Reply to the comments from the Editor and Reviewers on the manuscript (NO. hess-2020-680) "Insights into isotopic mismatch between soil water and *Salix matsudana* Koidz xylem water from root water isotope measurements".

Dear Editor and Reviewers,

We greatly appreciate the efforts that you and both Reviewers have made to provide further critical feedback regarding our manuscript. We strongly believe that your suggestions have helped us to improve our manuscript. We have carefully considered them and tried to revise the manuscript accordingly, then provided detailed point-by-point replies to your and two reviewers' comments. These comments are presented in blue. Passages changed in specific responses to the comments are presented (together with page and line number) in quotation marks and italic font. All the changes in the revised manuscript have been marked by change-tracking.

Point-by-point by responses to Editor's comments

1. Please consider rephrasing the end of your introduction, because the paper could still benefit from a more specific primary hypothesis.

Reply: Thanks for your suggestion. We have rephrased the hypothesis to make it clearer, as follows (Page 3 lines 73-75):

"We hypothesize that soil water with various mobility is isotopically separated in the soil matrix, which brings about heterogeneity of the soil water, resulting in an isotopic deviation between the measured trunk water and potential water sources of S. matsudana *trees during water uptake."*

2. L147: were taken

Reply: We have revised this sentence in the revised manuscript, as follows (Page 4 lines 117-120):

"These samples were taken to the laboratory to determine their particle size using a MS 2000 Laser Particle Size Analyzer (Malvern Instruments, Malvern, UK), and to obtain their water retention curves using a CR21G high-speed centrifuge (Hitachi, Japan)."

3. L293: "homogeneous" is maybe not the correct expression. I suggest changing it to "bulk soil water had little horizontal variation within 80 cm…"

Reply: We have revised this sentence in the revised manuscript, as suggested (Page 8 lines 226-227):

"Bulk soil water had little horizontal variation within 80 cm from tap roots."

4. Fig 2: I would suggest to add the schematic plot in the new Fig. 2 to the original Figure 1. There, the schematic graph can replace the map with the Loess Plateau, which is not necessary to show for your study.

Reply: Thanks for your suggestion. We have added the schematic plot in the new Figure 2 to the original Figure 1 as follows:

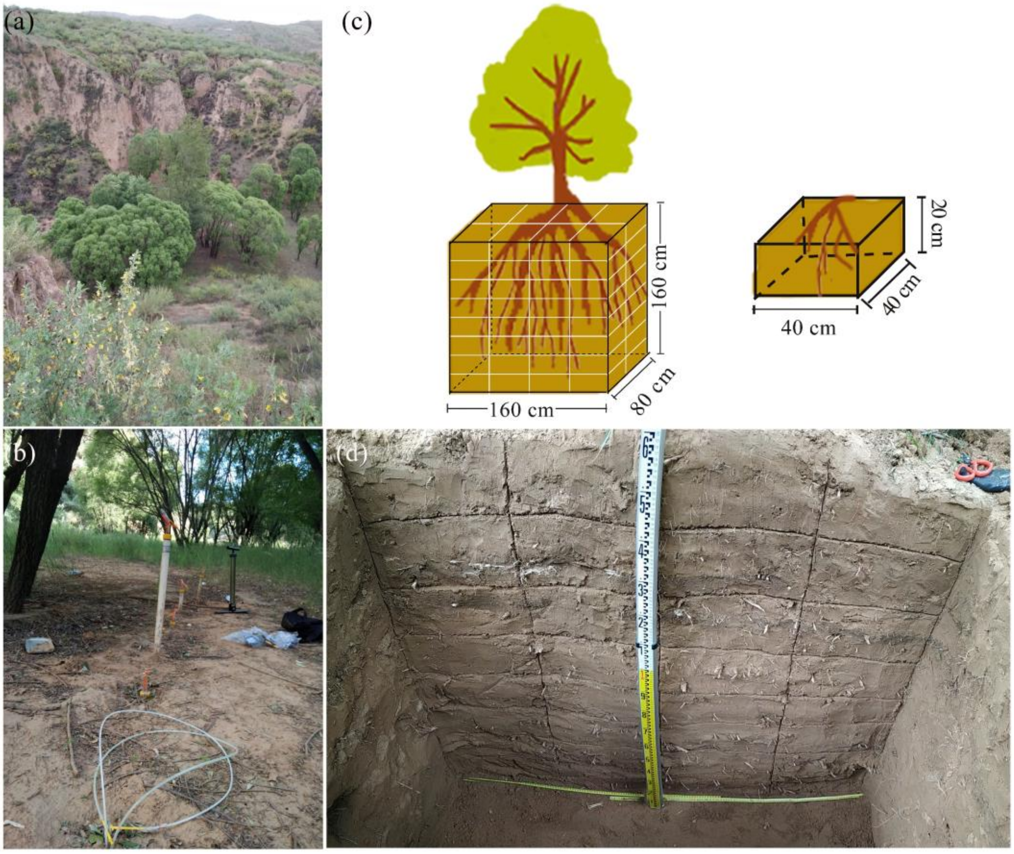

Figure 1: (a) Photograph of *Salix matsudana* Koidz, our sampling tree, (b) mobile water collection using suction lysimeters (white plastic tubes) (with application of 60 kPa tension), (c) schematic diagram of root excavations and measurements as described in Section 2.2, and (d) profile of the soil cuboid (length, width and depth: 160, 80 and 160 cm, respectively) being dug to obtain root isotopic data, and the soil cuboid was divided into 64 sub-cuboids and root isotope in each sub-cuboid (length, 40 cm; width, 40 cm; height, 20 cm) were collected separately.

5. Figure 4, 5, Table 2: The unit is missing for lc-excess (it's permill).

Reply: We thank you for alerting us to this error and we have added the unit to Figures 3-4 (the original Figures 4-5) and Table 2, as suggested.

6. Fig 8: Please add in the caption that the insert graph is a conceptual dual isotope plot.

Reply: We have added a new sentence to the caption of Figure 7 (the original Figure 8) as suggested:

"The upper left insert is a conceptual dual isotope plot."

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

1. This revised version has addressed the issues that were apparent in the original manuscript. The paper could still benefit from a more specific primary hypothesis. However, I do not see the need for further revisions.

Reply: We further thank the reviewer for the positive feedback and many well-founded points that have certainly helped us improve the manuscript in the process of revision.

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

1. The current study initially limited isotopic mismatches between plant xylem water and its potential water sources to two phases, either root water uptake or water transmission from root to stem. In order to identify which phase was the cause, the authors extensively collected samples of bulk soil water, mobile water, groundwater, plant trunk water and root water in different depths, in a field condition. The main results included: mobile water was different from bulk soil water; root water was also different from bulk soil water; root water at 100-160 cm depths were similar to plant trunk water. Then, the authors concluded that the

isotopic offset between bulk soil water and plant trunk water “reflected” an isotopic mismatch between root water and bulk soil water.

The conclusion of this study sounds reasonable and supported by the related results. However, I find two weak points that associated with this study.

Reply: We thank the reviewer for the constructive comments and suggestions. We have carefully considered them and explained the weaknesses and ambiguities mentioned below one by one.

2. 1\ In Fig. 3 (the most important figure throughout the study), isotope values of different types of water (such as bulk soil water, less mobile water, root water and plant water) were plotted separately in different dual-plots, which prohibited the main result generation. Moreover, there was lack of a clear figure that shows the offset between bulk soil water and plant water, and the match between root water and plant water. Furthermore, most of the other figures (such as Figs.4, 5, 6, 7) are not that important to the main results.

Reply: As shown in Figure 2 (the original Figure 3) below, Figure 2a is a general graph, which shows water stable isotopes for all water samples including bulk soil water, mobile water, root water, trunk water, less mobile water, and groundwater, allowing us to compare their whole distributions on the dual isotope plot. Since these water samples came from different soil depths (i.e. 20, 30, 50, 100, 150 cm) or tree heights (150, 250, 350, 150 cm), a single graph cannot be able to effectively express such a wealth of information. To make it clearer, we have plotted Figure 2b-f to portray the differences in the isotopic composition of each water source at indicated depths or heights.

We agree that the Figure 2 (original Figure 3) is the most important figure throughout the study, but other figures help us to better understand relationships between the isotopic composition of soil water of various mobility, root water and trunk water, supporting the test of our working hypothesis. For example, Figure 3 (original Figure 4) shows the isotopic offsets between soil water with different mobility (i.e. bulk soil water, mobile water, less mobile water) through the lc-excess values at indicated depths (20, 30, 50, 100 and 150 cm) in the soil matrix, indicating the heterogeneity of soil water. We further examined the relationship between lc-excess values of soil water with different mobility and gravimetric water content (GWC) to clarify the influence of GWC on the isotopic offsets of bulk soil water and mobile water through Figure 4 (the original Figure 5). Figure 5 (the original Figure 6) depicts the isotopic composition of root water and bulk soil water at indicated depths and horizontal distances, which can determine whether isotopic deviation occurs during the root water uptake at the root-soil interface. Furthermore, we tested whether there is consistency in hydrogen and oxygen isotopic offsets between bulk soil water and root water, and between bulk soil water and trunk water after isotopic offsets occurring in these water sources through Figure 6 (the original Figure 7). Finally, we summarized isotopic variations along the soil-root-trunk continuum through the schematic diagram of Figure 7 (the original Figure 8).

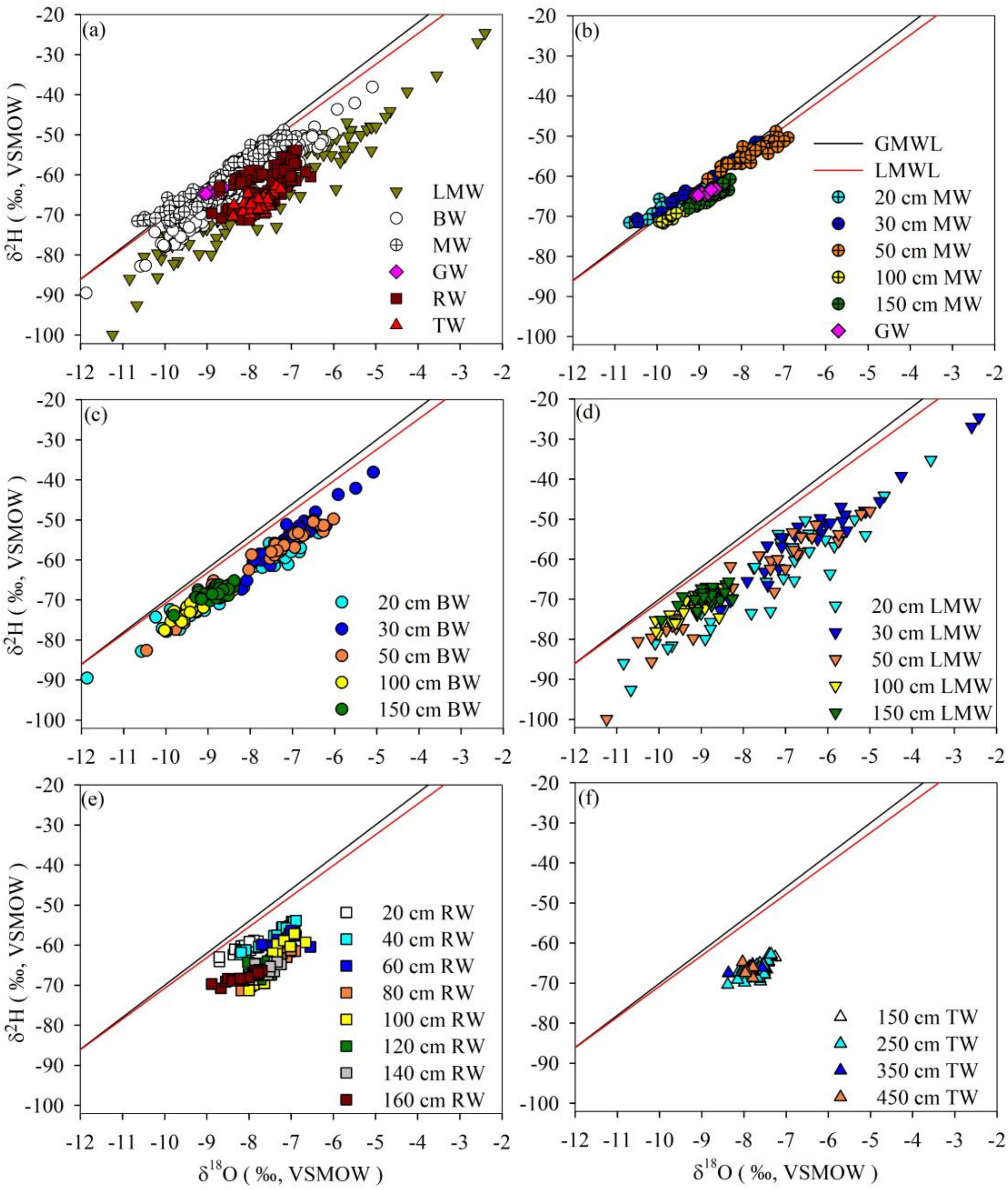

Figure 2 (a) $\delta^{18}O$ and $\delta^{2}H$ isotopic composition collected from August 4 to September 15, 2019. Plotted values include bulk soil water (BW), mobile water (MW), root water (RW), trunk water (TW), less mobile water (LMW) and groundwater (GW). (b) $\delta^{18}O$ and $\delta^{2}H$ isotopic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

= 5.91 + 7.67 $\delta^{18}O$, R^2 = 0.96). The black line represents the global meteoric water line (GMWL, $\delta^2H = 10 + 8\ \delta^{18}O$).

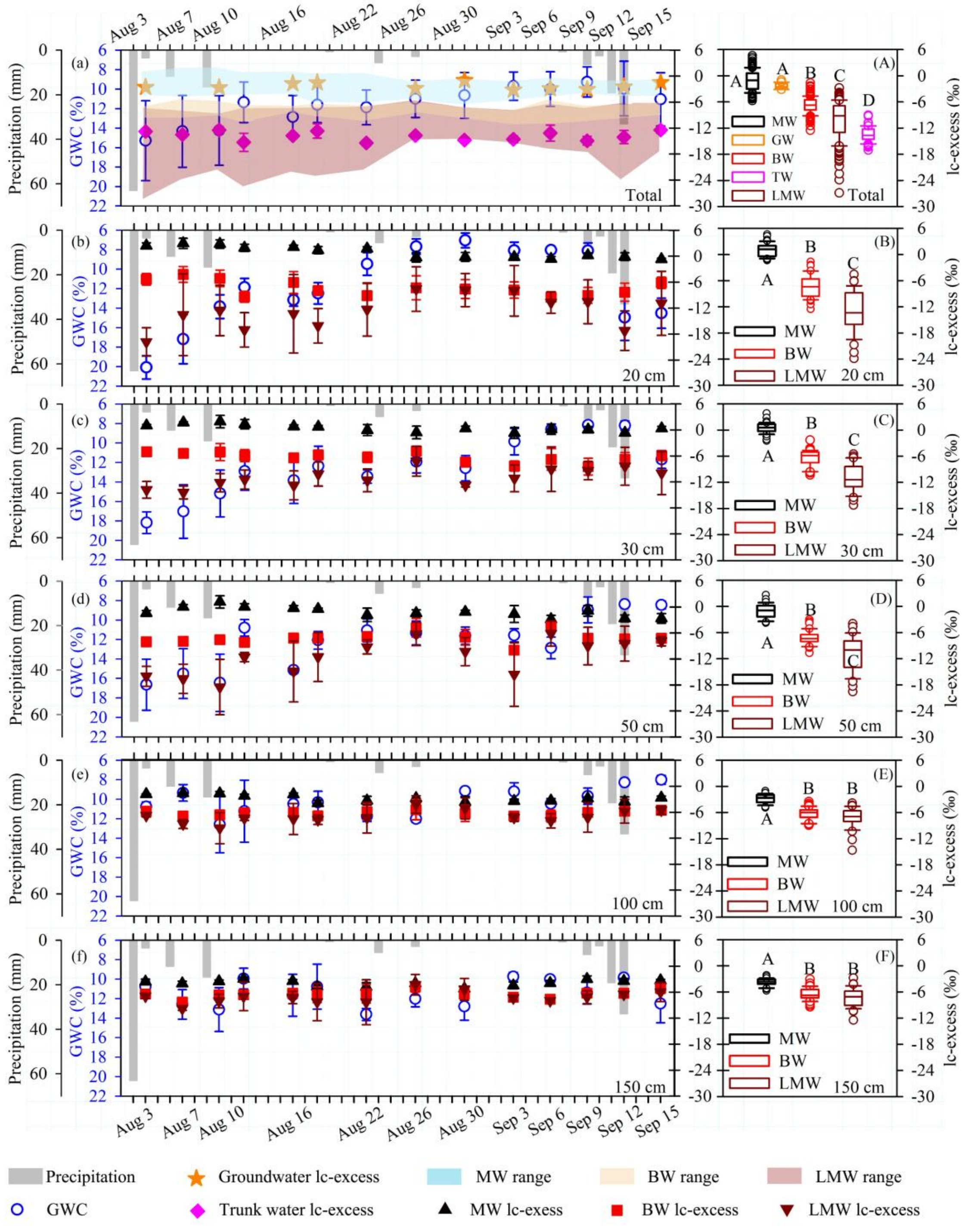

Figure 3 (a-f) Temporal dynamics of hydrological conditions (precipitation and gravimetric water content, GWC) and lc-excess values (these values are means and standard deviations for three sites) of groundwater (GW), trunk water (TW), mobile water (MW), bulk soil water (BW) and less mobile water (LMW)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

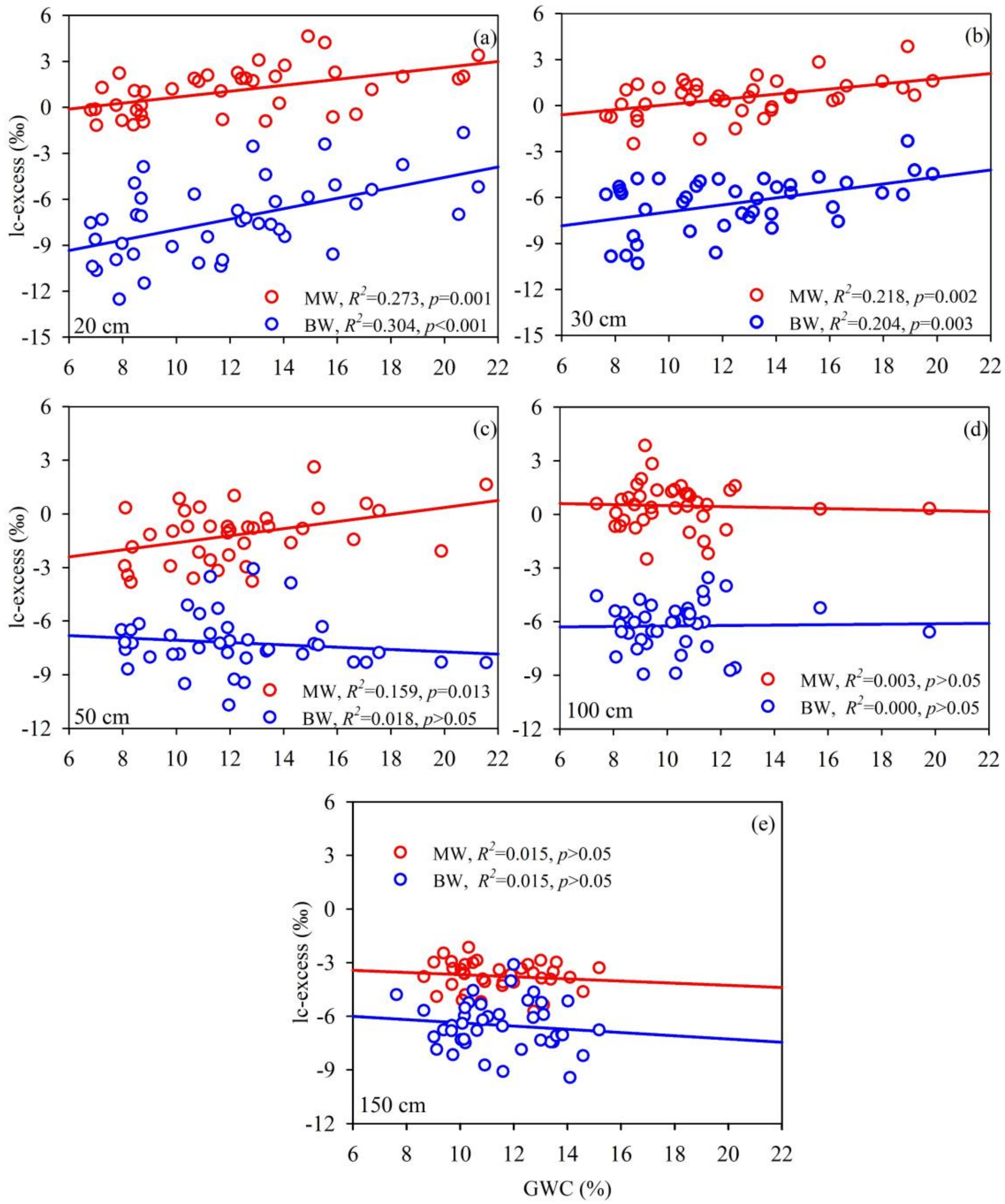

Figure 4 Relationships between gravimetric water content (GWC) and (a) lc-excess values at 20 cm depth, (b) lc-excess values at 30 cm depth, (c) lc-excess values at 50 cm depth, (d) lc-excess values at 100 cm depth and (e) lc-excess values at 150 cm depth. Data from lc-excess values of mobile water (MW) and bulk soil water (BW) are shown in red and blue circles, respectively. The insets show the fitness of the linear regressions.

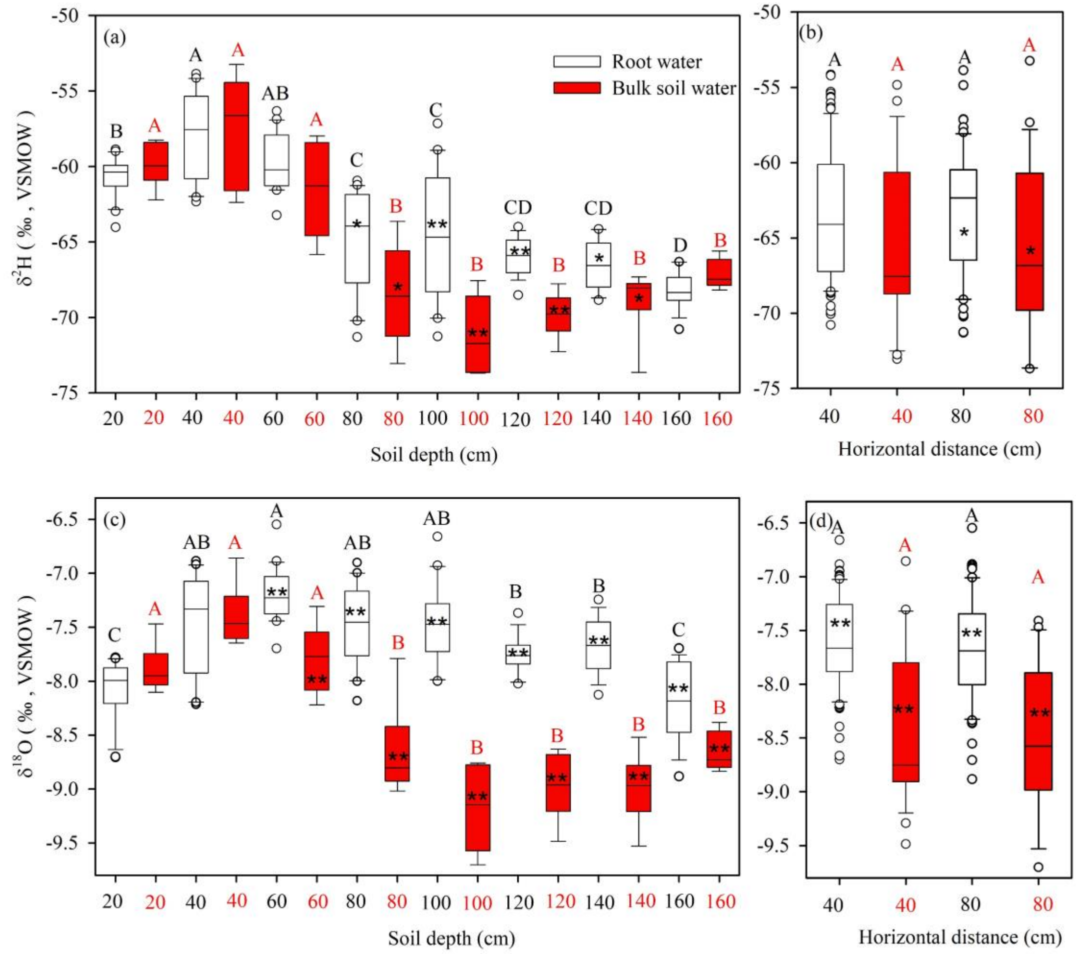

Figure 5 Boxplots of root water and bulk soil water stable isotopes (δ^2H and $\delta^{18}O$) at indicated depths (a, c) and horizontal distances from the tap root of the focal root system (b, d). The top and bottom of each box are the 25th and 75th percentiles of the samples, respectively. The black line in each box is the sample median. Asterisks indicate significantly differing isotopic composition between soil water and root water (* and **: $p < 0.05$ and $p < 0.01$, respectively, according to two-tailed tests). Plant root water stable isotopes or bulk soil water stable isotopes at different depths that do not share a letter are significantly different ($p < 0.05$, Tukey-Kramer HSD).

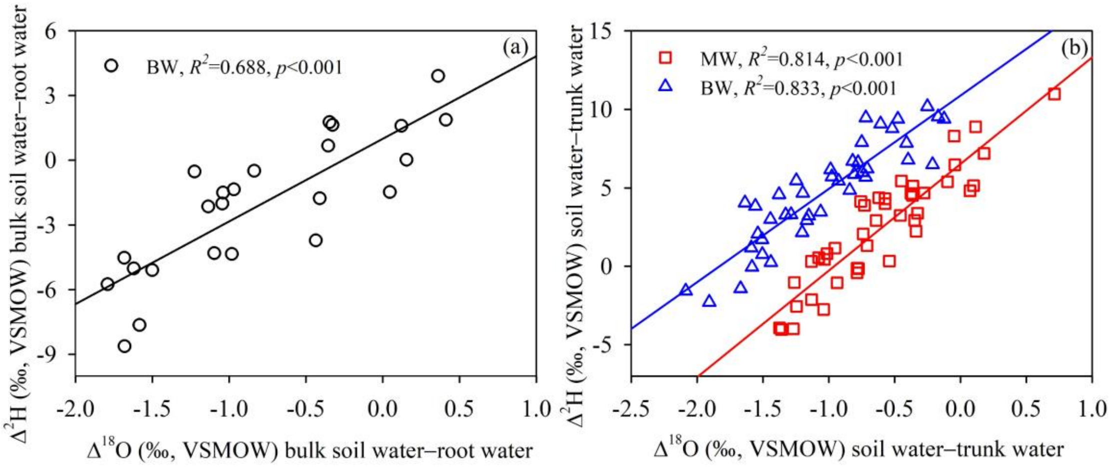

Figure 6 (a) Relationship of hydrogen isotope offset (Δ^2H, $\Delta^2H = \delta^2H_{soil} - \delta^2H_{root}$) and oxygen isotope offset ($\Delta^{18}O$, $\Delta^{18}O = \delta^{18}O_{soil} - \delta^{18}O_{root}$) between bulk soil water and root water, according to analyses of samples of bulk soil water (BW) and root water collected from 0-160 cm depths on August 18, 2019. (b) Relationship of hydrogen isotope offset (Δ^2H, $\Delta^2H = \delta^2H_{soil} - \delta^2H_{trunk}$) and oxygen isotope offset ($\Delta^{18}O$, $\Delta^{18}O = \delta^{18}O_{soil} - \delta^{18}O_{trunk}$) between soil water and trunk water, according to analyses of samples for bulk soil water, mobile water (MW) and trunk water collected from August 4 to September 15, 2019. The insets show the fitness of the linear regressions (a-b).

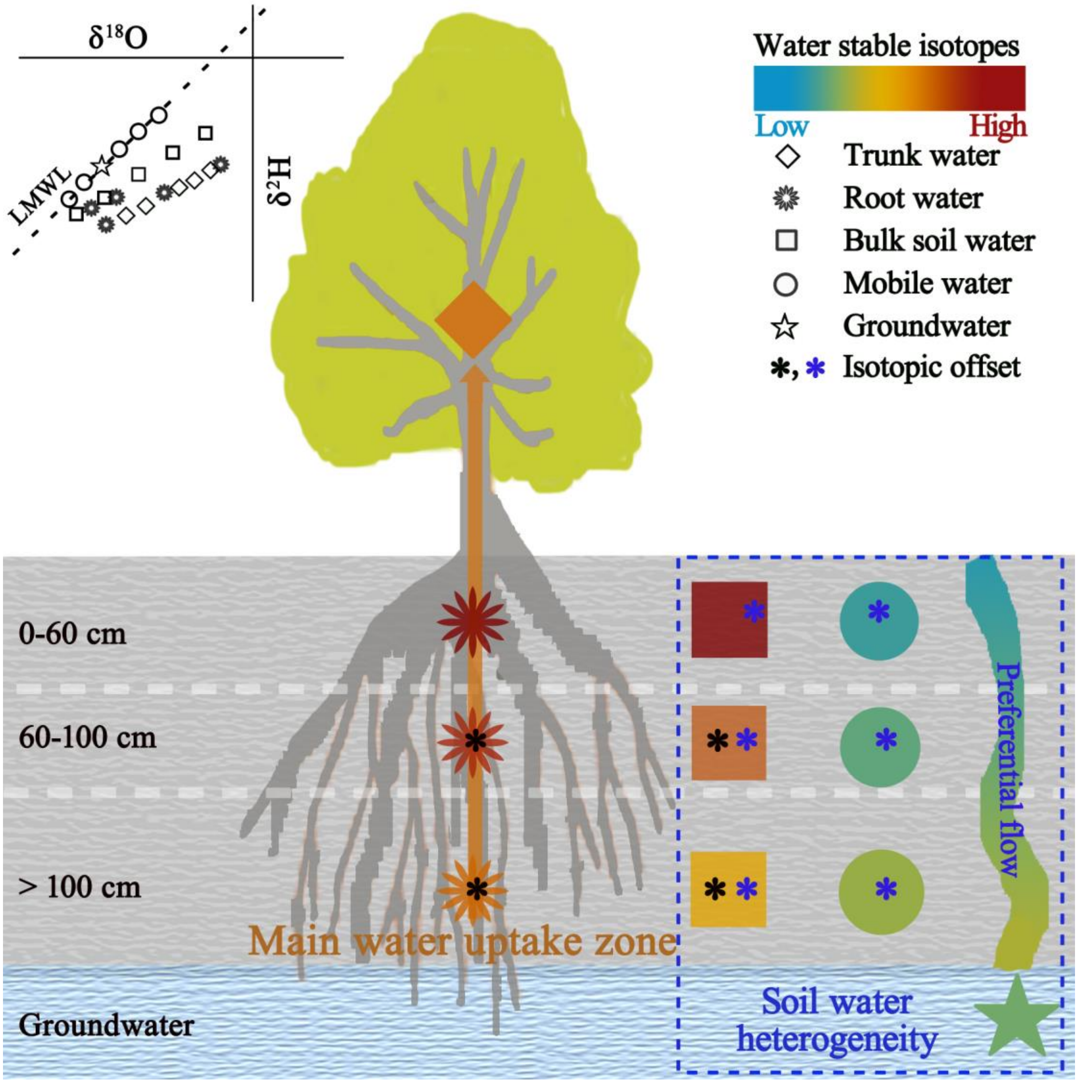

Figure 7 Schematic diagram of isotopic dynamics along the soil-root-trunk continuum. Color codes indicate isotopic composition of mobile water, bulk soil water and root water at indicated depths, groundwater, and trunk water (from blue to brown representing low to high). The upper left insert is a conceptual dual isotope plot. 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 ($p < 0.05$).

3. 2\ The title of this manuscript concentrated on the "isotopic mismatch between bulk soil water and trunk water" of a specific tree species, which fitted the content very well. On the other hand, the Introduction part reviewed all possible mechanisms but lack of concentration. Additionally, as commented above, related results (represented by these figures) were not properly organized.

Reply: We are grateful for the reviewer's suggestion. In the Introduction, we summarized various possible mechanisms that result in the isotopic deviation between plant trunk water and the potential water sources, suggesting contributions of these mechanisms to the isotopic deviations are uncertain. We emphasized that 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. Especially, plants' root systems clearly play a major role in transmission of water from soil to trunk, and thus potentially in isotopic variation. However, little attention has been paid to the isotopic composition of root water due to the assumption of non-fractionation and general inaccessibility of roots, leading to a lack of key information to explain the mismatch. Therefore, this study limited isotopic mismatch between plant trunk water and its potential water sources to three parts, that is, the heterogeneity of the soil water in the soil matrix, root water uptake at the root-soil interface and water transmission from root to trunk, to identify more specifically the sites and causes of the isotope deviations. As mentioned in our reply to comment 2, the current results have been reasonably expressed through these figures, which can be used to test our working hypothesis.