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

Ying Zhao[1,2], Li Wang[1,3]

[1]State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and the Ministry of Water Resources, Yangling 712100, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, China

Correspondence to: Li Wang (wangli5208@nwsuaf.edu.cn)

Abstract. Increasing numbers of field studies have detected isotopic mismatches between plant ~~xylem~~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 ~~xylem~~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, ~~xylem~~trunk water~~,~~ and root water of *Salix matsudana* Koidz trees) with about three-day~~at high temporal~~ resolution ~~to analyze isotopic dynamics~~ in the soil-root-~~xylem~~trunk continuum. We report three main findings. First, we detected clear separation between mobile water and bulk soil water isotopic ~~signals~~composition, but the distinction between~~ supporting the 'two water worlds' (TWW) hypothesis.~~ mobile water and bulk soil water gradually decreased with increasing soil depth. Second, ~~the isotopic composition~~root water deviated from bulk soil water isotopic composition, but it ~~of bulk soil water was closest to and~~ overlapped with the composition derived for less mobile water. ~~that of root water at 0-60 cm depths, but δ^2H and δ^{18}O values of root water at 80-160 cm depths deviated significantly from that of bulk soil water at the root soil interface. This was likely due to separation of mobile and tightly bound soil water (as in the TWW hypothesis) and plant fractionation.~~ 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, ~~xylem~~trunk water was only isotopically similar to root water at 100-160 cm depths~~ and these root layers provided 74% of the xylem water.~~, 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.

~~In conclusion, isotopic offset occurred at the interface between the soil and *S. matsudana* roots, and it can be attributed to a combination of plant fractionation and TWW type separation of bound and mobile soil water. Our study contributes to the~~

~~body of knowledge on isotopic dynamics in the soil-root-xylem continuum and provides potentially valuable insights regarding isotopic offsets between soil water and xylem water of *S. matsudana* tree and other species in similar conditions.~~

1 Introduction

35   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~~s~~. In terrestrial ecosystem~~s~~, plant transpiration accounts for more than 60% of total evapotranspiration and returns approximately 39% of incident precipitation to the atmosphere (Schlesinger and

40   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~~isotopes in water~~ ($\delta^{18}O$ 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.,

45   2020). This application relies on the assumption that RWU is generally a non-fractionating process (Ehleringer and Dawson, 1992), so the isotopic composition of ~~xylem~~trunk water effectively reflects that of water sources. Thus, by comparing $\delta^{18}O$ and $\delta^{2}H$ values of plant ~~xylem~~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., 2018;~~ Wang et al., 2020; Zhao et al., 2021). However, a growing body of evidence indicates that

50   there is an isotopic offset between ~~xylem~~trunk water and potential plant water sources, that is, the isotopic composition of ~~xylem~~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 implies that water isotope composition changes in the movements from soil to root and then to xylem, which might not be solely due to isotopic fractionation (Poca et al., 2019), but also to ecohydrological separation (Brooks et al., 2010), water isotope heterogeneity (Barbeta et al., 2020). Observed offsets could also be at least partly due to~~

55   ~~the water extraction technology used (Orlowski et al., 2018). The contributions of these factors to the isotopic deviations are uncertain.Analyses of isotopic signals ($\delta^{2}H$ and $\delta^{18}O$) within watersheds have suggested that groundwater is isolated from water sources used by plants, a phenomenon called ecohydrological separation or the 'two water worlds' (TWW) hypothesis (Brooks et al., 2010; McDonnell, 2014). This hypothesis is broadly supported on a global scale by enrichment of $\delta^{2}H$ and $\delta^{18}O$ values in soil water and xylem water, but not groundwater and streams (Evaristo et al., 2015). The isotopic offset between~~

60   ~~plant xylem water and~~This phenomenon ~~groundwater~~ has been attributed, at least in some areas, to isotopic heterogeneities across soil water pools (~~Evaristo et al., 2016; Bowling et al., 2017~~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

65 mainly derived from the less mobile water (89% ± 6), different to the more mobile water component in the soil matrix. ~~two water pools in the soil matrix: a tightly bound water pool used by plants and a mobile water pool related to infiltration and groundwater recharge via preferential flow (Evaristo et al., 2016; Bowling et al., 2017).~~ These studies mentioned above ~~The TWW hypothesis~~ relies on the ~~mentioned~~ 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). ~~For~~

70 ~~example,~~ Lin and Sternberg (1993) and Ellsworth and Williams (2007) found evidence that hydrogen ~~isotope~~ isotopic fractionation occurs during RWU of halophytic and xerophytic plants. ~~In addition,~~ Poca et al. (2019) reported that arbuscular mycorrhizal fungi can enhance ~~isotope~~ isotopic fractionation during RWU, resulting in up to −24.6‰ and −2.9‰ differences in δ^2H and $\delta^{18}O$ values, ~~respectively,~~ between soil and plant ~~xylem~~trunk water, respectively. ~~However, Barbeta et al. (2020) concluded that isotopic mismatches between soil water and xylem water are less likely to be caused by plants' fractionation~~

75 ~~than by water isotope heterogeneities in plant tissues and soil pores.~~ In addition, effects of extraction technology between cryogenically extracted trunk water and source water must be considered (Chen et al., 2020). ~~For example, i~~Incomplete extraction of water during cryogenic distillation ~~could~~ 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 ~~stem~~trunk water ~~cryogenically extracted~~ and source water in nine woody plant species and demonstrated that this offset stems

80 from ~~a cryogenic extraction associated~~ methodological artifac~~t~~s during cryogenic vacuum extraction. Thus, the extracted water does not properly represent the water available to plants, and may contribute to apparent ~~xylem~~trunk-soil water isotopic offsets.

Explanation of the isotopic offset between soil and ~~xylem~~trunk water is essential, but identifying roles of specific process~~es~~ 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

85 to have strongly interactive effects and may act on any compartment along the soil-root-~~xylem~~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. ~~leading to the variation in water isotopes. In addition, plants' roots transmit water from soil to xylem, and thus may play key roles in isotopic variation, e.g., root preferentially use tightly bound water according to the TWW hypothesis (Brooks et~~

90 ~~al., 2010) and mycorrhizal fungi may contribute to fractionation (Poca et al., 2019).~~ However, much more attention has been paid to ~~the~~ isotopic composition of plant ~~xylem~~trunk water and potential water sources (Chang et al., 2019; Kuhnhammer et al., 2020) than to isotopic ~~signatures~~ 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 isotop~~ice~~ic composition ~~dynamics~~ of

95 each component in the ~~water transport process along the~~ soil-root-~~xylem~~trunk continuum. More specifically, we exploited the specific isotop~~e fingerprints~~ic composition (δ^2H and $\delta^{18}O$ values) of mobile water, bulk soil water, groundwater ~~and xylem water of *Salix matsudana* trees~~, and derived ~~line conditioned excess (lc excess) values~~less mobile water to test the

heterogeneity of soil water~~assess the TWW hypothesis~~. 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 ~~xylem~~trunk water _of Salix matsudana_ trees ~~during water transport from root to xylem~~, to identify more specifically the sites and causes of the ~~isotope~~ isotopic deviation. ~~Finally, we used the SIAR model to calculate contributions of root water and soil water to xylem water.~~ We hypothesize that mobile water is isotopically separate from bulk soil water in the soil matrix and ~~there is an~~ isotopic deviation occurs between ~~xylem~~trunk water of _S. matsudana_ trees and their potential water sources due to heterogeneity of the soil water~~(the first hypothesis), and that this deviation might be due to a combination of multiple factors (the second hypothesis)~~.

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 °C, 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 °C in January to 26.20 °C in July. The Liudaogou catchment is in the 'water-wind erosion crisscross area' of the ~~plateau~~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, ~~catchment~~ designated sites 1, 2 and 3, located 50, 80 and 100 m upstream of the dam, respectively~~designated sites 1, 2 and 3, 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~~was chose for~~ sampling tree. The average age and height of the trees are about 30 years and 12 m, respectively. The soil at the site ~~includes~~ consists of sandy loam and loam according to the USDA classification system (Table 1), with ~~and its~~ bulk density ~~ranges~~ ranging from 1.4 to 1.6 g cm^{-3}. Water retention curves ~~of different soil layers~~at 20, 30, 50, 100 and 150 cm soil depths ~~in a~~at sampling ~~plot~~ 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 ~~the~~ sampling ~~plot~~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 ~~the~~ root samples from one *S. matsudana* tree and soil samples ~~of *S. matsudana* tree~~ at selected soil depths (0-160 cm with 20 cm intervals) at each of the three sampling site~~s~~, on August 18, 2019, to measure ~~roots'~~ 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). We then divided the cuboid into 64 sub-cuboids (length~~:~~, 40 cm~~,~~; width~~:~~, 40 cm~~,~~; height~~:~~, 20 cm) (Fig. ~~1d~~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°C.~~The coarse roots (> 2 mm in diameter) in each sub-cuboid was collected and measured its isotopic composition.~~ 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 we~~also collected disturbed soil samples at 10 cm intervals from 0 to 100 cm depths and 20 cm intervals from 100-160 cm depths~~at 0-160 cm depths~~ using a soil auger to measure soil particle size at sampling site 1~~,~~. ~~and~~ We also collected undisturbed soil samples at 20, 30, 50, 100 and 150 cm depths using cutting rings (100 cm^3 ~~in~~ volume) to obtain water retention curves at the same sampling site. These samples were took~~We then took the samples~~ to the laboratory and to determine~~d~~ their particle size and bulk density using a MS 2000 Laser Particle Size Analyzer (Malvern Instruments, Malvern, UK), and obtained 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 δ^{18}O) analysis

Previously unpublished data we obtained~~Our previous results~~ have shown that the isotopic composition of ~~xylem~~trunk water of *S. matsudana* tree~~s~~ 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~~Thus, to assess the TWW hypothesis that separation of the bound water used by plants and mobile water strongly contributes to isotopic deviation (Brooks et al., 2010)~~, 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 ~~isotope~~isotopic deviation ~~within~~ during the period when mobile water was available (i.e. from August 4 to September 15 2019)~~when mobile water was available~~. Soil water from 0-160 cm depths (bulk soil water, N=247; mobile water, N=191), groundwater (N=22), plants' ~~xylem~~trunk water (N=61) and root water (N=156) were collected for the hydrogen and oxygen isotopic analyses. ~~For these analyses, p~~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°C. 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°C 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 sampling campaignes. 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 brancheswas peeled from the twigs and all leaves were removed to avoid perturbance of xylemtrunk water isotopic signatures composition by fractionation. Pieces of the de-barked and de-leaved twigs, 30 mm long, were then immediately placed in 10 mL vials, and the vials were sealed with caps then the caps were secured with wrapped in parafilmParafilm. These samples were also kept in a cool box until storage in the lab at 4°C. In addition, samples of xylem at selected tree heights (150, 250, 350, 450 cm) and root samples at selected soil depths (0-160 cm with 20 cm intervals) and horizontal distances (0-80 cm with 40 cm intervals, excavated as described in Section 2.2) were collected on August 18, 2019. Similarly, of 30 mm long pieces of the de-barked twigs were immediately placed in 10 mL vials and wrapped in parafilm.

2.4 Stable isotope analysis

A LI-2100 automated vacuum distillation system (LICA Inc., Beijing, China) was used to extract water from the soil, xylemtrunk 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 °C (heating temperature, 130 °C; cooling trap, −95 °C) 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 isotopes 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:

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

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

2.5 Calculations of le-excess of source water and less mobile water ~~Methods for assessing ecohydrological separation and determining plant water sources~~

~~To test the TWW hypothesis, w~~We calculated the line-conditioned excess (lc-excess) values of bulk soil water, mobile water, groundwater and trunk water, following ~~(~~Landwehr and Coplen, (2006~~))~~ ~~of bulk soil water, mobile water, groundwater and xylem water~~. 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:

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

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 $\delta^2 H$ 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 $\delta^2 H = 7.67 \delta^{18}O + 5.91$.

In addition, following Sprenger et al. (2019), ~~we estimated $\delta^2 H$ and $\delta^{18}O$ values of tightly bound water to test the TWW hypothesis. W~~we determined the maximum value of ~~tightly bound~~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 ~~tightly bound~~less mobile water. Based on an isotope mass balance approach, the isotopic composition of ~~tightly bound~~less mobile water was calculated as follows:

$$\delta_{\text{~~TL~~LMW}} = \frac{\delta_{BW} \cdot \theta_{BW} - \delta_{MW} \cdot \theta_{MW}}{\theta_{\text{~~TL~~LMW}}} \tag{4}$$

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

~~To compare the isotopic composition of root and soil water at the same depth, we calculated contributions of root water and soil water to xylem water of S. matsudana tree. For this, we employed Stable Isotope Analysis in R (SIAR, version 4.2) to quantify the sources of water taken up by the trees. This is a package designed to solve mixing models for isotopic data in a Bayesian framework (Parnell et al., 2010). Based on results of the soil water and root water isotopes analyses ($\delta^2 H$ and $\delta^{18}O$ values) (Fig. 5), soil water sources available for root uptake were divided into four categories when running the SIAR model: groundwater and soil water at 0-60, 60-100, 100-160 cm depths. In parallel, root water sources available for root uptake were divided into the same four categories when running this model. In the SIAR model, the trophic enrichment factor was set to 0 for both $\delta^2 H$ and $\delta^{18}O$ because plant water use does not generally cause fractionation of hydrogen and oxygen isotopes (Ehleringer and Dawson, 1992). This model was run with 500,000 iterations (discarding the first 50,000 iterations) and the~~

7

most likely contribution (mean of the posterior distribution) of a water source (root water or soil water) to xylem water of *S. matsudana* trees on August 18, 2019 was obtained.

2.6 Statistical analysis

All statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, USA). Shapiro-Wilk and Levene's tests
230 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 ~~soil~~ less mobile water, root water, mobile water, and bulk soil water isotopic composition. Presented diagrams were generated using SigmaPlot 12.5.

3 Results

235 ## 3.1 Dual-isotope plots

~~Plots of t~~She stable isotopic composition (δ^2H and $\delta^{18}O$) of all water samples ~~in dual isotope space~~ are shown in Fig. ~~2a~~3a 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
240 (Fig. 3b).~~at 20, 30 and 50 cm depths ($\delta^2H = 6.70\ \delta^{18}O - 2.10$, $R^2 = 0.998$, N = 113, p < 0.001) typically plotted within the 95% confidence interval of precipitation (Fig. 2b), while mobile water at 100 and 150 cm depths ($\delta^2H = 5.91\ \delta^{18}O - 13.05$, $R^2 = 0.94$, N = 73, p < 0.001) slightly deviated from the LMWL. Groundwater was isotopically similar to mobile water at 150 cm depth.~~ Bulk soil water ~~($\delta^2H = 7.25\ \delta^{18}O - 4.35$, $R^2 = 0.95$, N = 199, p < 0.001)~~ partly overlapped isotopically with mobile water but it generally plotted below mobile water (Fig. ~~2a~~ 3a and c). Less mobile water deviated from the LMWL and
245 overlapped with root water and trunk water (Fig. 3a and d). ~~Root water ($\delta^2H = 5.47\ \delta^{18}O - 21.46$, $R^2 = 0.33$, N = 156, p < 0.001) strongly deviated from the LMWL and was generally isotopically enriched compared to mobile water and bulk soil water except that root water at 0-60 cm depths overlapped with bulk soil water (Fig. 2d). Neither mobile water nor bulk soil water matched the isotope composition of xylem water. Xylem~~ Trunk water ~~($\delta^2H = 3.69\ \delta^{18}O - 38.20$, $R^2 = 0.31$, N = 61, p < 0.001)~~ was ~~only~~ isotopically similar to root water at 100-160 cm depths (Fig. ~~2~~3a and e-~~f~~e).
250 **Fig. ~~2~~3**

Table 2

8

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

Fig. ~~3~~4

As shown in Fig. ~~3~~4 and Table 2, ~~the mean lc-excess values were −1.27 ± 2.10, −2.35 ± 0.62, −6.72 ± 1.24 and −13.82 ± 1.01 for mobile water, groundwater, bulk soil water and xylem water, respectively. The~~ 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 ~~xylem~~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-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 $0.9 \pm 1.1‰$ and $−6.8 \pm 1.6‰$, 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 $−3.3 \pm 1.1‰$, $−6.5 \pm 1.4‰$ and $−7.4 \pm 2.1‰$, respectively (Fig. 4c-e). ~~, indicating the existence of isotopic separation and supporting the TWW hypothesis. The lc-excess value of bulk soil water was generally higher than that of xylem water (p < 0.05), suggesting that xylem water was isolated from all potential water sources. This hypothesis is corroborated by tightly bound water data (Fig. S3), as lc-excess of xylem water is generally within the range of lc-excess of tightly bound water, indicating that plants might preferentially use tightly bound water.~~

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~~during the whole sampling period ~~(August 4 to September 15, 2019),~~ (Fig. 4A-E). 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 $1.1 \pm 1.5‰$, $−7.3 \pm 2.5‰$ and $−12.8 \pm 4.3‰$, respectively. ~~GWC at 20 cm depth was strongly affected by precipitation and evaporation, ranging from 7.4 to 20.8% (mean: 11.9%). The lc-excess values of bulk soil water and mobile water were always significantly different under all soil moisture conditions (p < 0.05). GWC at 150 cm depth remained relatively stable, ranging from 9.3 to 15.3% (mean: 11.7%). There was a significant isotopic difference between mobile and bulk soil water in this layer (p < 0.05), but it was smaller than the corresponding difference in other soil layers.~~ 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. ~~At every sampling depth, the lc-excess of mobile water was always higher than that of bulk soil water (p < 0.05),~~ but ~~the Δlc-excess was most pronounced in the 20-50 cm depths. A~~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. ~~4a~~5a-c). ~~While, n~~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. ~~4~~5

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

290 **Fig. ~~5~~6**

As shown in Fig. ~~5b~~6b and d, there were no significant differences (p > 0.05) in isotopic composition (δ^2H and δ^{18}O) of either root water or bulk soil water between 40 cm and 80 cm horizontal distance~~s~~ from selected tree trunks, suggesting that isotopic composition of the bulk soil water was horizontally homogenous ~~>~~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 δ^{18}O values in root water

295 (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 δ^{18}O at 60-160 cm depths (p < 0.01) (Fig. ~~5a~~6a, c). The maximum differences between bulk soil water and root water were −8.6 and −1.8‰ for Δ^2H and Δ^{18}O, respectively. Although δ^2H and δ^{18}O values of root water and bulk soil water behaved

300 differently, a strong correlation was observed between Δ^{18}O (Δ^{18}O = δ^{18}O$_{soil}$ − δ^{18}O$_{root}$) and Δ^2H (Δ^2H = δ^2H$_{soil}$ − δ^2H$_{root}$) for soil-root offset (Fig. ~~6a~~7a) 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 between Δ^{18}O (Δ^{18}O = δ^{18}O$_{soil}$ − δ^{18}O$_{\text{~~xylem~~trunk}}$) and Δ^2H (Δ^2H = δ^2H$_{soil}$ − δ^2H$_{\text{~~xylem~~trunk}}$) soil-~~xylem~~trunk offsets during August 4 to September 15 (bulk soil water-~~xylem~~trunk water: y = 6.80x + 6.52, R^2 = 0.83, N = 42, p < 0.001; mobile water-~~xylem~~trunk water: y = 5.93x + 10.87, R^2 = 0.81, N = 42, p < 0.001) (Fig. ~~6b~~7b). ~~These results~~

305 ~~show that water isotopes, especially hydrogen isotopes, changed between root water and soil water, and between soil water and xylem water, supporting our first hypothesis.~~

Fig. ~~6~~7

~~3.4 Contributions~~

~~Fig. 7~~

310 ~~Potential sources of plant xylem water were determined using a Bayesian mixing model approach. Fig. 7 shows contributions of potential water sources calculated from root water and bulk soil water isotopic signatures. According to root water isotopic data, root water at 0-60, 60-100, and 100-160 cm depths and groundwater accounted for 3 ± 3% (mean ± 1SD), 17 ± 9%, 74 ± 10% and 6 ± 4% of xylem water, respectively. According to bulk soil water isotopic data, bulk soil water at 0-60, 60-100, 100-160 cm depths and groundwater accounted for 26 ± 6%, 32 ± 10%, 33 ± 12% and 9 ± 8% of xylem water,~~

315 ~~respectively. Such large differences in contributions also suggest that an isotopic offset occurs at the root-soil interface.~~

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

320   At our study site during the ~~covered~~ experimental period (August 4 to September 15, 2019), a clear isotopic separation between mobile and bulk soil water was observed (Fig. 4 and 8). 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

325   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)

330   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 $17.4 \pm 2.7\%$ and $10.8 \pm 1.5\%$ 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

335   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

340   decreased. The lc-excess values of mobile and bulk soil water ~~at all measured depths~~ 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).

345   ~~A key question is why mobile water separates from bulk soil water isotopically? Some studies indicate that mobile and bulk soil water might be derived from precipitation falling at different times (e.g., Gierke et al., 2016; Allen et al., 2019). In some environments, small pores that contain tightly bound water are preferentially filled by snowmelt from winter and spring, whereas mobile water from summer thunderstorms infiltrates quickly through macropores along preferential flow paths. Due~~

11

355 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, ~~for the following reasons. T~~the effect of soil evaporation on bulk soil water gradually weakens with increas~~es in~~ing soil depth~~s~~. Thus, the enriched isotopic ~~signals~~ composition formed by evaporation in bulk soil water gradually decline or even disappear. ~~In addition~~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).

360 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~~mobile water mixes with tightly bound water~~ (Sprenger et al., 2016; Kubert et al., 2020). ~~Although the mixing of mobile and tightly bound water conflicts with the original hypothesis of Brooks et al. (2010), evidence~~ Evidence of this ~~phenomenon~~ mixing has been provided by Vargas et al. (2017), who found that 75 to 95% of ~~tightly bound~~ less mobile

365 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 ~~bound~~ less mobile ~~soil~~ water isotop~~e ratios~~ic composition are affected by soil texture and mineralogy (e.g., smectite and clay contents). The extent to which ~~tightly bound~~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. ~~These findings suggest that mobile and bulk soil water at~~

370 ~~all measured depths were continuously separated in the soil matrix, which might be related more to fundamental processes that drive isotopic changes (e.g., soil evaporation and water flow paths) than to soil water conditions. This result is consistent with findings by Evaristo et al. (2016) and Dubbert et al. (2019) that the isotopic distinction in the TWW hypothesis may be driven by spatiotemporal dynamics of soil water profiles associated with soil evaporation.~~

4.1.2 Isotopic offset between bulk soil water and root water

375 We compared the isotopic composition of root water and bulk soil water at the same depth (Fig. 6). Contrary to expectations, ~~The~~the root water and bulk soil water at 0-60 cm depths showed ~~enriched isotopic signals (δ^2H and δ^{18}O values) that were~~ consistent ~~with bulk soil water δ^2H and δ^{18}O values~~isotopic signals at the same depth and distinct from mobile water ~~isotopic signals (Fig. 2 and 5)~~. ~~In contrast~~However, at 80-160 cm depths, δ^2H and δ^{18}O values of root water deviated significantly from those of bulk soil water ~~and mobile water, especially δ^2H values~~. ~~These results showed that the isotopic~~

380 ~~offset between plant root water and soil water occurred at the root-soil interface (Fig. 8).~~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

12

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

385 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

390 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,

395 measurements of the δ^2H and $\delta^{18}O$ of soil, trunk and root water from potted *Fagus sylvatica* 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.

~~Fig. 8~~Overall, the most plausible~~An alternative~~ 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

400 of bulk soil water. As shown in Fig. 3, less mobile water overlapped isotopically with root water after removing the influence of mobile water.~~plant rely predominantly on immobile tightly bound water in accordance with the TWW hypothesis (Brooks et al., 2010; McDonnell, 2014).~~ The rapidity of mobile water's passage through soil reduces its contact with mineral surfaces, and hence its nutrient concentrations~~of mobile water~~ (McDonnell, 2017; Sprenger et al., 2019). Thus, plants may have used~~preferentially use~~ large amounts of ~~tightly bound~~less mobile water that ~~is~~was strongly affected by evaporative effects in

405 the presented study, isotopically distinct from mobile water and groundwater, and with ~~showing~~ similar ~~enriched~~ isotopic ~~signals~~composition ~~and resulting in isotopic separation from mobile water and groundwater~~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

410 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.

~~This hypothesis is corroborated by the overlap in isotopic composition between root and bulk soil water at 0–60 cm depths~~

415 ~~(Fig. 2 and 5). However, our results showed that bulk soil water did not match root water isotopes at soil depths greater than~~

13

80 cm. 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. As shown in Fig. S4, root water does not match tightly bound water isotopes well at same depth. These results suggest that other factors may also contribute to isotopic offsets in addition to ecohydrological separation. Another possible explanation is that isotope fractionation occurs during uptake by roots, as previously reported for halophytic and xerophytic plant species (Lin and Sternberg, 1993; Ellsworth and Williams, 2007; Barbeta et al., 2019). Isotopic fractionation can explain isotopic mismatches between root water and bulk soil water at 80-160 cm depths. We speculate that fractionation also occurs at 0-60 cm depths, but bulk soil water in this layer is strongly affected by evaporation, resulting in the same enriched isotope signals, so isotopically enriched signals caused by soil evaporation mask plant fractionation signals. In contrast, soil evaporation is weaker in deep layers (80-160 cm), leading to a dampened isotopic enrichment signal in bulk soil water, while, root water isotopes are still relatively enriched due to the fractionation effect. Therefore, the isotopic offset between root water and bulk soil water was only observed in the deep soil layer. The maximum differences between bulk soil water and root water were −8.6 and −1.8‰ for Δ^2H and Δ^{18}O, respectively: lower than the differences between bulk soil water and xylem water reported by Barbeta et al. (2020) (ca. −11 and −2‰ for Δ^2H and Δ^{18}O, respectively) and Poca et al. (2019) (−24.6 and −2.9‰ for Δ^2H and Δ^{18}O, respectively). The inconsistency in results may be related to variations in arbuscular mycorrhiza (Poca et al., 2019), soil water loss and soil type (Vargas et al., 2017), fractionation in root xylem water transport (Martin Gomez et al., 2017) and plant species (Dubbert et al., 2019). We also detected a positive linear relationship between Δ^{18}O and Δ^2H in both soil-root and soil-xylem offsets (Fig. 6), suggesting that both hydrogen and oxygen isotopes changed simultaneously, in accordance with findings by Vargas et al. (2017) and Barbeta et al. (2020). Overall, these findings suggest that the isotopic offset between soil water and root water is likely governed by ecohydrological separation and plant fractionation, and may be not due solely to either of them, supporting our second hypothesis.

4.2 Root water and ~~xylem~~trunk water ~~isotopes~~isotopic composition

We found that ~~xylem~~trunk water mainly overlapped isotopically with root water at 100-160 cm depths (Fig. ~~2~~3), while the isotopically enriched root water at 0-80 cm depths was not reflected in the ~~xylem~~trunk water ~~isotopes~~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 δ^{18}O values of trunk water during our high frequency (ca. 3-day) sampling period from August 4 to September 15, 2019 (Fig. 4) and found that δ^2H and δ^{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, ~~isotope enrichment may have been present in the unsampled branches~~ we collected ~~xylem~~trunk water at ~~different~~150-450 cm tree heights ~~(150-450 cm)~~ on August 18,

14

2019, and found no significant differences (p > 0.05) (Fig. S~~5~~3). These results indicated that the trees always used a stable
water source during the study period~~Thus, we used root water isotopes to quantify the proportional use of soil water at different~~ ~~depths, and found that water from 100-160 cm layers accounted for 74% of the total (Fig. 7).~~ ~~Under the assumption that plant~~ ~~fractionation does not occur, o~~One possibility is that trees preferentially use ~~more~~ 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). ~~Furthermore~~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 isotop~~e signals~~ic 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 ~~xylem~~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). 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.

~~Another possibility is that water isotope heterogeneity in plant tissues may contribute to the isotopic deviation. Xylem water might not reflect current root water isotopic signals because it takes time to transport water from roots to branches and water also has residence times in branches and roots (Penna et al., 2018; Allen et al., 2019). For example, using deuterated water (D₂O) and heat as tracers to analyze water transport from the base of the trunk to the upper crown, Meinzer et al. (2016) found that transit times ranged from 2.5 to 21 days and residence times ranged from 36 to 79 days. However, we found that δ²H and δ¹⁸O values of xylem water remained stable (mean values: −66.68 ± 1.61 and −7.71 ± 0.24‰, respectively) during our high frequency (ca. 3 day) sampling period from August 4 to September 15, 2019 (Fig. 3), which does not support this interpretation. Moreover,~~ ~~to test the possibility that isotope enrichment may have been present in the unsampled branches we collected xylem water at different tree heights (150-450 cm) on August 18, 2019, and found no significant differences (p > 0.05) (Fig. S5).~~ ~~In addition, previous studies have also provided indications that xylem water isotope was more enriched than that of potential water sources due to fractionation effect during water transport in the xylem. This phenomenon has been reportedly associated with temporal declines in sap flow rates (Martin-Gomez et al., 2017), water exchange between phloem and xylem (Cernusak et al., 2005) and leafless or newly leafed deciduous species (e.g., Quercus laevis and Carya floridana) (Ellsworth and Sternberg, 2015). However, we found that the xylem water contained more unenriched isotopic signal from deep roots than enriched isotopic signal from shallow roots. The result show that there was no fractionation during water transport from root to xylem (Fig. 8).~~ ~~Thus, we used root water isotopes to quantify the proportional use of soil water at different depths, and found that water from 100-160 cm layers accounted for 74% of the total (Fig. 7).~~ ~~In conclusion, replacement of soil water by root water in analyses provides a means for accurately quantifying plant water sources.~~

15

5 Conclusion

At our study site during the ~~covered~~ 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.~~xylem water of *S. matsudana* trees was isotopically isolated from bulk soil water, mobile water and groundwater, supporting our first hypothesis that isotopic offset occurred between xylem water and potential water sources. We further detected the cause and location of this mismatch.~~ In the soil matrix, bulk soil water generally had ~~higher~~ lower δ^2H and $\delta^{18}O$ 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~~water flow paths, following the isotopic patterns in the TWW hypothesis~~. 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.

~~The isotopic composition of root water overlapped with that of bulk soil water at 0-60 cm depths, in association with plants' use of bulk soil water as in the TWW framework. However, root water deviated significantly from bulk soil water isotopically at 80-160 cm depths and the maximum difference between bulk soil water and root water was −8.6 and −1.8‰ for δ^2H and $\delta^{18}O$, respectively. These findings suggest isotopic offset occurred at root-soil interface, probably due to a combination of ecohydrological separation, as in the TWW hypothesis, and plant fractionation, supporting our second hypothesis. In contrast, no isotopic fractionation occurred during root to xylem water transport. Isotopically, xylem water of *S. matsudana* trees mainly overlapped with root water isotopes at 100-160 cm depths and the contribution of these root layers to xylem water reached 74%. Our results challenge current understanding of the behavior of H and O isotopes, extending our knowledge of isotopic signals in the soil-root-xylem continuum and providing valuable insights into fundamental ecohydrological process.~~

16

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

525 Adams, R.E., Hyodo, A., SantaMaria, T., Wright, C.L., Boutton, T.W., and West, J.B.: Bound and mobile soil water isotope ratios are affected by soil texture and mineralogy, whereas extraction method influences their measurement, Hydrol. Process., 34, 991-1003, 2020.

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.

530 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.

535 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.

Bowling, D.R., Schulze, E.S., and Hall, S.J.: Revisiting streamside trees that do not use stream water: can the two water worlds hypothesis and snowpack isotopic effects explain a missing water source? Ecohydrol., 10, e1771, 2017.

Brandes, E., Wenninger, J., Koeniger, P., Schindler, D., Rennenberg, H., Leibundgut, C., Mayer, H., and Gessler, A.: Assessing
540 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.

Brooks, J.R., Barnard, H.R., Coulombe, R., and McDonnell, J.J.: Ecohydrologic separation of water between trees and streams in a Mediterranean climate, Nat. Geosci., 3, 100-104, 2010.

Cernusak, L.A., Farquhar, G.D., and Pate, J.S.: Environmental and physiological controls over oxygen and carbon isotope
545 composition of Tasmanian blue gum, *Eucalyptus globulus*, Tree Physiol., 25, 129-146, 2005.

Chang, E,H., Li, P., Li, Z.B., Xiao, L., Zhao, B.H., Su, Y.Y., and Feng, Z.H.: Using water isotopes to analyze water uptake during vegetation succession on abandoned cropland on the Loess Plateau, China, Catena, 181, 104095, 2019.

Chen, Y.L., Helliker, B.R., Tang, X.H., Li, F., Zhou, Y.P., and Song, X.: Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water, Proc. Natl. Acad. Sci. U. S. A., 202014422, 2020.

550   Craig, H.: Isotopic variations in meteoric waters. Science, 133, 1702-1703, 1961.

Dubbert, M., Caldeira, M.C., Dubbert, D., and Werner, C.: A pool-weighted perspective on the two-water-worlds hypothesis, New Phytol., 222, 1271-1283, 2019.

Ehleringer, J.R., and Dawson, T.E.: Water uptake by plants: perspectives from stable isotope composition, Plant Cell Environ., 15, 1073-1082, 1992.

555   ~~Ellsworth, P.Z., and Sternberg, L.S.L.: Seasonal water use by deciduous and evergreen woody species in a scrub community is based on water availability and root distribution, Ecohydrol., 8, 538-551, 2015.~~

Ellsworth, P.Z., and Williams, D.G.: Hydrogen isotope fractionation during water uptake by woody xerophytes, Plant Soil, 291, 93-107, 2007.

~~Evaristo, J., Jasechko, S., and McDonnell, J.J.: Global separation of plant transpiration from groundwater and streamflow,~~
560   ~~Nature, 525, 91-94, 2015.~~

Evaristo, J., McDonnell, J.J., Scholl, M.A., Bruijnzeel, L.A., and Chun, K.P.: Insights into plant water uptake from xylem-water isotope measurements in two tropical catchments with contrasting moisture conditions, Hydrol. Process., 30, 3210-3227, 2016.

Evaristo, J., Kim, M., van Haren, J., Pangle, L.A., Harman, C.J., Troch, P.A., and McDonnell, J.J.: Characterizing the fluxes
565   and age distribution of soil water, plant water and deep percolation in a model tropical ecosystem. Water Resour. Res., 55, 3307-3327, 2019.

Gaj, M., Kaufhold, S., Koeniger, P., Beyer, M., Weiler, M., and Himmelsbach, T.: Mineral mediated isotope fractionation of soil water, Rapid Commun. Mass Spectrom., 31, 269-280, 2017.

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

Gong, T.X., Zhu, Y.J, and Shao, M.A.: Spatial distribution of caliche nodules in surface soil and their influencing factors in the Liudaogou catchment of the northern Loess Plateau, China, Geoderma, 329, 11-19, 2018.

Good, S.P., Noone, D., and Bowen, G.: Hydrologic connectivity constrains partitioning of global terrestrial water fluxes, Science, 349, 175-177, 2015.

575   Jiang, P.P., Wang, H.M., Meinzer, F.C., Kou, L., Dai, X.Q., and Fu, X.L.: Linking reliance on deep soil water to resource economy strategies and abundance among coexisting understory shrub species in subtropical pine plantations, New Phytol., 225, 222-233, 2020.

Knighton, J., Kuppel, S., Smith, A., Soulsby, C., Sprenger, M., and Tetzlaff, D.: Using isotopes to incorporate tree water storage and mixing dynamics into a distributed ecohydrologic modelling framework, Ecohydrol., 13, e2201, 2020a.

580   Knighton, J., Singh, K., and Evaristo, J.: Understanding catchment-ccale forest root water uptake strategies across the continental United States through inverse ecohydrological modeling, Geophys. Res. Lett., 47, e2019GL085937, 2020b.

18

Kubert, A., Paulus, S., Dahlmann, A., Werner, C., Rothfuss, Y., Orlowski, N., and Dubbert, M.: Water stable isotopes in ecohydrological field research: comparison between in situ and destructive monitoring methods to determine soil water isotopic signatures. Front. Plant Sci., 11, 387, 2020.

585 Kuhnhammer, K., Kubert, A., Bruggemann, N., Diaz, P.D., van Dusschoten, D., Javaux, M., Merz, S., Vereecken, H., Dubbert, M., and Rothfuss, Y.: Investigating the root plasticity response of *Centaurea jacea* to soil water availability changes from isotopic analysis, New Phytol., 226, 98-110, 2020.

Landwehr, J.M., and Coplen, T.B.: Line-conditioned excess: a new method for characterizing stable hydrogen and oxygen isotope ratios in hydrologic systems, in: International Conference on Isotopes in Environmental Studies, Aquatic Forum,
590 Monte-Carlo, Monaco, IAEA, Vienna, 132-134, 2006.

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.

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.

595 McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D.L., Helmisaari, H.S., Hobbie, E.A., Iversen, C.M., Jackson, R.B., Leppalammi-Kujansuu, J., Norby, R.J., Phillips, R.P., Pregitzer, K.S., Pritchard, S.G., Rewald, B., and Zadworny, M.: Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes, New Phytol., 207, 505-518, 2015.

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

McDonnell, J.J.: The two water worlds hypothesis: ecohydrological separation of water between streams and trees? WIREs Water 1, 323-329, 2014.

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

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

Oerter, E.J., and Bowen, G.: In situ monitoring of H and O stable isotopes in soil water reveals ecohydrologic dynamics in managed soil systems, Ecohydrology, 10, e1841, 2017.

Orlowski, N., Breuer, L., Angeli, N., Boeckx, P., Brumbt, C., Cook, C.S., Dubbert, M., Dyckmans, J., Gallagher, B., Gralher,
610 B., Herbstritt, B., Herve-Fernandez, P., Hissler, C., Koeniger, P., Legout, A., Macdonald, C.J., Oyarzun, C., Redelstein, R., Seidler, C., Siegwolf, R., Stumpp, C., Thomsen, S., Weiler, M., Werner, C., and McDonnell, J.J.: Inter-laboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water, Hydrol. Earth Syst. Sci., 22, 3619-3637, 2018.

Parnell, A.C., Inger, R., Bearhop, S., and Jackson, A.L.: Source partitioning using stable isotopes: coping with too much
615 variation, Plos One, 5, e9672, 2010.

Penna, D., Geris, J., Hopp, L., and Scandellari, F.: Water sources for root water uptake: Using stable isotopes of hydrogen and oxygen as a research tool in agricultural and agroforestry systems, Agric. Ecosyst. Environ., 291, 106790, 2020.

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.

Rothfuss, Y., and Javaux, M.: Reviews and syntheses: isotopic approaches to quantify root water uptake: a review and comparison of methods, Biogeosciences, 14, 2199-2224, 2017.

Schlesinger, W.H., and Jasechko, S.: Transpiration in the global water cycle, Agric. For. Meteorol., 189-190, 115-117, 2014.

Sprenger, M., and Allen, S.T.: What ecohydrologic separation is and where we can go with it, Water Resour. Res., 56, e2020WR027238, 2020.

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., Leistert, H., Gimbel, K., and Weiler, M.: Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes. Rev. Geophs., 54, 674-704, 2016.

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.

Wang, D.D., Yu, Z.T., Peng, G., Zhao, C.Y., Ding, J.L., and Zhang, X.L.: Water use strategies of Populus euphratica seedlings under groundwater fluctuation in the Tarim River Basin of Central Asia, Catena, 166, 89-97, 2018.

Wang, J., Fu, B.J., Wang, L.X., Lu, N., and Li, J.Y.: Water use characteristics of the common tree species in different plantation types in the Loess Plateau of China, Agric. For. Meteorol., 288-289, 108020, 2020.

Xiang, W., Si, B.C., Biswas, A., and Li, Z.: Quantifying dual recharge mechanisms in deep unsaturated zone of Chinese Loess Plateau using stable isotopes, Geoderma, 337, 773-781, 2019.

Yang, F.T., Feng, Z.M., Wang, H.M., Dai, X.Q., and Fu, X.L.: Deep soil water extraction helps to drought avoidance but shallow soil water uptake during dry season controls the inter-annual variation in tree growth in four subtropical plantations, Agric. For. Meteorol., 234-235, 106-114, 2017.

Zhao, L.J., Wang, L.X., Cernusak, L.A., Liu, X.H., Xiao, H.L., Zhou, M.X., and Zhang, S.Q.: Significant difference in hydrogen isotope composition between xylem and tissue water in *Populus Euphratica*, Plant Cell Environ., 39, 1848-1857, 2016.

650   Zhao, Y., and Wang, L.: Plant water use strategy in response to spatial and temporal variation in precipitation patterns in China: a stable isotope analysis, Forests, 9, 123, 2018.

Zhao, Y., Wang, L., Knighton, J., Evaristo, J., and Wassen, M.: Contrasting adaptive strategies by *Caragana korshinskii* and *Salix psammophila* in a semiarid revegetated ecosystem. Agric. For. Meteorol., 300, 108323, 2021.

Zheng, W.B., Wang, S.Q., Sprenger, M., Liu, B.X., and Cao, J.S.: Response of soil water movement and groundwater recharge
655   to extreme precipitation in a headwater catchment in the North China Plain, J. Hydrol., 576, 466-477, 2019.

Zhu, Y.J., and Shao, M.A.: Variability and pattern of surface moisture on a small-scale hillslope in Liudaogou catchment on the northern Loess Plateau of China, Geoderma, 147, 185-191, 2008.

660
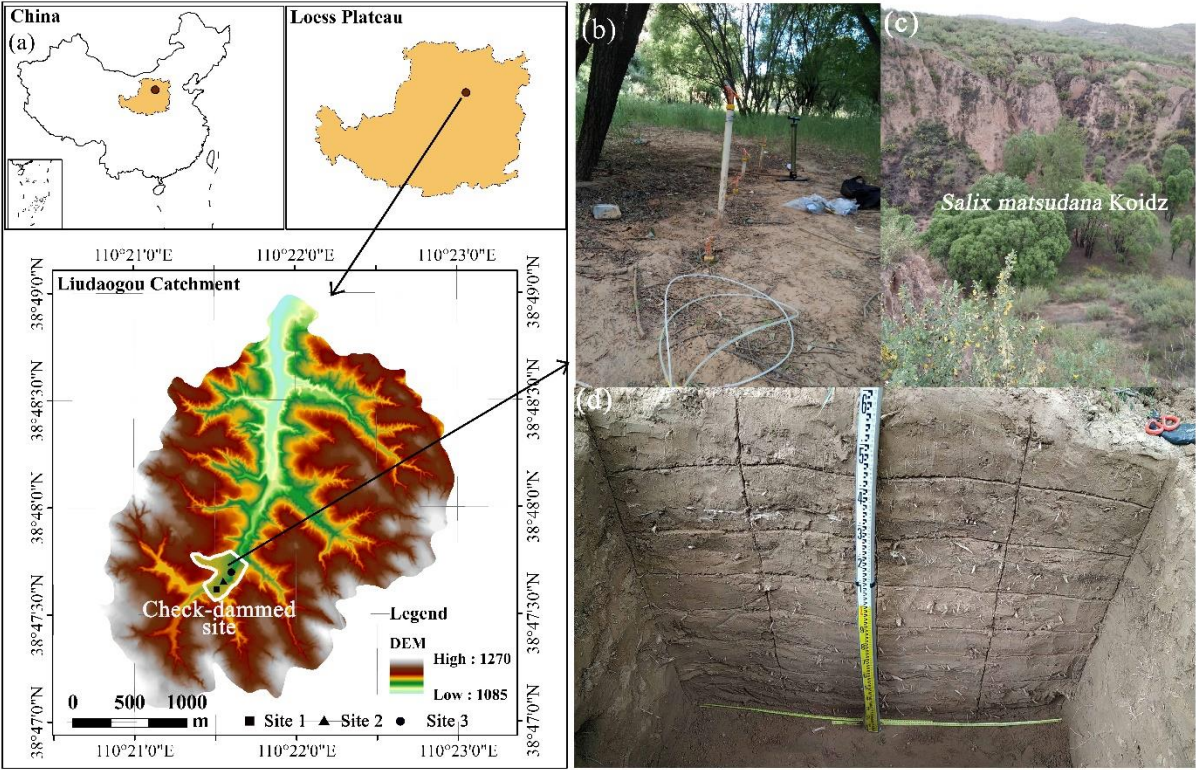
Figure 1: (a) Location of the study area on the Loess Plateau, China. (b) Photograph of mobile water collection using suction lysimeters (white plastic tubes) (with application of 60 kPa tension), and (c) *Salix matsudana* Koidz, our sampling tree. (d) Profile of the soil cuboid (length, width and depth: 160, 80 and 160 cm, respectively) being dug to
665   **obtain root isotopic data. 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.**
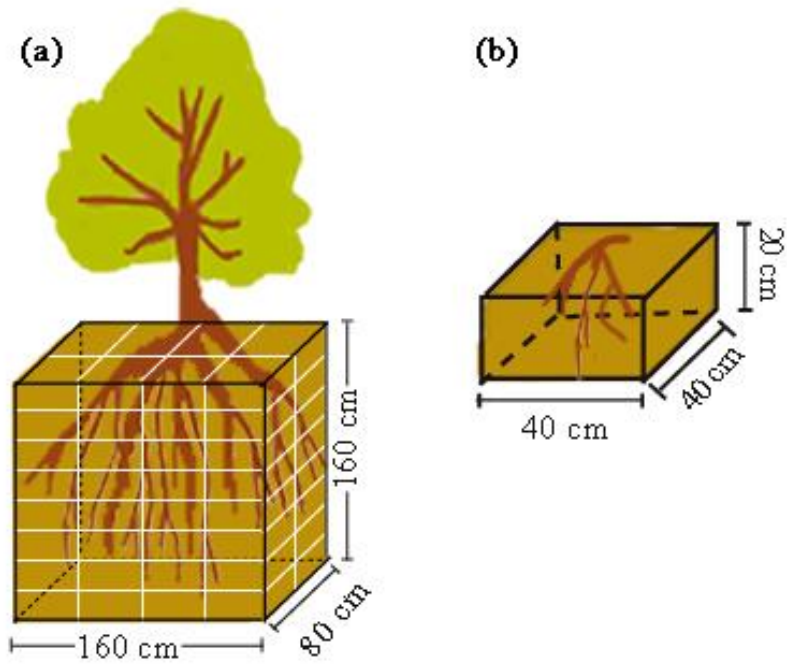
Figure 2: Schematic diagram of root excavations (a) and measurements (b) as described in Section 2.2

670

675
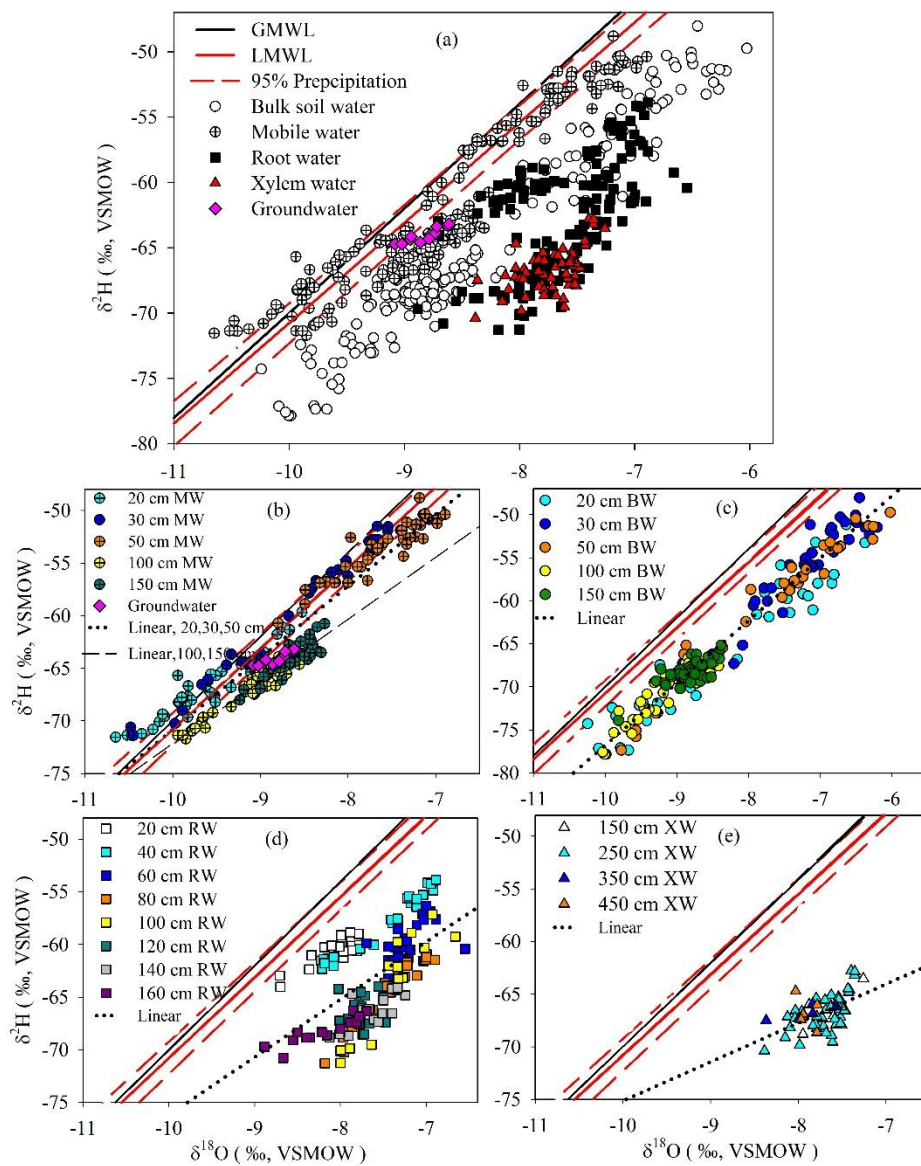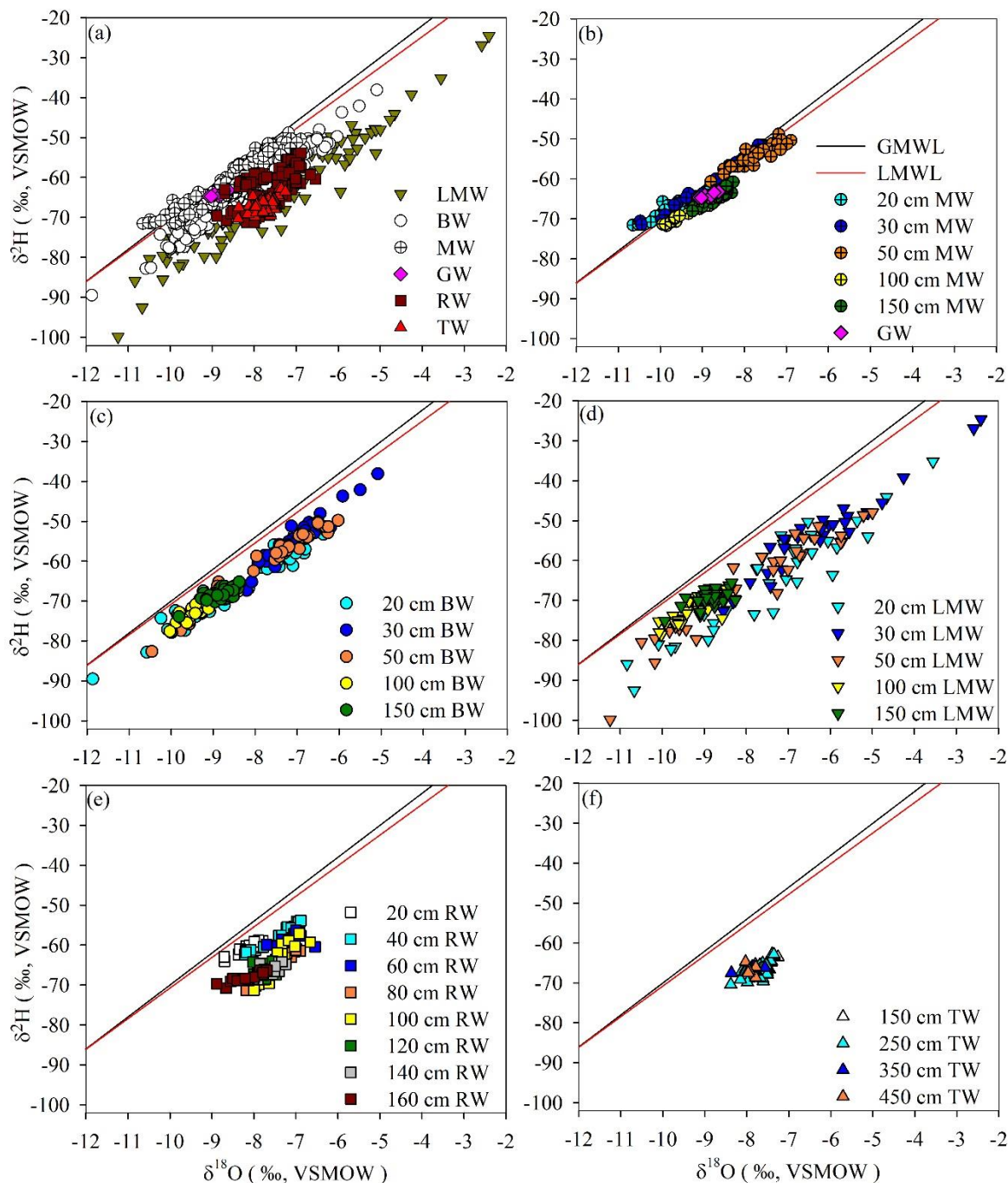
Figure 2 3 (a) δ¹⁸O and δ²H isotope valuesic composition collected from August 4 to September 15, 2019. Plotted values include bulk soil water (BW), mobile water (MW), root water (RW), xylem trunk water (XWTW), less mobile water (LMW) and groundwater (GW). (b) δ¹⁸O and δ²H isotope isotopic values composition of groundwaterGW, and MW collected from different depths, (c) BW

24

collected from different depths, (d) <u>LMW collected from different depths, (e)</u> RW collected from different depths, and (e<u>f</u>) ~~XW~~ <u>TW</u>
collected from different tree heights. The red line represents the 2016-2019 local meteoric water line (LMWL, $\delta^2H = 5.91 + 7.67 \, \delta^{18}O$,
$R^2 = 0.96$) ~~and 95% confidence interval of precipitation~~. 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.~~
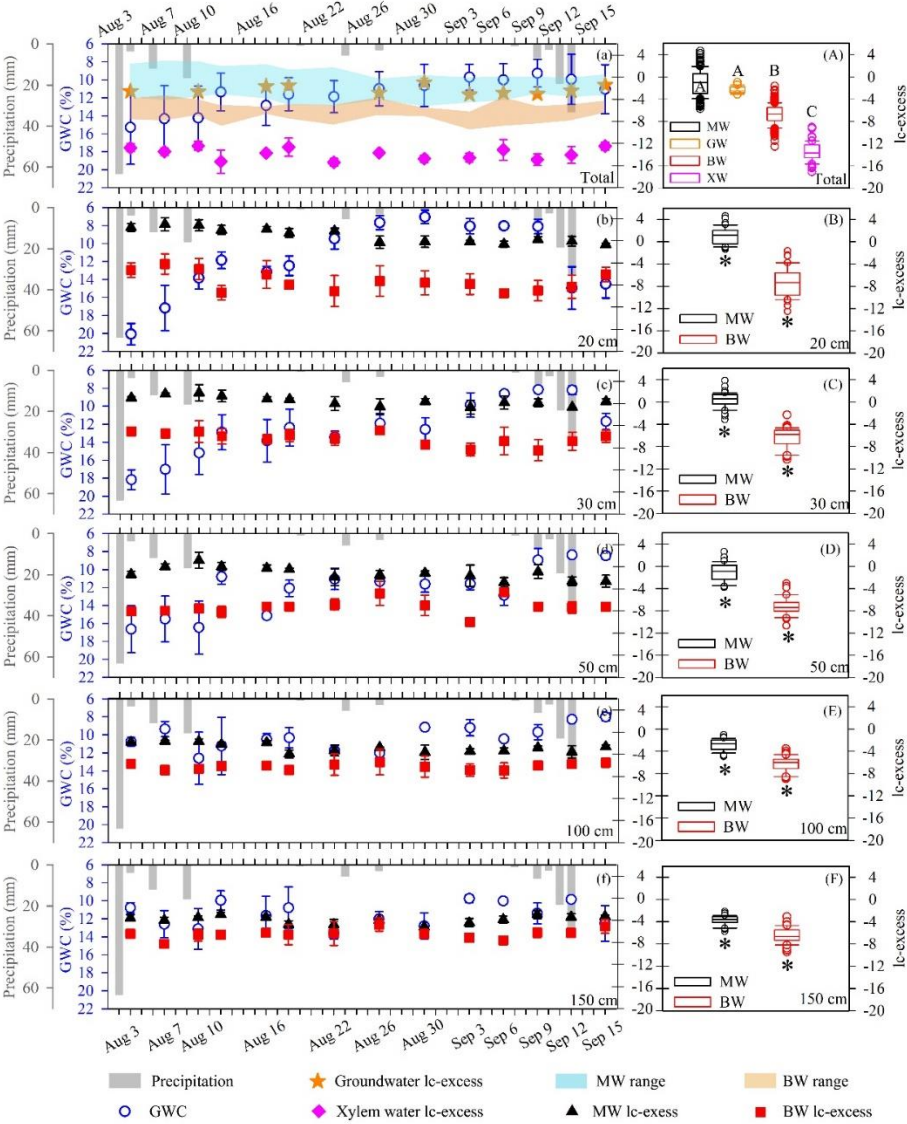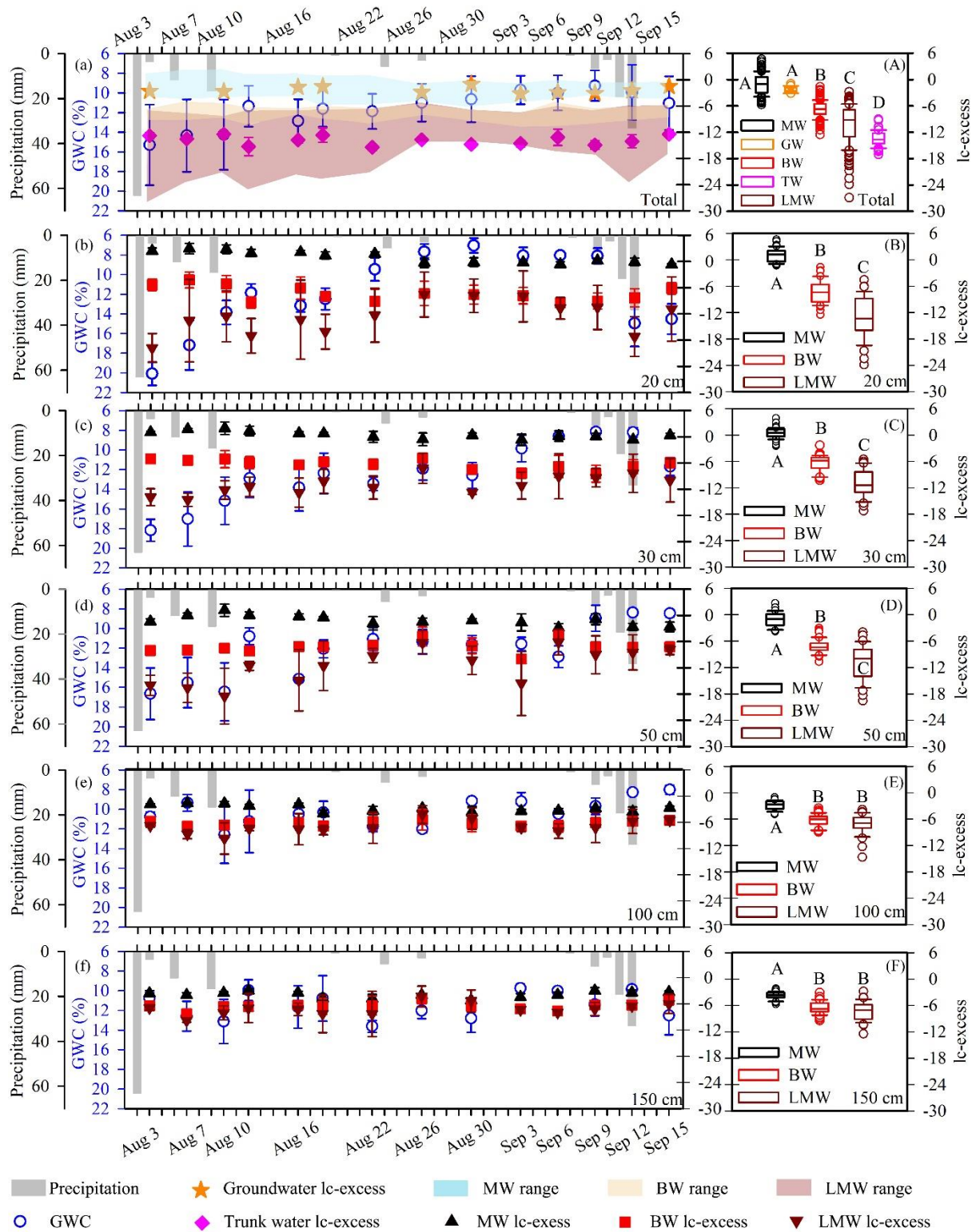
685 **Figure ~~3~~ 4 (a-f) Temporal dynamics of hydrological conditions (precipitation and gravimetric water content, GWC) and lc-excess values (these values are means and standard deviations for three sites) of groundwater (GW), ~~xylem~~ trunk water (~~XW~~TW), mobile water (MW) ~~and,~~ bulk soil 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 ~~XW~~ LMW (N=~~61~~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;**

690 **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. ~~Xylem~~ Trunk water and potential water sources that do not share a letter are significantly different (p < 0.05, Tukey-Kramer HSD). ~~Asterisks show significantly differing lc-excess values between mobile water and bulk soil water at the same depth (p < 0.05).~~**

695

28
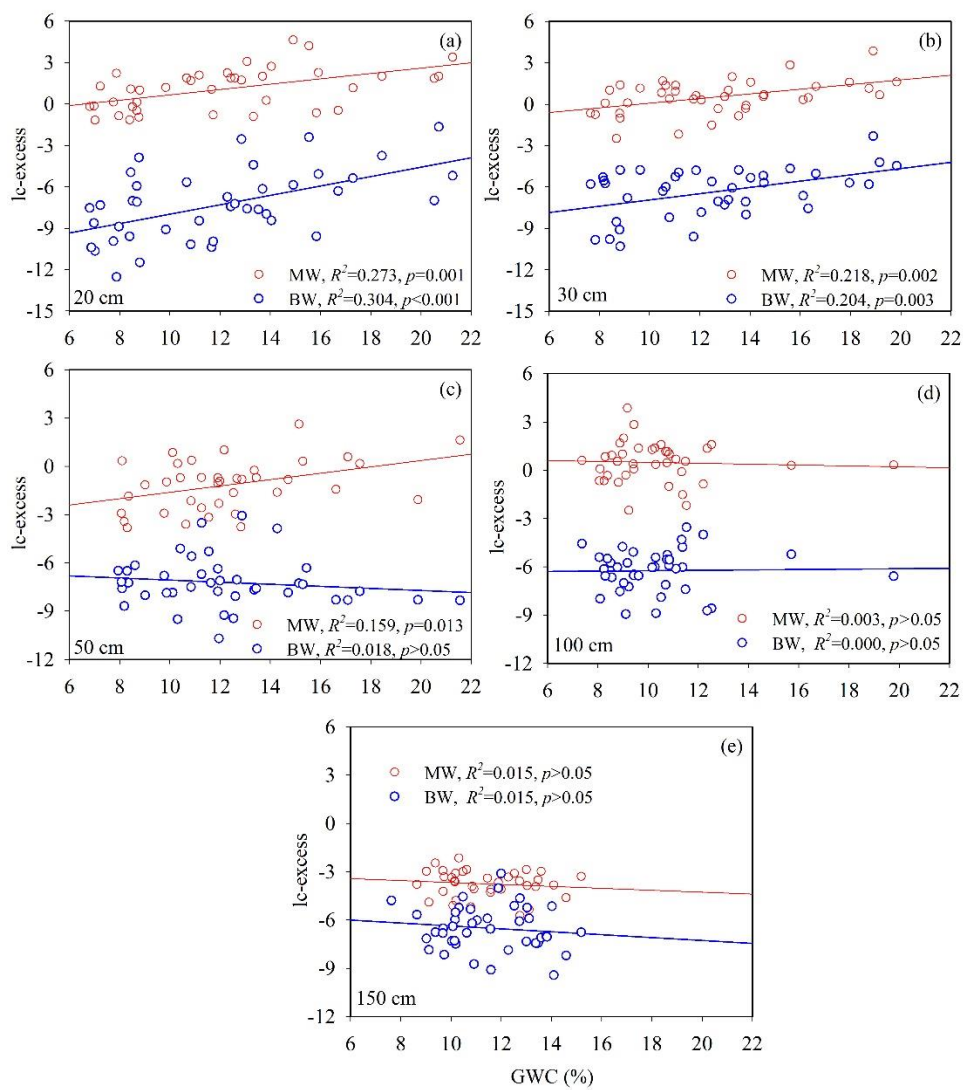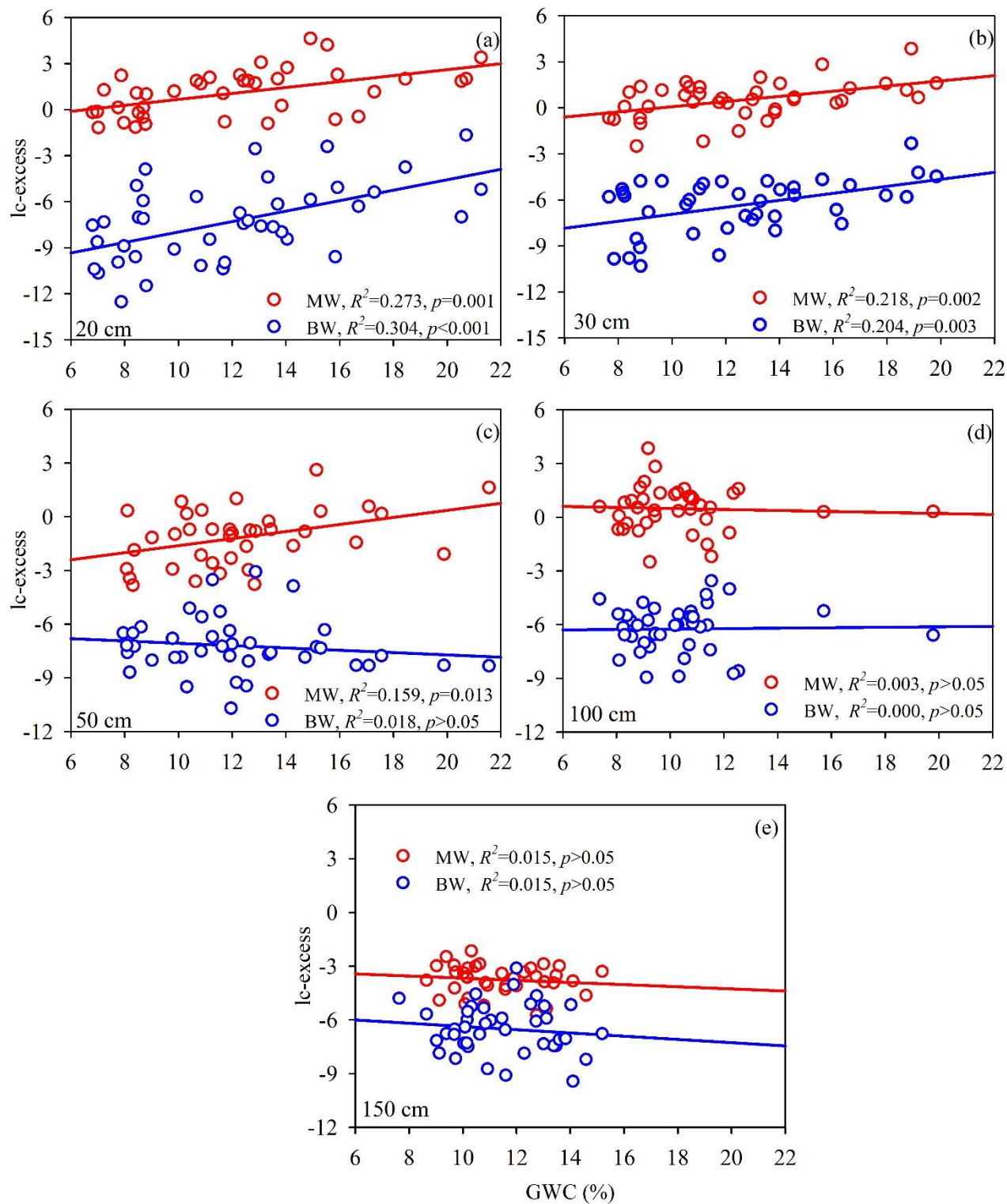
Figure 4-5 Relationships between gravimetric water content (GWC) and (a) lc-excess values at 20 cm depth, (b) lc-excess values at 30 cm depth, (c) lc-excess values at 50 cm depth, (d) lc-excess values at 100 cm depth and (e) lc-excess values at 150 cm depth. Data from lc-excess values of mobile water (MW) and bulk soil water (BW) are shown in red and blue circles, respectively. The insets show the fitness of the linear regressions.
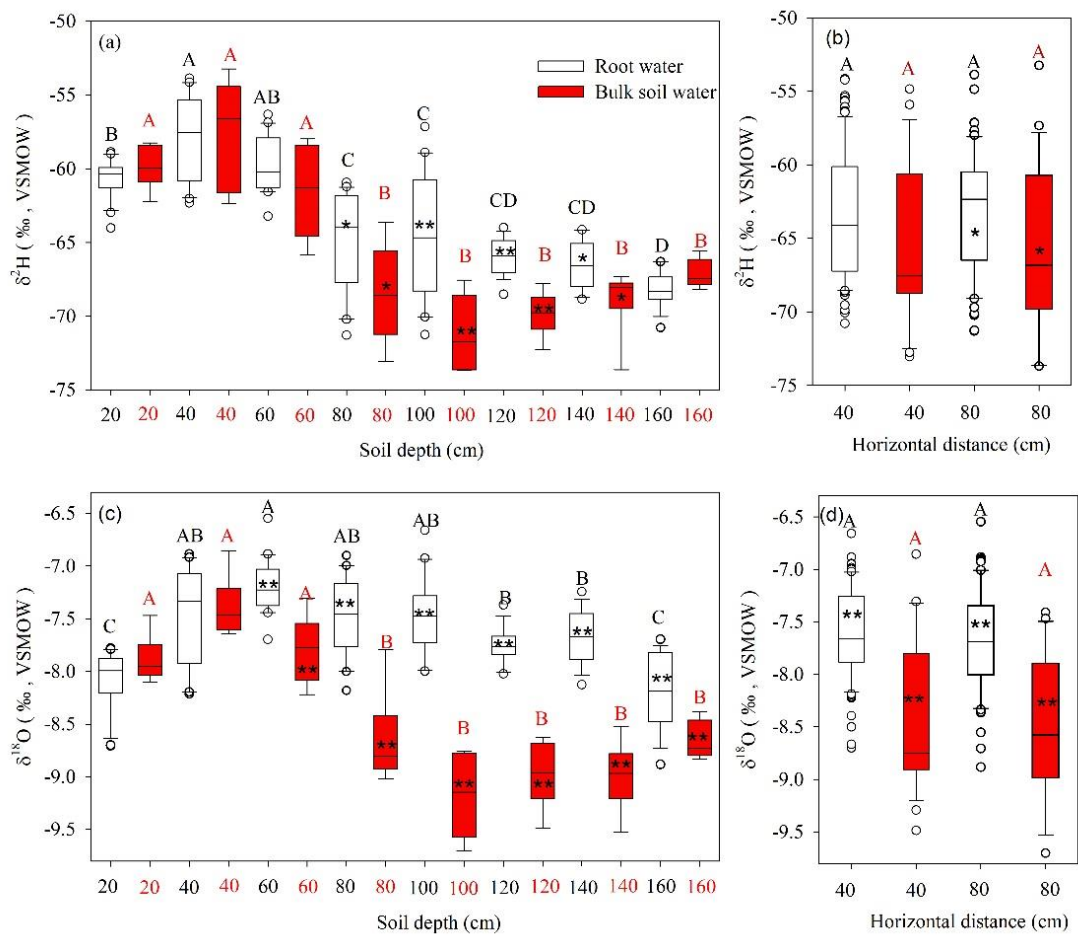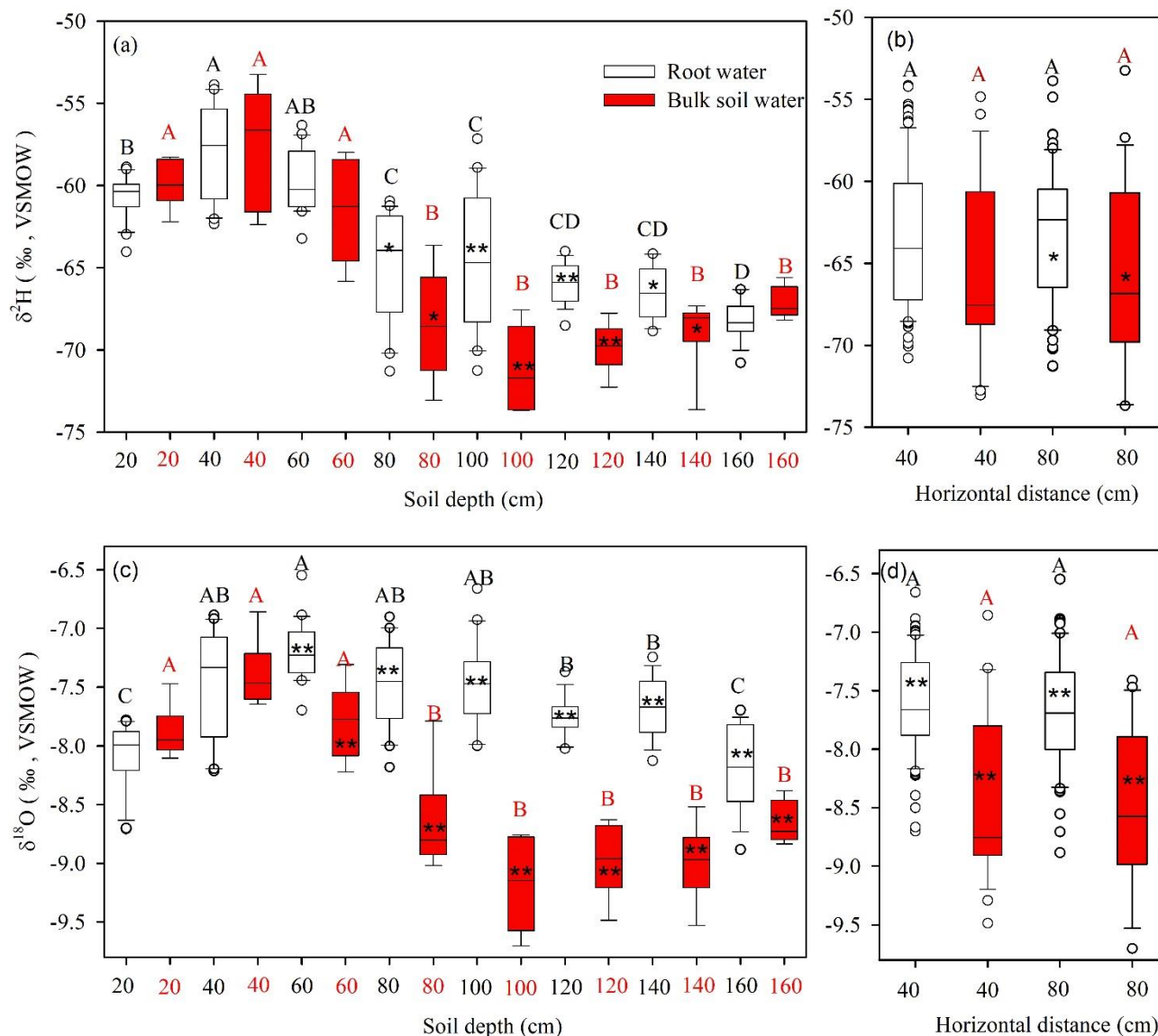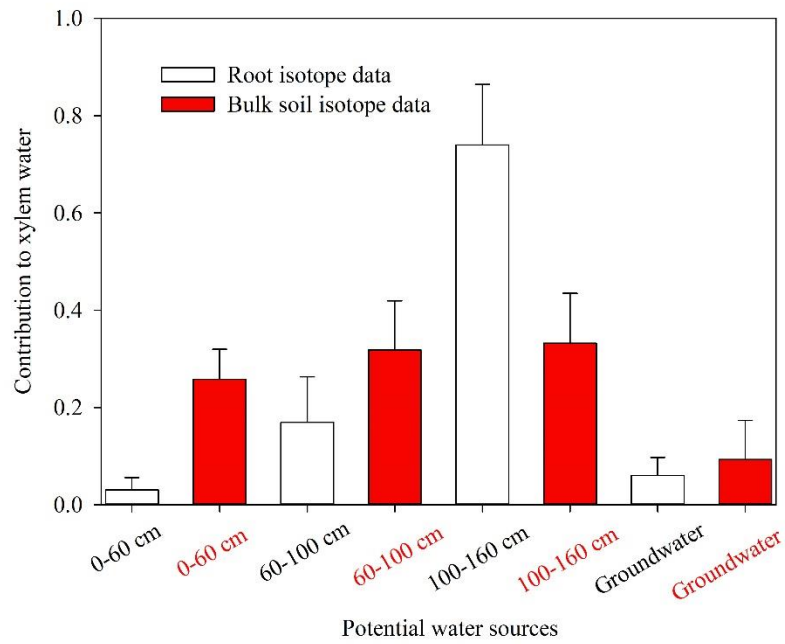
Figure 5 6 Boxplots of root water and bulk soil water stable isotopes composition (δ^2H and $\delta^{18}O$) at indicated depths (a, c) and horizontal distances from the tap root of the focal root system (b, d). The top and bottom of each box are the 25th and 75th percentiles of the samples, respectively. The black line in each box is the sample median. Asterisks indicate significantly differing isotopic values composition between soil water and root water (* and **: $p < 0.05$ and $p < 0.01$, respectively, according to two-tailed tests). Plant root water isotopes 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).
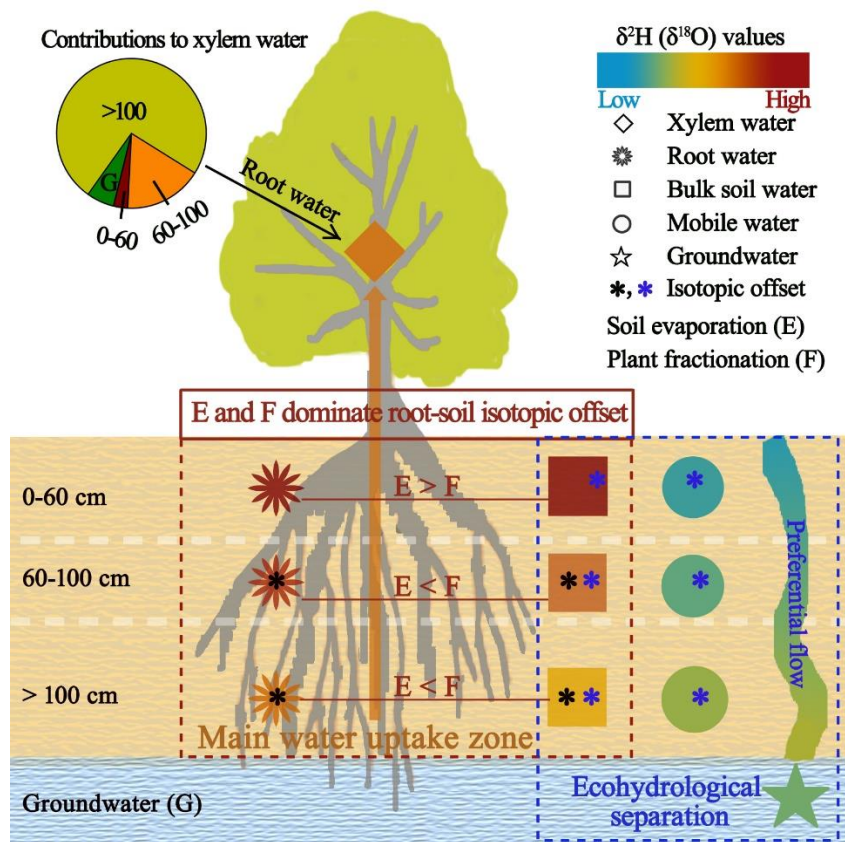
31
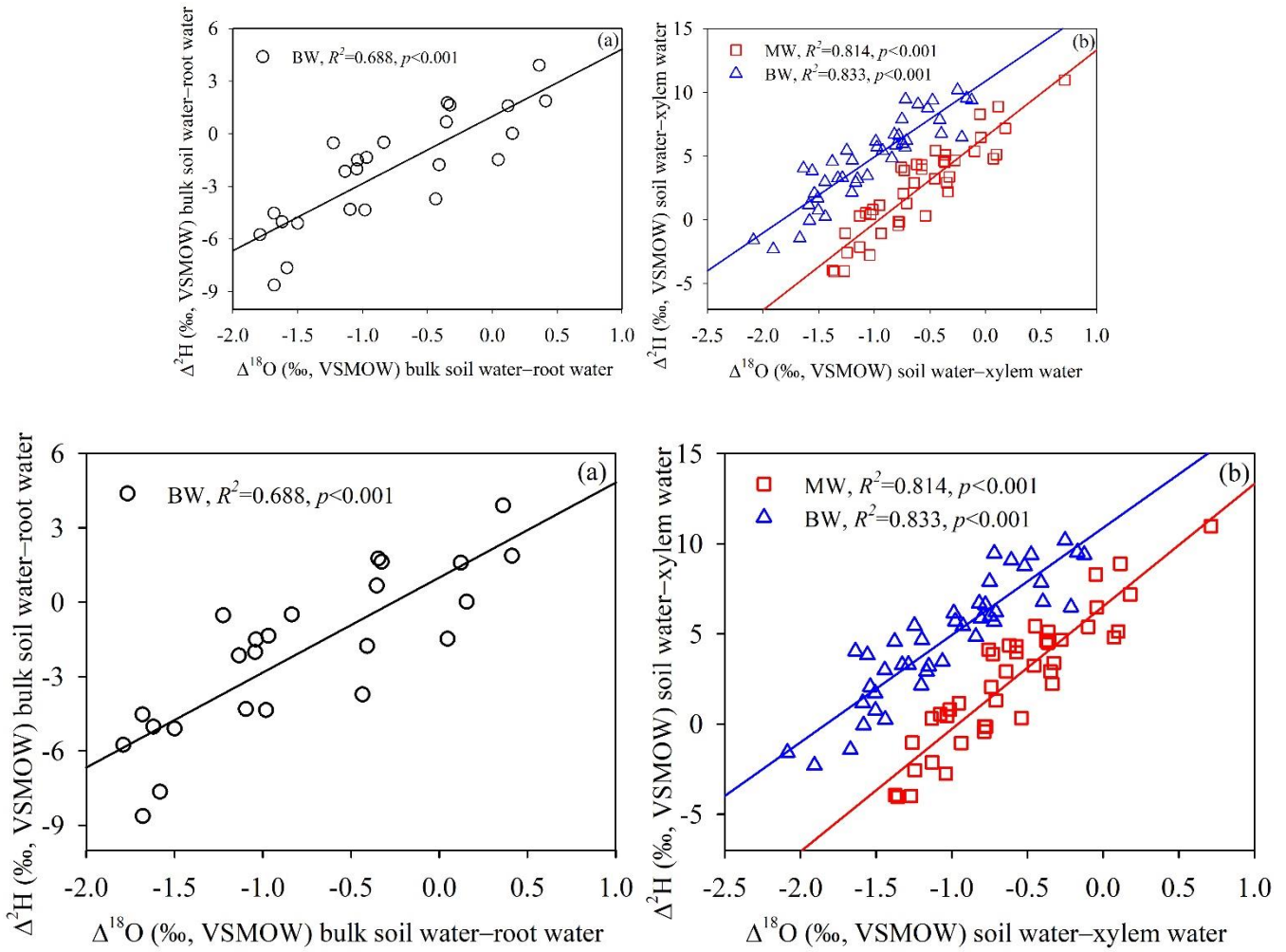
Figure 6 7 (a) Relationship of hydrogen isotope offset (Δ^2H, Δ^2H = δ^2H$_{soil}$ − δ^2H$_{root}$) and oxygen isotope offset (Δ^{18}O, Δ^{18}O = δ^{18}O$_{soil}$ − δ^2O$_{root}$^{18}O$_{root}$) between bulk soil water and root water, according to analyses of samples of bulk soil water (BW) and root water collected from 0-160 cm depths on August 18, 2019. (b) Relationship of hydrogen isotope offset (Δ^2H, Δ^2H = δ^2H$_{soil}$ − δ^2H$_{xylem}$^{2}H$_{trunk}$) and oxygen isotope offset (Δ^{18}O, Δ^{18}O = δ^{18}O$_{soil}$ − δ^2O$_{xylem}$^{18}O$_{trunk}$) between soil water and xylem trunk water, according to analyses of samples for bulk soil water, mobile water (MW) and xylem trunk water collected from August 4 to September 15, 2019. The insets show the fitness of the linear regressions (a-b).

715

720

32

725 Figure 7 Contributions of potential water sources to plant xylem water, based on analyses of samples of root water and bulk soil water isotopes collected on August 18, 2019.
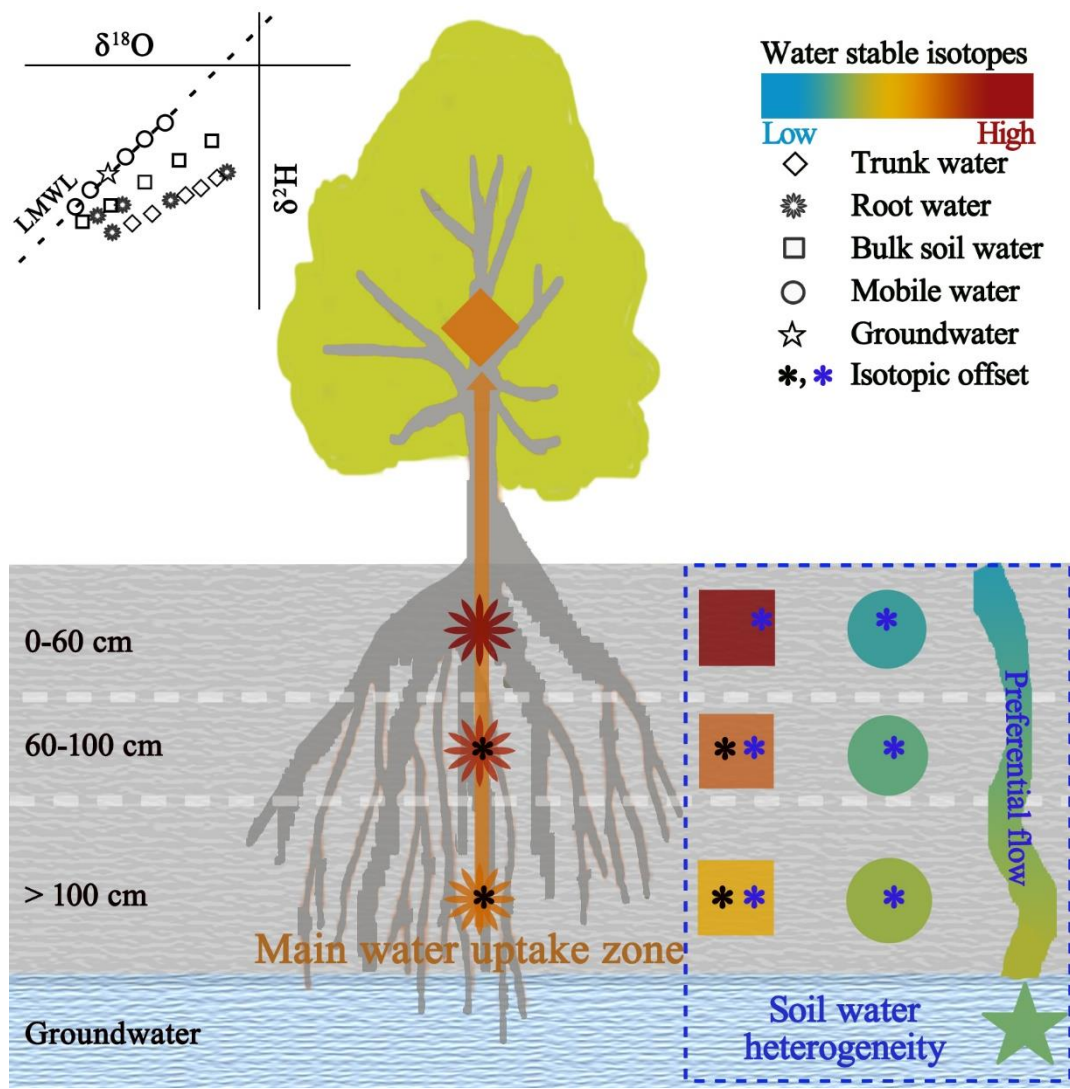
Contributions to xylem water

>100

G

0-60 60-100

Root water

δ^2H ($\delta^{18}O$) values

Low   High

◇ Xylem water
✳ Root water
□ Bulk soil water
○ Mobile water
☆ Groundwater
✱, ✱ Isotopic offset

Soil evaporation (E)
Plant fractionation (F)

E and F dominate root-soil isotopic offset

0-60 cm   E > F

60-100 cm   E < F

> 100 cm   E < F

Main water uptake zone

Groundwater (G)

Ecohydrological
separation

Preferential flow

Figure 8 Schematic diagram of isotopic dynamics along the soil-root-~~xylem~~ trunk continuum. Color codes indicate isotopic ~~signals~~
composition of mobile water, bulk soil water and root water at indicated depths, groundwater and ~~xylem~~ trunk water (from blue to high
representing low to high). ~~The pie chart represents the contributions of potential water sources to xylem water obtained using the~~
~~Bayesian mixing model SIAR based on both δ²H and δ¹⁸O values of root and groundwater (G) samples.~~ 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) and lc-excess values (Fig. 4) 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)