**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 will be added to the revised manuscript.

"A long polyvinyl chloride pipe was inserted into the holes at the target depth before injecting 300 mL tracer solution ($\delta$D = 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.."

"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 various 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., 2021), which should be not less than 98%."

**References**

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, 2021.

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 $\delta^2$H and $\delta^{18}$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.

(2) Information on isotopic fractionation will be added to the Introduction and Discussion.

**Introduction**

"Although some recent studies found isotopic fractionation along the soil–root–stem–twig–leaf pathway(e.g., Barbeta et al., 2019; Poca et al., 2019; Vargas et al., 2017), the factors affecting such fractionation remain unclear (Barbeta 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), yet CVD is the most common methodology for water extraction to date (De La Casa et al., 2022)."

**Discussion**

"Isotopic offsets between plants and their potential water sources have been 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 found that isotopic fractionation during root water uptake could be attributed to the existence of Casparian strips, resulting in isotope enrichment in root water and depletion in xylem water (Naseer et al., 2012; Vargas et al., 2017). Seeger and Weiler (2021) suggested whether xylem water was completely renewed by newly absorbed soil water is another important factor affecting isotopic offset."

**References**

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

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.

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.

Orlowski N., Breuer L., 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.

Orlowski N., Pratt D.L., 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.

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., 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.

Poca M., Coomans O., Urcelay C., Zeballos S.R., Bodé S., Boeckx P.: Isotope fractionation during root water uptake by Acacia caven is enhanced by arbuscular mycorrhizas, Plant Soil, 441, 485-497, DOI:10.1007/s11104-019-04139-1, 2019.

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., Yuhong L., da Silveira Lobo Sternberg 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.

Zhao L., Wang L., Cernusak L.A., Liu X., Xiao H., Zhou M., 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.

(3) The following section will be added to the revised manuscript.

**"4.4 Uncertainty caused by isotopic offset"**

"Isotopic offsets between plants and their potential water sources have been 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 found that isotopic fractionation during root water uptake could be attributed to the existence of Casparian strips, resulting in isotope enrichment in root water and depletion in xylem water (Naseer et al., 2012; Vargas et al., 2017). Seeger and Weiler (2021) suggested whether xylem water was completely renewed by newly absorbed soil water is another important factor affecting 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 $\delta$D values of xylem water, ensuring they match those of soil water. In comparison, although we did not collect soil water isotope samples in the isotope labeling experiments, and thus could not correct the $\delta$D values in xylem water of labeled apple trees, this may have little effect on determining the soil layer depths from which trees derive their water source due to the high $\delta$D 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. In addition, CVD may mask or exaggerate the isotopic offset, although it was the most common methodology (Orlowski et al., 2016a,b, 2018). 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., 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.

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.

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.

Orlowski N., Breuer L., 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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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., Yuhong L., da Silveira Lobo Sternberg 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.

Zhao L., Wang L., Cernusak L.A., Liu X., Xiao H., Zhou M., 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.

**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 will be added to the Introduction.

"Although some recent studies found isotopic fractionation along the soil–root–stem–twig–leaf pathway(e.g., Barbeta et al., 2019; Poca et al., 2019; Vargas et al., 2017), the factors affecting such fractionation remain unclear (Barbeta 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), yet CVD is the most common methodology for water extraction to date (De La Casa et al., 2022)."

**References**

Barbeta A., Burlett R., Martín-Gómez P., Fréjaville B., Devert N., Wingate L., Domec J.-C., 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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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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Poca M., Coomans O., Urcelay C., Zeballos S.R., Bodé S., Boeckx P.: Isotope fractionation during root water uptake by Acacia caven is enhanced by arbuscular mycorrhizas, Plant Soil, 441, 485-497, DOI:10.1007/s11104-019-04139-1, 2019.

Vargas A.I., Schaffer B., Yuhong L., da Silveira Lobo Sternberg 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.

Zhao L., Wang L., Cernusak L.A., Liu X., Xiao H., Zhou M., 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.

**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 will change the manuscript as follows.

"Orlowski et al. (2016a,b, 2018) suggested that the fractionation is mainly related to cryogenic vacuum distillation (CVD), yet CVD is 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., Pratt D.L., 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.

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., 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 100 m deep on average, which cannot be reached by plant roots. We will amend the manuscript.

**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 will make changes in the revised manuscript.

**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 will be 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 orchard.

| 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 will be added to the revised manuscript.

"A long polyvinyl chloride pipe was inserted into the holes at the target depth before injecting 300 mL tracer solution ($\delta$D = 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 150000‰.

**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 layout of unlabeled trees and labeled trees will be shown in Figure S1.

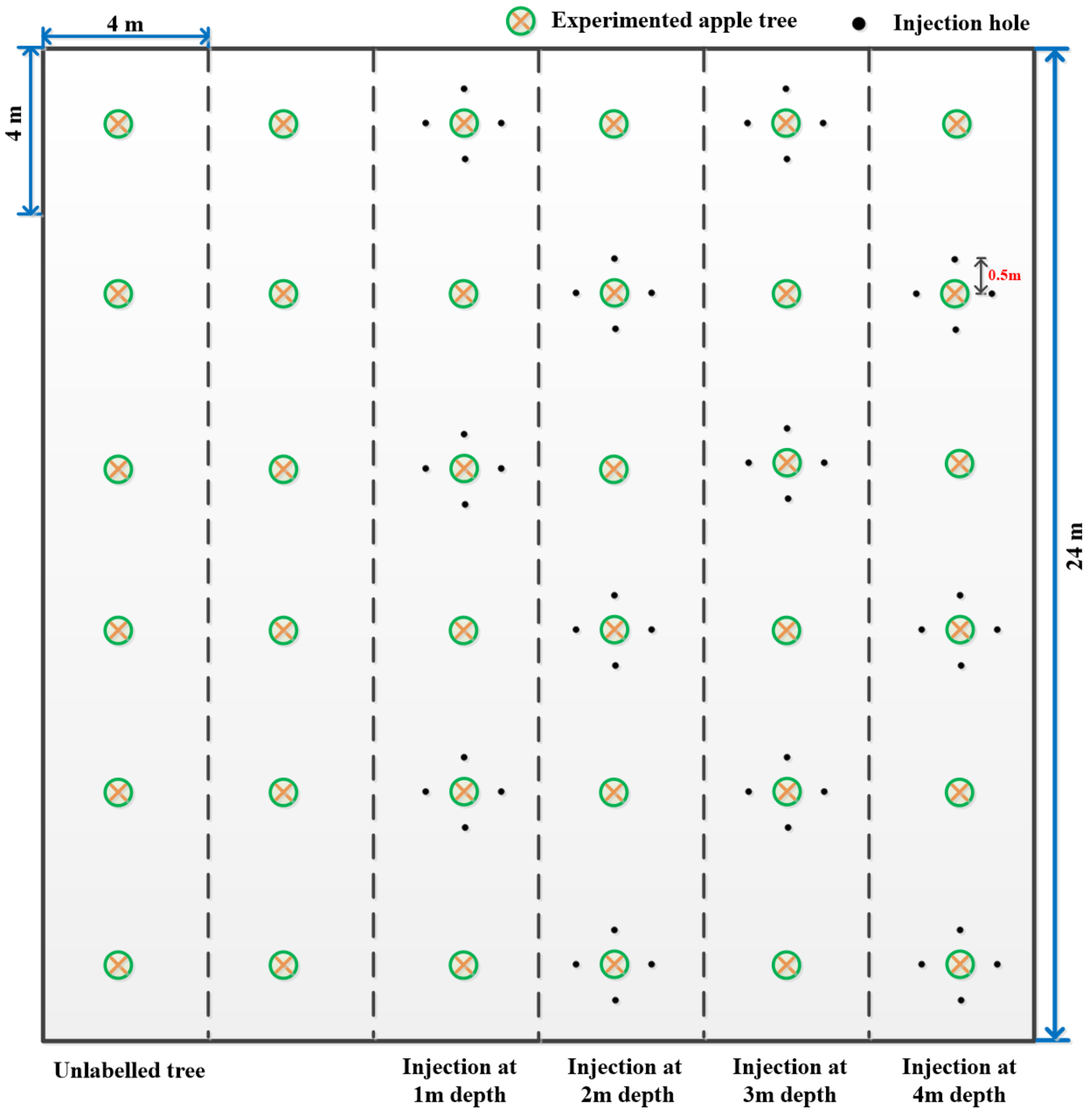


**Figure S1** The layout of apple trees in experiment plot.

**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 will be revised.

"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 various 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 will be added to the revised manuscript.

“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 various 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 $\delta^2$H and $\delta^{18}$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 $\delta^{18}$O and $\delta$D 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 will be added to the revised manuscript.

**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):

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

where $a_s$ and $b_s$ are the slope and intercept of soil water line (SWL), respectively; $\delta D$ 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 $\delta D$ and $\delta^{18}O$ values for each potential water source were used as source data; the $\delta D$ after subtracting the SW-excess and $\delta^{18}O$ values for xylem water were used as mixture data. $\delta D$ 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 will make changes to the revised manuscript.

**References**

Barbeta A., Jones S.P., Clavé L.,Wingate L., Gimeno T.E., Fréjaville B., Wohl S., 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 will be deleted in the revised manuscript.

**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 ($\delta D$ = 714,000‰) is much higher than it in xylem water, it cannot be shown in the Figure 5. We will add the isotopic

composition of the tracer solution to Figure 5's title.

(2) The background value without 2 SD will be added to Figure 5.

(3) The sample size will be added to Figure 5's title.

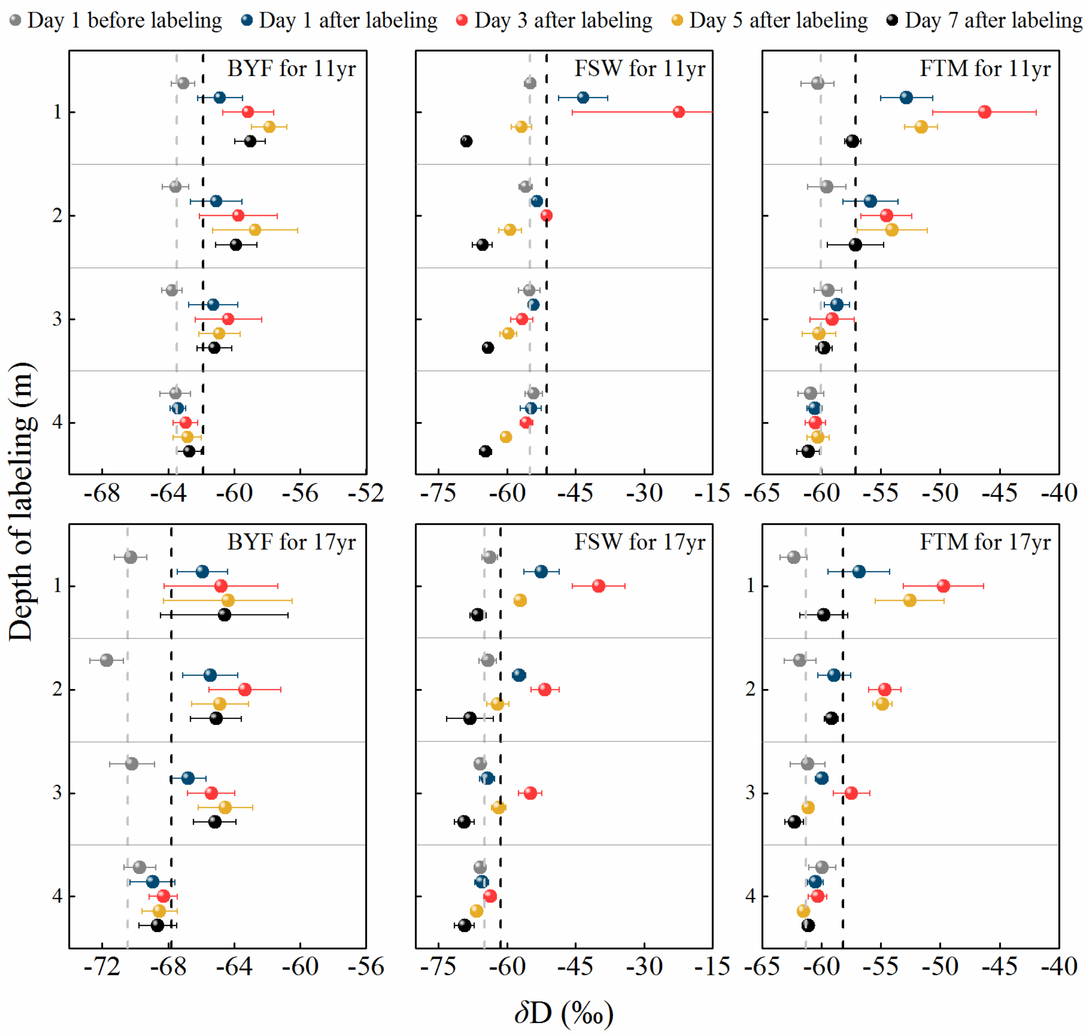


**Figure 5:** Temporal dynamics of $\delta D$ values in xylem water for 11- and 17-year-old apple trees. Sample collection started on day 1 before $D_2O$ tracer solution ($\delta D$ = 714000‰) injection and commenced until day 7 (N=6). Gray dashed lines represent the background value (mean $\delta D$ 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; $\delta D$ 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 $\delta D$ after subtracting the SW-excess and $\delta^{18}O$ values for xylem water were used as mixture data for the Bayesian isotope mixing model.

(2) The soil water line and xylem water line will be 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.

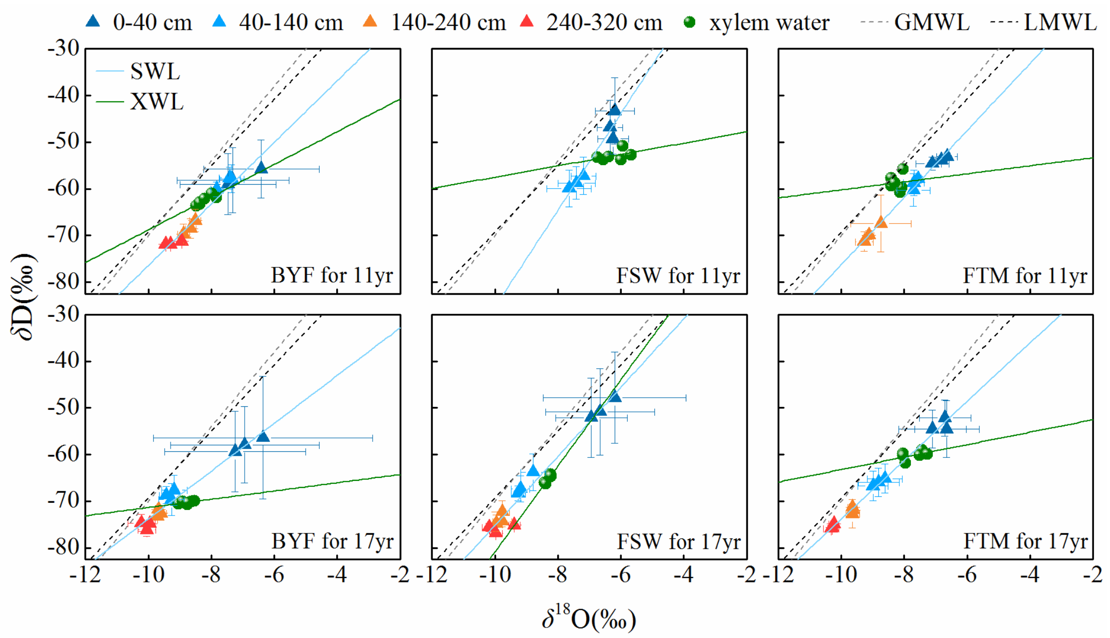


**Figure 7:** $\delta D$ and $\delta^{18}O$ values in xylem water and different soil layers (0–40 cm, 40–140 cm, 140–240 cm, 240–320 cm) for 11- and 17-year-old apple trees (±SD). The GMWL and LMWL represents the global and local meteoric water lines. LMWL: $\delta D = 7.1\delta^{18}O+2.1$. The SWL and

XWL represents the soil water line and xylem water line.

(3) The LMWL Equation will be added. "LMWL: $\delta D = 7.1\delta^{18}O+2.1$".

Details on sample size and sample timing will be added to the "Materials and Methods" and Figure 2.

"Rain 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."

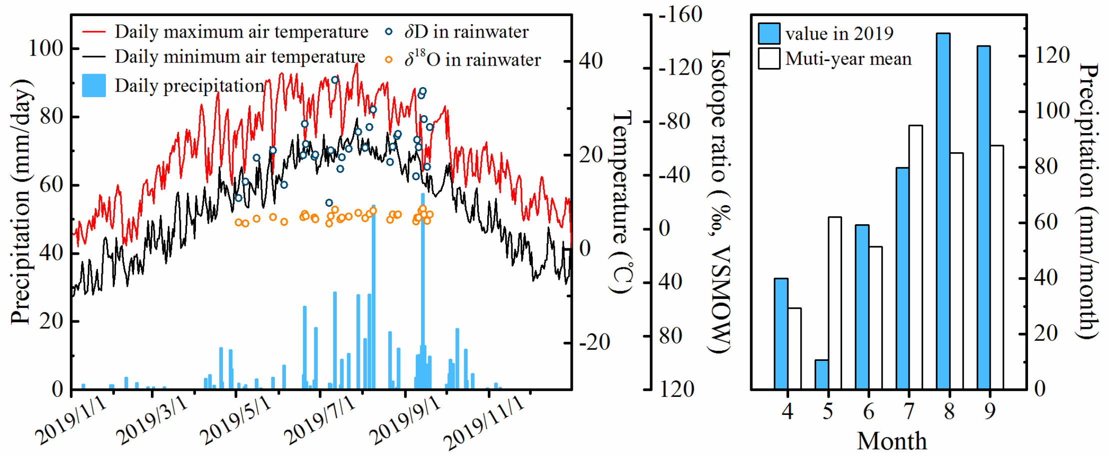


**Figure 2:** Time series of meteorological data and rainwater isotopic values in 2019 and monthly precipitation in 2019 and multi-year mean, respectively.

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

**Response:** Agreed. The regression line will be removed in the revised manuscript.

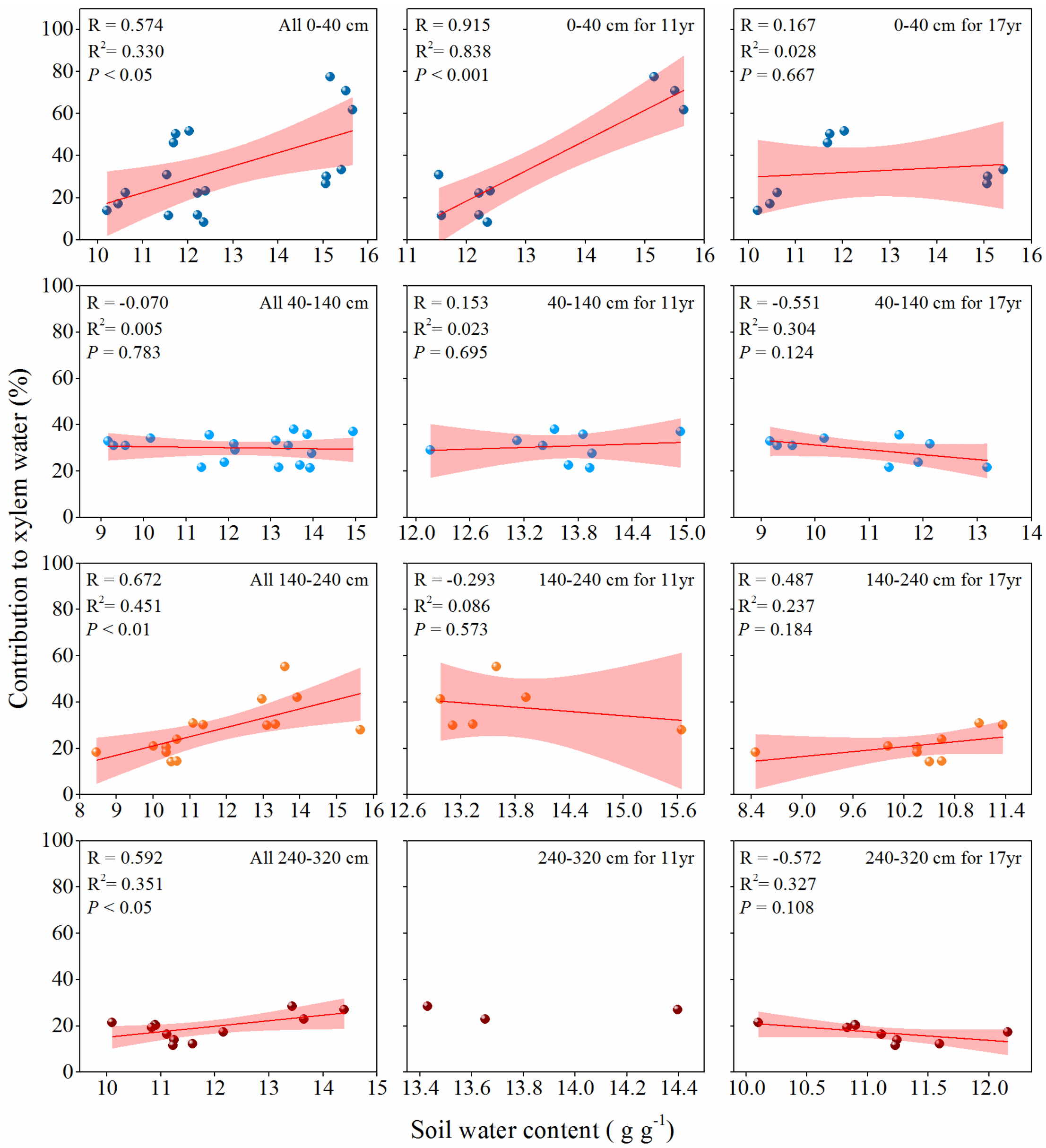


**Figure 9:** Relationship between the contribution of water sources and soil water content in different soil layers of 11- and 17-year-old apple orchards.

**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 will be deleted in the revised manuscript.

**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 will add a new section to the revised manuscript.

**"4.4 Uncertainty caused by isotopic offset"**

"Isotopic offsets between plants and their potential water sources have been 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 found that isotopic fractionation during root water uptake could be attributed to the existence of Casparian strips, resulting in isotope enrichment in root water and depletion in xylem water (Naseer et al., 2012; Vargas et al., 2017). Seeger and Weiler (2021) suggested whether xylem water was completely renewed by newly absorbed soil water is another important factor affecting 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 $\delta$D values of xylem water, ensuring they match those of soil water. In comparison, although we did not collect soil water isotope samples in the isotope labeling experiments, and thus could not correct the $\delta$D values in xylem water of labeled apple trees, this may have little effect on determining the soil layer depths from which trees derive their water source due to the high $\delta$D 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. In addition, CVD may mask or exaggerate the isotopic offset, although it was the most common methodology (Orlowski et al., 2016a,b, 2018). 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."

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Zhao L., Wang L., Cernusak L.A., Liu X., Xiao H., Zhou M., 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:** Agreed. We will change the revised manuscript.

"Analytical techniques based on stable isotopes ($\delta$D and $\delta^{18}$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 mismatch 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.

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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:** Agreed. We will change the revised manuscript.

"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:** Agreed. We will change the revised manuscript.

"**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:** Agreed. We will change the revised manuscript.

"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:** Agreed. We will change the revised manuscript.

"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:** Agreed. We will change the revised manuscript.

"**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 (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)."