Response to Reviewer 1, 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 #1 for thoughtfully and critically reviewing our manuscript. We greatly appreciate the positive feedback and many well-founded points that have certainly helped us to improve the manuscript. Overall, we agree with these suggestions and have made targeted amendments, as described in the detailed point-by-point replies to the Reviewer' comments below. The reviewer's comments are presented in blue, and passages changed in specific responses to the comments are presented in quotation marks and italic font.

Point-by-point by responses to Reviewer 1's comments

Major points:

1. The manuscript hess-2020-680: "Insights into isotopic mismatch between soil water and *Salix matsudana* Koidz xylem water from root water isotope measurements" by Zhao and Wang investigates potential reasons for an observed mismatch between soil and xylem water stable isotope values by measuring water stable isotope ratios in different soil water pools (mobile, bulk soil and bound) across soil depths, as well as in roots (across depth) and xylem of three rigorously sampled tree individuals. The authors conclude that the observed isotopic differences between xylem and bulk soil water arise from a combination of ecohydrological separation, i.e. isotopic differences in mobile and bound soil water and plant fractionation during root water uptake.

Root water uptake depth is routinely determined by comparing the isotope composition of xylem water with that of soil water in different depths (and other water sources like stream and groundwater) assuming that extracted water from bulk soil samples represents available water sources. This long-standing principle is lately repeatedly questioned and a lot of uncertainty persists on potential reasons and underlying causes. This work contributes to the discussion and provides new insights. I especially liked, that the authors conducted an experiment under natural conditions. I do not know of any other study that sampled water sources, xylem water and also systematically investigated root xylem within a field experiment to tackle this question and I applaud the authors for conducting this surely very labour-intensive work. The manuscript is well-structured and understandable. Generally, I am in favour of publishing this work. However, I think that a number of critical points (see major points below) should be addressed beforehand in a revised version. I also suggest an English native speaker to proof-read the manuscript and help to further improve some of the expressions.

Reply: We thank the reviewer for the constructive comments and suggestions, we have carefully considered them and tried our best to address the highlighted weaknesses in the manuscript. In addition, we will invite a professional native English-speaking editor with a PhD in a relevant discipline to edit the next version.

2. You state that plant fractionation, i.e. change of isotope values during root water uptake, is one of two main causes for the observed mismatch. This was observed before in xerophytic and halophytic plants and for plants in symbiosis with arbuscular mycorrhiza (e.g. Ellsworth & Williams 2007, Poca et al. 2019). However, previous studies on plant fractionation reported depleted (more negative) isotope values in plant xylem as compared to soil water,

hence plants discriminated against the heavy isotope (mostly ^{2}H). This is not in line with enriched (less negative) xylem values reported here. While it cannot be ruled out completely that water would get enriched in heavy isotopes during rwu, this was not reported before and considering other potential reasons, namely isotopic heterogeneities across soil water pools as well as temporal variability and methodological artefacts, this seems unlikely to me. If you decide to keep it in the manuscript, this discrepancy to other studies should be pointed out and discussed in detail.

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, 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 $\delta^{2}H$ values than bulk soil water (up to 8.6‰) as suggested. Second, as pointed out by Reviewer #2, 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. 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."

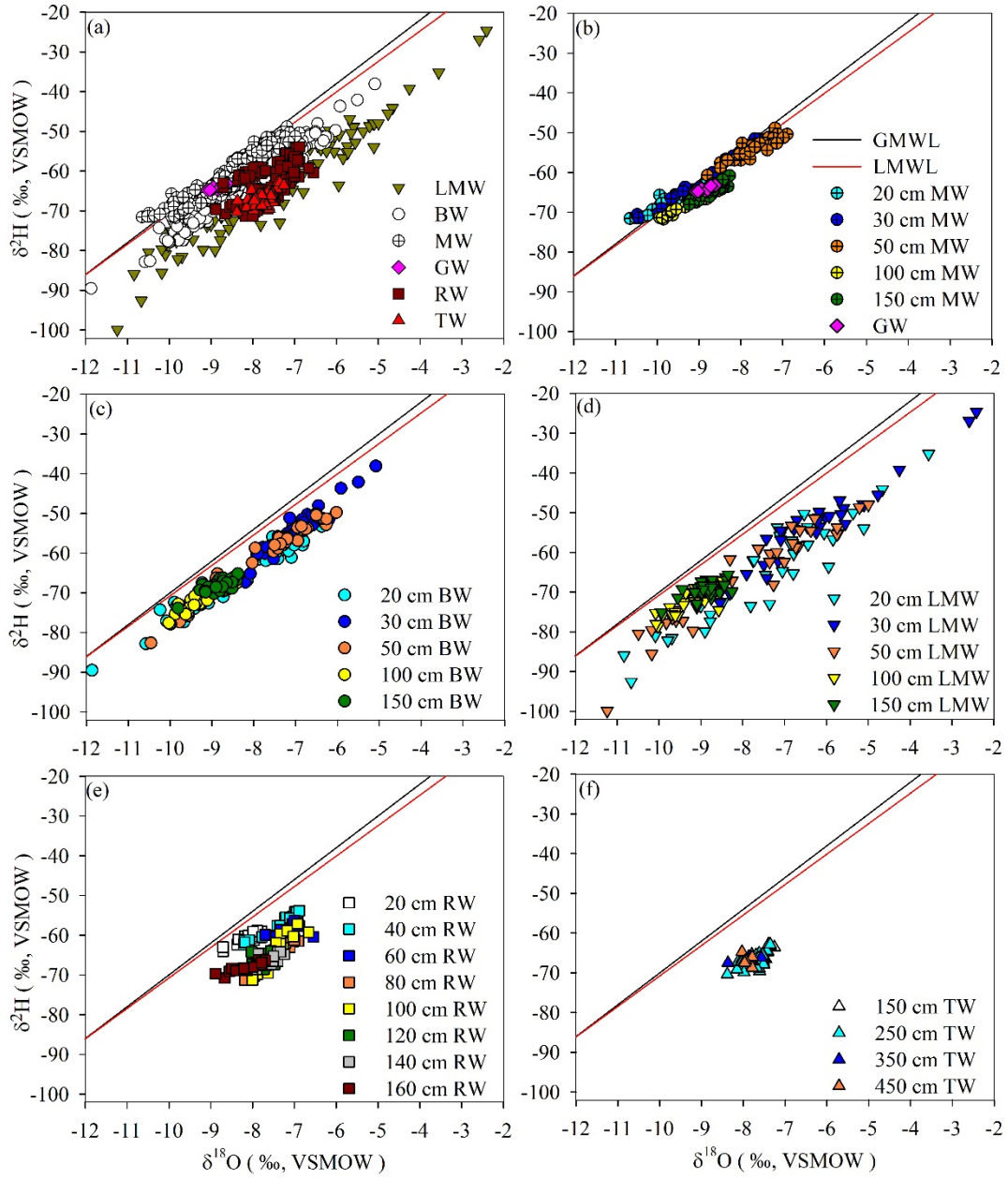

Figure 1 (a) $\delta^{18}O$ and $\delta^{2}H$ isotopic composition collected from August 4 to September 15, 2019. Plotted values include bulk soil water (BW), mobile water (MW), root water (RW), trunk water (TW), less mobile water (LMW) and groundwater (GW). (b) $\delta^{18}O$ and $\delta^{2}H$ isotopic 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.

3. Building on that, some root and all stem xylem samples show an evaporative enrichment in the dual isotope plot (Fig. 2). This is discussed in the manuscript and related to an enriched signal of bound soil water. I think this argument would be strengthened if you provide further description on the sampling procedure. Specifically, I wondered if evaporation during root sample collection could potentially influence obtained results. Did you sample roots right from the soil profile wall or exclude the first few centimetres? How fast was sampling conducted after digging the hole? Also: Was soil thoroughly removed from sampled roots? Regarding xylem sampling, you write “Bark was peeled from the twigs and all leaves were removed to avoid perturbance of xylem water isotopic signatures by fractionation.” (L 136-137). So, did you sample twigs that had leaves directly attached to them? Could the enriched

signal hence arise from back-diffusion or any other exchange with enriched leave water before sampling? Were twigs fully suberized or were they green and hence photosynthetically active?

Reply: We would like to add details about the root and trunk water (changing xylem water to trunk water, as suggested) sampling and schematic diagram of root excavations (Figure 2) in the *Materials and methods* section, 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. 2a). We then divided the cuboid into 64 sub-cuboids (length, 40 cm; width, 40 cm; height, 20 cm) (Fig. 2b) 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 ℃."

Regarding trunk sampling

"Tree samples were collected simultaneously with the soil samples. These consisted of twigs collected from the south-facing side of three S. matsudana *trees at 250 cm height on each*

sampling occasion. In addition, samples of trunk at selected tree heights (150, 250, 350, 450 cm) were collected on August 18, 2019. Bark and phloem were peeled from fully suberized branches to avoid perturbance of trunk water isotopic composition by fractionation. Pieces of the de-barked and de-leaved twigs, 30 mm long, were then immediately placed in 10 mL vials, the vials were sealed with caps then the caps were secured with Parafilm. These samples were also kept in a cool box until storage in the lab at 4°C."

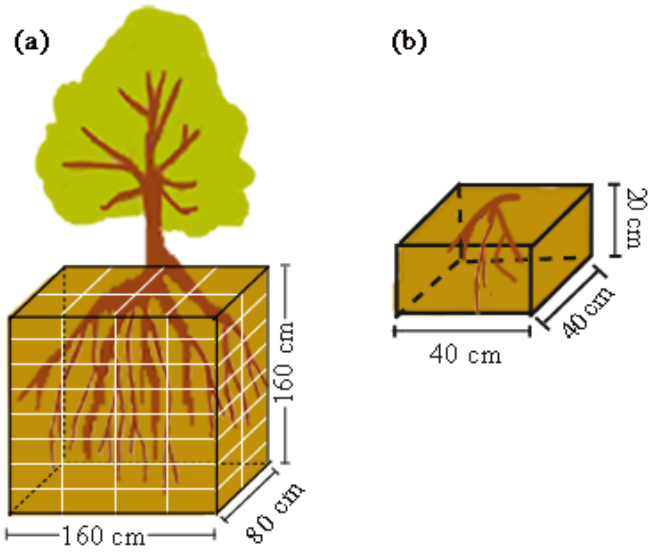

Figure 2: Schematic diagram of root excavations (a) and measurements (b).

4. In my personal opinion, I would not put so much emphasise on the TWW hypothesis. If a reader is not familiar with it, it might be a bit confusing (especially in the abstract). I think it is good that you refer your results to it but I would reduce the importance it has in your manuscript, e.g the amounts of mentions.

   Also, I think the term "tightly bound water" is misleading. At least in my perception it suggests that plants use an exceptionally tightly bound water pool (as compared simply to bound water). However, in my opinion, they probably just use the water that is available to them and mobile water might infiltrate beyond the root zone too quickly to be available in the

long term. I imagine that the sampled mobile water (in lysimeters) mainly originates from percolation during precipitation events, when relative humidity is high, solar radiation is low and plants hence do not transpire a lot. I suggest to use “less mobile water” or simply “bound water”. However, I am aware that it is also termed “tightly bound water” in the TWW hypothesis.

Reply: We agree that references to the TWW hypothesis should be reduced. Moreover, the short experimental period and the focus on phenomenon that are not directly related to the TWW hypothesis hinder concise, meaningful discussion of the hypothesis and ecohydrological separation, as pointed out by Reviewer #2. 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 suggested) at the same depths, and the impact of this heterogeneity on plant water uptake in the next version.

5. You took the time to calculate the isotopic composition of tightly bound soil water (from measured bulk and mobile soil water) and you repeatedly argue that plants preferentially use this water source. However, this data is only incorporated into one supplemental figure. I think it would strengthen the story if those values were incorporated into the main figures as well (e.g. Figure 2 and 5).

Reply: We plan to add data on the less mobile water stable isotopes in the supplemental figures to the main figures as suggested. Please see Figure 1 above and Figure 3 below.

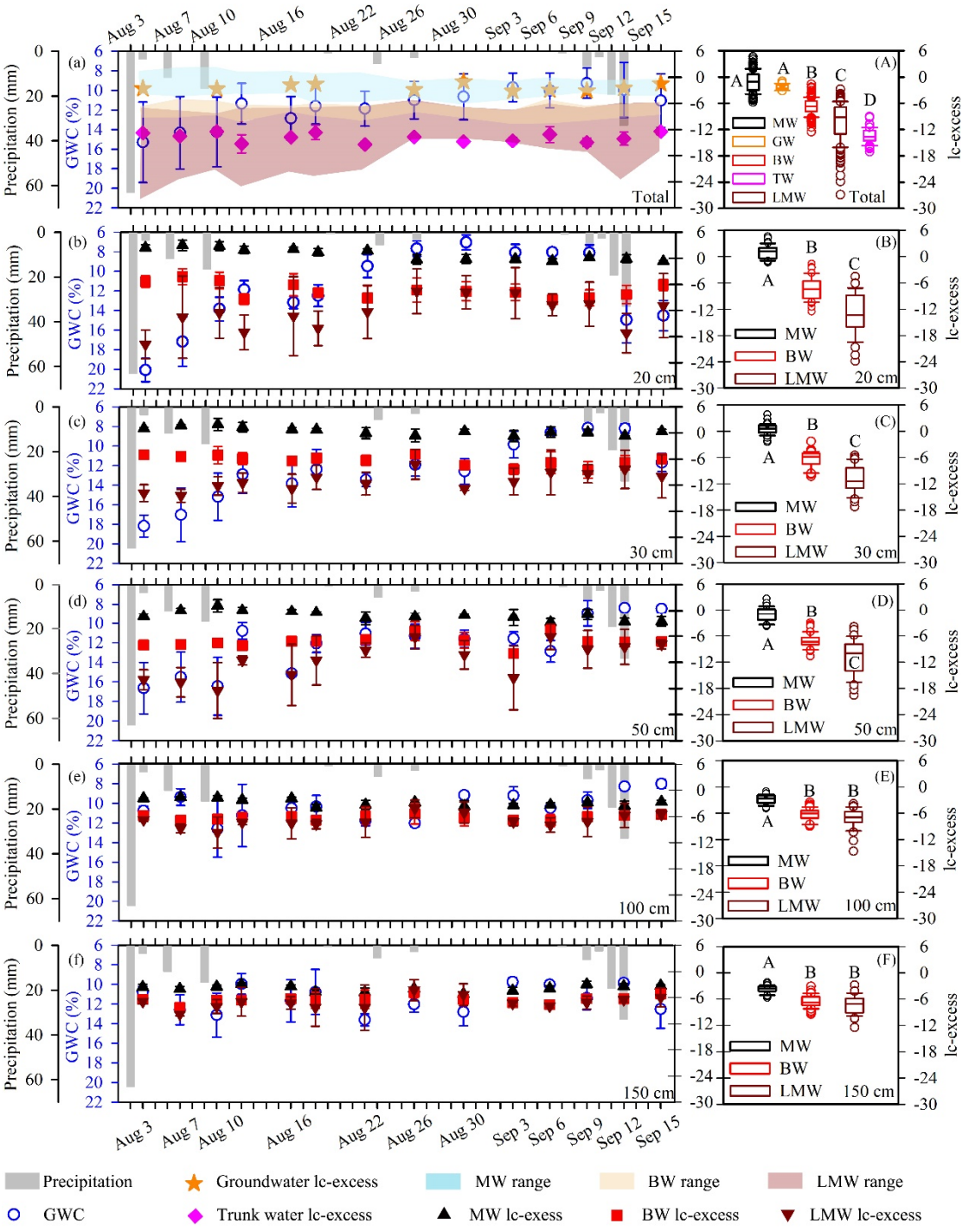

Figure 3 (a-f) Temporal dynamics of hydrological conditions (precipitation and gravimetric water content, GWC) and lc-excess values (these values are means and standard deviations for three sites) of groundwater (GW), trunk water (TW), mobile water (MW), less mobile water (LMW) and bulk soil water (BW) 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).

6. SIAR modelling: In my opinion, this does not strengthen the story. The isotopic composition of water sources incorporated are quite similar to each other. Did you check if the modelling results change substantially when running the calculations multiple times? Also, you use bulk soil water in different soil depths as available water sources. Contradictorily, these do not match with observed xylem values and you argue in the text that plants use soil water with differing isotopic composition. Additionally, measurement uncertainty in sources and plant xylem should be considered (see e.g. Kühnhammer et al. 2020). Having that said, I like that you compare rwu fractions derived with bulk soil samples and sampled roots as water sources. If you decide to keep the SIAR modelling, it would be interesting, in my opinion, to also look at tightly bound water as a potential set of sources and see how this changes the results. You could then also discuss the weaknesses of those purely statistical models and make use of your data to communicate potential issues with the usual approach (e.g. comparing bulk soil water and xylem water) to the scientific community.

Reply: We would like to delete the calculation of plant water source contributions based on SIAR modeling as suggested, but keep the conclusion that root water at 100-160 cm depths was the main water source for the sampled plants. We believe that root water can reflect the water source of

trees better than bulk soil water (which has been more extensively used), for reasons detailed in our response to comment 2.

7. As you sampled roots across soil depths, do you also have information on root length density across the profile or any other measure of root quantity across soil depths? I think this could add some interesting insights into the trees' water uptake strategies.

Reply: Unfortunately, we did not collect other root information such as root length density due to the large workload.

Technical comments:

8. As you are discussing isotopic differences of soil water pools sampled as a reason for the observed mismatch to xylem water, you should pay close attention to specifying which soil water pool you are talking about. I suggest, either you always specify this or you clarify once that when talking about soil water in general you always refer to bulk soil water.

Reply: Thanks for your suggestions. We will identify the soil water we are referring to in the revised version.

9. In the copy I received for review, the figure quality seems too low, axis labels and names seem a bit blurry. It should be verified that all labels and names are easily readable.

Reply: The resolution of the figures was reduced by conversion from Word to pdf format. We plan to address to this issue in the revised manuscript. In addition, we will replot the figures to improve their resolution.

10. Check for consistency of isotope terminology and avoid using (too many) different ones if it does not contribute to the readability of the text. You use all of the following: "isotopic

composition", "isotope composition", "isotopes in water", "isotopic signature", "isotope signals", "isotopic signals", "isotope fingerprints", "H and O isotopes", "hydrogen and oxygen isotopes", "water isotopes", "isotopic values", "isotopic patterns"

I know that sometimes it makes sense to mix it up a bit to avoid too many repetitions but I think you can eliminate at least a few of them :-D

Also I would use the term "water stable isotopes" instead of "water isotopes"

Reply: Thanks for your suggestions. We will apply more consistent isotope terminology in the next version.

11. You use xylem to refer to measurements in the trunk. However, water within roots is also transported in the xylem (which you sampled to obtain the root isotopic values). I suggest to specify the use of words here or use trunk instead of xylem

Reply: We will change xylem water to trunk water as suggested in the next version.

Specific comments:

12. Title: "insights into [an] isotopic mismatch between [bulk] soil water and *Salix matsudana* Koidz xylem water from root water isotope measurements"

Reply: As advised, we intend to revise the title in the next version, as follows:

"Insights into isotopic mismatch between bulk soil water and Salix matsudana *Koidz trunk water from root water stable isotope measurements"*

13. Figure 2: I think the axis limits should be the same in all subplots (x-axis different for panel c).

Reply: We intend to standardize the x- and y-axis scales of the plots (see Figure 1 above) in the next version as suggested.

14. Figure 4 (caption): remove repetition of lc-excess

Reply: We will also remove the repetition of lc-excess in the next version as suggested.

15. Figure 7: SD should also be displayed in the opposite direction, are the obtained distributions (of a RWU fraction at a certain depth) normally-distributed? If not, display uncertainty in a different way. Maybe it would make sense to display those distributions as boxplots

Reply: As mentioned in our reply to comment 6, we would like to delete Figure 7 due to the removal of references to the SIAR model.

16. Figure 8: I like the colour coding depicting the isotopic composition of the different compartments studied. Maybe this would come out even clearer if tree and soil background colours were a little more subtle (maybe grey scale?). Also see my general comment 1 to revaluate if you should emphasize fractionation as the main cause for the isotopic mismatch

Reply: We will replot Figure 8 in the next version, as follows:

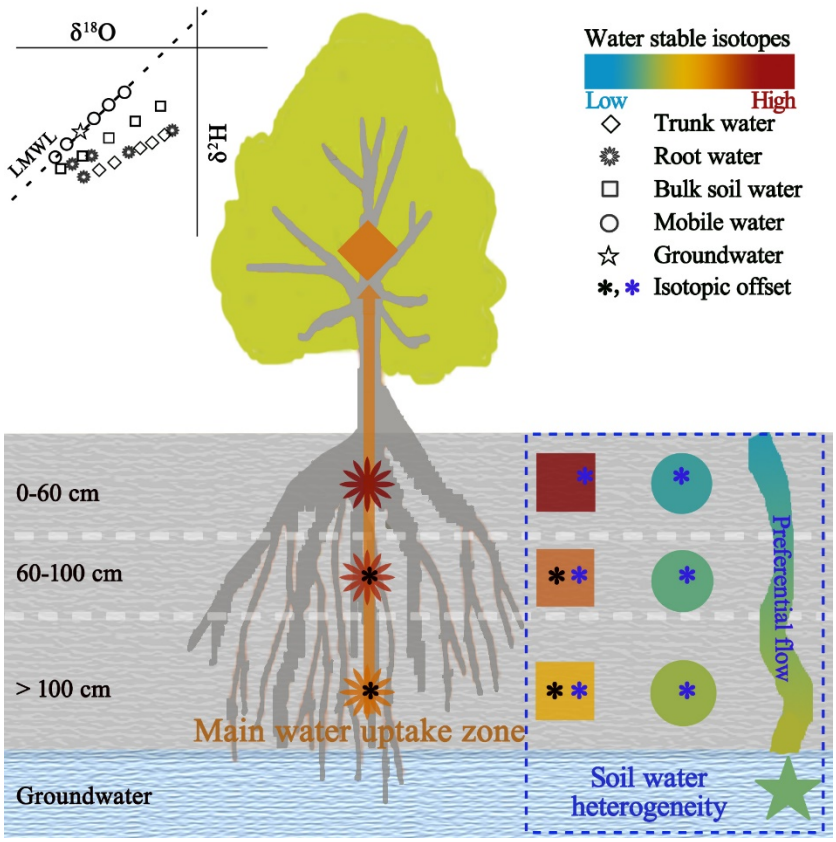

Figure 4 Schematic diagram of isotopic dynamics along the soil-root-trunk continuum. Color codes indicate isotopic composition of mobile water, bulk soil water and root water at indicated depths, groundwater and trunk water (from blue to brown representing low to high). The black asterisks indicate significant differences in the isotopic offset between root water and bulk soil water at the same depth ($p < 0.05$). The blue asterisks indicate significant differences in the isotopic offset between mobile water and bulk soil water at the same depth ($p < 0.05$).

17. Figure S2: Symbols are different sizes in different subplots

Reply: We will replot Figure S2 and keep the same size for each subplot as suggested.

18. Figure S3: What is displayed? The figure legends and part of the caption states you display bulk water (BW) and tightly bound water (TW), but the caption also mentions mobile water (MW) – I assume that is a typo. I would also suggest changing the colours in Figure S3 to match with the colours in Figure 3, i.e. bulk soil water should always have the same colour

Reply: We would like to delete Figure S3 and add the isotopic data in it to Figure 3 (see above).

19. Figure S4: the colours of the boxplots are hard to distinguish

Reply: We would like to delete Figure S4 due to changes in the text.

20. Line 12: "at high temporal resolution" it reads as if all before mentioned parameters were sampled over time. However, this is not true for root xylem (only sampled on one occasion). Also, I think the perception of high temporal resolution is quite different depending on who you ask, especially with new in situ methods evolving. Maybe specify that you sampled twice a week/every X days?

Reply: We plan to clarify this as follows:

"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."*

21. Line 12-13: "to analyze isotopic dynamics in the soil-root-xylem continuum"

I don't really see a lot of focus on (temporal) isotope dynamics in your manuscript. You do not really discuss the variations over time (and only sampled roots once), maybe apart from the influence of GWC on soil water isotopes

Reply: We would like to revise this sentence in the next version, as follows:

"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."*

22. Line 17: I personally would not mention the TWW here again. “and plant fractionation” see my major point 1)

Reply: We would like to rephrase the Abstract in the next version because of the changes in conclusions such as those regarding the TWW hypothesis (see our response to major point 4) and plant fractionation (see our response to major point 2), 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."

23. Line 20: "isotopic offset occurred at the interface between the soil and *S. matsudana* roots"

    This statement is a bit misleading I think because if different soil water pools were not isotopically well mixed, the offset does not occurred at the interface between soil and roots but because plants only access a certain soil water pool

Reply: We will amend the sentence as follows:

"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."*

24. Line 28: "in [the] global hydrological cycle" or "in hydrological cycles", "terrestrial ecosystem[s]"

Reply: We plan to amend this sentence, as follows:

"RWU also controls partitioning of infiltrated soil water between groundwater recharge and local atmospheric return through evapotranspiration (Knighton et al., 2020a; Knighton et al., 2020b), and thus plays a key role in the global hydrological cycle. In terrestrial ecosystems, plant transpiration accounts for more than 60% of total evapotranspiration and returns approximately 39% of incident precipitation to the atmosphere."

25. Line 42: exchange "in the movements" with "along the pathway"

Reply: We would like to delete this sentence due to changes in the text.

26. Line 43: "but also [due] to ecohydrological separation (Brooks et al., 2010) [and] water isotope …"

Reply: We plan to delete all content regarding the TWW hypothesis (see our response to major point 4).

27. Line 44-45: add reference to Chen et al. (2020) paper

Reply: We will add the reference in the next version as suggested.

28. Line 57: put "respectively" at the end of the sentence?

Reply: We will revise this sentence in the next version as suggested, as follows:

"Poca et al. (2019) reported that arbuscular mycorrhizal fungi can enhance isotopic fractionation during RWU, resulting in up to −24.6‰ and −2.9‰ differences in δ^2H and $\delta^{18}O$ values, between soil and plant trunk water, respectively."

29. Line 60: "incomplete extraction of water during cryogenic distillation could fractionate water isotopes" due to Rayleigh fractionation during the extraction process an incomplete extraction could not only fractionate water stable isotopes in the sample but surely does!

Reply: We will revise this sentence in the next version as suggested:

"For example, incomplete extraction of water during cryogenic distillation fractionates water stable isotopes (Gaj et al., 2017; Orlowski et al., 2018)."

30. Line 61-62: "between [cryogenically extracted] stem water and source water"

Reply: We will adjust the sentence as suggested:

"Chen et al. (2020) found the common presence of significant isotopic deviations between cryogenically extracted trunk water and source water in nine woody plant species and demonstrated that this offset stems from methodological artifacts during cryogenic vacuum extraction."

31. Line 63: “cryogenic extraction-associated methodological artifact” sounds a bit overly complicated

suggestion: “methodological artifacts during cryogenic vacuum extraction”

Reply: We will adjust the sentence as suggested:

“Chen et al. (2020) found the common presence of significant isotopic deviations between cryogenically extracted trunk water and source water in nine woody plant species and demonstrated that this offset stems from methodological artifacts during cryogenic vacuum extraction.”

32. Line 65: “specific process[es]”

Reply: We plan to amend this sentence, as follows:

“Explanation of the isotopic offset between soil and trunk water is essential, but identifying roles of specific processes is generally hindered by the diversity of mechanisms that may be involved.”

33. Line 67: “along [the] soil-root-xylem continuum

Reply: We will adjust the sentence as suggested:

“Moreover, these mechanisms tend to have strongly interactive effects and may act on any compartment along the soil-root-trunk continuum such as soil matrix or soil-root interface or plant woody tissues.”

34. Line 68: “leading to the variation in water isotopes” I don’t understand the statement of this subordinate clause.

Reply: We will rephrase this sentence and delete the subordinate clause, as follows:

"Moreover, these mechanisms tend to have strongly interactive effects and may act on any compartment along the soil-root-trunk continuum such as soil matrix or soil-root interface or plant woody tissues."

35. Line 69: "roots preferentially use tightly bound water according to the TWW hypothesis"

In my opinion this statement is misleading as it might be attributed to the long-term availability of water in the soil. Mobile water (transported in big pores) percolates quickly below the plant rooting zone and therefore is only shortly plant available (during precipitation when rh is usually high and solar radiation low). Hence, roots do not prefer the more tightly bound water, they just use the water they have access to. I know however that it is termed like this in the TWW papers and different people might interpret this statement differently.

Reply: We plan to delete all content regarding the TWW hypothesis (see our response to major point 4).

36. Line 78-79: I would delete "during water transport from root to xylem" as you did not measure isotopes during the transport but at two locations (roots and trunk xylem)

Reply: We will delete the clause in the next version as suggested.

37. Line 80-82: We hypothesize that 1) there is an isotopic deviation between xylem water of S. matsudana trees and their potential water sources, and that 2) this deviation might be due to a combination of multiple factors.

I think the second hypothesis is quite unspecific and that this is the case is already clear from your literature review. Be more precise on what you investigated and how your research contributes to disentangle combination of multiple contributing factors

Reply: We will amend the sentence as suggested:

"We hypothesize that mobile water is separate from bulk soil water isotopically in the soil matrix and trunk water of S. matsudana *trees isotopically deviates from their potential water sources due to the heterogeneity of soil water."*

38. Line 98-99: We selected three sampling sites in the check-dammed channel of the Liudaogou catchment. Designated sites 1, 2 and 3 are located 50, 80 and 100 m upstream of the dam, respectively.

Reply: We intend to amend these lines as follows:

"We selected three sampling sites in the check-dammed channel of the Liudaogou catchment, designated sites 1, 2 and 3, located 50, 80 and 100 m upstream of the dam, respectively."

39. Line 100: "was chose for sampling tree" correct grammar

Reply: We intend to amend these lines as follows:

*"*Salix matsudana *Koidz, is one of the main tree species in the check-dammed catchment, so we chose* S. matsudana *as the sampling tree".*

40. Line 101: "includes" do you mean "consists of"?

Reply: Yes, we will amend the sentence as follows:

"The soil at the site consists of sandy loam and loam according to the USDA classification system, with bulk density ranging from 1.4 to 1.6 g cm^{-3}*."*

41. Line 102: “different soil [depths]” in soil science layers refer to differences in stratification, “in a sampling plot” which sampling plot? As you introduced the numbers before you could just specify

Reply: We intend to clarify this, as follows:

“Water retention curves at 20, 30, 50, 100 and 150 cm soil depths at sampling site 1 are shown in Fig. S1.”

42. Line 104: “from the sampling plot[s]” or “sampling area”? or are you referring to a specific plot?

Reply: We intend to clarify this, as follows:

“Meteorological data on precipitation and air temperature (with 30-min resolution) were obtained from a weather station located about 500 m from sampling site 1.”

43. Line 109: We collected root samples of [one] *S. matsudana* tree at each [of the three] sampling site[s]

Reply: We intend to clarify these points as follows:

“We collected root samples from one S. matsudana *tree and soil samples at selected soil depths (0-160 cm with 20 cm intervals) at each of the three sampling sites”*

44. Line 112-113: “was collected and measured its isotopic composition” correct grammar

Reply: We will correct this sentence, as follows:

“2-3 coarse roots (> 2 mm diameter) from each sub-cuboid were randomly selected and the roots from the top few centimeters of the topsoil were not artificially removed.”

45. Line 113: “collected disturbed soil samples at 0-160 cm depths” at what interval? Table one suggests 10 cm increments

Reply: We will clarify this, as follows:

"Moreover, we collected disturbed soil samples at 10 cm intervals from 0 to 100 cm depths and 20 cm intervals from 100-160 cm depths using a soil auger to measure soil particle size at sampling site 1."

46. Line 114-116: Did you measure particle size for both disturbed and undisturbed samples? Or was this only done for disturbed samples and samples in cutting rings were used for water retention curves?

Reply: We will clarify this, as follows:

"We also collected undisturbed soil samples at 20, 30, 50, 100 and 150 cm depths using cutting rings (100 cm^3 volume) to obtain water retention curves at the same sampling point."

47. Line 119: "Our previous results have shown" This reads as it was referring to a previous (published) study. Either you are missing a reference here or you should rephrase the sentence to make clear that this refers to a previous campaign and is up to now unpublished data

Reply: We will clarify this, as follows:

"Previously unpublished data we obtained have shown that the isotopic composition of trunk water of S. matsudana *trees did not match bulk soil water in the dual-isotope space from May to September 2018."*

48. Line 120-121: I am having difficulty to understand this sentence (referring to TWW)

Reply: We will delete this sentence in the next version because it is related to the TWW hypothesis (see our response to major point 4).

49. Line 126: delete “For these analyses” otherwise it reads like precipitation samples are connected to beforementioned sample analysis

Reply: We will delete “for these analyses” in the next version as suggested.

“Precipitation samples were collected as soon as a rain event ended from a polyethylene funnel and bottle, with a plastic ball placed in the funnel to reduce evaporation.”

50. Line 124: “within the period when mobile water was available [i.e. from August 4 to September 15 2019].

Reply: We will amend the sentence accordingly, as follows:

“So, high frequency sampling (ca. 3-day temporal resolution) was applied to analyze the causes and locations of isotopic deviation during the period when mobile water was available (i.e. from August 4 to September 15 2019).”

51. Line 128: do you know the depth of the groundwater table at the study site? At which depth did you sample groundwater?

Reply: We will add details about the groundwater table in the next version as suggested:

“At our study site, the mean groundwater table depth was 3.6 m and groundwater samples were collected at ca. 30 cm depth from its surface.”

52. Line 129-131: How were soil samples (for isotopic analysis) stored? How was evaporation from samples prevented?

Reply: We intend to clarify these points as follows:

“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 ℃.”

53. Line 138: “placed in 10 mL vials and wrapped in parafilm” I guess you sealed the vials with caps and then secured the caps with parafilm? Parafilm is not 100% gas tight but permeable to water vapor.

Reply: We intend to clarify this, as follows:

“Pieces of the de-barked and de-leaved twigs, 30 mm long, were then immediately placed in 10 mL vials, the vials were sealed with caps then the caps were secured with Parafilm. These samples were also kept in a cool box until storage in the lab at 4°C.”

54. Line 141-142: “Similarly, of 30 mm long pieces of the de-barked twigs were immediately placed in 10 mL vials and wrapped in parafilm.” Is this a repetition or did you want to specify the sampling in different tree heights? Maybe streamline to avoid redundancies

Reply: This sentence describes the collection of trunk water at different sampling heights. We will revise these sentences to make them clearer, as suggested.

“Tree samples were collected simultaneously with the soil samples. These consisted of twigs collected from the south-facing side of three S. matsudana *trees at 250 cm height on each sampling occasion. In addition, samples of trunk at selected tree heights (150, 250, 350, 450 cm) were collected on August 18, 2019. Bark and phloem were peeled from fully suberized branches to avoid perturbance of trunk water isotopic composition by fractionation. Pieces of the de-barked and de-leaved twigs, 30 mm long, were then immediately placed in 10 mL vials, the vials were sealed with caps then the caps were secured with Parafilm. These samples were also kept in a cool box until storage in the lab at 4°C.”*

55. Line 163: b does not appear in your lc-excess formula (is essentially the lc-excess)

Reply: We thank the reviewer for alerting us to this error and we will revise the lc-excess formula in the next version, as follows:

"$lc - excess = \delta^2 H_s - a\delta^{18} O_s - b$ "

56. Line 166-167: In my opinion you do not need the link to the TWW here

Reply: We will delete this sentence because of the removal of references to TWW hypothesis (see our response to major point 4).

57. Line 174: "To compare the isotopic composition of root and soil water at the same depth"

Actually you only indirectly compare root and bulk soil water with this. If you aim at comparing those two, I think there is better, different approaches. I would rather say you compare their contribution to xylem water. See also my major comment 5) here.

Reply: We will delete this sentence because of the removal of references to SIAR model (see our response to major point 6).

58. Line 177: "Based on results of the soil water and root water isotope analysis"

I do not get what the decision criteria was here. Please specify.

Reply: We will delete this sentence because of the removal of references to SIAR model (see our response to major point 6).

59. Line 178: "soil [and root] water sources were divided into …"

Then you can delete the next sentence

Reply: We will delete this sentence because of the removal of references to SIAR model (see our response to major point 6).

60. Line 181: "because plant water use does not generally cause fractionation of hydrogen and oxygen isotopes"

This contradicts with the statement in your paper that fractionation during rwu influences the xylem isotope values. I would rephrase and write: "assuming no fractionation during plant water uptake"

Reply: We will delete this sentence because of the removal of references to SIAR model (see our response to major point 6).

61. Line 202: "overlapped with bulk soil water (Fig 2d)" Actually one needs to look at Figure 2a and d to see the overlap

Reply: We intend to clarify this in the next version, as suggested.

62. Line 208: unit ‰ is missing

Reply: The information expressed in this sentence is limited, thus we would like to delete it, but we plan to 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 (‰)	$\delta^{18}O$ 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)

63. Line 211: I would not mention TWW here, as it is part of the discussion

Reply: We will delete this sentence because of the removal of references to TWW hypothesis (see our response to major point 4).

64. Line 212-213: “suggesting that xylem water was isolated from all potential water sources.” Actually, it only means that xylem water did not reflect bulk soil water sources and not that it is independent from all potential water sources. Also contradicts with the next sentence and is strictly speaking already discussing the results.

Reply: We would like to delete this sentence, but add a brief discussion about this issue in Section 4.1.2 of the Discussion “Isotopic offset between bulk soil water and root water”, as follows:

“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.”

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

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.

65. Line 213-214: streamline sentence to avoid repetition of tightly bound water, also includes discussion of results already

Reply: We will delete this sentence because it is more suitable for the *Discussion* than the *Results*.

66. Line 232: "[horizontal] distance"

Reply: We will amend the sentence as suggested:

"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."

67. Line 233: exchange "> 80 cm" with "within 80 cm"

Reply: We will amend the sentence as suggested:

"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, suggesting that isotopic composition of the soil was horizontally homogenous within 80 cm from tap roots."

68. Line 239 & 241: δ^2O should be $\delta^{18}O$ I assume, also stay consistent with the subscripts, here the subscript soil refers to bulk soil water but subscript BW also exists

Reply: We thank the reviewer for alerting us to this error and will revise this sentence in the next version as follows:

"Similarly, a strong correlation was observed between $\Delta^{18}O$ ($\Delta^{18}O = \delta^{18}O_{soil} - \delta^{18}O_{trunk}$) and Δ^2H ($\Delta^2H = \delta^2H_{soil} - \delta^2H_{trunk}$) soil-trunk offsets during August 4 to September 15."

69. Line 243: "These results show that water isotopes, especially hydrogen isotopes, changed between…"

Water stable isotopes do not change, the ratios of the isotopes change

Reply: We will delete this sentence because of the changes in content.

70. Line 244: “supporting our first hypothesis” belongs into discussion

Reply: We will delete this sentence in the next version because it is related to the TWW hypothesis (see our response to major point 4).

71. Line 246: Specify heading of subsection, contribution of what?

Reply: We would like to delete Section 3.4 of the Results, “Contributions”, because of the removal of references to the SIAR model (see our response to major point 6).

72. Line 248: “Potential sources of plant xylem water were determined using…”

you do not determine potential sources but the contribution of these sources to rwu

Reply: We will delete this sentence because of the removal of references to SIAR model (see our response to major point 6).

73. Line 256: “Separation of mobile water and bulk soil water in the soil matrix”

Your manuscript deals with this separation a lot and it is also a big part of your study design. Why is this not one of your hypothesis/aims?

Reply: Thanks for your suggestion. We will change it to one of our hypotheses in the next version, as follows:

“We hypothesize that mobile water is isotopically separate from bulk soil water in the soil matrix and isotopic deviation occurs between trunk water of S. matsudana *trees and their potential water sources due to heterogeneity of the soil water.”*

74. Line 257: exchange “covered” with “experimental”

Reply: We will revise this sentence in the next version as suggested:

“At our study site during the experimental period (August 4 to September 15, 2019), a clear isotopic separation between mobile and bulk soil water was observed.”

75. Line 261: “clear [isotopic] separation”

Reply: We will add “isotopic”, as suggested:

“The lc-excess values of mobile and bulk soil water consistently differed significantly, although GWC varied greatly, suggesting a clear isotopic separation between mobile and bulk soil water that is not affected by GWC.”

76. Line 266-268: Reference for statement missing

Reply: We will add references in the next version, 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.

77. Line 268-269: “Due to the seasonal variation in precipitation, winter and summer precipitation have different isotope signals”

Both half sentences state the same thing. Seasonal variation is not the cause for isotopic differences between winter and summer precipitation

Reply: Following the suggestion we will amend this sentence, as follows:

“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).”

78. Line 272: place “at our study site” at the beginning of the sentence

Reply: We will amend the sentence as suggested:

“At our study site, precipitation in winter (December-February) and summer (June-September) accounted for 2% and 77% of total average annual precipitation (464 mm) from 2003 to 2019, respectively.”

79. Line 274: “caused by other factors, and not necessarily by seasonal variation in precipitation”

Observed differences could also stem from isotopic differences of individual precipitation events. Did you check if with high and low intensities are systematically different? If so, high intensity precipitation events might percolate faster into the soil and also contribute bigger ´water quantities to sampled mobile water.

Reply: Thanks for your suggestions. In the next version we would like to change our discussion of this issue based on the antecedent precipitation events, as follows:

"Notably, there was a major rainstorm the day before the sampling (August 3), with 63 mm precipitation. The mean GWC in 0-50 and 100-150 cm layers reached 17.4 ± 2.7% and 10.8 ± 1.5% between August 4 and August 7, respectively. These results imply that precipitation greatly supplemented water in the upper soil layer. So mobile water collected by suction lysimeters during this period contained a considerable proportion of water from the rain event on August 3. In contrast, bulk soil water contained not only mobile water from this rain event, but also antecedent less mobile water that could not be extracted by a suction lysimeter, resulting in the isotopic separation between mobile water and bulk soil water."

80. Line 277: "with [increasing] soil depth"

Reply: We will add "increasing" as suggested:

"The effect of soil evaporation on bulk soil water gradually weakens with increasing soil depth."

81. Line 281-282: "Although the mixing of mobile and tightly bound water conflicts with the original hypothesis of Brooks et al. (2010)"

Does it really disagree? Or is it more a question of the degree of mixing or rather that mixing between soil water pools is not complete as previously assumed?

Reply: We will delete this sentence because of the removal of references to TWW hypothesis (see our response to major point 4).

82. Line 282-285: Increased mixing with soil depth was e.g. also observed/mentioned by Sprenger et al. (2016) and Kübert et al. (2020)

https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/2015RG000515

https://www.frontiersin.org/articles/10.3389/fpls.2020.00387/full

Reply: Thanks for this suggestion. We will add these references in the next version, as follows:

"Both mobile water and less mobile water in deep layers are more fully mixed than in shallow layers (Sprenger et al., 2016; Kubert et al., 2020)."

83. Line 290-291: "driven by spatiotemporal dynamics of soil water profiles associated with soil evaporation"

What do you mean with that? The temporal (and spatial) differences in infiltration and evaporation and how those influence the sampled soil water sources?

Reply: We will delete this sentence because of the removal of references to TWW hypothesis (see our response to major point 4).

84. Line 296-297: "These results showed that the isotopic offset between plant root water and soil water occurred at the root-soil interface."

I think this statement is misleading. I think this statement is misleading. In my opinion it points towards the explanation that bulk soil water is not representative of available plant water sources (see next paragraph). This heterogeneity does however not only apply to the root-soil interface. Or am I missing something here?

Reply: As mentioned in our response to comment 2, we would like to revise this sentence in the next version as follows:

"These findings are not consistent with the greater ^{2}H 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."

85. Line 302: “that is strongly affected by evaporative effects [in the presented study], isotopically separated from mobile water and groundwater and shows similar enriched isotopic signals [than xylem water]”

Reply: We will amend the sentence as suggested:

“Plants may use large amounts of less mobile water that is strongly affected by evaporative effects in the presented study, isotopically separated from mobile water and groundwater and shows similar enriched isotopic composition than trunk water.”

86. Line 303-304: “This hypothesis is corroborated by the overlap in isotopic composition between root and bulk soil water at 0-60 cm depths (Fig. 2 and 5).”

Why? As I understand it, this speaks against the statement in the sentence before.

Reply: We will delete this sentence in the next version because it is related to the TWW hypothesis (see our response to major point 4).

87. Line 304-309: “We considered whether bulk soil water isotopes can represent isotopic values of tightly bound water used by plants. Generally, the water designated ‘bulk soil water’ includes mobile and tightly bound water due to limitations of water extraction technology when assessing the TWW hypothesis. Thus, the proportion of mobile water in the bulk soil water increases as soil moisture increases, resulting in isotopic deviation between root water and bulk soil water.”

I do not understand the argument here. Maybe rephrasing helps :-D

Reply: We will delete this sentence in the next version because it is related to the TWW hypothesis (see our response to major point 4).

88. Line 331-332: “Under the assumption that plant fractionation does not occur”

Do you mean that the isotopic composition of water does not change during within plant transport? Fractionation is mainly believed to occur during rwu I think. However, as you sampled the roots, you eliminate this influencing factor.

Reply: We will delete this sentence in the next version because of the changes in content.

89. Line 341: "also has residence times in branches and roots"

What do you mean with this? How is it different from the time lag due to transport from roots to branches? Do you refer to an influence of xylem water storage?

Reply: We intend to clarify this, 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).*"

Meinzer, F.C., Woodruff, D.R., Marias, D.E., Smith, D.D., Mcculloh, K.A., Howard, A.R., and Magedman, A.L.: Mapping 'hydroscapes' along the iso- to anisohydric continuum of stomatal regulation of plant water status, Ecol. Lett., 19, 1343-1352, 2016.

90. Line 346: "that isotope enrichment may have been present in the unsampled branches"

Why would you measure a potential enrichment caused by unsampled branches by sampling at different heights? What do you mean with enrichment present in unsampled branches?

Reply: We will revise this sentence in the next version as follows:

"Moreover, to test the possibility that isotopic composition of trunk water may be heterogeneous at different tree heights, we collected trunk water at 150-450 cm tree heights on August 18, 2019, and found no significant differences ($p > 0.05$) (Fig. S3)."

91. Line 348: "xylem water was [isotopically] more enriched than…"

Reply: We will amend the sentence 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.

92. Line 352-353: "However, we found that the xylem water contained [more of the depleted] isotopic signal of deep roots than [of the] enriched signal from shallow roots. The results show that there was no isotopic fractionation during water transport from root to xylem"

I am not sure if this allows the conclusion that no fractionation during transport took place. However, I agree that it strongly suggests it. I would also specify here that you did not observe an enrichment (fractionation could go both ways) during transport as other authors suggested.

Reply: We plan to amend the sentence 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). However, we did not find that trunk water of the trees we sampled had higher $\delta^{18}O$ values than root water, Thus, we believe it reflects the selective utilization of water source rather than isotopic fractionation within woody tissues."

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.

93. Line 358: exchange "covered" with "experimental" or write "period covered"

Reply: We will revise this sentence in the next version as suggested:

"At our study site during the experimental period, the isotopic offset existed between trunk water of S. matsudana *trees and bulk soil water."*

94. Line 359-360: "isotopic offset [exists] between xylem water and [bulk soil water]"

As you elaborate in your manuscript, bulk soil water might not reflect all available soil water sources.

Reply: We would like to rephrase the Conclusion in the next version because of the changes of in conclusions such as those regarding the TWW hypothesis (see our response to the major point 4) and plant fractionation (see our response to major point 2), as follows:

"At our study site during the experimental period, there was an isotopic offset between trunk water of S. matsudana *trees and bulk soil water. We explored causes of the mismatch and sources of water taken up by the trees by analyzing the stable isotope composition of soil water with various mobility, root water and trunk water. In the soil matrix, bulk soil water generally had lower lc-excess values than mobile water, due to effects of soil evaporation and mixture of newly infiltrated mobile and less mobile water with increasing depth. Root water did not match bulk soil water at the same depth completely, due to the complexity of root systems and soil water heterogeneity. The maximum differences in δ^2H and $\delta^{18}O$ between bulk soil water and root water were −8.6 and −1.8‰, respectively. Overall, the δ^2H and $\delta^{18}O$ values derived for less mobile water overlapped with those of root water and trunk water, and the trunk water values mainly overlapped with those of root water at 100-160 cm depths. These findings suggest that the isotopic offset between bulk soil water and trunk water was due to isotopic mismatch between root water and bulk soil water associated with heterogeneity of the soil water. 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."*

95. Line 361: "and water flow paths" what do you mean with that? Infiltration along preferential flow paths?

Reply: We intend to clarify this, as follows:

"In the soil matrix, bulk soil water generally had lower lc-excess values than mobile water, due to effects of soil evaporation and mixture of newly infiltrated mobile and less mobile water with increasing depth."

96. Line 369: "the [estimated] contribution of roots in these depths to xylem water [was] 74%."

Reply: We will delete this sentence because of the removal of references to SIAR model (see our response to major point 6).