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

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Abstract. 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 δ^{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 δ^{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.

1 Introduction

Root water uptake (RWU) is the main mechanism through which plants obtain the water they require for photosynthesis, metabolism and maintenance (McCormack et al., 2015). 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 (Schlesinger and

Jasechko, 2014; Good et al., 2015). However, although the pivotal role of RWU has been long recognized, there is limited understanding and quantification of RWU because of the opaque nature of the soil and variability in time and space of the RWU process.

Analyses of water stable isotopes ($\delta^{18}O$ and $\delta^{2}H$) have been extensively applied in attempts to determine the sources of water used by plants, providing useful insights into the RWU process (Rothfuss and Javaux, 2017; Penna et al., 2020). This application relies on the assumption that RWU is generally a non-fractionating process (Ehleringer and Dawson, 1992), so the isotopic composition of trunk water effectively reflects that of water sources. Thus, by comparing $\delta^{18}O$ and $\delta^{2}H$ values of plant trunk water to those of potential contributory water sources (e.g., water from different soil layers, groundwater and precipitation), the relative contributions of these water sources to RWU can be estimated (Rothfuss and Javaux, 2017; Wang et al., 2020; Zhao et al., 2021). However, a growing body of evidence indicates that there is an isotopic offset between trunk water and potential plant water sources, that is, the isotopic composition of trunk water does not match any of the considered water sources in the dual-isotope space (Bowling et al., 2017; Vargas et al., 2017; Barbeta et al., 2019). This phenomenon has been attributed, at least in some areas, to isotopic heterogeneities across soil water pools (Oerter and Bowen, 2017; Dubbert et al., 2019). For example, the isotopic data of mobile water, bulk soil water, groundwater, stream water and derived less mobile water from Sprenger et al. (2019) suggested mobile water and less mobile water were continuously separated in a Scots pine forest over the 8-month experimental period. Based on a 9-month drought and rewetting experiment, Evaristo et al. (2019) found root water uptake is mainly derived from the less mobile water (89% ± 6), different to the more mobile water component in the soil matrix. These studies mentioned above relies on the assumption that no isotopic fractionation occurs during RWU, but some studies indicate that such fractionation probably does contribute to the isotopic offset (Vargas et al., 2017; Barbeta et al., 2019). Lin and Sternberg (1993) and Ellsworth and Williams (2007) found evidence that hydrogen isotopic fractionation occurs during RWU of halophytic and xerophytic plants. 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 $\delta^{2}H$ and $\delta^{18}O$ values, between soil and plant trunk water, respectively. In addition, effects of extraction technology between cryogenically extracted trunk water and source water must be considered (Chen et al., 2020). Incomplete extraction of water during cryogenic distillation fractionates water stable isotopes (Gaj et al., 2017; Orlowski et al., 2018). 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. Thus, the extracted water does not properly represent the water available to plants, and may contribute to apparent trunk-soil water isotopic offsets.

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 (e.g., water isotopic heterogeneities, isotopic fractionation, and water extraction technology used) (Sprenger and Allen, 2020). 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 (Sprenger et al., 2019; Poca et al., 2019; Barbeta et al., 2019). Thus, it is necessary to systematically analyze isotopic composition of each component along the pathway from soil to root and then to trunk. However,

much more attention has been paid to isotopic composition of plant trunk water and potential water sources (Chang et al., 2019; Kuhnhammer et al., 2020) than to isotopic composition of root water due to the inaccessibility of roots (Zhao et al., 2016), leading to a lack of key information to explain observed mismatches.

Therefore, the aim of the study presented here was to analyze hydrogen and oxygen isotopic composition of each component in the soil-root-trunk continuum. More specifically, we exploited the specific isotopic composition (δ^2H and $\delta^{18}O$ values) of mobile water, bulk soil water, groundwater, and derived less mobile water to test the heterogeneity of soil water. We compared the isotopic composition of root and soil water at root-soil interface at 0-160 cm depths, as well as the isotopic composition of root and trunk water of *Salix matsudana* trees, to identify more specifically the sites and causes of the isotopic deviation. 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. ~~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.~~

2 Materials and methods

2.1 Site description

The study was conducted in the Liudaogou catchment (38°47′-38°49′N, 110°21′-110°23′E) on Loess Plateau of China ~~(Fig. 1)~~. The area and altitude of the catchment are 6.89 km^2 and 1081-1274 m, respectively. The regional climate is classified as semi-arid with cool dry winters and most of precipitation occurs during the warm summer season. The mean annual precipitation and temperature in the catchment are 464 mm and 8.4 ℃, respectively. The study area received less than usual precipitation (426 mm) in the study year (2019). During this year, the seasonal distribution of precipitation was uneven, mostly concentrated in July to September (70%), and the average daily temperature ranged from −13.48 ℃ in January to 26.20 ℃ in July. The Liudaogou catchment is in the 'water-wind erosion crisscross area' of the Plateau. The soil erosion modulus for this area is reportedly 15,040 t km^{-2} a^{-1} (Gong et al., 2018). Severe soil erosion has caused strongly fragmented landforms, with gullies accounting for ca. 38% of the total area (Zhu and Shao, 2008). Both vegetation and engineering measures (check dams) are used here to mitigate soil erosion. Common species used in reforestation of the area include *Salix matsudana* Koidz, *S. psammophila*, *Caragana korshinskii* and *Medicago sativa* L. Check dams are usually built in gullies and other channels in the area to trap runoff and sediments from steep slopes and improve agricultural yields.

Fig. 1

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 ~~(Fig. 1)~~. *Salix matsudana* Koidz, is one of the main tree species in the check-dammed catchment, so we chose *S. matsudana* as the sampling tree (Fig. 1a). The average age and height of the trees are about 30 years and 12 m, respectively. The soil at the site consists of sandy loam and loam according to the USDA

classification system (Table 1), with bulk density ranging from 1.4 to 1.6 g cm^{-3}. Water retention curves at 20, 30, 50, 100 and 150 cm soil depths at sampling site 1 are shown in Fig. S1. 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. Precipitation was measured using TE525 rain gauges (Campbell Scientific Inc.), which provide ± 1 percent accuracy at rates up to 25.4 mm hr^{-1}. Air temperature was measured using HMP45D probes, which have ± 0.2 °C accuracy at 20 °C (Vaisala Inc.).

Table 1

2.2 Measurements of roots and soil properties

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, on August 18, 2019, to measure their isotopic composition. 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. ~~1d and Fig. 2a~~1c-d). We then divided the cuboid into 64 sub-cuboids (length, 40 cm; width, 40 cm; height, 20 cm) (Fig. ~~2b~~1c) 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°C. 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°C. 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. 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 site. These samples were taken~~took~~ to the laboratory ~~and~~ to determine their particle size ~~and bulk density~~ using a MS 2000 Laser Particle Size Analyzer (Malvern Instruments, Malvern, UK), and to obtain~~ed~~ their water retention curves ~~for them~~ using a CR21G high-speed centrifuge (Hitachi, Japan).

~~**Fig. 2**~~

2.3 Water sampling for stable isotope (δ^2H and $\delta^{18}O$) analysis

Previously unpublished data we obtained have shown that the isotopic composition of trunk water of *S. matsudana* trees did not match soil water in the dual-isotope space from May to September 2018 (Fig. S2). To assess the impact of soil water heterogeneity on root water uptake, we collected mobile and bulk soil water in 2019. Due to effects of drought, mobile water samples could not be obtained continuously from May to July 2019 (Table S1). 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). Soil water from 0-160 cm depths (bulk soil water, N=247; mobile

water, N=191), groundwater (N=22), plants' trunk water (N=61) and root water (N=156) were collected for the hydrogen and oxygen isotopic analyses. 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. Groundwater samples were collected at a water well located about 300 m from the soil and root sampling plot. 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. Soil samples were collected at 10 cm intervals from 0-100 cm depths and 20 cm intervals from 100-160 cm depths at each of the three sampling sites (Site1, N=68; Site 2, N=69; Site 3, N=62). These soil samples were rapidly placed in 10 mL vials and sealed with caps then the caps were secured with Parafilm, then kept in a cool box until storage in the lab at −20℃. The soil samples of each layer were divided into two groups: one for isotopic analysis and the other for determination of gravimetric soil water content (GWC, %) by the drying method (105℃ for 12 h). In parallel, mobile water was sampled at 20, 30, 50, 100 and 150 cm depths using suction lysimeters when water was present. Each lysimeter consisted of a porous cup with two inserted tubes that allowed creation of the vacuum in the lysimeter and sampling of soil water by injecting air into the lysimeter (Fig. 1b). A tension of 60 kPa was applied to each suction lysimeter.

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℃.

2.4 Stable isotope analysis

A LI-2100 automated vacuum distillation system (LICA Inc., Beijing, China) was used to extract water from the soil, trunk and root samples. This system is similar to cryogenic vacuum distillation systems that are widely used elsewhere (Gaj et al., 2017), except that it uses a compressor refrigeration unit and not liquid nitrogen. Samples were subjected to the maximum allowed vacuum pressure of 1500 Pa and temperature differential of 225 ℃ (heating temperature, 130 ℃; cooling trap, −95 ℃) for 180 min during extraction, in efforts to ensure that more than 99% of the water was collected from them. The δ^2H and $\delta^{18}O$ values for all samples were determined using an Isoprime 100 Stable Isotope Ratio Mass Spectrometer (Isoprime Ltd Inc., Cheadle, UK) at the Institute of Water-saving Agriculture in Arid Areas of China, Northwest A&F University. The precision of the analyses of H and O isotopic composition was 0.5 and 0.1‰, respectively. The isotopic composition (^2H to ^1H and ^{18}O to ^{16}O ratios) of the samples was normalized relative to the V-SMOW (Vienna Standard Mean Ocean Water) standard set by the International Atomic Energy Agency. The resulting ratios were then expressed in delta notation (δ^2H and $\delta^{18}O$ values), calculated as follows:

$$\delta^2H(‰) = \left(\frac{R_{sample}}{R_{standard}}\right) - 1 \quad (1)$$

$$\delta^{18}O(‰) = (\frac{R_{sample}}{R_{standard}}) - 1 \quad (2)$$

2.5 Calculations of le-excess of source water and less mobile water

We calculated the line-conditioned excess (lc-excess) values of bulk soil water, mobile water, groundwater and trunk water, following Landwehr and Coplen, (2006). The lc-excess values were used to identify the degree of 'offset' of environmental waters from precipitation. A negative lc-excess that exceeds the standard deviation of the local meteoric water line (LMWL) indicates that water has undergone evaporative isotopic enrichment (Evaristo et al., 2016). The lc-excess values of samples were calculated as follows:

$$lc - excess = \delta^2 H_s - a\delta^{18} O_s - b \quad (3)$$

where the subscript 's' represents the sample and a and b are the slope and intercept of the LMWL, respectively. The LMWL shows the relationship between δ^2H and $\delta^{18}O$ in precipitation, and according to analysis of the precipitation (N=89) from 2016 to 2019 at our study site, this was $\delta^2H = 7.67\ \delta^{18}O + 5.91$.

In addition, following Sprenger et al. (2019), we determined the maximum value of less mobile water (here defined as water that could not be accessed by suction lysimeter) at selected depths (20, 30, 50, 100 and 150 cm), that is, the GWC determined by application of 60 kPa suction (the tension applied to obtain mobile water). The mobile fraction of soil water was calculated from the difference between the measured bulk soil water and less mobile water. Based on an isotope mass balance approach, the isotopic composition of less mobile water was calculated as follows:

$$\delta_{\mathrm{LMW}} = \frac{\delta_{\mathrm{BW}} \cdot \theta_{\mathrm{BW}} - \delta_{\mathrm{MW}} \cdot \theta_{\mathrm{MW}}}{\theta_{\mathrm{LMW}}} \quad (4)$$

Here, δ and θ represent the isotopic composition and GWC of samples, respectively, while, the subscripts 'LMW', 'BW' and 'MW' represent less mobile water, bulk soil water and mobile water, respectively.

2.6 Statistical analysis

All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, USA). Shapiro-Wilk and Levene's tests were respectively used to check that the data met normality of distribution and homogeneity of variance requirements for planned analyses. One-way ANOVA followed by Tukey's test was used to detect significant differences in the variation in depth of less mobile water, root water, mobile water, and bulk soil water isotopic composition. Presented diagrams were generated using SigmaPlot 12.5.

3 Results

3.1 Dual-isotope plots

Stable isotopic composition (δ^2H and $\delta^{18}O$) of all water samples ~~are~~is shown in Fig. ~~3a~~ 2a and Table 2. 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. ~~3b~~2b). Bulk soil water partly overlapped isotopically with mobile water but it generally plotted below mobile water (Fig. ~~3a~~ 2a and c). Less mobile water deviated from the LMWL and overlapped with root water and trunk water (Fig. ~~3a~~ 2a and d). Trunk water was isotopically similar to root water at 100-160 cm depths (Fig. ~~3a~~ 2a and e-f).

Fig. ~~3~~2

Table 2

3.2 The lc-excess of mobile water, bulk soil water and less mobile water

Fig. ~~4~~3

As shown in Fig. ~~4~~ 3A and Table 2, 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 (August 4 to September 15, 2019). The lc-excess of trunk water was generally lower than that of bulk soil water but it was within the range of lc-excess of less mobile water. A heavy rain event occurred the day before the sampling (August 3), with 63 mm precipitation. Shallow GWC (20-30 cm) was sensitive to this rain event and decreased gradually from August 4 to September 9 (Fig. ~~4a~~3b-c ~~b~~). Although GWC varied greatly, mobile water and bulk soil water at 20-30 cm depths remained relatively stable during this period, with average lc-excess values of 0.9 ± 1.1‰ and −6.8 ± 1.6‰, respectively. While, the lc-excess values of less mobile water at the same depths gradually increased and stabilized, ranging from −23.9 to −4.6‰. GWC at deep layers (i.e. 100 and 150 cm) was less affected by precipitation, ranging from 8.0 to 13.6%. Similarly, the mean lc-excess values of mobile water, bulk soil water and less mobile water at 100-150 cm layers fluctuated slightly from August 4 to September 9, with the average values of −3.3 ± 1.1‰, −6.5 ± 1.4‰ and −7.4 ± 2.1‰, respectively (Fig. ~~4c~~3e-f ~~e~~).

At every sampling depth, the mean lc-excess of mobile water was always higher than that of bulk soil water and less mobile water (Tukey-Kramer HSD, $p < 0.05$) during the whole sampling period (Fig. ~~4A~~3B-~~E~~F). Particularly, the most significant difference between mobile water, bulk soil water and less mobile water appeared in the 20 cm soil layer, with average lc-excess values of 1.1 ± 1.5‰, −7.3 ± 2.5‰ and −12.8 ± 4.3‰, respectively. No correlation between Δlc-excess (lc-excess difference between measured mobile water and bulk soil water) and GWC was detected at 20-150 cm depths, but a strong positive correlation between lc-excess value and GWC was observed at 20, 30 and 50 cm depth for mobile water (20 cm, $y = 0.19x - 1.27$, $R^2 = 0.27$, N = 40, p = 0.001; 30 cm, $y = 0.17x - 1.61$, $R^2 = 0.22$, N = 40, p = 0.002; 50 cm, $y = 0.20x - 3.59$, $R^2 = 0.16$, N = 38, p = 0.013) and 20-30 cm depths for bulk soil water (20 cm, $y = 0.34x - 11.39$, $R^2 = 0.30$, N =42, $p < 0.001$; 30 cm, $y = 0.23x - 9.21$, $R^2 = 0.20$, N =40, p = 0.003) (Fig. ~~5a~~4a-c). No correlation between these variables was detected (Fig. 4d ~~and~~ e) at 100 and 150 cm depths for mobile water and for bulk soil water.

Fig. ~~5~~4

3.3 Comparison between root water and bulk soil water isotopic composition at different depths

Fig. ~~6~~5

As shown in Fig. ~~6b~~ 5b and d, 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 bulk soil water ~~was horizontally homogenous~~had little horizontal variation within 80 cm from tap roots. However, isotopic variations with depth were detected in both root water and bulk soil water. Generally higher δ^2H and $\delta^{18}O$ values in root water (mean values and standard deviations for three soil profiles: −65.90 ± 2.92 and −7.66 ± 0.40‰, respectively) than in bulk soil water (mean values and standard deviations for three soil profiles: −69.09 ± 2.50 and −8.89 ± 0.38‰, respectively) were observed at 80-160 cm depths. Root water and bulk soil water significantly differed in δ^2H at 80-140 cm depths ($p < 0.05$) and $\delta^{18}O$ at 60-160 cm depths ($p < 0.01$) (Fig. ~~6a~~5a and~~,~~ c). The maximum differences between bulk soil water and root water were −8.6 and −1.8‰ for Δ^2H and $\Delta^{18}O$, respectively. Although δ^2H and $\delta^{18}O$ values of root water and bulk soil water behaved differently, a strong correlation was observed between $\Delta^{18}O$ ($\Delta^{18}O = \delta^{18}O_{soil} - \delta^{18}O_{root}$) and Δ^2H ($\Delta^2H = \delta^2H_{soil} - \delta^2H_{root}$) for soil-root offset (Fig. ~~7a~~6a) at 0-160 cm depths (bulk soil water-root water: $y = 3.83x + 0.99$, $R^2 = 0.69$, $N = 24$, $p < 0.001$). Similarly, a strong correlation was observed bet ween $\Delta^{18}O$ ($\Delta^{18}O = \delta^{18}O_{soil} - \delta^{18}O_{trunk}$) and Δ^2H ($\Delta^2H = \delta^2H_{soil} - \delta^2H_{trunk}$) soil-trunk offsets during August 4 to September 15 (bulk soil water-trunk water: $y = 6.80x + 6.52$, $R^2 = 0.83$, $N = 42$, $p < 0.001$; mobile water-trunk water: $y = 5.93x + 10.87$, $R^2 = 0.81$, $N = 42$, $p < 0.001$) (Fig. ~~7b~~6b).

Fig. ~~7~~6

4 Discussion

4.1 Isotopic dynamics at the root-soil interface

4.1.1 Separation of mobile water and bulk soil water in the soil matrix

Fig. ~~8~~7

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 (Figs. ~~4~~ 3 and ~~8~~7). A key question is why mobile water separates from bulk soil water isotopically? 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). 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. Such small amounts of winter precipitation might not be able to fill the small pores. Notably, there was a major rainstorm the day before the sampling (August 3), with 63 mm precipitation. The mean GWC in 0-50 cm 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 the 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. Furthermore, the lc-excess values of both mobile and bulk soil water were positively correlated with GWC at 20 and 30 cm depths. When GWC increased due to precipitation, the lc-excess values of mobile and bulk soil water increased. Similarly, when GWC decreased due to evaporation, their lc-excess values also decreased. 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. This result is consistent with the finding by Evaristo et al. (2016) that ecohydrological separation was consistently present in two tropical catchments with contrasting moisture conditions (Luquillo and Susua catchments in Puerto Rico, with mean annual precipitations of 3700 and 1200 mm, respectively).

We also found the degree of separation between the lc-excess of mobile and bulk soil water gradually decreased as the soil depth increased (e.g., 100 cm and 150 cm). On the one hand, the effect of soil evaporation on bulk soil water gradually weakens with increasing soil depth. Thus, the enriched isotopic composition formed by evaporation in bulk soil water gradually decline or even disappear. On the other hand, mobile water in deep layers is more likely to be recharged by both preferential and matrix flows than by preferential flow alone (Xiang et al., 2019). Under matrix flow conditions, newly infiltrated water displaces existing 'old water', pushing it deeper into the soil profile and eventually into groundwater (Zheng et al., 2019), so 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). Evidence of this mixing has been provided by Vargas et al. (2017), who found that 75 to 95% of less mobile water isotopically exchanged with mobile water in a glasshouse experiment with potted *Persea americana* in two contrasting soil types. In addition, Adams et al. (2020) found that mobile and less mobile water isotopic composition are affected by soil texture and mineralogy (e.g., smectite and clay contents). The extent to which less mobile water mixes with mobile water is unclear at our study site, but such exchange might be one of the reasons for the weakening of the separation between mobile and bulk soil water in deep layers.

4.1.2 Isotopic offset between bulk soil water and root water

We compared the isotopic composition of root water and bulk soil water at the same depth (Fig. ~~6~~5). Contrary to expectations, the root water and bulk soil water at 0-60 cm depths showed consistent δ^2H and $\delta^{18}O$ values. 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 the 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 sources 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 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 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. ~~3~~2a, 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.

4.2 Root water and trunk water isotopic composition

We found that trunk water mainly overlapped isotopically with root water at 100-160 cm depths (Fig. ~~3~~2a and e-f), while the isotopically enriched root water at 0-80 cm depths was not reflected in the trunk water isotopic composition. 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). We thus 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 (Fig. ~~4~~3a) and found that δ^2H and $\delta^{18}O$ values of trunk water remained stable (mean values: −66.68 ± 1.61 and −7.71 ± 0.24‰, respectively) during this period. 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). These results indicated that the trees always used a stable water source during the study period. One possibility is that trees preferentially use much deeper soil water and groundwater than fluctuating shallow soil water, which is a less stable and reliable water source because it is subject to rapid evaporation and seasonal precipitation (Zhao and Wang, 2018). Deep soil water can make a significant contribution to drought avoidance during dry periods (Yang et al., 2017) and increasing capacity for deep soil water utilization was positively correlated with intrinsic water use efficiency (Jiang et al., 2020). Moreover, *S. matsudana*'s deep water use strategy may provide favorable water conditions for shallow-rooted herbaceous species, facilitating stable coexistence. Roots at 0-80 cm depths absorb less water with enriched isotopic composition than deep roots. A small proportion of the isotopically enriched root water fully mixes with isotopically depleted root water in deep layers, resulting in the disappearance of isotopically enriched signals in the trunk water. 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 ^{18}O values than root water (Fig. ~~3~~2a and e-f). Therefore, in this study, root water partially overlapped with trunk water isotopic composition, we believe it reflects the selective utilization of water source rather than isotopic fractionation within woody tissues.

5 Conclusion

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.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

Author contributions

LW conceptualized this research. YZ collected the data. Both authors contributed to the writing of the manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgments

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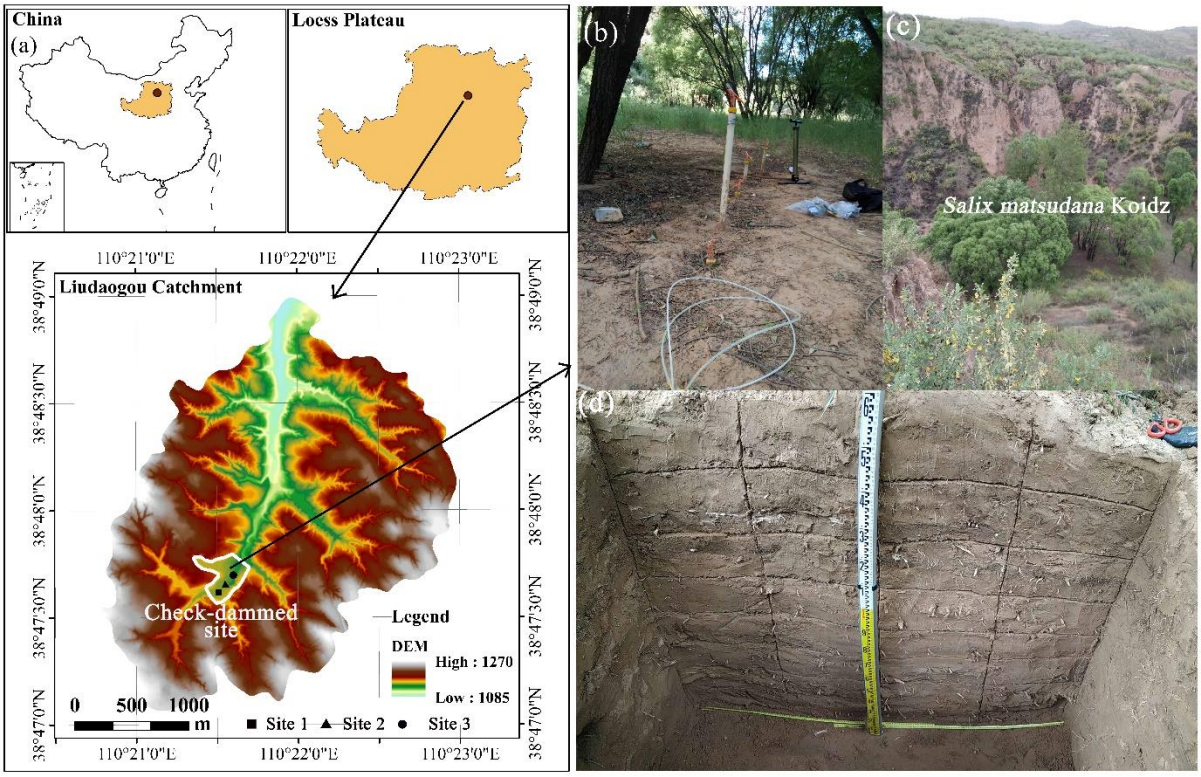


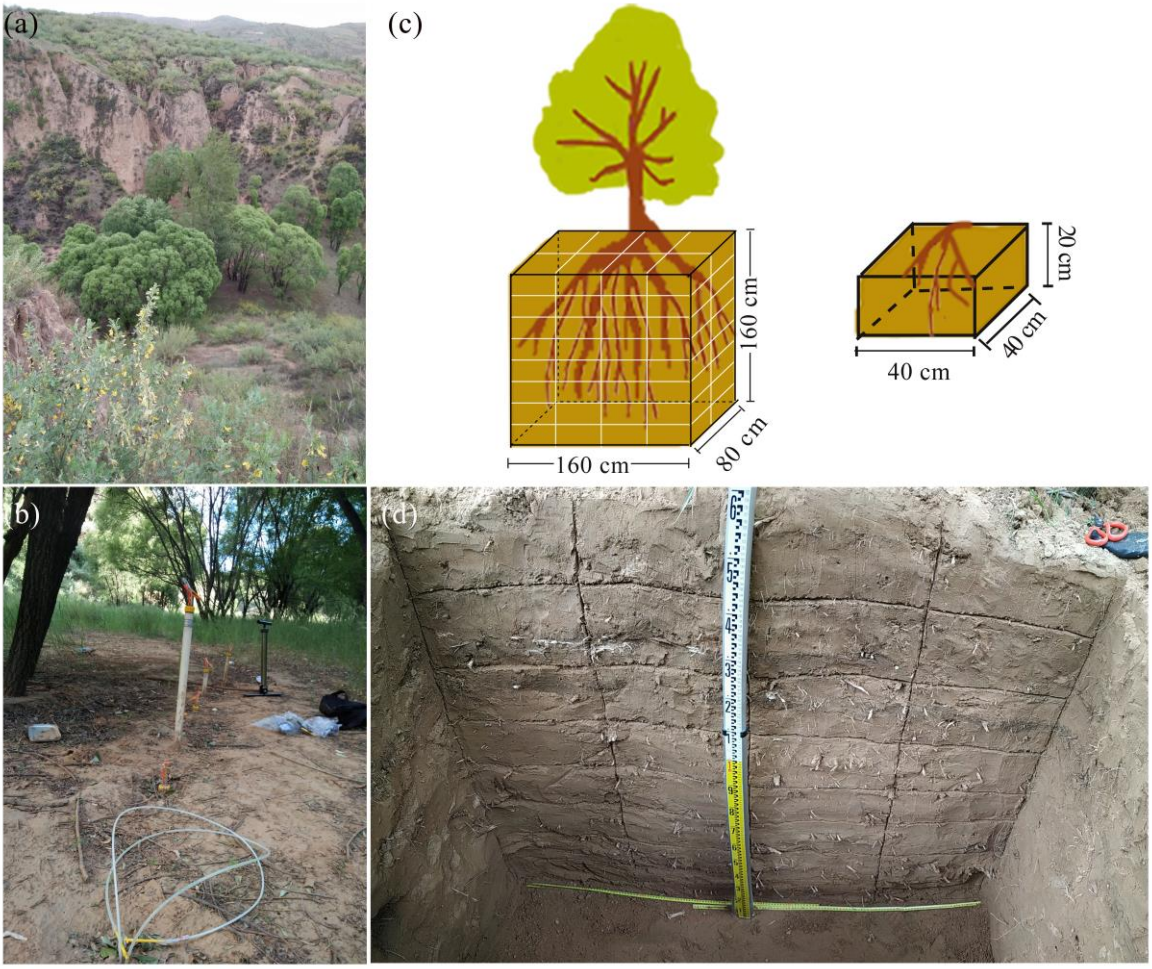
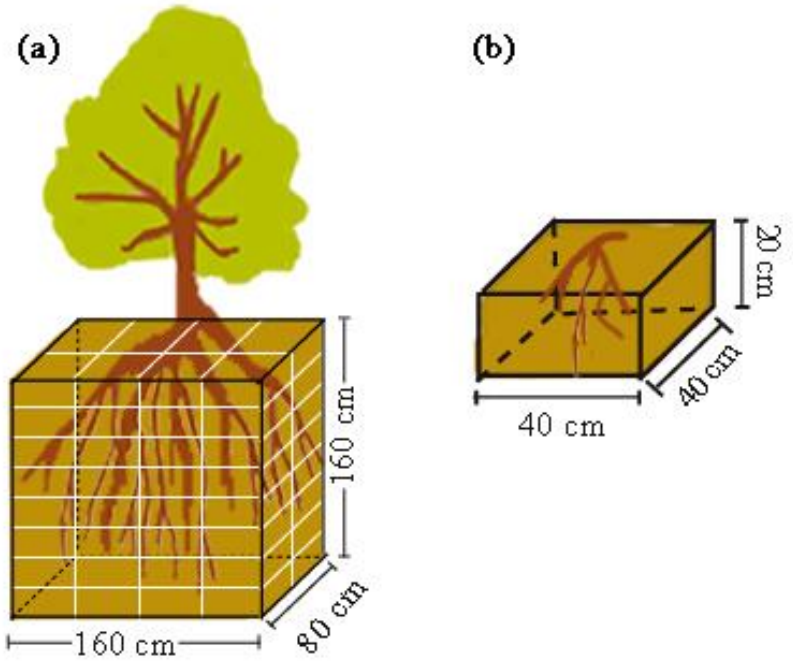


Figure 1~~:~~ (a)~~ Location of the study area on the Loess Plateau, China.~~ Photograph of *Salix matsudana* Koidz, our sampling tree, (b) ~~Photograph of~~ mobile water collection using suction lysimeters (white plastic tubes) (with application of 60 kPa tension),~~ and~~ (c) schematic diagram of root excavations and measurements as described in Section 2.2~~*Salix matsudana* Koidz, our sampling tree.~~ , and (d) ~~Profile~~ profile of the soil cuboid (length, width and depth: 160, 80 and 160 cm, respectively) being dug to obtain root isotopic data~~. T~~, 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.



500 **~~Figure 2: Schematic diagram of root excavations (a) and measurements (b) as described in Section 2.2~~**

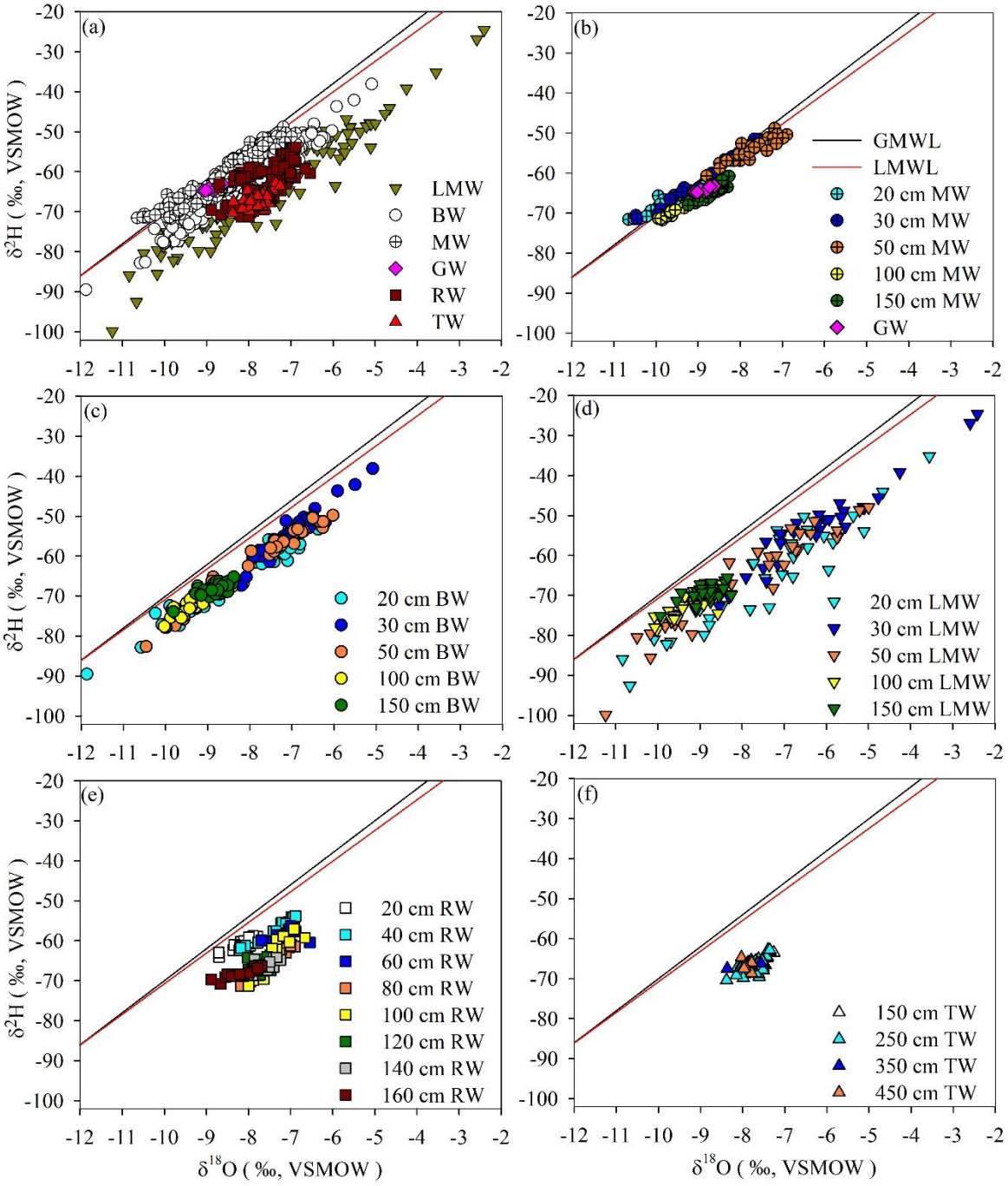


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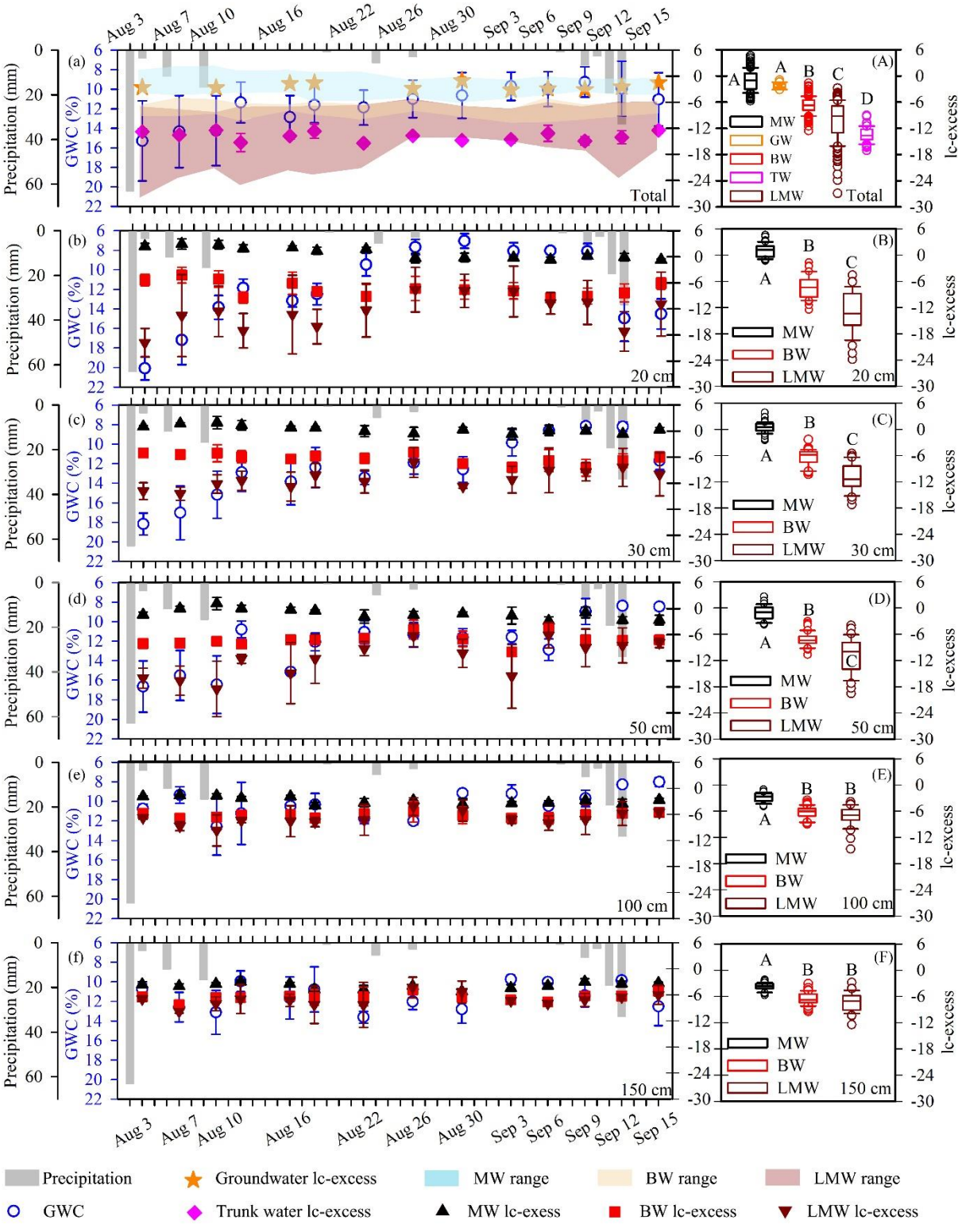
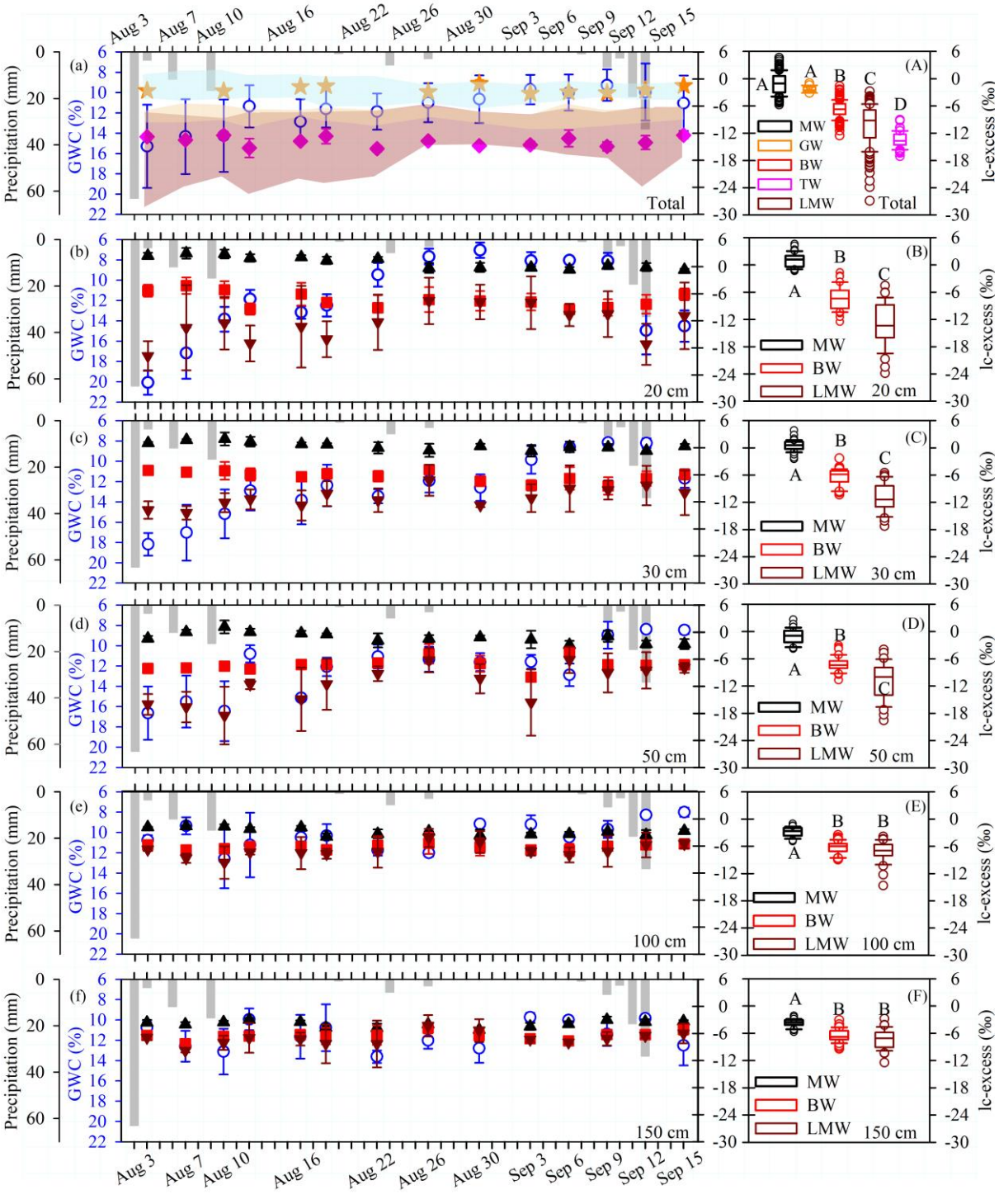
Figure ~~3~~ 2 (a) $\delta^{18}O$ and $\delta^{2}H$ isotopic composition collected from August 4 to September 15, 2019. Plotted values include bulk soil water (BW), mobile water (MW), root water (RW), trunk water (TW), less mobile water (LMW) and groundwater (GW). (b) $\delta^{18}O$ and $\delta^{2}H$ 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, $\delta^2H = 5.91 + 7.67\ \delta^{18}O$, $R^2 = 0.96$). The black line represents the global meteoric water line (GMWL, $\delta^2H = 10 + 8\ \delta^{18}O$).


Aug 3
Aug 7
Aug 10
Aug 16
Aug 22
Aug 26
Aug 30
Sep 3
Sep 6
Sep 9
Sep 12
Sep 15
Precipitation (mm)
GWC (%)
lc-excess
(a)
Total
(A)
MW
GW
BW
TW
LMW
Total
(b)
20 cm
(B)
(c)
30 cm
(C)
(d)
50 cm
(D)
(e)
100 cm
(E)
(f)
150 cm
(F)
Precipitation
Groundwater lc-excess
MW range
BW range
LMW range
GWC
Trunk water lc-excess
MW lc-exess
BW lc-excess
LMW lc-excess


Aug 3
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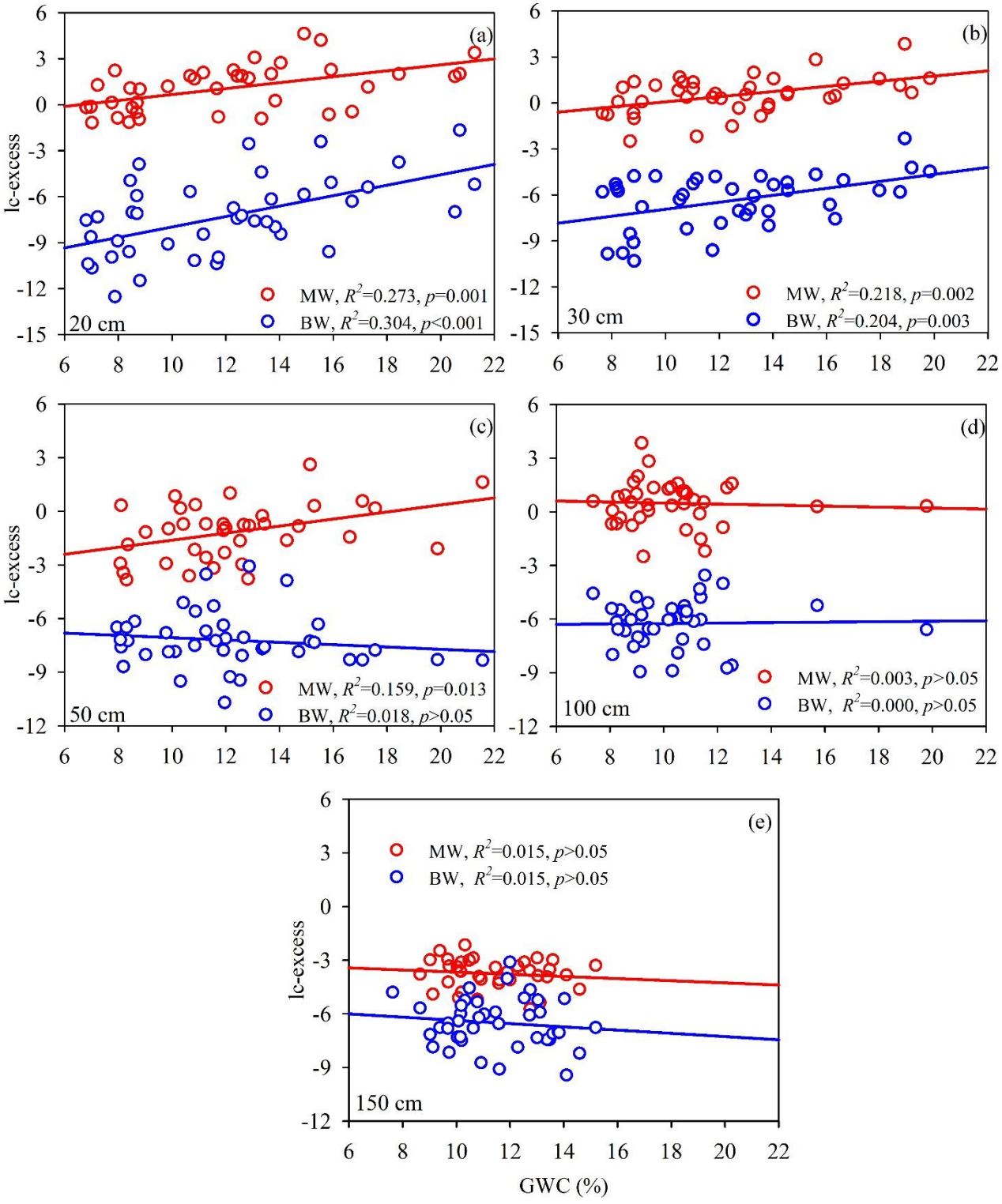
Aug 26
Aug 30
Sep 3
Sep 6
Sep 9
Sep 12
Sep 15
Precipitation (mm)
GWC (%)
lc-excess (‰)
(a)
Total
(A)
MW
GW
BW
TW
LMW
(b)
20 cm
(B)
(c)
30 cm
(C)
(d)
50 cm
(D)
(e)
100 cm
(E)
(f)
150 cm
(F)
Precipitation
Groundwater lc-excess
MW range
BW range
LMW range
GWC
Trunk water lc-excess
MW lc-exess
BW lc-excess
LMW lc-excess

Figure ~~4~~ 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).


(a)
20 cm
MW, R^2=0.273, p=0.001
BW, R^2=0.304, p<0.001
(b)
30 cm
MW, R^2=0.218, p=0.002
BW, R^2=0.204, p=0.003
(c)
50 cm
MW, R^2=0.159, p=0.013
BW, R^2=0.018, p>0.05
(d)
100 cm
MW, R^2=0.003, p>0.05
BW, R^2=0.000, p>0.05
(e)
150 cm
MW, R^2=0.015, p>0.05
BW, R^2=0.015, p>0.05
lc-excess
GWC (%)

525

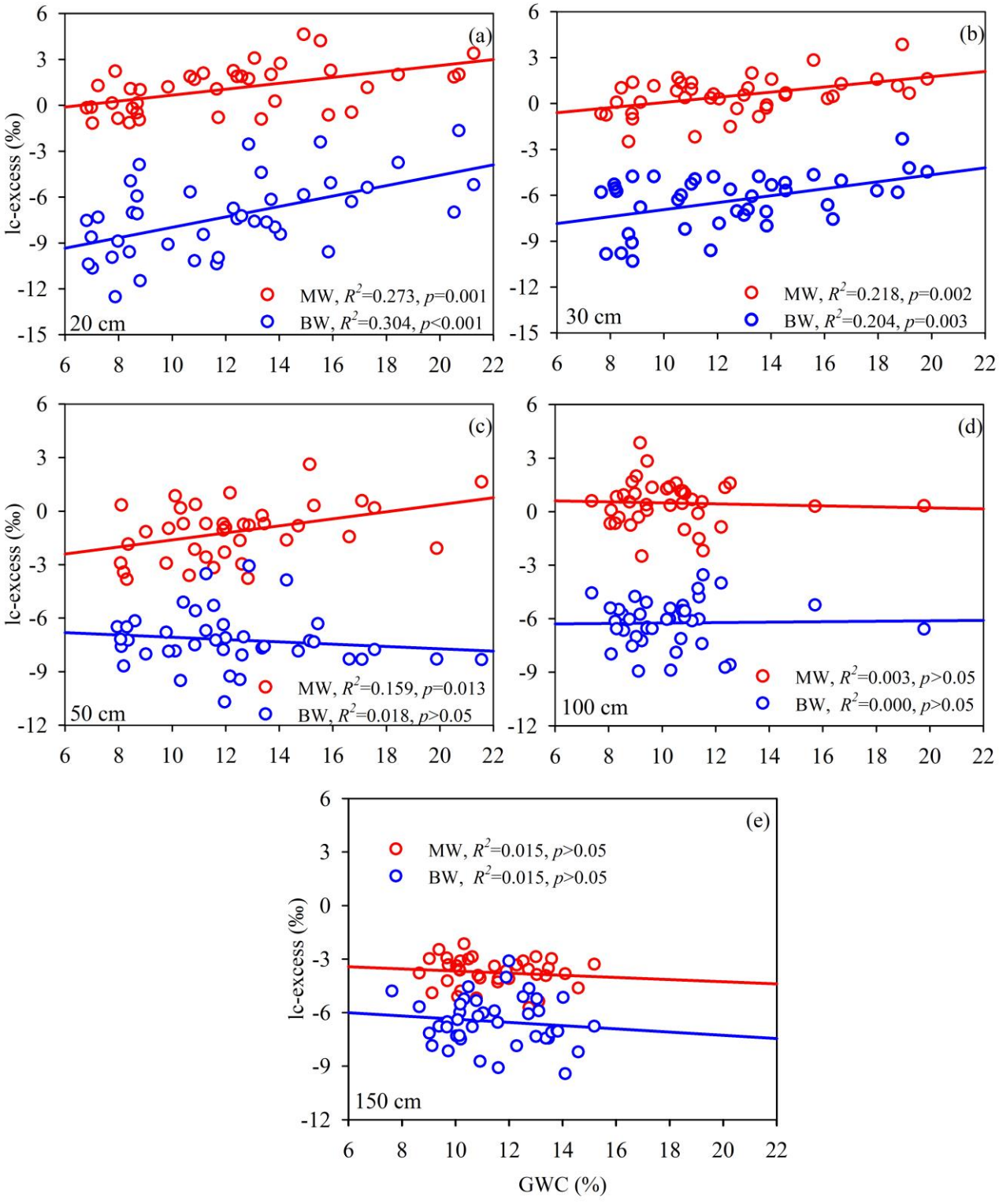
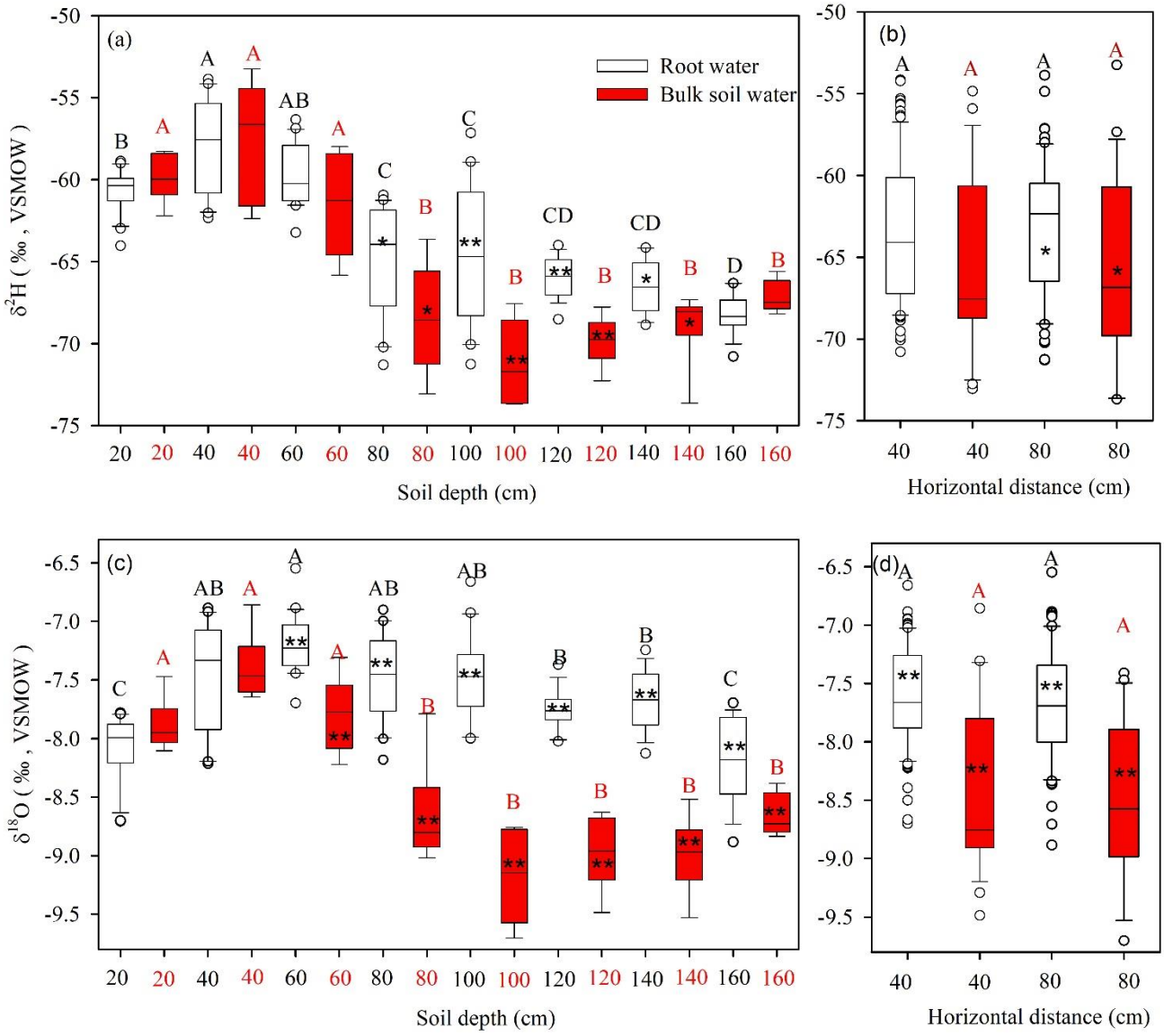

(a)
20 cm
MW, R^2=0.273, p=0.001
BW, R^2=0.304, p<0.001
(b)
30 cm
MW, R^2=0.218, p=0.002
BW, R^2=0.204, p=0.003
(c)
50 cm
MW, R^2=0.159, p=0.013
BW, R^2=0.018, p>0.05
(d)
100 cm
MW, R^2=0.003, p>0.05
BW, R^2=0.000, p>0.05
(e)
150 cm
MW, R^2=0.015, p>0.05
BW, R^2=0.015, p>0.05
lc-excess (‰)
GWC (%)

Figure ~~5~~ 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.


(a)
(b)
(c)
(d)
Root water
Bulk soil water
δ2H (‰ , VSMOW)
δ18O (‰ , VSMOW)
Soil depth (cm)
Horizontal distance (cm)
20 20 40 40 60 60 80 80 100 100 120 120 140 140 160 160
40 40 80 80

535

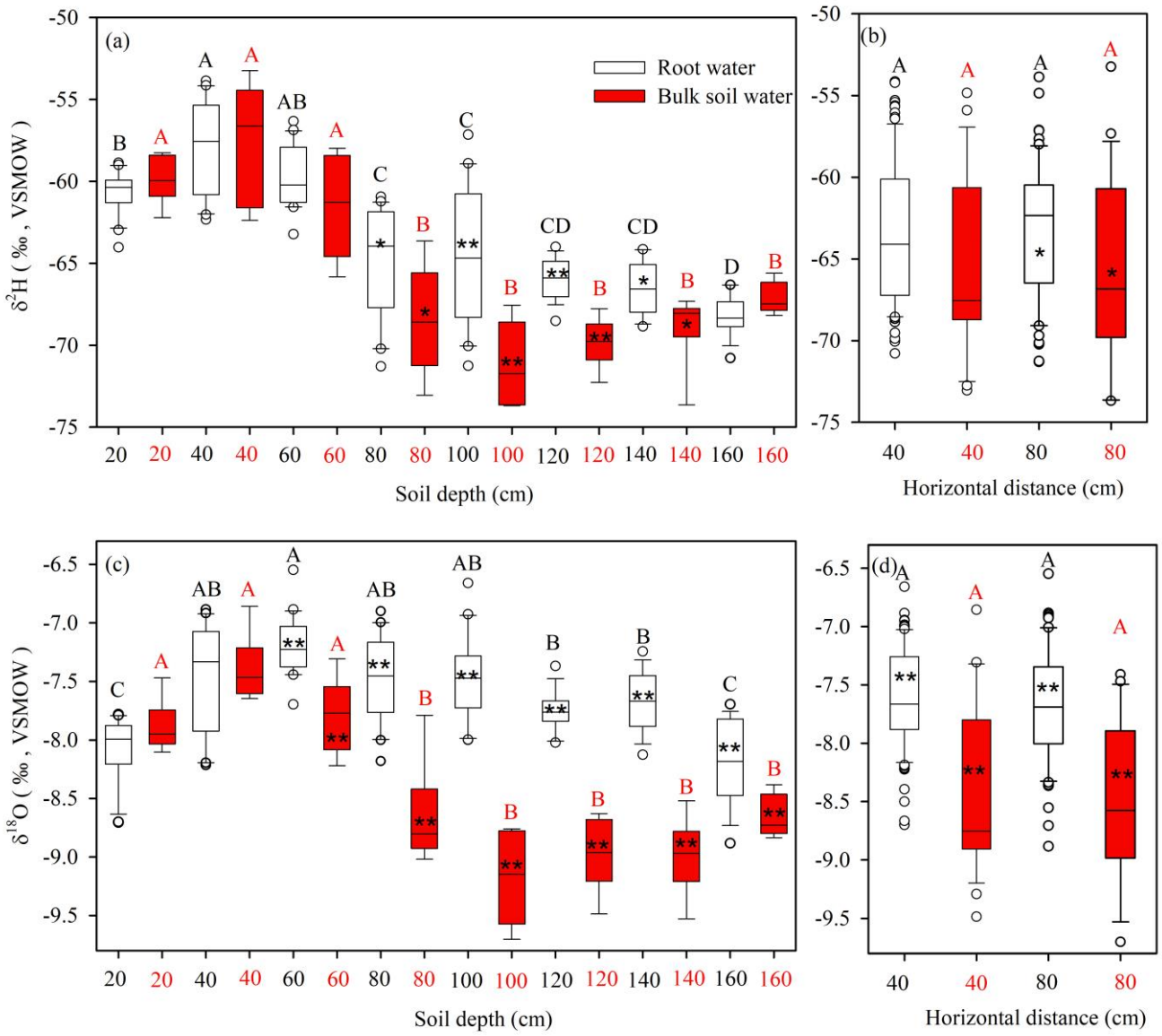


Figure ~~6~~ 5 Boxplots of root water and bulk soil water stable isotopes (δ^2H and $\delta^{18}O$) at indicated depths (a, c) and horizontal distances from the tap root of the focal root system (b, d). The top and bottom of each box are the 25th and 75th percentiles of the samples, respectively. The 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).

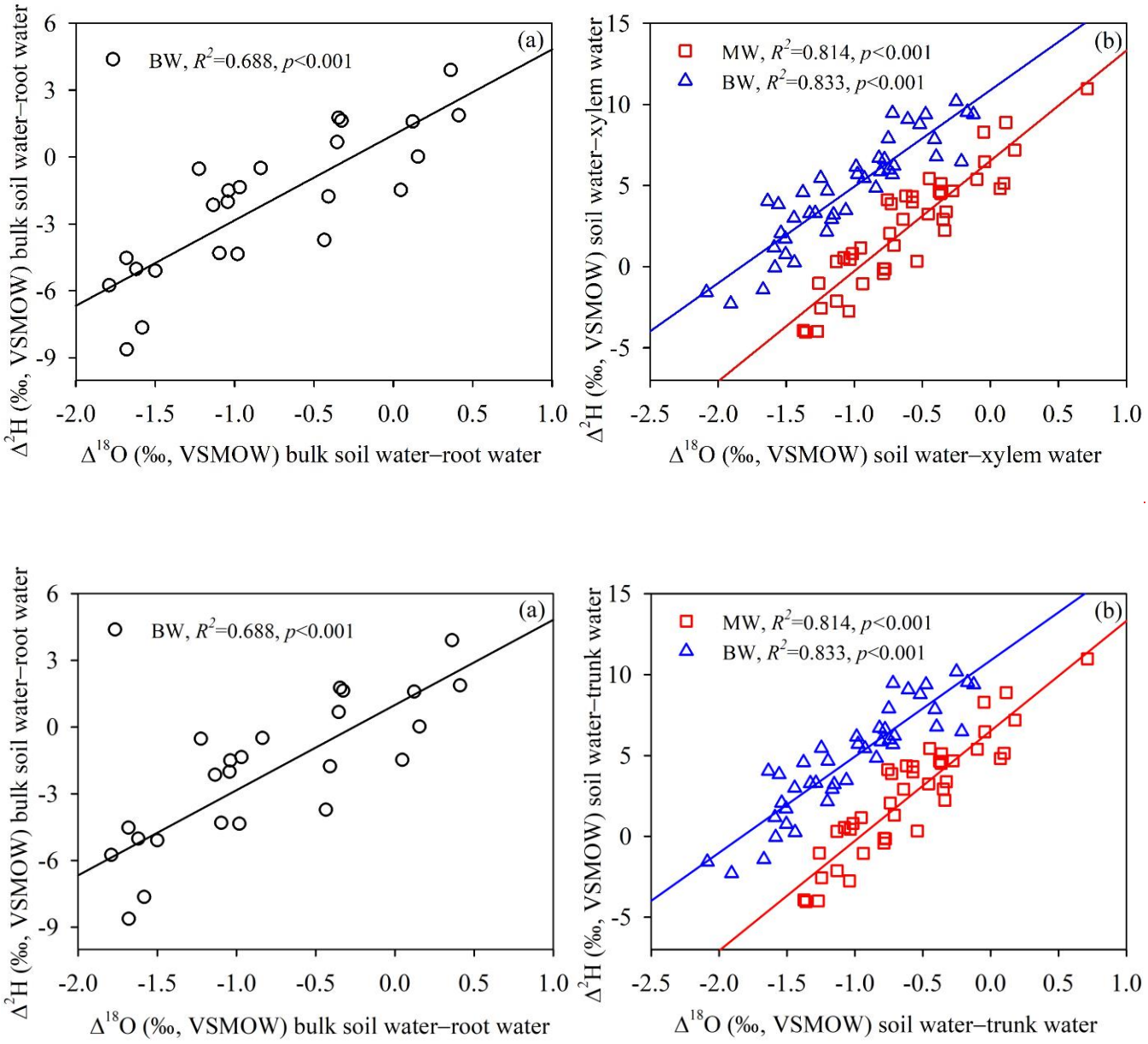


Figure ~~7~~ 6 (a) Relationship of hydrogen isotope offset (Δ^2H, $\Delta^2H = \delta^2H_{soil} - \delta^2H_{root}$) and oxygen isotope offset ($\Delta^{18}O$, $\Delta^{18}O = \delta^{18}O_{soil} - \delta^{18}O_{root}$) between bulk soil water and root water, according to analyses of samples of bulk 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).

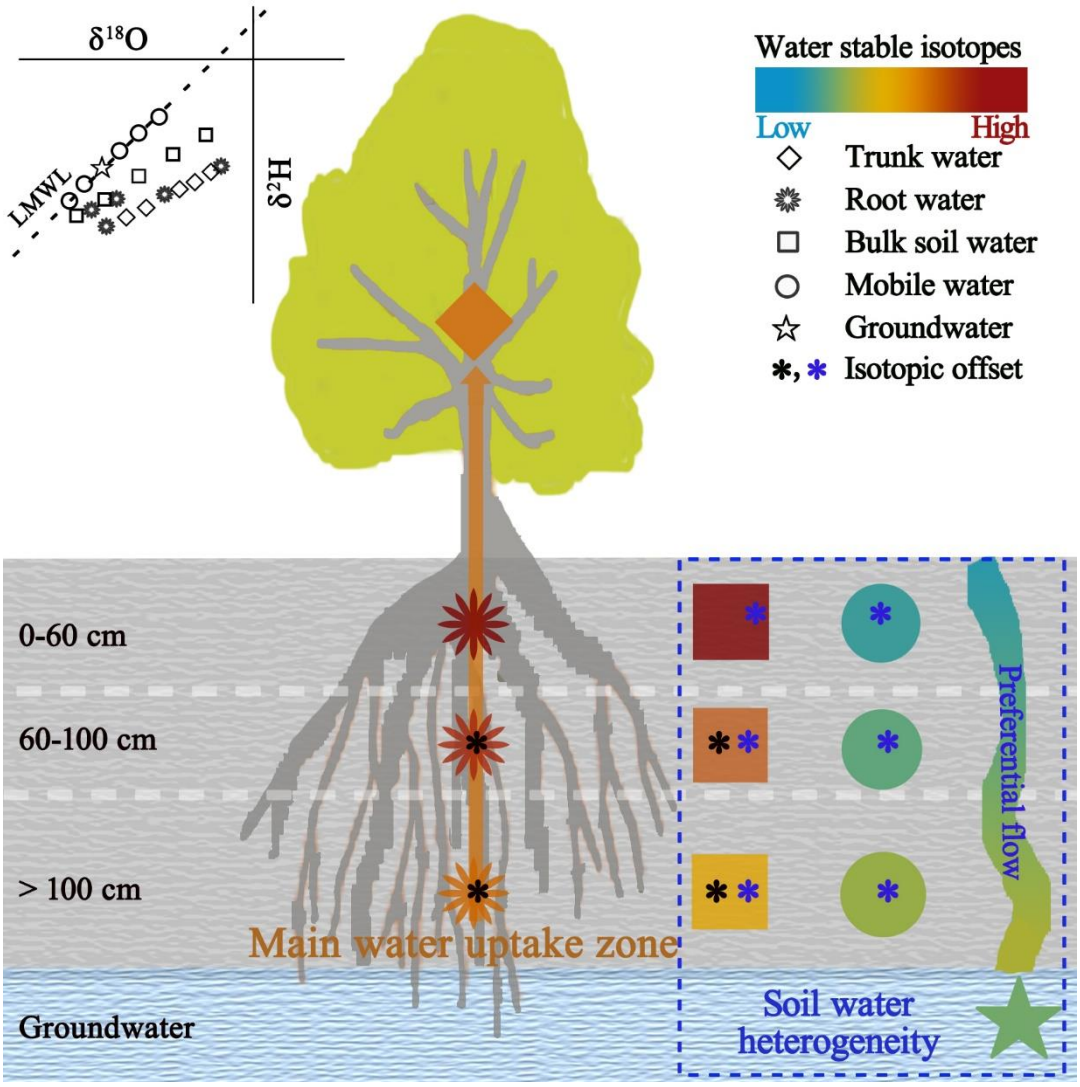


Figure ~~8~~ 7 Schematic diagram of isotopic dynamics along the soil-root-trunk continuum. Color codes indicate isotopic composition of mobile water, bulk soil water and root water at indicated depths, groundwater, and trunk water (from blue to brown representing low to high). The upper left insert is 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 ($p < 0.05$).

Table 1 Distribution of soil particle composition according to the USDA soil texture classification system

Soil depth (cm)	Soil particle composition (%)			Soil texture
	Sand	Silt	Clay	
10	56.76	34.78	8.46	Sandy loam
20	64.45	28.31	7.24	Sandy loam
30	67.65	25.69	6.66	Sandy loam
40	53.20	37.96	8.84	Sandy loam
50	60.67	31.18	8.15	Sandy loam
60	39.07	48.44	12.50	Loam
70	60.54	31.22	8.24	Sandy loam
80	50.55	40.38	9.07	Loam
90	51.06	39.06	9.88	Loam
100	61.05	30.43	8.52	Sandy loam
120	63.81	29.38	6.82	Sandy loam
140	51.31	39.17	9.52	Loam
160	45.29	44.09	10.61	Loam

Table 2 Water stable isotopes (see Fig. ~~3~~2) and lc-excess values (Fig. ~~4~~3) for all water samples. Range values show min, max (mean)

Water samples	N	$\delta^{2}H$ 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)