HESS-2022-142

Title: The natural abundance of stable water isotopes method may overestimate deep-layer soil water use by trees

Author(s): Shaofei Wang et al.

MS type: Research article

Iteration: Major revision

Comments from handling editor:

Dear authors,

the comments of the two Referees are somewhat split, but it seems to me that the issues raised by Reviewer #2 are serious and should be carefully addressed.

However, from your response to the comments received, I can see that you are going to thoroughly revise your manuscript.

I look forward to receiving the revised manuscript for further review.

Best regards

Roberto Greco

Response: Thank you for your letter and the referees' comments concerning our manuscript entitled "The natural abundance of stable water isotopes method may overestimate deep-layer soil water use by trees" (hess-2022-142). Those comments are valuable and very helpful. We have read through comments carefully and have made corrections. Based on the instructions provided in your letter, we uploaded the file of the revised manuscript. Revisions in the text are shown using red highlight for additions, and strikethrough font for deletions. The responses to the reviewer's comments are marked in blue and presented following.

Anonymous Referee #1

With great pleasure I read your manuscript, where you investigate the water use strategy of apple trees by different ages. You injected labeled water (D_2O) and studied at which depth the trees withdraw their waters. This analysis was carried out for three different growing stages. The paper is very well structured, easy to read, informative figures and in good English language. The applied method is correct and I have no comments about the conclusion. Hence my comments limit mostly to technical issues, except from the following two comments:

Response: Thank you very much for the constructive and encouraging comments and giving us an opportunity to revise this paper. Corrections have been made based on the recommendations, and the detailed response to each comment is presented as follow.

Comment 1

From a scientific point of view your work is really interesting, but the question remains what we can do with this information (social relevance). Furthermore, the study only looks at one growing cycle and ignores that water use strategies change depending on water availability (or climate). In case plants experience dry spells, their roots develop differently in comparison to plant that do not experience dry spells. So there is also a long-term strategy, where plants (sometimes) can adapt to climate change. Could you discuss on this topic?

Response: Thanks for your comments. Here are our responses.

(1) On the Loess Plateau, apple trees are the dominant cash tree plantations. Over recent decades, the cultivated area of apple trees increased continuously, with the Plateau becoming the largest apple tree cultivation zone globally, accounting for more than one-quarter of global coverage and production (Gao et al., 2021). The apple industry has become the backbone of the local rural economy, involving more than 10 million farmers (Gao et al., 2021). The apple trees on the Loess Plateau are heavily dependent on soil water in deep layers due to low annual precipitation (400-600 mm) and abundant soil water resource in deep vadose zone (Wang et al., 2021; Yang et al., 2022). Therefore, it is of great significance to determine the utilization time of deep-layer soil water for orchard water management and apple yield improvement. We have added the social relevance of the findings to the revised manuscript (L 83-89 and 364-365).

References

Gao, X., Zhao, X., Wu, P., Yang, M., Ye, M., Tian, L., Zou, Y., Wu, Y., Zhang, F., and Siddique, K. H. M.: The economic–environmental trade-off of growing apple trees in the drylands of China: A conceptual framework for sustainable intensification, J. Clean Prod., 296, 126497, https://doi.org/10.1016/j.jclepro.2021.126497, 2021.

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(2) We agree that water use strategy can be changed by climate. The apple trees are expected to use more soil water in deeper soils in dry spells. We have added more details to the Discussion section to explain this point (L 367-386).

"Our results show that apple trees switch their water sources between different soil layers to adapt to the changing water environments on the Loess Plateau, which is particularly important in the context of climate change. Special attention should be directed to water consumption in deep soils—we found that apple trees absorbed the most water from deep soils during the BYF stage, with 17-year-old apple trees consuming more water in these layers than 11-year-old trees throughout the growing season. This result is in accordance with previous observations in this region that soil water availability gradually decreased with increasing stand age, and then apple trees absorbed more water from deeper soil layers (Li et al., 2019). Similarly, Barbeta et al. (2015) found that trees increased their use proportion of deep soil water and groundwater following a long-term (12 years) experimental drought. However, this water-use strategy may not be sustainable for trees in DVZ regions where deep soil water is difficult to be recharged. The result of the tritium peak method suggested that it took more than 50 years for soil water migration to 6 m depth in apple orchards in DVZ regions (Li et al., 2018). Thus, once DLSW is depleted, it cannot be replenished within a short timeframe, reducing the tree's ability to resist water stress. Also, Wu et al. (2021) observed that soil water generated by precipitation was the primary water source for apple trees when deep soil water was depleted, dominating their transpiration. In this case, trees were likely to encounter irreversible embolism, increasing the risk of drought-induced mortality, threatening the sustainable development of vegetation and changing regional hydrological cycle (Brodribb et al., 2020; Zhang et al., 2020). Therefore, we suggest long-term and high-frequency monitoring of isotopes in soil and xylem water, especially at a large geographical scale, to further understand the long-term changes in plant water use strategy and evaluate their adaptability under climate change."

References

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Zhang, Z., Huang, M., Yang, Y., and Zhao, X.: Evaluating drought-induced mortality risk for Robinia pseudoacacia plantations along the precipitation gradient on the Chinese Loess Plateau, Agr. Forest Meteorol., 284, 107897, https://doi.org/10.1016/j.agrformet.2019.107897, 2020.

Comment 2

Data availability: I don't think the current data-statement is sufficient. Data that is used in publications should preferable be available online and not "upon request". The latter is only possible in exceptional cases. If this is the case, this should be justified.

Response: Agreed. The text has been revised (L 419).

“The data that support the findings of this study has been made publicly available in Zenodo (https://doi.org/10.5281/zenodo.7169689).”

Technical issues:

L60: BYF is note explained in the main text (only in the abstract). I think it's good practice to define abbreviations the first time you mention them in the main text.

Response: Agreed. We have amended the manuscript (L 66).

“Wang et al. (2020) argued that the absorption of deep soil water only occurred during the blossom and young fruit (BYF) stage in apple orchards”.

L88: unit of annual rainfall in mm/y.

Response: Done (L 96).

“Mean annual precipitation in the study region is 507.9 mm/y”.

Table1: I would recommend to change the way the units are provided. I would skip the '/' and use brackets.

Response: Agreed. The text has been revised (L 106).

Table 1. General information on the two apple orchards.

Stand age (a)	Longitude	Latitude	Altitude (m)	Height (cm)	Trunk diameter* (cm)	Crown size (cm)
11	109°50'13"	35°20'5"	863.8	355	12.0	405×352
17	109°50'18"	35°19'58"	862.9	395	14.4	450×380

L155-and further: all variables/parameters should be in italic.

Response: Done (L 190-192).

"Figure 2 shows the total precipitation (P_t) and growing season (April to September) precipitation (P_g). P_t and P_g in 2019 were 522.1 mm and 442.3 mm, respectively, similar to the multiyear (1999–2018) mean (507.9 mm/y for P_t and 407.5 mm/y for P_g)."

L156: "mean annual P_t": this is long-term P_t? If so, provide period. Furthermore unit should be mm/y.

Response: Yes, "mean annual P_t" is long-term P_t. The period has been added to the revised manuscript (L 191).

"P_t and P_g in 2019 were 522.1 mm and 442.3 mm, respectively, similar to the multiyear (1999–2018) means (507.9 mm/y for P_t and 407.5 mm/y for P_g)."

L156-157: but the monthly rainfall can differ a lot (see figure2b). So is 2019 a normal year?

Response: The study area is located in China's Loess Plateau, its most significant climatological characteristics are distinctly seasonal precipitation, approximately 55-78% of which falls in June through September (Fu et al., 2017; Jia et al., 2017). In 2019, 74.9% of the precipitation in study area fell in June through September, according with the seasonal distribution characteristics of precipitation in the Plateau. In addition, the total precipitation (P_t) and growing season (April to September) precipitation (P_g) in 2019 were 522.1 mm and 442.3 mm, respectively, similar to the multiyear (1999–2018) mean (507.9 mm/y for P_t and 407.5 mm/y for P_g). In this way, the year of 2019 was considered a normal precipitation year. We have made changes in the revised manuscript (L 190-194).

References

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Jia, X., Shao, M., Zhu, Y., and Luo, Y.: Soil moisture decline due to afforestation across the Loess Plateau, China, J. Hydro., 546, 113-122, DOI:10.1016/j.jhydrol.2017.01.011, 2017.

Fig 2: unit of precipitation is mm/day (LEFT) and mm/month (RIGHT).

Response: Done (L 198).

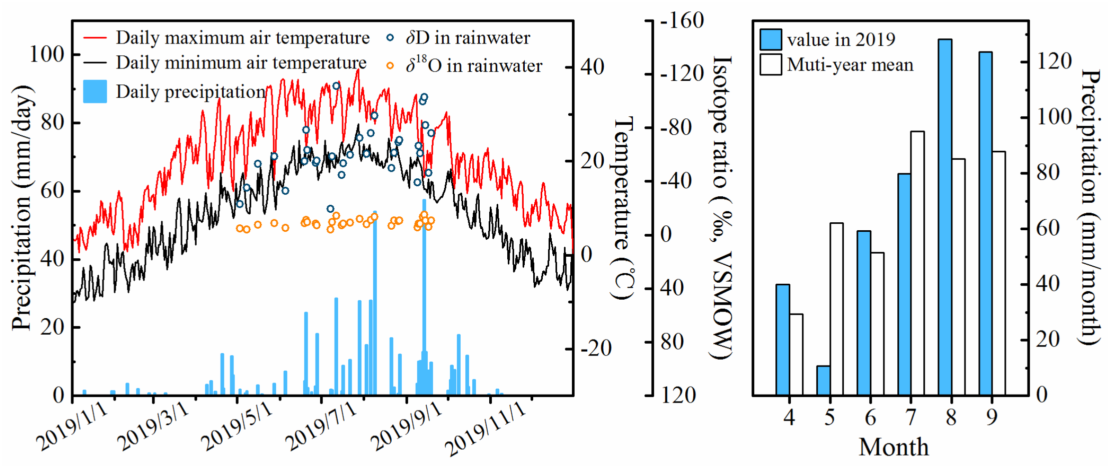

Figure 2: Time series of meteorological data and rainwater isotopic values in 2019, monthly precipitation in 2019, and multi-year mean, respectively.

Fig 5: I would rotate this figure 90 degrees, so you can more easily compare the figures with fig 3, 4, and 6.

Response: Agreed. The Figure 5 has been revised (L 227).

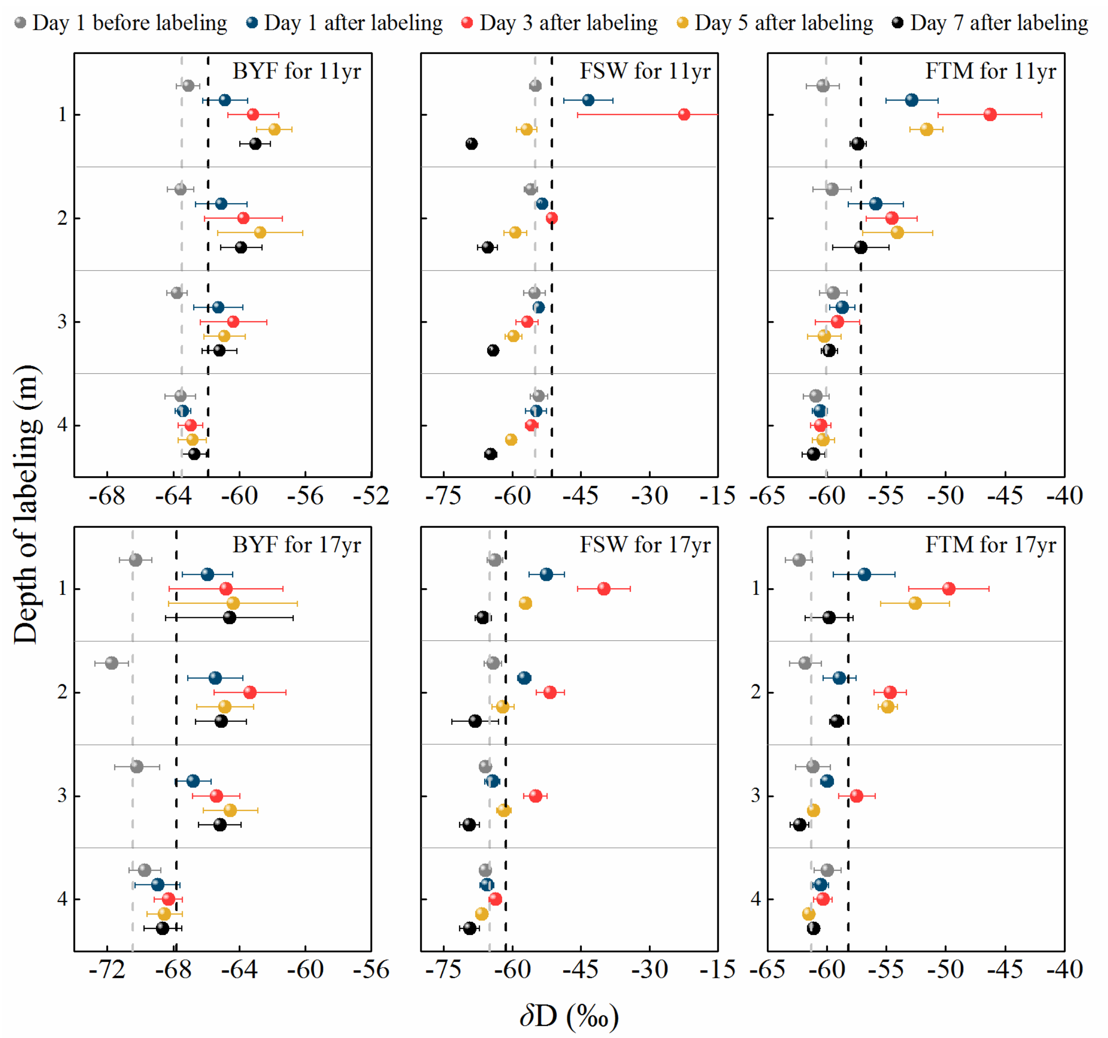

Figure 5: Temporal dynamics of δD values in xylem water for 11- and 17-year-old apple trees. Sample collection started on day 1 before D_2O tracer solution (δD = 714000‰) injection and commenced until day 7 (N=6). Gray dashed lines represent the background value (mean δD values in xylem water on day 1 before labeling) and black dashed lines represent 2 SD above the background value.

L212: "with relative higher reliance": I am not fully understand this sentence. Could you explain?

Response: It means that the contribution proportion of water from 140–320 cm soil layer both exceeded 48% in BYF stage for 11- and 17-year-old apple trees, which was higher than other stages. To clarify it, we have reorganized the sentence (L 254).

"The BYF stage produced more negative isotopic values in xylem water for 11- and 17-year-old apple trees (Fig. 7), which mainly utilized water from 140–320 cm soil layer (more than 48%)".

Anonymous Referee #2

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.

Response: Thank you for your constructive and encouraging comments and giving us an opportunity to revise this paper. Corrections have been made based on the recommendations, with detailed response to each comment presented below.

(1) Methodological details have been added to the revised manuscript (L 119-121, 125-126 and 149-161).

"A long polyvinyl chloride pipe was inserted into the holes at the target depth before injecting 300 mL tracer solution (δD = 714,000‰, 30 mL 99.99% D_2O plus 270 mL tap water) into each hole. The total amount of injected solution was 1,200 mL for each tree."

"Two xylem samples were collected for each tree, with a total sample size of six for each treatment in a single sampling."

"A CVD system (Li-2000; LICA United Technology Limited, Beijing, China) was used to extract water under a heating temperature of 95 ℃ and a pressure of 0.2 Pa, which has been applied in previous studies (Huo et al., 2020; Tao et al., 2021a; Wang et al., 2021b; Zhao e al., 2020). The extraction time of soil water and xylem water samples were 90 min and 120 min respectively. Samples were weighed before and after extraction and again after oven-drying for 24 h to calculate the extraction efficiency (Wang et al., 2021b), which should be not less than 98%. The stable hydrogen and oxygen isotope compositions of extracted soil water and xylem water were determined using a TIWA-45EP isotope ratio infrared spectroscopy analyzer (Los Gatos Research, Mountain View, USA) and Stable Isotope Ratio Mass Spectrometer (Isoprime Limited, UK), respectively. The measurement precision of δ^{18}O and δD is 0.2‰ and 1.0‰ for the TIWA-45EP isotope ratio infrared spectroscopy analyzer and 0.3‰ and 2.0‰ for the Stable Isotope Ratio Mass

Spectrometer, respectively. Each isotopic sample was repeatedly measured six times. The first three measurements were discarded to mitigate the memory effect of isotopic measurement, and the mean value of the last three measurements was taken as the isotopic value of sample."

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(2) Information on isotopic fractionation has been added to the Introduction and Discussion (L 50-54 and 395-402).

Introduction

"Although some recent studies found isotopic offset along the soil–root–stem–twig–leaf pathway (e.g., Barbeta et al., 2019; Poca et al., 2019; Vargas et al., 2017), the mechanism of the fractionation remain in debate (Barbeta et al., 2022; Chen et al., 2020; Wen et al., 2022; Zhao et al., 2016). Orlowski et al. (2016a,b, 2018) suggested that the fractionation is mainly related to cryogenic vacuum distillation (CVD). However, the CVD is still the most common methodology for water extraction to date (De La Casa et al., 2022)."

Discussion

"Recently, isotopic offsets between plants and their potential water sources have been also found in various ecosystems, which may hinder the unambiguous identification of water sources and influence the accurate assessment of DLSW utilization (Barbeta et al., 2022; De La Casa et al., 2022; Zhao et al., 2016). Some studies argued that isotopic fractionation during root water uptake could be attributed to the existence of Casparian strips which can lead to isotope enrichment in root water and depletion in xylem water (Naseer et al., 2012; Vargas et al., 2017). Seeger and Weiler (2021) questioned whether xylem water was completely renewed by newly absorbed soil water, thus affecting isotopic offset. Furthermore, CVD may mask or exaggerate the isotopic offset, although it was the most common methodology (Chen et al., 2020;

Orlowski et al., 2016a,b, 2018; Wen et al., 2022)."

References

Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L., Domec, J.-C., and Ogée, J.: 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, 2022.

Barbeta, A., Jones, S. P., Clavé, L., Wingate, L., Gimeno, T. E., Fréjaville, B., Wohl, S., and Ogée, J.: Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest, Hydrol. Earth Syst. Sci., 23, 2129-2146, DOI:10.5194/hess-23-2129-2019, 2019.

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Naseer, S., Lee, Y., Lapierre, C., Franke, R., Nawrath, C., and Geldner, N.: Casparian strip diffusion barrier in Arabidopsis is made of a lignin polymer without suberin, Proc. Natl. Acad. Sci. U. S. A., 109(25), 10101-10106, https://doi.org/10.1073/pnas.1205726109, 2012.

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(3) The following section has been added to the revised manuscript (L 389-405).

"4.4 Uncertainty caused by isotopic offset"

"In this study, isotopic offset between xylem and soil water was observed for both 11- and 17-year-old unlabeled apple trees (Fig.7 and Table S2). We used the isotopic composition of soil water to correct δD values of xylem water, ensuring they match those of soil water. Although we did not collect soil water isotope samples in the isotope labeling experiments, this may have little effect on determining the soil layer depths from which trees derive their water source due to the high δD values in the injected solution. It should be noted that isotopic spatial heterogeneity related to destructive sampling (xylem and soil water) could lead to an isotopic mismatch between xylem and soil water. Recently, isotopic offsets between plants and their potential water sources have been also found in various ecosystems, which may hinder the unambiguous identification of water sources and influence the accurate assessment of DLSW utilization (Barbeta et al., 2022; De La Casa et al., 2022; Zhao et al., 2016). Some studies argued that isotopic fractionation during root water uptake could be attributed to the existence of Casparian strips which can lead to isotope enrichment in root water and depletion in xylem water (Naseer et al., 2012; Vargas et al., 2017). Seeger and Weiler (2021) questioned whether xylem water was completely renewed by newly absorbed soil water, thus affecting isotopic offset. Furthermore, CVD may mask or exaggerate the isotopic offset, although it was the most common methodology (Chen et al., 2020; Orlowski et al., 2016a,b, 2018; Wen et al., 2022). When quantifying the water use strategies of plants, the isotopic measurement bias related to CVD should be considered. As a whole, there are various trends and causes of isotopic offset; further research about offset is urgently needed to better understand root water uptake processes."

References

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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-twig-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).

Response: Agreed. Information on isotopic fractionation has been added to the

Introduction (L 50-54).

"Although some recent studies found isotopic offset along the soil–root–stem–twig–leaf pathway (e.g., Barbeta et al., 2019; Poca et al., 2019; Vargas et al., 2017), the mechanism of the fractionation remain in debate (Barbeta et al., 2022; Chen et al., 2020; Wen et al., 2022; Zhao et al., 2016). Orlowski et al. (2016a,b, 2018) suggested that the fractionation is mainly related to cryogenic vacuum distillation (CVD). However, the CVD is still the most common methodology for water extraction to date (De La Casa et al., 2022)."

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Chen, Y., Helliker, B. R., Tang, X., Li, F., Zhou, Y., and Song, X.: Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water, Proc. Natl. Acad. Sci. U. S. A., 117, 33345, 10.1073/pnas.2014422117, 2020.

De la Casa, J., Barbeta, A., Rodriguez-Una, A., Wingate, L., Ogee, J., and Gimeno, T. E.: Isotopic offsets between bulk plant water and its sources are larger in cool and wet environments, Hydrol. Earth Syst. Sci., 26, 4125-4146, 10.5194/hess-26-4125-2022, 2022.

Orlowski, N., Breuer, L., and McDonnell, J. J.: Ecohydrology Bearings – Invited Commentary Critical issues with cryogenic extraction of soil water for stable isotope analysis, Ecohydrology, 9, 3-10, DOI:10.1002/eco.1722, 2016a.

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Lines 50-52: The authors should mention limitations due to the extraction technique applied to soils (e.g., cryogenic vacuum distillation, Orlowski et al. (2016a,b, 2018)).

Response: Agreed. We have changed the manuscript as follows (L 52-54).

"Orlowski et al. (2016a,b, 2018) suggested that the fractionation is mainly related to cryogenic vacuum distillation (CVD). However, the CVD is still the most common methodology for water extraction to date (De La Casa et al., 2022)."

References

De la Casa, J., Barbeta, A., Rodriguez-Una, A., Wingate, L., Ogee, J., and Gimeno, T. E.: Isotopic offsets between bulk plant water and its sources are larger in cool and wet environments, Hydrol. Earth Syst. Sci., 26, 4125-4146, 10.5194/hess-26-4125-2022, 2022.

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Orlowski, N., Breuer, L., Angeli, N., Boeckx, P., Brumbt, C., Cook, C. S., Dubbert, M., Dyckmans, J., Gallagher, B., Gralher, B., Herbstritt, B., Hervé-Fernández, P., Hissler, C., Koeniger, P., Legout, A., Macdonald, C. J., Oyarzún, C., Redelstein, R., Seidler, C., Siegwolf, R., Stumpp, C., Thomsen, S., Weiler, M., Werner, C., and McDonnell, J. J.: Inter-laboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water, Hydrol. Earth Syst. Sci., 22, 3619-3637, DOI:10.5194/hess-22-3619-2018, 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?

Response: The groundwater level in the study area is over 50 m deep on average, which cannot be reached by plant roots. We have amended the manuscript (L 97).

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?

Response:

(1) The plant and row spacing for the two orchards was 4 × 4 m, with all trees planted at the same interval.

(2) The results of two-dimensional root distribution of apple tree roots from Huo et al. (2021) showed that apple trees in shallow soil layers had relatively high root density and wide horizontal root distribution. With soil depth and horizontal distance increasing, the root density of apple trees significantly decreased. The tree root density was low and horizontal distribution was narrow in deep soil layers. Therefore, tree distance had little effect on root density and water uptake.

We have made changes in the revised manuscript (L 101).

References

Huo, G., Gosme, M., Gao, X., Dupraz, C., Yang, J., and Zhao, X.: Dynamics of interspecific water relationship in vertical and horizontal dimensions under a dryland apple-*Brassica* intercropping Quantifying by experiments and the 3D Hi-sAFe model, Agr. Forest Meteorol., 310, 108620, 10.1016/j.agrformet.2021.108620, 2021.

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.

Response: The height, trunk diameter (TD) and crown size (CS) were mean values for 20 trees in each orchard. The maximum and minimum values for all characteristics are shown in Table S1.

Table S1 The maximum and minimum values of the height (H), trunk diameter (TD) and crown size (CS) for apple trees in two orchards.

Stand age (a)	H_{max} (cm)	H_{min} (cm)	TD_{max} (cm)	TD_{min} (cm)	CS_{max} (cm)	CS_{min} (cm)
11	403	320	12.8	11.5	430 × 400	360 × 320
17	450	355	15.2	13.8	475 × 420	390 × 340

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.

Response: The isotopic composition of the tracer solution and total amount of injected solution have been added to the revised manuscript (L 119-121).

“A long polyvinyl chloride pipe was inserted into the holes at the target depth before injecting 300 mL tracer solution (δD = 714,000‰, 30 mL 99.99% D_2O plus 270 mL tap water) into each hole. The total amount of injected solution was 1,200 mL for 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.

Response:

(1) The results from Huo et al. (2020) showed that 300 mL of aqueous solution wet 400 cm^3 of the soil on the Loess Plateau, equivalent to a <1% change in SWC (within the measurement error), so the impact on soil hydrological processes was negligible.

(2) Unlike soil water content, the isotopic composition of soil water changed obviously after injection due to the high isotopic value of D (714,000‰) in the injected solution, which could reach to 150,000‰.

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 unlabeled trees are located compared to the trees used for the experiment.

Response: Agreed. Two xylem samples were collected for each tree, with a total sample size of six for each treatment in a single sampling. The background value was an average of six xylem samples from unlabeled trees. The text has been revised (L 125-127). The layout of unlabeled trees and labeled trees are shown in Figure S1.

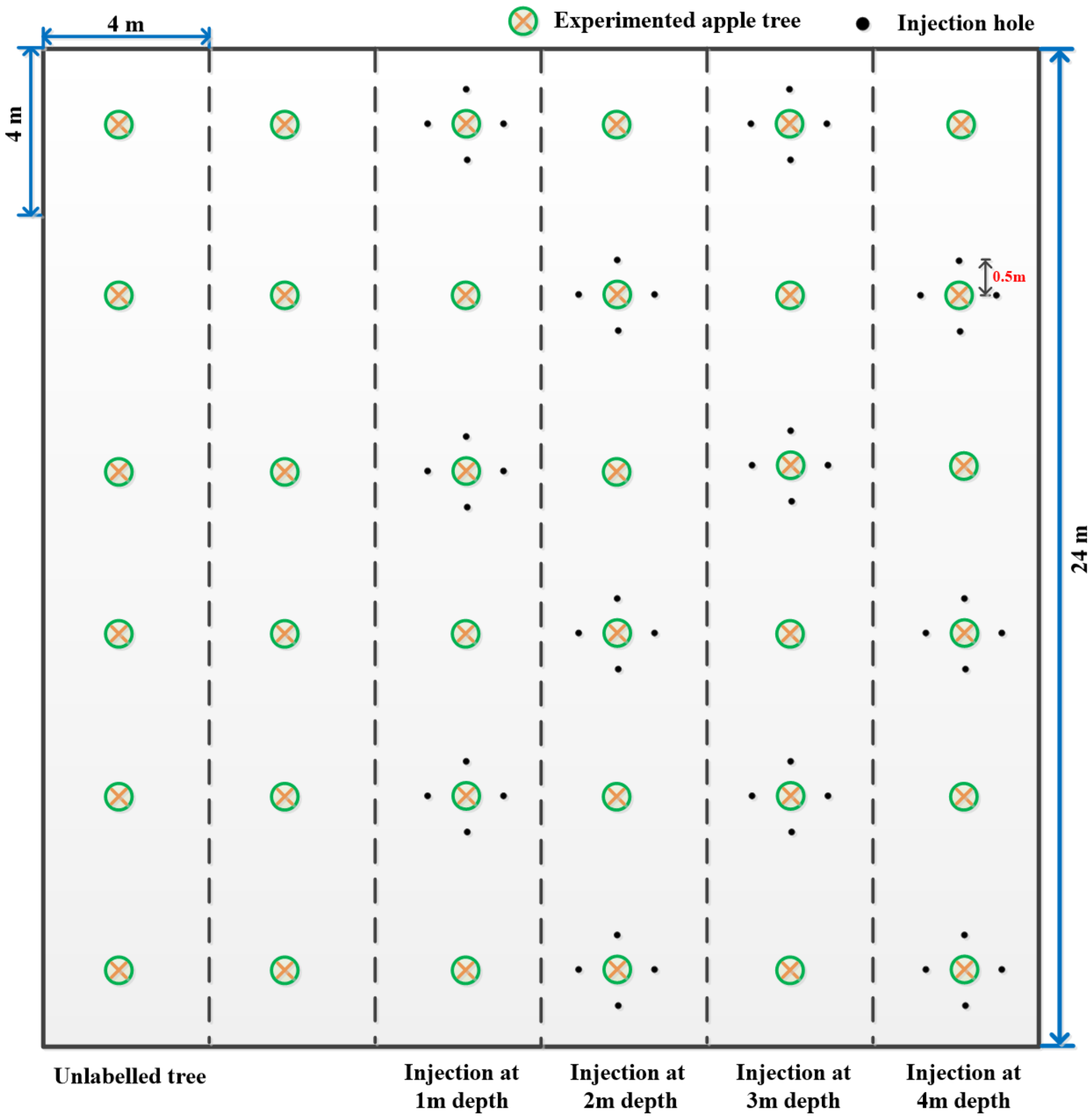

Figure S1 The layout of apple trees in experiment plot.

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.

Response:

(1) The manufacturer of the cryogenic vacuum distillation system has been revised (L 149-151).

"A cryogenic vacuum distillation system (Li-2000; LICA United Technology Limited, Beijing, China) was used to extract water under a heating temperature of 95 ℃ and a pressure of 0.2 Pa, which has been applied in previous studies (Huo et al., 2020; Tao et al., 2021a; Wang et al., 2021b; Zhao e al., 2020)."

(2) A detailed description of the cryogenic vacuum distillation method and its

extraction efficiency has been added to the revised manuscript (L 149-153).

"A cryogenic vacuum distillation system (Li-2000; LICA United Technology Limited, Beijing, China) was used to extract water under a heating temperature of 95 ℃ and a pressure of 0.2 Pa, which has been applied in previous studies (Huo et al., 2020; Tao et al., 2021a; Wang et al., 2021b; Zhao e al., 2020). The extraction time of soil water and xylem water samples were 90 min and 120 min respectively. Samples were weighed before and after extraction and again after oven-drying for 24 h to calculate the extraction efficiency (Wang et al., 2021b), which should be not less than 98%."

Reference

Huo, G., Zhao, X., Gao, X., and Wang, S.: Seasonal effects of intercropping on tree water use strategies in semiarid plantations: Evidence from natural and labelling stable isotopes, Plant Soil, 10.1007/s11104-020-04477-5, 2020.

Tao, Z., Neil, E., and Si, B.: Determining deep root water uptake patterns with tree age in the Chinese loess area, Agric. Water Manage., 249, 106810, https://doi.org/10.1016/j.agwat.2021.106810, 2021b.

Wang, H., Jin, J., Cui, B., Si, B., Ma, X., and Wen, M.: Technical note: Evaporating water is different from bulk soil water in delta δ^2H and δ^{18}O and has implications for evaporation calculation, Hydrol. Earth Syst. Sci., 25, 5399-5413, 10.5194/hess-25-5399-2021, 2021.

Zhao, Y., Wang, Y., He, M., Tong, Y., Zhou, J., Guo, X., Liu, J., and Zhang, X.: Transference of *Robinia pseudoacacia* water-use patterns from deep to shallow soil layers during the transition period between the dry and rainy seasons in a water-limited region, For. Ecol. Manage., 457, 10.1016/j.foreco.2019.117727, 2020.

Lines 133-135: Details about the uncertainty in the isotopic analyses for each instrument should be added here. Did the authors use specific practices to mitigate the memory effect in the isotopic measurements?

Response:

(1) The measurement precision of δ^{18}O and δD is 0.2‰ and 1.0‰ for the TIWA-45EP isotope ratio infrared spectroscopy analyzer and 0.3‰ and 2.0‰ for the Stable Isotope Ratio Mass Spectrometer, respectively.

(2) Yes. "Each isotopic sample was repeatedly measured six times. The first three measurements were discarded to mitigate the memory effect of isotopic measurement, and the mean value of the last three measurements was taken as the isotopic value of sample."

The relevant content has been added to the revised manuscript (L 157-161).

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?

Response: In general, soil water is the primary water source for trees on the Loess Plateau. We assessed the isotopic offset between xylem water and soil water using soil water line conditioned excess (SW-excess) proposed by Barbeta et al. (2019):

$$\text{SW-excess} = \delta D - a_s\delta^{18}O - b_s \qquad (1)$$

where a_s and b_s are the slope and intercept of soil water line (SWL), respectively; δD and $\delta^{18}O$ are the isotopic compositions of xylem water. A positive SW-excess value means that xylem water plots above SWL in a $\delta D - \delta^{18}O$ diagram (i.e. D in xylem water is more enriched than SWL), while a negative value means that xylem water plots below SWL in a $\delta D - \delta^{18}O$ diagram (i.e. D in xylem water is more depleted than SWL).

In the Bayesian isotope mixing model, the δD and $\delta^{18}O$ values for each potential water source were used as source data; the δD after subtracting the SW-excess and $\delta^{18}O$ values for xylem water were used as mixture data. δD values of xylem water corrected by SWL can match those of soil water. Thus, the fractionation factor in this model was set to zero, assuming no isotope fractionation during root water uptake.

We have made changes to the revised manuscript (L 163-168 and 174-177).

References

Barbeta, A., Jones, S. P., Clavé, L., Wingate, L., Gimeno, T. E., Fréjaville, B., Wohl, S., and Ogée, J.: Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest, Hydrol. Earth Syst. Sci., 23, 2129-2146, DOI:10.5194/hess-23-2129-2019, 2019.

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.

Response: The index S represented the sensibility of the response of root water uptake to soil water content increase. A higher S value means a faster response of root water uptake and greater sensitivity to moisture changes. As the index is likely to lead to misunderstanding, it has been deleted in the revised manuscript (L 180-183).

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.

Response:

(1) Because the concentration of D in tracer solution (δD = 714,000‰) is much higher than it in xylem water, it cannot be shown in the Figure 5. We have added the isotopic composition of the tracer solution to Figure 5's title (L 229).

(2) The background value without 2 SD has been added to Figure 5 (L 227).

(3) The sample size has been added to Figure 5's title (L 229).

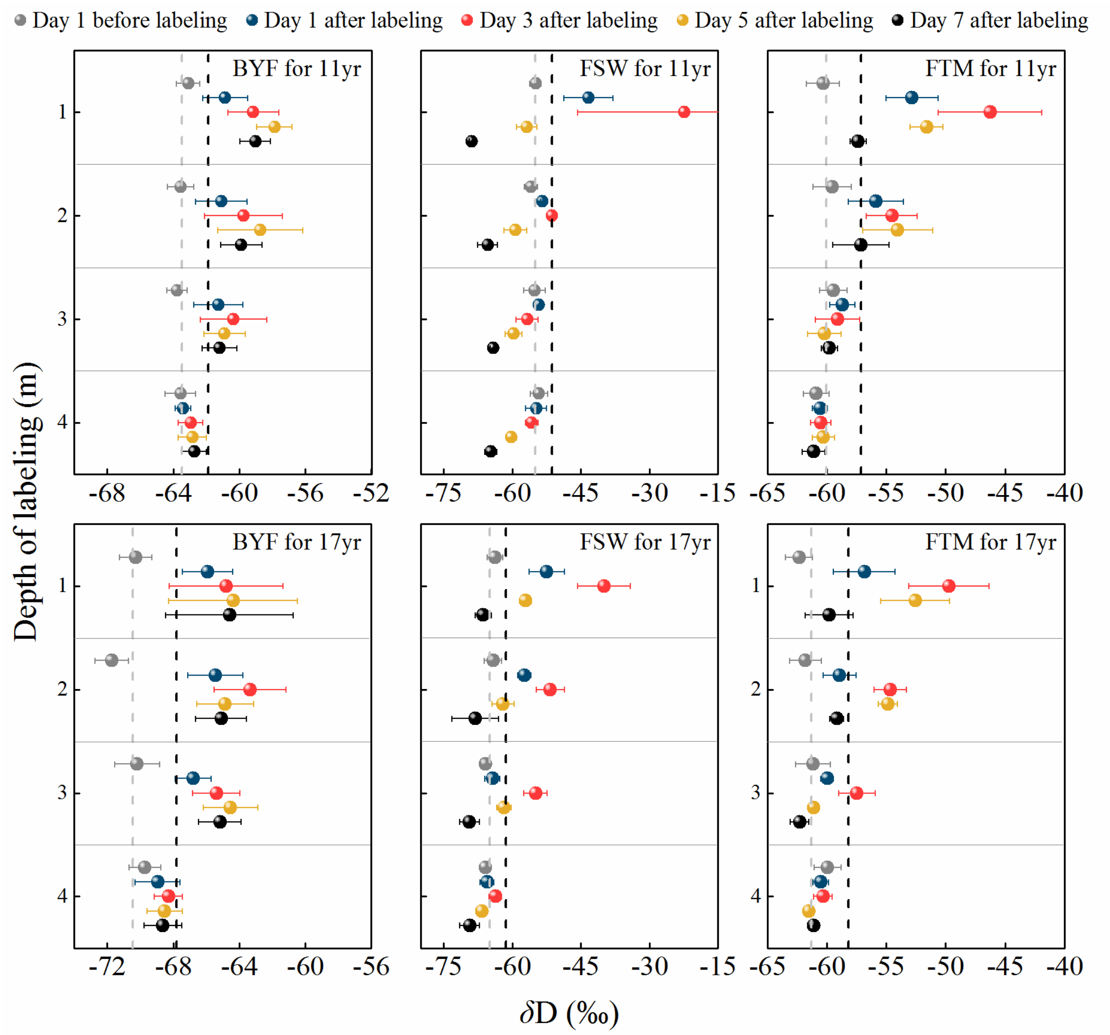

Figure 5: Temporal dynamics of δD values in xylem water for 11- and 17-year-old apple trees. Sample collection started on day 1 before D_2O tracer solution (δD = 714000‰) injection and commenced until day 7 (N=6). Gray dashed lines represent the background value (mean δD values in xylem water on day 1 before labeling) and black dashed lines represent 2 SD above the background value.

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.

Response:

(1) We assessed the isotopic offset between xylem water and soil water using soil water line conditioned excess (SW-excess) proposed by Barbeta et al. (2019):

$$\text{SW-excess} = \delta D - a_s\delta^{18}O - b_s \quad (1)$$

where a_s and b_s are the slope and intercept of soil water line (SWL), respectively; δD and $\delta^{18}O$ are the isotopic compositions of xylem water.

Table S2 Mean (±SD, n=6) soil water excess (SW-excess, ‰) for 11- and 17-year-old apple trees.

Stand age (a)	Growth stage		
	BYF	FSW	FTM
11	2.09 ±0.83	-6.80 ±3.56	4.78 ±1.85
17	-2.75 ±075	-2.57 ±0.29	-0.80 ±1.72

The results showed that SW-excess was negative in FSW stage for 11yr and BYF stage for 17yr trees, which meant that xylem water plots below SWL in a $\delta D - \delta^{18}O$ diagram (i.e. D in xylem water is more depleted than SWL). Therefore, the δD after subtracting the SW-excess and $\delta^{18}O$ values for xylem water were used as mixture data for the Bayesian isotope mixing model.

The text has been revised (L 162-168 and 174-177).

(2) The soil water line and xylem water line have been added to Figure 7. We did not show the isotopic composition of injected water because the soil water and xylem water in these plots were from unlabeled trees (L 264).

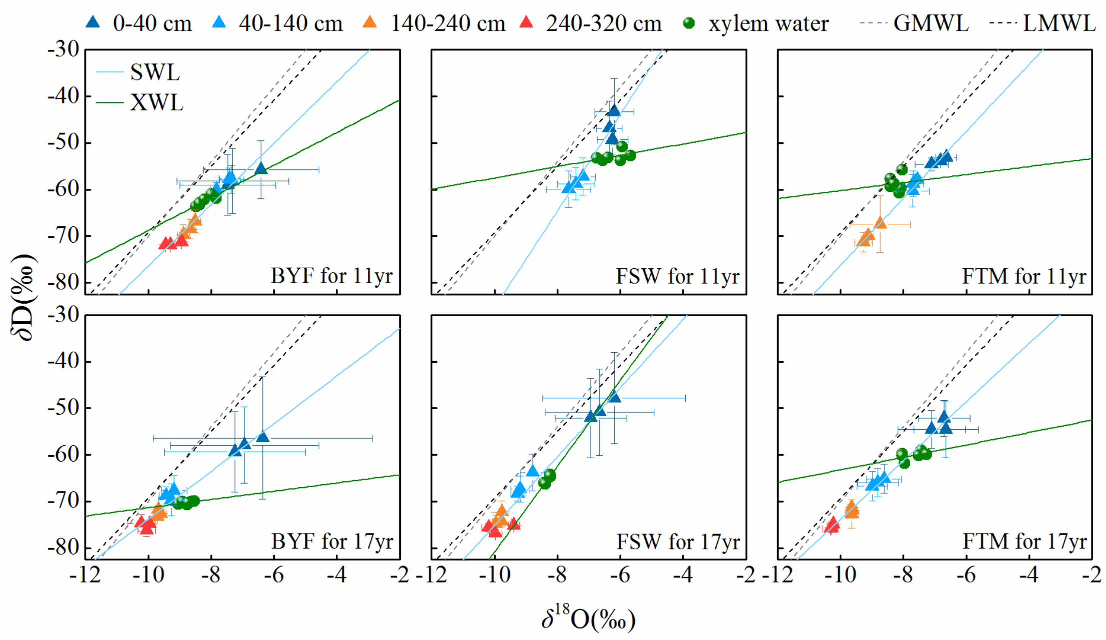

Figure 7: δD and δ^{18}O values in xylem water and different soil layers (0–40 cm, 40–140 cm, 140–240 cm, 240–320 cm) for 11- and 17-year-old apple trees (±SD). The GMWL and LMWL represents the global and local meteoric water lines. LMWL: $\delta D = 7.1\delta^{18}O+2.1$. The SWL and XWL represents the soil water line and xylem water line, respectively.

(3) The LMWL Equation has been added (L 266). "LMWL: $\delta D = 7.1\delta^{18}O+2.1$".

Details on sample size and sample timing have been added to the "Materials and Methods" and Figure 2 (L137-140).

"Rainwater samples (N = 32) were collected using a combined device of polyethylene bottle and funnel during rainfall events between May and September. A plastic ball was placed on the funnel to prevent evaporation. The collected rainwater samples were immediately sealed into vials by parafilm and stored at 4°C for isotopic determination."

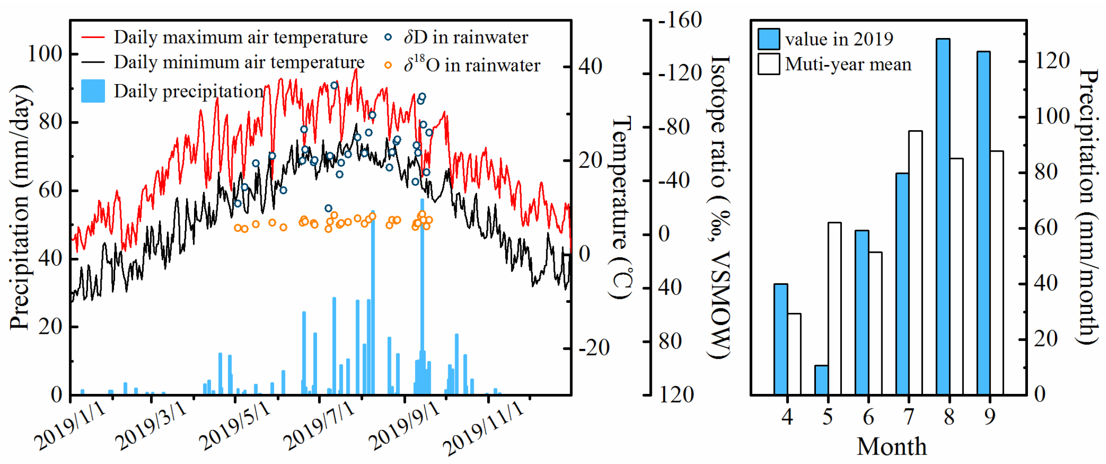

Figure 2: Time series of meteorological data and rainwater isotopic values in 2019 and monthly precipitation in 2019 and multi-year mean, respectively.

Figure 9: Please remove the regression line when there are only three samples used for the analysis.

Response: Done (L 283).

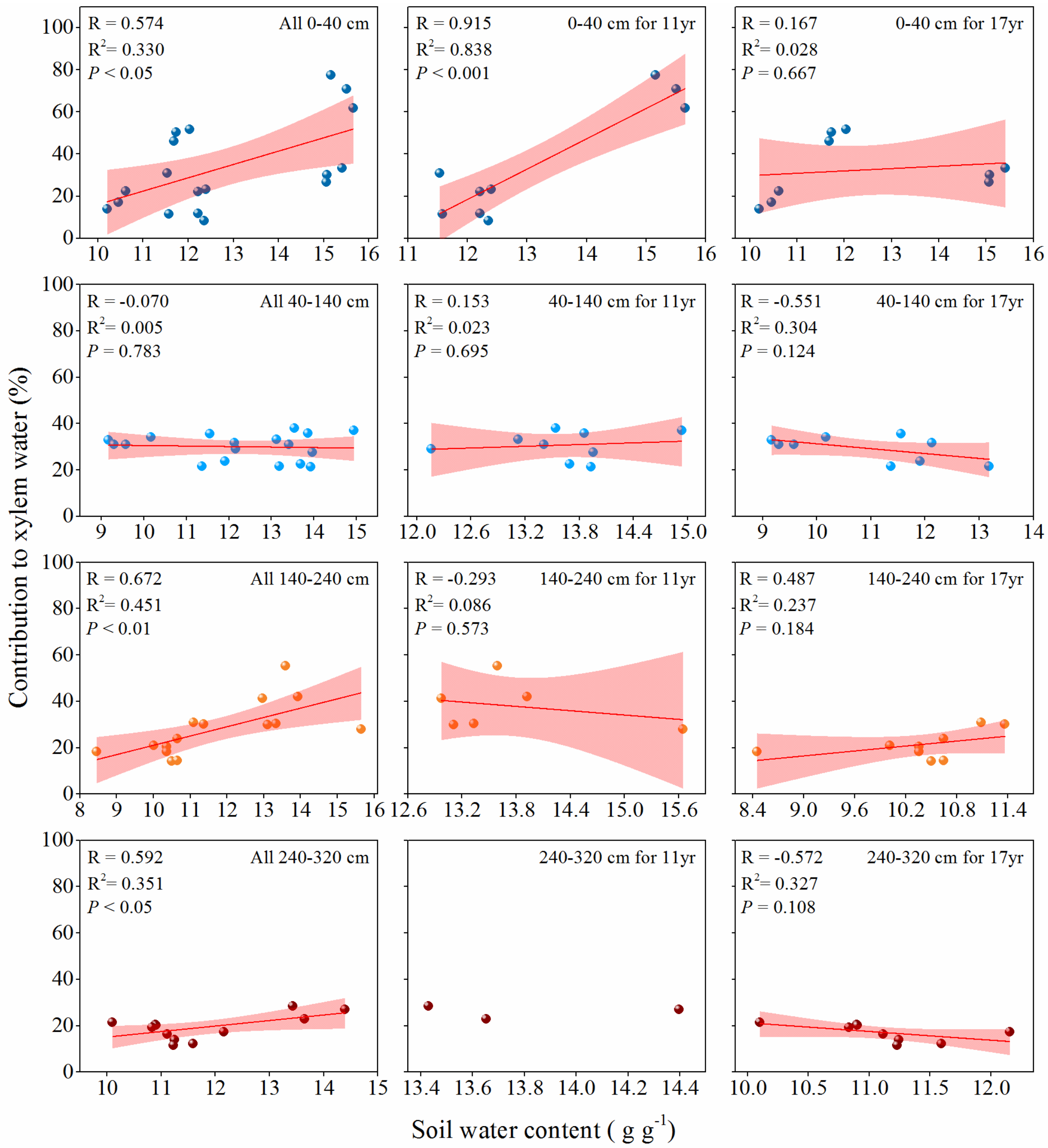

Figure 9: Relationship between the contribution of water sources and soil water content in different soil layers of 11- and 17-year-old apple orchards.

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.

Response: Agreed. The description of S has been deleted in the revised manuscript (L 180-183).

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 unlabeled and labeled trees, and to the extraction method (cryogenic vacuum distillation system).

Response: We have added a new section to the revised manuscript (L 389-405).

"4.4 Uncertainty caused by isotopic offset"

"In this study, isotopic offset between xylem and soil water was observed for both 11- and 17-year-old unlabeled apple trees (Fig.7 and Table S2). We used the isotopic composition of soil water to correct δD values of xylem water, ensuring they match those of soil water. Although we did not collect soil water isotope samples in the isotope labeling experiments, this may have little effect on determining the soil layer depths from which trees derive their water source due to the high δD values in the injected solution. It should be noted that isotopic spatial heterogeneity related to destructive sampling (xylem and soil water) could lead to an isotopic mismatch between xylem and soil water. Recently, isotopic offsets between plants and their potential water sources have been also found in various ecosystems, which may hinder the unambiguous identification of water sources and influence the accurate assessment of DLSW utilization (Barbeta et al., 2022; De La Casa et al., 2022; Zhao et al., 2016). Some studies argued that isotopic fractionation during root water uptake could be attributed to the existence of Casparian strips which can lead to isotope enrichment in root water and depletion in xylem water (Naseer et al., 2012; Vargas et al., 2017). Seeger and Weiler (2021) questioned whether xylem water was completely renewed by newly absorbed soil water, thus affecting isotopic offset. Furthermore, CVD may mask or exaggerate the isotopic offset, although it was the most common methodology (Chen et al., 2020; Orlowski et al., 2016a,b, 2018; Wen et al., 2022). When quantifying the water use strategies of plants, the isotopic measurement bias related to CVD should be considered. As a whole, there are various trends and causes of isotopic offset; further research about offset is urgently needed to better understand root water uptake processes."

References

Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L., Domec, J.-C., and Ogée, J.: 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, 2022.

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Naseer, S., Lee, Y., Lapierre, C., Franke, R., Nawrath, C., and Geldner, N.: Casparian strip diffusion barrier in Arabidopsis is made of a lignin polymer without suberin, Proc. Natl. Acad. Sci.

U. S. A., 109(25), 10101-10106, https://doi.org/10.1073/pnas.1205726109, 2012.

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Orlowski, N., Pratt, D. L., and McDonnell, J. J.: Intercomparison of soil pore water extraction methods for stable isotope analysis, Hydrol. Process., 30, 3434-3449, DOI:10.1002/hyp.10870, 2016b.

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Seeger, S. and Weiler, M.: Temporal dynamics of tree xylem water isotopes: in situ monitoring and modeling, Biogeosciences, 18, 4603-4627, 10.5194/bg-18-4603-2021, 2021.

Vargas, A. I., Schaffer, B., Li, Y., and Sternberg, L. d. S. L.: Testing plant use of mobile vs immobile soil water sources using stable isotope experiments, New Phytol., 215, 582-594, DOI:10.1111/nph.14616, 2017.

Wen, M., He, D., Li, M., Ren, R., Jin, J., and Si, B.: Causes and Factors of Cryogenic Extraction Biases on Isotopes of Xylem Water, Water Resour. Res., 58, e2022WR032182, 10.1029/2022wr032182, 2022.

Zhao, L., Wang, L., Cernusak, L. A., Liu, X., Xiao, H., Zhou, M., and Zhang, S.: Significant difference in hydrogen isotope composition between xylem and tissue water in *Populus Euphratica*, Plant Cell Environ., 39, 1848-1857, DOI:10.1111/pce.12753, 2016.

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.).

Response: Corrected (L 43-46 and 394-395).

“Analytical techniques based on stable isotopes (δD and δ^{18}O) can be applied to study plant water use based on the assumption that no isotope fractionation occurs during root water uptake (Dawson et al., 2002; Ehleringer and Dawson, 1992; Evaristo et al., 2015; Rothfuss and Javaux, 2017).”

“It should be noted that isotopic spatial heterogeneity related to destructive sampling (xylem and soil water) could lead to an isotopic offset between xylem and soil water.”

References

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., and Tu, K. P.: Stable Isotopes in Plant Ecology, Annu. Rev. Ecol. Syst., 33, 507-559, 10.1146/annurev.ecolsys.33.020602.095451, 2002.

Ehleringer, J. R. and Dawson, T. E.: WATER-UPTAKE BY PLANTS - PERSPECTIVES FROM STABLE ISOTOPE COMPOSITION, Plant Cell Environ., 15, 1073-1082, 10.1111/j.1365-3040.1992.tb01657.x, 1992.

Evaristo, J., Jasechko, S., and McDonnell, J. J.: Global separation of plant transpiration from groundwater and streamflow, Nature, 525, 91-94, 10.1038/nature14983, 2015.

Rothfuss, Y. and Javaux, M.: Reviews and syntheses: Isotopic approaches to quantify root water uptake: a review and comparison of methods, Biogeosciences, 14, 2199-2224, 10.5194/bg-14-2199-2017, 2017.

Line 51: Please replace “confusion of” with “unclear”.

Response: Done (L 57).

“However, it is challenging to quantify where in the soil profile the roots extract water due to limitations in monitoring technologies and unclear physical processes such as preferential flow.”

Title of section 2.2: I suggest changing it with “Sample collection”.

Response: Done (L 109).

“**2.2** Sample collection”

Title of section 2.2.2: I suggest changing it with “Collection of soil and vegetation samples for isotopic analysis”.

Response: Done (L 129).

“2.2.2 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).

Response: Done (L 213).

“Figure 4: Vertical distribution of soil water content (SWC) before the tracer injection in 11-year-old (A) and 17-year-old (B) apple orchards. Values are means ± SD (N=3).”

Figure 8: Please remove from the caption "Seasonal patterns of" because the results refer only to three specific tracer injections.

Response: Done (L 270).

"**Figure 8:** The contribution of four potential water sources to xylem water in 11-year-old (A) and 17-year-old (B) apple trees. Error bars indicate standard errors of the means (N=3). Asterisks represent significant differences between growing stages (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)."