Response to Reviewer 2, re: "Insights into isotopic mismatch between soil water and *Salix matsudana* Koidz xylem water from root water isotope measurements", in review in HESSD (NO. hess-2020-680).

We thank Reviewer #2 for the time and effort spent providing us with valuable feedback, which can certainly help us to strengthen the manuscript. We agree with the suggestions and have made targeted amendments, as detailed in the point-by-point responses below. The reviewer's comments are presented in blue, while passages changed in specific responses to the comments are presented in quotation marks and italic font.

Point-by-point by responses to Reviewer 2's comments

Major points:

1. In the manuscript entitled, "Insights into isotopic mismatch between soil water and *Salix matsudana* Koidz xylem water from root water isotope measurements", results from a field study show isotopic differences between different soil water and plant water pools. The dataset is interesting and results from intensive measurements. I think some of the findings are potentially interesting and warrant publication. However, the current presentation has some weaknesses and ambiguities that need to be addressed. Ultimately, the imprecise use of jargon obscures the interpretation and implications of the study.

Reply: We thank the reviewer for the constructive comments and suggestions, we have carefully considered them and tried our best to reduce the highlighted weaknesses and ambiguities in the manuscript, especially the problem about the “two water worlds” hypothesis.

2. Throughout, references are made to ecohydrological separation and the two-water-worlds (TWW) hypothesis. However, I am not sure how the authors define these phenomena; their definitions seem different from my own, different from the literature that they are citing, and they may change throughout. At times, it seems that these terms just mean “there are isotopic differences”, which is not particularly novel to identify. The paper would greatly benefit from less use of jargon. It should be more explicitly stated what is being tested. From my reading of this, the key questions of this paper are “Does root water isotopic composition match that of soils’ bulk, mobile, and bound water fractions of soils at the same depths”. Then there is a second question “How does choice of stem sample (from four heights) or choice of soil versus roots influence inferences of water uptake depths”. Neither of these questions is especially related to TWW or ecohydrological separation. Frankly, the duration of the study is too short to assess either TWW or ecohydrological separation because any observed differences between plant water and groundwater isotope ratios might be a product of lags, rather than the use of fundamentally different sources. The process Brooks et al referred to as ecohydrological separation was only observable through using measurements across multiple seasons.

Reply: We agree that the short experimental period and the focus on phenomena that are not directly related to the “two water worlds” (TWW) hypothesis hinder concise, meaningful discussion of the hypothesis and ecohydrological separation. Reviewer 1 also suggested that references to the TWW hypothesis should be reduced. Therefore, we would like to delete all content about the TWW hypothesis and pay more attention to the soil water’s heterogeneity through the comparison of mobile water, bulk soil water, and derived characteristics of less mobile water (we intend to change 'tightly bound water' to 'less mobile water', as advised by Reviewer 1) at the same depths, and the impact of this heterogeneity on plant water uptake in the next version.

3. The isotopic differences between root water and soil water is key to the conclusions made in this paper. The authors seem to suggest that the water in roots should match the water in soils around them. They did not, and this was interpreted as potential fractionation. However, roots can transport water from different locations. I would only expect similarity between roots and surrounding soils if fine roots were sampled. For coarser roots, as used in this study, I would expect those roots to transport water from much deeper depths and integrate large volumes of soil water. Thus, it is not clear that "a combination of plant fractionation and TWW-type separation" (which, again, needs to be clarified) is needed to explain the observations here. This needs to be further discussed. Potentially, additional excavations may be warranted to identify whether the size of root samples could include fine roots that extend substantially deeper.

Reply: Thanks for your suggestions. We would like to change the conclusion that isotopic fractionation leads to the observed mismatch between root water and bulk soil water at the same depth in the manuscript, for two reasons. First, the water in the sampled coarse roots (> 2 mm diameter) does not necessarily match the bulk soil water around them because sampled coarse roots can transport and mix water from different locations, as suggested. Second, as suggested by Reviewer 1, recent studies on isotopic fractionation have found stronger ^{2}H depletion in trunk water/root water than in bulk soil water (e.g., Poca et al., 2019; Vargas et al., 2017). However, these findings are not consistent with our finding that root water had higher δ^2H values than bulk soil water (up to 8.6‰). Most importantly, we found that the isotopic composition of root water deviated from that of bulk soil water, but overlapped with the values derived for less mobile water (see Figure 1 below). Thus, we concluded that soil-root isotopic offsets are more likely to be caused by the complexity of root systems and the heterogeneity of bulk soil water than isotopic fractionation during root water uptake. Hence, we would like to add the following discussion regarding this issue in the next version:

"We compared the isotopic composition of root water and bulk soil water at the same depth. Contrary to expectations, the root water and bulk soil water at 0-60 cm depths showed consistent δ^2H and $\delta^{18}O$ isotopic composition. However, at 80-160 cm depths, δ^2H and $\delta^{18}O$ values of root water deviated significantly from those of bulk soil water. An alternative explanation for isotopic mismatch at the same depth is that it is due to the complexity of root systems and difficulties in unambiguously determining root traits and functions at specific depths because of the opaque nature of soil. For example, if collected roots are close to the absorptive roots like fine roots (< 2 mm diameter), they may have similar isotopic composition to bulk soil water at the same depth. In contrast, if they are closer to transport roots like taproots, much of their water content may be from different positions, thereby resulting in inconsistent isotopic composition between root water and surrounding bulk soil water. Nevertheless, although it is difficult to assess the importance of sampled roots for a whole root system's water uptake, root water may reflect the water source of trees better than bulk soil water (which has been more extensively used), for two reasons. First, bulk soil water is commonly collected in cores of 50 cm^3 or more (Sprenger et al., 2015; Penna et al., 2018). It is possible to determine the fractions and isotopic composition of bulk soil water held

under specific tension ranges, but information on the spatiotemporal heterogeneity of pore sizes within the cores, and associated effects on uptake patterns, is lost (McCutcheon et al., 2016). Root water is not subject to this deficiency as it consists of water absorbed by fine roots distributed in pores of various sizes. In addition, we systematically collected coarse roots (with > 2 mm diameter) within 80 cm of the main trunk at 20 cm intervals from 0 to 160 cm depths of soil to reduce the potential errors caused by the lack of representativeness of some root water. Our results suggest that trunk water was isotopically closer to root water than bulk soil water. Similarly, measurements of the δ^2H and $\delta^{18}O$ of bulk soil, trunk and root water from potted Fagus svlvatica *saplings under control and drought treatments by Barbeta et al. (2020) showed that the δ^2H of trunk water consistently matched the δ^2H of root water, and deviated significantly from the δ^2H of bulk soil water under both treatments.*

Overall, the most plausible explanation for isotopic mismatch between root water and bulk soil water in dual-isotope plots is that bulk soil water is not representative of available plant water sources because of the heterogeneity of bulk soil water. As shown in Fig. 1, less mobile water overlapped isotopically with root water after removing the influence of mobile water. The rapidity of mobile water's passage through soil reduces its contact with mineral surfaces, and hence its nutrient concentrations (McDonnell, 2017; Sprenger et al., 2019). Thus, plants may have used large amounts of less mobile water that was strongly affected by evaporative effects in the presented study, isotopically distinct from mobile water and groundwater, and with similar isotopic composition to trunk water. In addition, isotopic offsets between bulk soil water and root/trunk water caused by isotopic fractionation have been previously reported (Lin and Sternberg, 1993; Vargas et al., 2017; Barbeta et al., 2019). Vargas et al. (2017) found that isotopic *fractionation caused more ^2H depletion in trunk water than in bulk soil water. Similarly, Poca et al. (2019) found that trunk water was significantly more depleted in ^2H than bulk soil water (by up to −15.6‰) and this isotopic fractionation occurred during transmembrane water transport by aquaporins. However, these findings are not consistent with the greater ^2H enrichment in root water than in bulk soil water (differences up to 8.6‰) we detected, suggesting that soil-root isotopic offsets are more likely to be caused by the complexity of root systems and heterogeneity of bulk soil water than isotopic fractionation during root water uptake."*

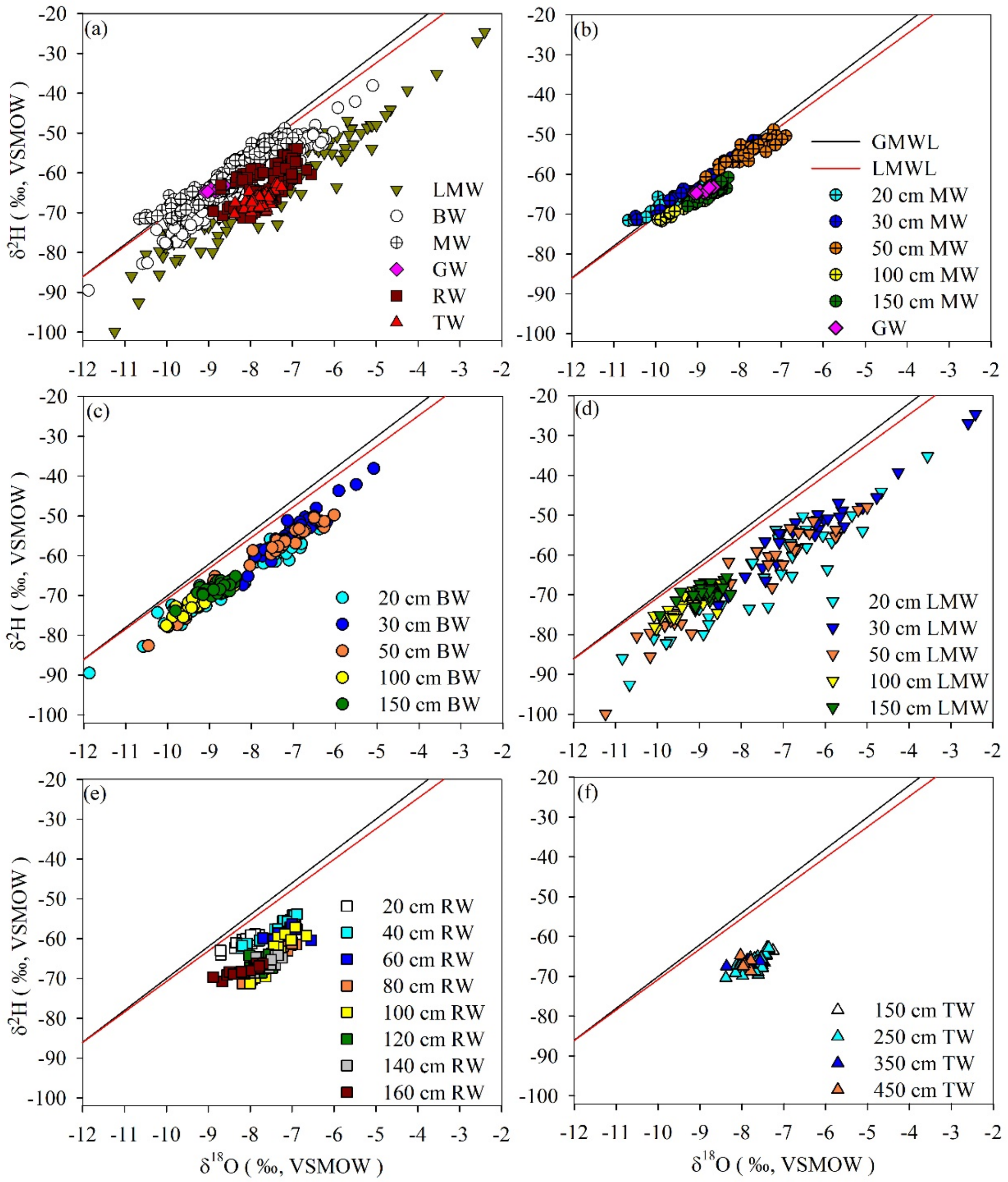

Figure 1 (a) $\delta^{18}O$ and $\delta^{2}H$ isotopic composition collected from August 4 to September 15, 2019. Plotted values include bulk soil water (BW), mobile water (MW), root water (RW), trunk water (TW), less mobile water (LMW) and groundwater (GW). (b) $\delta^{18}O$ and $\delta^{2}H$ isotopic composition of groundwater, and MW collected from different depths, (c) BW collected from different depths, (d) LMW collected from different depths, (e) RW collected from different depths, and (f) TW collected from different tree heights. The red line represents the 2016-2019 local meteoric water line (LMWL, $\delta^2H = 5.91 + 7.67\ \delta^{18}O$, $R^2 = 0.96$). The black line represents the global meteoric water line (GMWL, $\delta^2H = 10 + 8\ \delta^{18}O$). The dotted black lines represent the linear regressions.

Barbeta, A., Gimeno, T.E., Clave, L., Frejaville, B., Jones, S.P., Delvigne, C., Wingate, L., and Ogee, J.M.: An explanation for the isotopic offset between soil and stem water in a temperate tree species, New Phytol., 227, 766-779, 2020.

Barbeta, A., Jones, S.P., Clave, L., Wingate, L., Gimeno, T.E., Frejaville, B., Wohl, S., and Ogee, 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, 2019.

Lin, G.H., and Sternberg, L.D.S.L.: Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants, In Stable isotopes and plant carbon-water relations, 497-510, 1993.

McCutcheon, R.J., McNamara, J.P., Kohn, M.J., and Evans, S.L.: An evaluation of the ecohydrological separation hypothesis in a semiarid catchment. Hydrol. Process. 31, 1-17, 2016.

McDonnell, J.J.: Beyond the water balance, Nat. Geosci., 10, 396-396, 2017.

Penna, D., Hopp, L., Scandellari, F., Allen, S.T., Benettin, P., Beyer, M., Geris, J., Klaus, J., Marshall, J.D., Schwendenmann, L., Volkmann, T.H.M., von Freyberg, J., Amin, A., Ceperley, N., Engel, M., Frentress, J., Giambastiani, Y., McDonnell, J.J., Zuecco, G., Llorens, P., Siegwolf, R.T.W., Dawson, T.E., and Kirchner, J.W.: Ideas and perspectives: tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes-challenges and opportunities from an interdisciplinary perspective, Biogeosciences, 15, 6399-6415, 2018.

Poca, M., Coomans, O., Urcelay, C., Zeballos, S.R., Bode, S., and Boeckx, P.: Isotope fractionation during root water uptake by *Acacia caven* is enhanced by arbuscular mycorrhizas, Plant Soil, 441, 485-497, 2019.

Sprenger, M., Herbstritt, B., and Weiler, M.: Established methods and new opportunities for pore water stable isotope analysis. Hydrol. Process. 29, 5174-5192, 2015.

Sprenger, M., Llorens, P., Cayuela, C., Gallart, F., and Latron, J.: Mechanisms of consistently disjunct soil water pools over (pore) space and time. Hydrol. Earth Syst. Sci., 23, 2751-2762, 2019.

Vargas, A.I., Schaffer, B., Yuhong, L., and Sternberg, L.S.L.: Testing plant use of mobile vs immobile soil water sources using stable isotope experiments, New Phytol., 215, 582-594, 2017.

Specific comments:

4. 14, 18, 77: I think the author is referring to ecohydrological separation, not the "two water worlds hypothesis", which is about streamflow. However, I am generally unclear on this.

Reply: We agree that the short experimental period and the focus on phenomena that are not directly related to the "two water worlds" (TWW) hypothesis hinder concise, meaningful discussion of the hypothesis and ecohydrological separation (see our response to major point 2). Thus, we plan to delete all content regarding the TWW hypothesis and ecohydrological separation

5. 20: From the abstract, it is not clear why this is "in conclusion"

Reply: We would like to rephrase the abstract, as follows:

"Increasing numbers of field studies have detected isotopic mismatches between plant trunk water and its potential sources. However, the cause of these isotopic offsets is not clear and it is uncertain whether they occur during root water uptake or during water transmission from root to trunk. Thus, we measured the specific isotopic composition (δ^2H and $\delta^{18}O$) of each component (e.g., bulk soil water, mobile water, groundwater, trunk water and root water of Salix matsudana *Koidz trees) with about three-day resolution in the soil-root-trunk continuum. We report three main findings. First, we detected clear separation between mobile water and bulk soil water isotopic composition, but the distinction between mobile water and bulk soil water gradually decreased with increasing soil depth. Second, root water deviated from bulk soil water isotopic composition, but it overlapped with the composition derived for less mobile water. The maximum differences in δ^2H and $\delta^{18}O$ between bulk soil water and root water were −8.6 and −1.8‰, respectively. Third, trunk water was only isotopically similar to root water at 100-160 cm depths, and it remained stable during the experimental period, suggesting that the trees consistently used the stable deep water source. In conclusion, the isotopic offset between bulk soil water and trunk water of* S. matsudana *reflected an isotopic mismatch between root water and bulk soil water associated with heterogeneity of the soil water. Our results illuminate relationships between the isotopic composition of soil water of various mobility, root water and trunk water that may be useful for advancing our understanding and representation of root water uptake and transport."*

6. 21-23: It is better to say what that contribution is, and what those insights are, rather than just mentioning that they exist.

Reply: We would like to revise this sentence as suggested, as follows:

"Our results illuminate relationships between the isotopic composition of soil water of various mobility, root water and trunk water that may be useful for advancing our understanding and representation of root water uptake and transport."

7. 41-44 If movement alone creates the change, I'd argue that this statement violates laws of mass conservation. Any changes must be matched by equal and opposite changes elsewhere. Ecohydrological separation is not a change from soil to root to xylem.

Reply: We thank the reviewer for alerting us to this error and intend to delete this sentence in the next version.

8. 46-48 See comments above. Please re-read Brooks et al.

Reply: We would like to delete all the content regarding the "two water worlds" hypothesis in the next version as suggested (see our response to major point 2). We will also read papers concerning the "two water worlds" hypothesis more carefully in the future.

9. 48-49 How would the hypothesis be supported by groundwater and streams? This does not make sense to me. The hypothesis is related to plant-available soil water.

Reply: We would like to delete this sentence in the next version because it is related to the "two water worlds" hypothesis.

10. 51: What does "related to infiltration" mean? Is "tightly bound" water not related to infiltration?

Reply: We would like to delete this sentence in the next version because it is related to the "two water worlds" hypothesis.

11. 120-124: This is a short period for assessing ecohydrological separation, especially in a dry-climate region. Identifying ecohydrological separation phenomena requires detecting bypassing of stored waters.

Reply: We agree that the short experimental period hinders concise, meaningful discussion of ecohydrological separation. Therefore, we will pay more attention to the soil water's heterogeneity through the comparison of mobile water, bulk soil water, and derived characteristics of less mobile water at the same depths, and the impact of this heterogeneity on plant water uptake in the next version.

12. 180-182 Given the interest in fractionation upon uptake, why not set it to a value more consistent with others in the literature.

Reply: Thanks for your suggestions. We would like to change the conclusion that the observed isotopic mismatch between root water and bulk soil water at the same depth was caused by isotopic fractionation, as advised by both reviewers (please see our response to main point 3). Moreover, application of the SIAR model does not strengthen the story, as pointed out by Reviewer #1, so we intend to delete the calculation of plant water source contributions based on the SIAR model.

13. 193-205 I do not understand why slopes in dual-isotope space are being used here. I don't think they are the most effective way to make the comparisons that are being made. First, were these slopes fit orthogonally? They should be. Second, when one line is compared to another, is that the result of an ANCOVA test? Third, are the p values the fits of the lines, or p values for the comparisons being described? I do not understand why fitted lines, rather than

the actual values, are being compared; it seems that the interpretations relate to the actual values.

Reply: We plan to use actual values instead of the slopes for analysis in accordance with this comment in the next version, as follows:

"The isotopic composition (δ^2H and $\delta^{18}O$) of all water samples are shown in Fig. 1a (see Figure 1 above) and Table 1. The slope and intercept of the local meteoric water line (LMWL, $\delta^2H = 7.67\ \delta^{18}O + 5.91$, $R^2 = 0.96$) were lower than those of the global meteoric water line (GMWL, $\delta^2H = 8\ \delta^{18}O + 10$) (Craig, 1961). Mobile water at all depths (i.e. 20, 30, 50, 100 and 150 cm) typically fell on the LMWL and groundwater was isotopically similar to mobile water at 150 cm depth (Fig. 1b). Bulk soil water partly overlapped isotopically with mobile water but it generally plotted below mobile water (Fig. 1a and c). Less mobile water deviated from the LMWL and overlapped with root water and trunk water (Fig. 1a and d). Trunk water was isotopically similar to root water at 100-160 cm depths (Fig. 1a and e-f)."

We will also add another table (see Table 1 below), showing the water stable isotopes and lc-excess values for all water samples.

Table 1 Water stable isotopes and lc-excess values for all water samples. Range values show min, max (mean).

Water samples	N	δ^2H range (‰)	δ^{18}O range (‰)	lc-excess range (‰)
Groundwater	22	−64.7, −63.2 (−64.1)	−9.1, −8.6 (−8.8)	−3.2, −1.0 (−2.4)
Mobile water	191	−71.7, −48.8 (−61.9)	−10.7, −6.9 (−8.7)	−5.7, 4.6 (−1.2)
Bulk soil water	203	−89.5, −38.1 (−64.5)	−11.9, −5.1(−8.3)	−12.5, −1.7(−6.7)
Less mobile water	176	−99.9, −24.6 (−65.1)	−11.2, −2.4 (−8.0)	−23.9, −2.8 (−9.9)
Root water	156	−71.3, −43.9 (−63.3)	−8.9, −6.5 (−7.6)	−16.9, −2.1 (−10.7)
Trunk water	61	−70.4, −62.8 (−66.7)	−8.4, −7.3 (−7.7)	−17.1, −9.0 (−13.5)

14. 208 What are these uncertainties: standard deviations? All depths are lumped together for soil water?

Reply: These numbers are in the form of "mean ± standard deviation". However, the information expressed in this sentence is limited, thus we would like to delete it, but add another table (see Table 1 above), showing the water stable isotopes and lc-excess values for all water samples.

15. 211 Ecohydrological separation was a process that could only be revealed from a long duration of sampling. How can this short period of measurement show ecohydrological separation?

Reply: Thanks for your suggestion. We agree that the short experimental period hinders concise, meaningful discussion of ecohydrological separation. Therefore, we would like to delete all content about ecohydrological separation and pay more attention to the soil water's heterogeneity through the comparison of mobile water, bulk soil water, and derived characteristics of less mobile water at the same depths, and the impact of this heterogeneity on plant water uptake in the next version.

16. 212 What is the statistical test used?

Reply: It was the Tukey-Kramer HSD test, as we intend to state in the next version.

"The mean lc-excess values of groundwater and mobile water did not significantly differ ($p > 0.05$), and they were significantly higher than those of bulk soil water, less mobile water and trunk water (Tukey-Kramer HSD, $p < 0.05$) during the sampling period."

17. 217 Does "always" mean every single value was different? I do not understand how one would test that.

Reply: The intended meaning is that at different sampling depths (i.e. 20 cm, 30 cm, 50 cm, 100 cm, 150 cm), there were significant differences between mobile water and bulk soil water lc-excess (see Figure 2A-F below). To clarify this, we intend to modify the sentence in the next version as follows:

"At every sampling depth, the mean lc-excess of mobile water was consistently higher than that of bulk soil water and less mobile water (Tukey-Kramer HSD test, $p < 0.05$) during the whole sampling period."

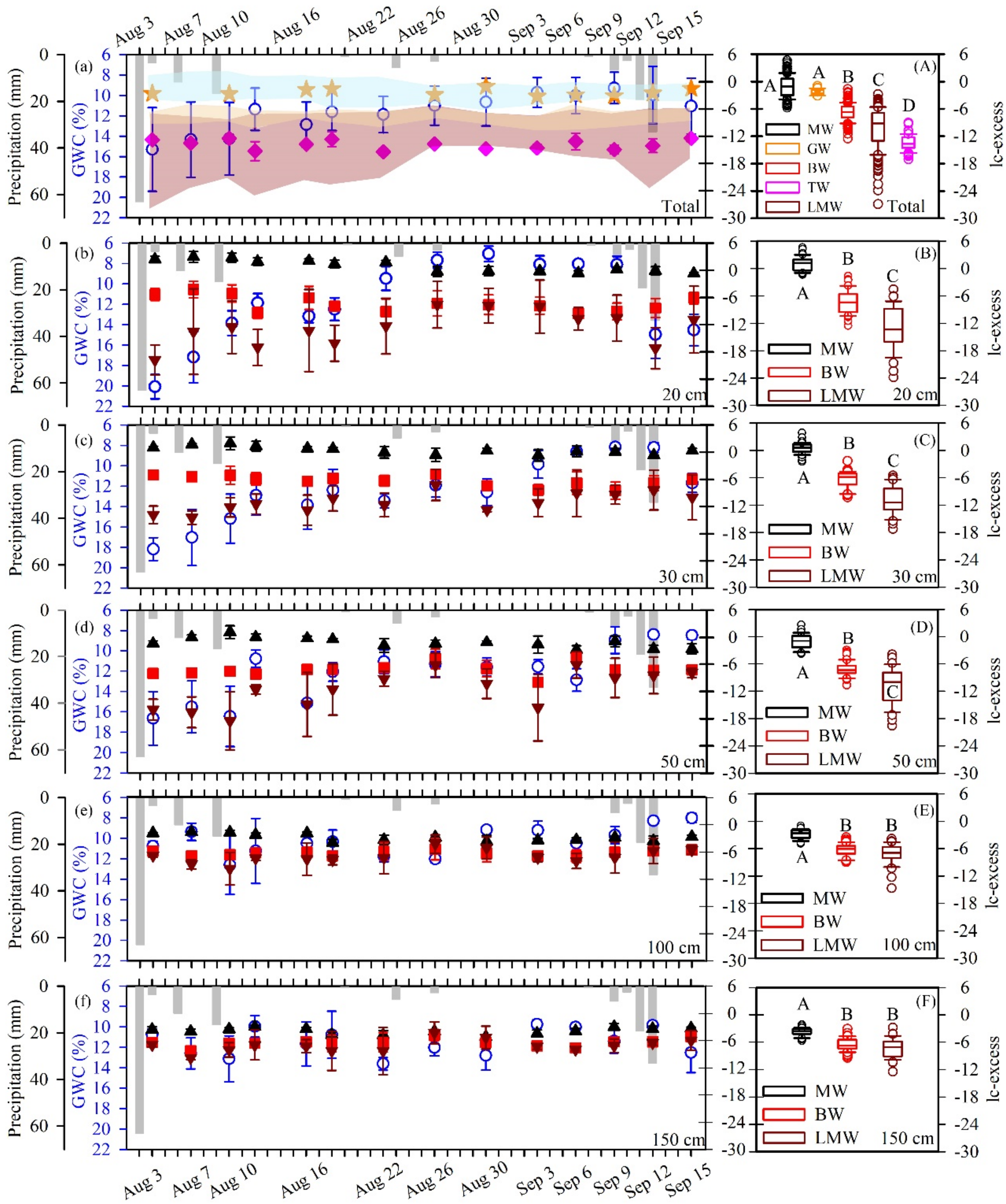

Figure 2 (a-f) Temporal dynamics of hydrological conditions (precipitation and gravimetric water content, GWC) and lc-excess values (these values are means and standard deviations for three sites) of groundwater (GW), trunk water (TW), mobile water (MW), less mobile water (LMW) and bulk soil water (BW) at indicated depths (20, 30, 50, 100 and 150 cm) during the period August 3 to September 15, 2019. (A) Boxplots of total MW (N=191), GW (N=22), BW (N=204), TW (N=61) and LMW (N=176) lc-excess values. (B-F) Boxplots of MW and BW at 20 cm (MW, N=40; BW, N=42; LMW, N=39), 30 cm (MW, N=40; BW, N=40; LMW, N=34), 50 cm (MW, N=38; BW, N=40; LMW, N=33), 100 cm (MW, N=36; BW, N=40; LMW, N=34) and 150 cm (MW, N=37; BW, N=42; LMW, N=36) depths. The top and bottom of each box are the 25th and 75th percentiles of the samples, respectively. The black line in each box is the sample median. Trunk water and potential water sources that do not share a letter are significantly different ($p < 0.05$, Tukey-Kramer HSD).

18. Section 3.3 Are these fine roots only? If not, I see no reason to think that roots should match soils at the same depths. A larger root is almost certainly going to integrate waters from zones larger than the soil surrounding it.

Reply: The roots we collected were coarse roots (> 2 mm diameter), as stated in section 3.3 of the Results "Comparison between root water and bulk soil water isotopes at different depths". We would like to add details regarding root collection, as advised by Reviewer #1. We will also add a brief discussion to the next version to make the results clearer, as follows:

Regarding root sampling:

"We excavated a soil cuboid with 160 cm depth, 80 cm width (horizontal distance) and 160 cm length with the main root of the selected tree at the center (Fig. 3a). We then divided the cuboid into 64 sub-cuboids (length, 40 cm; width, 40 cm; height, 20 cm) (Fig. 3b) and dug each sub-cuboid one by one to minimize risks of evaporation. 2-3 coarse roots (> 2 mm diameter) from each sub-cuboid were randomly selected and roots from the top few centimeters of the topsoil were not artificially removed. To minimize the influence of attached soil on root water, these sampled roots were rapidly peeled to remove bark, placed in 10 mL vials and sealed with caps then the caps were secured with Parafilm. Finally, these samples were kept in a cool box until storage in the lab at 4℃. To compare the isotopic composition of root and bulk soil water at the

same depths, we collected samples of soil around the sampled roots in each sub-cuboid. These soil samples were also rapidly placed in 10 mL vials that were sealed in the same manner as the root samples, then kept in a cool box until storage in the lab at −20 ℃."

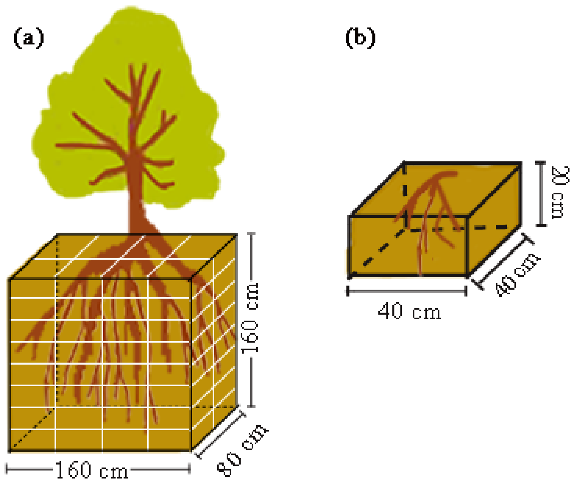

Figure 3: Schematic diagram of root excavations (a) and measurements (b).

Regarding the brief discussion:

"We compared the isotopic composition of root water and bulk soil water at the same depth. Contrary to expectations, the root water and bulk soil water at 0-60 cm depths showed consistent δ^2H and $\delta^{18}O$ isotopic composition. However, at 80-160 cm depths, δ^2H and $\delta^{18}O$ values of root water deviated significantly from those of bulk soil water. An alternative explanation for isotopic mismatch at the same depth is that it is due to the complexity of root systems and difficulties in unambiguously determining root traits and functions at specific depths because of the opaque nature of soil. For example, if collected roots are close to the absorptive roots like fine roots (< 2 mm diameter), they may have similar isotopic composition to bulk soil water at the same depth. In contrast, if they are closer to transport roots like taproots, much of their water content may be from different positions, thereby resulting in inconsistent isotopic composition between root water and surrounding bulk soil water."

19. 233 I do not understand this "horizontal homogeneity" discussion. Figure 3 shows that mobile water and bulk water at a given depth differ. That implies heterogeneity.

Reply: As shown in Figure 3 above, we sampled root water for stable isotope analysis at horizontal distances of 0-40 cm and 40-80 cm from selected tree trunks at the same depth. The results showed that there were no significant differences ($p > 0.05$) in isotopic composition (δ^2H and $\delta^{18}O$) of either root water or bulk soil water between 40 cm and 80 cm horizontal distance. To clarify this, we would like to revise the sentence in the next version, as follows:

"There were no significant differences (p > 0.05) in isotopic composition (δ^2H and $\delta^{18}O$) of either root water or bulk soil water between 40 cm and 80 cm horizontal distance from selected tree trunks, suggesting that isotopic composition of the soil was horizontally homogenous within 80 cm from tap roots."

20. 273 So if "separation" here is not the same as the "separation" that has been described throughout, it would be useful for the authors to use a more literal descriptor of the process that they are investigating.

Reply: We intend to rephrase these sentences, as follows:

"Gierke et al. (2016) examined the stable isotopic composition of precipitation, bulk soil water and trunk water in a high elevation watershed and their results suggested that mobile water was primarily associated with summer thunderstorms, and thus subject to minimal evaporative loss. In contrast, less mobile water was derived from snowmelt, filling small pores in the shallow soils. Allen et al. (2019) characterized the occurrence of winter and summer precipitation in plant trunk samples using a seasonal origin index and found that winter precipitation was the predominant water source for midsummer transpiration in sampled beech and oak trees. Due to seasonal

isotopic cycles in precipitation, there may be clear distinctions in the isotopic composition of mobile water and less mobile water derived from precipitation falling at different times (Bowen et al., 2019)"

Allen, S.T., Kirchner, J.W., Braun, S., Siegwolf, R.T.W., and Goldsmith, G.R.: Seasonal origins of soil water used by trees, Hydrol. Earth Syst. Sci., 23, 1199-1210, 2019.

Bowen, G.J., Cai, Z.Y., Fiorella, R.P., and Putman, A.L.: Isotopes in the water cycle: regional- to global-scale patterns and applications, Annu. Rev. Earth Planet. Sci., 47, 453-479, 2019.

Gierke, C., Newton, B.T., and Phillips, F.M.: Soil-water dynamics and tree water uptake in the Sacramento Mountains of New Mexico (USA): a stable isotope study, Hydrogeol. J., 24, 805-818, 2016.

21. 291 At this point, I've become overly confused with regard to what the authors consider "TWW" to be.

Reply: For reasons stated in our reply to comment 2, we are going to delete content related to this hypothesis and ecohydrological separation, and pay more attention to the impact of the heterogeneity of soil water on root water uptake in the next version.

22. 316 What does "mask" mean? Even if there is evaporation, fractionation upon uptake would result in different values between the roots and soils, regardless of the background (soil water) signal.

Reply: We plan to change the conclusion that the observed isotopic mismatch between root water and bulk soil water at the same depth was caused by isotopic fractionation, as advised by both reviewers (please see our response to main point 3). Thus, we will delete this sentence.

23. 343 The authors should probably specify that this is the duration of the detectable label and not the mean or median of the residence time distribution. Is that correct?

Reply: We will amend the sentence as follows:

"As the time required for isotopic tracer (D_2O) to move from the base of a trunk to the upper crown of a tree reportedly ranges from 2.5 to 21 days (Meinzer et al., 2016), the isotopic composition of trunk water may differ from that root water collected on the same day (August 18)."

24. 345 what is "this interpretation" referring to?

Reply: The intended meaning of "This interpretation" was that the root water we collected cannot reflect plant trunk water (xylem water has been changed to trunk water as advised by Reviewer 1) isotopic composition. We intend to revise the sentence to improve its clarity, as follows:

"As the time required for isotopic tracer (D_2O) to move from the base of a trunk to the upper crown of a tree reportedly ranges from 2.5 to 21 days (Meinzer et al., 2016), the isotopic composition of trunk water may differ from that root water collected on the same day (August 18). Thus, we measured δ^2H and $\delta^{18}O$ values of trunk water during our high frequency (ca. 3-day) sampling period from August 4 to September 15, 2019."

25. 352 It is best to not use "enriched" or "unenriched" unless specifying what they are enriched in (e.g., deuterium) and what they are enriched relative to (e.g., precipitation).

Reply: We intend to clarify these points as follows:

"Furthermore, previous studies have provided indications that trunk water becomes more enriched in ^{18}O due to the temporal declines in sap flow rates (Martin-Gomez et al., 2017) and the mixture of trunk water with leaf water (Brandes et al., 2007)."

Brandes, E., Wenninger, J., Koeniger, P., Schindler, D., Rennenberg, H., Leibundgut, C., Mayer, H., and Gessler, A.: Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. Plant Cell Environ., 30,113-127, 2007.

Martin-Gomez, P., Serrano, L., and Ferrio, J.P.: Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecohydrology, Tree Physiol., 37, 511-522, 2017.

26. 356 How is it known that root water is an accurate approach? This is important. It seems like root water tells you a different thing than soil water: the depth of roots contributing to transpiration, rather than the depth of soils contributing to transpiration.

Reply: We intend to delete the calculation of plant water source contributions based on the SIAR model, as advised by Reviewer #1, but keep the conclusion that root water at 100-160 cm depths was the main water source for plants. Although it is difficult to assess the importance of sampled roots for a whole root system's water uptake, root water may reflect the water source of trees better than bulk soil water (which has been more extensively used), for two reasons. First, bulk soil water is commonly collected in cores of 50 cm^3 or more (Sprenger et al., 2015; Penna et al., 2018). It is possible to determine the fractions and isotopic composition of bulk soil water held under specific tension ranges, but information on the spatiotemporal heterogeneity of pore sizes within the cores, and associated effects on uptake patterns, is lost (McCutcheon et al., 2016). Root water is not subject to this deficiency as it consists of water absorbed by fine roots distributed in pores of various sizes. In addition, we systematically collected coarse roots (with > 2 mm diameter) within 80 cm of the main trunk at 20 cm intervals from 0 to 160 cm depths of soil to reduce the potential errors caused by the lack of representativeness of some root water. Our results suggest that trunk water was isotopically closer to root water than bulk soil water. Similarly, measurements of the δ^2H and $\delta^{18}O$ of bulk soil, trunk and root water from potted Fagus svlvatica saplings under control and drought treatments by Barbeta et al. (2020) showed that the δ^2H of trunk water consistently matched the δ^2H of root water, and deviated significantly from the δ^2H of bulk soil water under both treatments.

27. 371 Please be more specific.

Reply: We plan to rephrase this sentence in the next version, as follows:

"The presented stable isotope data for bulk soil water, mobile water, less mobile water, root water and trunk water were highly valuable for analyzing the spatial heterogeneity of water fluxes in the root zone, and elucidating the water sources used by the plants."