their physiology?

We mean that the water flow will be disrupted when sampling tree cores on the same individual repeatedly to e.g. obtain a comprehensive understanding of water uptake over time. That leads to altered water transport patterns and subsequently alters the physiological functioning. We will elaborate on this in the revised manuscript.

Line 62: Add Kuhnhammer et al., 2021 along with Beyer et al., 2020 here as well. We will provide the suggested reference in the revised version.

Line 81: What was the volume of the standard water (in the larger vial)? Did the authors try different volumes of crimp neck sample vials? Why 50 ml was the selected volume?

The standard bottles were 250ml "Schott" lab bottles containing 50ml of the standard water. We will add this information to the revised manuscript.

As for the size of the crimp vials, see above. We tried 20ml vials first, but ultimately we were unhappy with the sample volume as it was too small to give a sufficiently long isotopic plateau during the measurements.

Line 139: Give the scientific name to the two species.

We will add the Latin names of the species to the revised manuscript.

Line 141: Give an estimate of the "several days" (e.g. ~ 5 days)

We waited 4 days between drilling the borehole and installing the fitting. We will add the information to the revised manuscript.

Line 143: It would be helpful to the reader if you already refer to the schematic figure here (Fig S1), and perhaps bring it to the main body of the paper (nice figure!).

We will add the figure to the main body in the revised manuscript and refer to it at this point.

L144: Maybe state clearly that one of the scots pine previously connected with the *in situ* system was monitored with the new system VSVS.

We will add the word previously to the sentence in the revised version of the manuscript.

L149: Was the borehole flushed again or any treatment used after the five weeks before the change in the system? Did the authors detect any wounding effect in the borehole? Since this is a technical note, these details should be clearer so others can replicate the method.

There was no further treatment of the borehole, except visual inspection regarding pit/resin production (there was none) before we connected it to the VSVS for the first time.

L161: What was the air temperature in the field during the vapour sampling? How does change in air temperature affect the sampling (e.g. from wood to air)? Or if sampling in days with different air temperatures? It would be important to include the first answer here since this is a methods paper and the later ones in the discussion.

Air and source (i.e. borehole) temperature was monitored constantly. However, until entering the crimped vial the PTFE tube was heated using a heating line and insulated. Because we re-heated the vials prior to measuring and during the measurement, we were not concerned about condensation that might occur during the storage period. Therefore, the source temperature defines the conversion between vapor and liquid isotope signatures. We will elaborate on this principle in the revised MM and mention it again in the discussion.

L175: It is not too clear why the in-situ n = 2. How was it determined? Please clarify this part or re-arrange the text to be more explicit.

The in-situ system was set up so that each tree was measured every 4 hours. During the time we sampled for the VSVS we disconnected the tree from the in-situ system and re-connected it to the in-situ system each time the trees measurement time came up in the schedule for the in-situ system. On that day we were able to make 2 observations using the in-situ system within the time we sampled for the VSVS, that equals n=2. We will rearrange the text to explain this in the revised manuscript.

L252-253: It would be helpful to show the atmospheric data in the supplementary information along with the samples.

We will add the required data to the revised manuscript.

L282: It would be helpful to see the data similarly to Figure 6. The raw along with the corrected for the field measurement.

We will adapt figure 7 accordingly in the revised manuscript.

L280-282: Didn't VSVS also fail to return the in-situ when compared with the other days (i.e. 1, 3, 7 and 14) and not only the "0-day"? Perhaps state it more directly.

We will revise this sentence to clearly show that it failed to return the oxygen composition regardless of storage time.

L290-295. "3.3 Time and Cost Efforts" – This is not a result per se but part of the discussion. For a more comprehensive comparison, one should also state what type of cryogenic extraction the authors refer to, as the reference is not enough as this is important to the reader. The time efficiency one should also discuss field-set uptime (e.g. how long does it take to set up the VSVS and in situ in the field?). This would be helpful to understand if short-term studies would still benefit from this approach.

We will move this to the discussion in the revised manuscript. We refer to using an extraction line following the Königer approach (Koeniger et al. 2011). The time effort includes the system setup, which takes longer for the in-situ than for the VSVS, since the latter only requires the fittings being installed, while the tubing is movable from tree to tree. It then takes longer to measure the samples, where the in-situ system does not require any additional work. This is how we ended up with the same amount of time needed for in-situ and VSVS. We will add more information to the table in the revised version.

L308-309: This is a bit of an overstatement for the field conditions. The VSVS proposed method results were not statistically similar to the defined "gold standard"/"true value" (*in situ*) for d18O and were somewhat similar for the d2H.

We will revise to something like: „sufficiently well in the lab and in the field study regarding the tracer detection in the hydrogen isotopes. We conclude with a call for more field data for this method to obtain field approval also for delta18O."

Fig. 6 Very minor comment: It would be nice to align the x-axis between the two plots.

We will revise the figure so that the axes align

Fig. 7 Very minor comment: Makes the asterisks larger; it is difficult to see them.

We will revise the figure so that the asterisks are larger.

References:

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Mortality under Rising Drought, CO2 and Vapour Pressure Deficit'. *Nature Reviews Earth & Environment*, March. https://doi.org/10.1038/s43017-022-00272-1.

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