

Hydrol. Earth Syst. Sci. Discuss., referee comment RC2  
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## Comment on hess-2022-142

Anonymous Referee #2

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Referee comment on "The natural abundance of stable water isotopes method may overestimate deep-layer soil water use by trees" by Shaofei Wang et al., Hydrol. Earth Syst. Sci. Discuss., <https://doi.org/10.5194/hess-2022-142-RC2>, 2022

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### General comment

The authors of this manuscript designed a tracer experiment based on stable isotopes of hydrogen and oxygen to identify the depth of soil water taken up by apple trees at three different stages (blossom and young fruit, fruit swelling and fruit maturation stage). The topic of this manuscript is timely and potentially interesting for the readers of Hydrology and Earth System Sciences. Overall, the manuscript is well written and structured, but I found that various and important methodological details (e.g., a detailed description of the extraction method used for soil and vegetation material, and the isotopic composition of the injected water) were not described in the manuscript. Furthermore, the authors should also have considered in the introduction and the discussion recent literature on isotopic fractionation and offset which presents the current technical limitations for the application of stable isotopes in ecohydrological studies. Finally, a section about the limitation of the methodological approach should be added before the conclusions.

### Specific comments

- Lines 44-45: The authors should mention in the introduction that isotopic fractionation has been observed in various studies (e.g., Poca et al., 2019; Vargas et al., 2017; Barbeta et al., 2019), along the soil-root-stem-twigh-leaf pathway. The factors affecting such fractionation/offset are still unclear, but they seem to be mainly related to the water extraction technique, and particularly to cryogenic vacuum distillation (e.g., Zhao et al., 2016; Barbeta et al., 2022).
- Lines 50-52: The authors should mention limitations due to the extraction technique applied to soils (e.g., cryogenic vacuum distillation, Orłowski et al. (2016a,b, 2018)).

- Lines 90-92: What is the depth to the water table in the study area? Do the roots of the apple trees have access to shallow groundwater?
- Lines 92-93: What is the average, minimum and maximum distance between the trees? Does the tree distance affect the root density and possibly the water uptake?
- Table 1: It is unclear whether height, trunk and crown size data represent an average or single values. The authors should add the minimum and maximum values for all characteristics, as well as the number of trees used in this study.
- Lines 108-109: The authors should report the isotopic composition of the water mixture used for injection/irrigation, as well as the total amount of irrigation water applied per each tree.
- Lines 109-111: I do not understand the aim of this sentence. Based on it, I understand that the authors may have injected a water amount that could be too small to detect a soil water content variation and perhaps, also an isotopic variation.
- Lines 112-113: Details about the sample size should be reported in the text here or in an additional table. Furthermore, more details are needed to understand how the background value was computed (is it an average of how many samples?), and where these unlabelled trees are located compared to the trees used for the experiment.
- Lines 132-133: I am not familiar with this cryogenic vacuum distillation system, and I am not sure it is manufactured by Los Gatos Research. I recommend adding here a detailed description of the extraction method, as well as information about the extraction efficiency.
- Lines 133-135: Details about the uncertainty in the isotopic analyses for each instrument should be added here. Did the authors use specific practises to mitigate the memory effect in the isotopic measurements?
- Line 143: Considering recent literature, the no isotopic fractionation assumption is a strong assumption that should be tested. Did the authors check whether their isotopic data present an offset compared to the isotopic composition of local water sources (e.g., precipitation, soil water before the tracer experiment and shallow groundwater) and the water used for the tracer experiment?
- Lines 145-148: I do not understand the purpose of this index,  $S$ . The description of this index should be improved, e.g. by adding what positive and negative values indicate, and the ranges used for the soil water content.
- Figure 5: The isotopic composition of the injected water should be plotted, and I suggest showing the background value also without the 2 SD. Furthermore, the sample size should be reported in the caption.
- Figure 7: In the plots for "FSW for 11yr" and "BYF for 17yr" there may be an isotopic offset for some xylem water samples; I suggest checking whether there is a significant deviation from the soil water isotopic line, by also considering the uncertainty due to the isotopic analyses. In these plots, the authors should add the isotopic composition of the background values (in soil and xylem waters) and of the injected water. Equation of the LMWL and details about sample size and when the samples were collected should be added in the caption.
- Figure 9: Please remove the regression line when there are only three samples used for the analysis.
- Section 3.6: I expected to read here the results concerning the application of the index  $S$ , but they are not reported. Therefore, I suggest adding here such results, or removing the description of  $S$  at Lines 145-148.
- Section 4.3: Please add in this section (or in a new one) the limitations of the experimental approach. I think the limitations of this work are mainly related to the assumptions of no isotopic fractionation and negligible spatial variability in the isotopic composition of unlabelled and labelled trees, and to the extraction method (cryogenic vacuum distillation system).

## Technical corrections

- Line 43: I think the authors should write "Analytical techniques based on stable isotopes...", and furthermore, they should consider that many sampling techniques are destructive because they require the collection of soil and vegetation material (e.g., leaves, twigs, wood cores etc.).
- Line 51: Please replace "confusion of" with "unclear".
- Title of section 2.2: I suggest changing it with "Sample collection".
- Title of section 2.2.2: I suggest changing it with "Collection of soil and vegetation samples for isotopic analysis".
- Figure 4: Please add in the caption when the soil water content was determined (before, during or after the tracer injection).
- Figure 8: Please remove from the caption "Seasonal patterns of" because the results refer only to three specific tracer injections.

## References

Barbeta A., Burlett R., Martín-Gómez P., Fréjaville B., Devert N., Wingate L., Domec J.-C., Ogée J., 2022. Evidence for distinct isotopic compositions of sap and tissue water in tree stems: consequences for plant water source identification. *New Phytol.*, 233, 1121-1132. DOI:10.1111/nph.17857

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