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

Ying Zhao¹,², Li Wang¹,³

¹State 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
²University of Chinese Academy of Sciences, Beijing 100049, China
³College 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 xylemtrunk 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 xylemtrunk. Thus, we measured the specific isotopic composition (δ²H and δ¹⁸O) of each component (e.g., bulk soil water, mobile water, groundwater, xylemtrunk water, and root water of Salix matsudana Koidz trees) with about three-day high temporal resolution to analyze isotopic dynamics in the soil-root-xylemtrunk continuum. We report three main findings. First, we detected clear separation between mobile water and bulk soil water isotopic signal 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 of 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 δ²H and δ¹⁸O 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 δ²H and δ¹⁸O between bulk soil water and root water were −8.6 and −1.8‰, respectively. Third, xylemtrunk 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

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 cycles. 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 in water (δ¹⁸O and δ²H) 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 xylem trunk water effectively reflects that of water sources. Thus, by comparing δ¹⁸O and δ²H 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 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). The contributions of these factors to the isotopic deviations are uncertain. Analyses of isotopic signals (δ²H and δ¹⁸O) 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 δ²H and δ¹⁸O values in soil water and xylem water, but not groundwater and streams (Evaristo et al., 2015). The isotopic offset between plant xylem water and 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 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 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 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 δ²H and δ¹⁸O values, respectively, between soil and plant xylemtrunk 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 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, incomplete extraction of water during cryogenic distillation could fractionate 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 stemtrunk water and cryogenically extracted and source water in nine woody plant species and demonstrated that this offset stems from a cryogenic extraction–associated methodological artifacts during cryogenic vacuum extraction. Thus, the extracted water does not properly represent the water available to plants, and may contribute to apparent xylemtrunk–soil water isotopic offsets.

Explanation of the isotopic offset between soil and xylemtrunk water is essential, but identifying roles of specific processes is generally hindered by the diversity of mechanisms that may be involved (e.g., water isotope 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–xylemtrunk 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 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 xylemtrunk 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 isotope composition dynamics of each component in the water transport process along the soil-root–xylemtrunk continuum. More specifically, we exploited the specific isotope–fingerprint composition (δ²H and δ¹⁸O values) of mobile water, bulk soil water, groundwater and xylem water of Salix matsudana trees, and derived line-conditioned excess (le-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 km² 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, 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 tree for sampling. The average age and height of the trees are about 30 years and 12 m, respectively. The soil at the site includes sandy loam and loam according to the USDA classification system (Table 1), with its bulk density 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.).
2.2 Measurements of roots and soil properties

We collected 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 sites, on August 18, 2019, to measure roots’ 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−1, width=40 cm−1, height=20 cm) (Fig. 1d and 1e) 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 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 1-2 and we also collected undisturbed soil samples at 20, 30, 50, 100 and 150 cm depths using cutting rings (100 cm3 in-volume) to obtain water retention curves at the same sampling site. These samples were took to the laboratory and determined 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).

2.3 Water sampling for stable isotope (δ2H and δ18O) analysis

Previously unpublished data we obtained have shown that the isotopic composition of xylem 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, 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 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, 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 campaigns. 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 was peeled from the twigs and all leaves were removed to avoid perturbation of xylem trunk water isotopic signatures 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 parafilm. 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, xylem 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 °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 δ²H and δ¹⁸O 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 is isotopic composition was 0.5 and 0.1‰, respectively. The isotopic composition (²H to ¹H and ¹⁸O to ¹⁶O 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 (δ²H and δ¹⁸O values), calculated as follows:
\[
\delta^2H(\%) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1
\]

\[
\delta^{18}O(\%) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1
\]

2.5 Methods for assessing ecohydrological separation and determining plant water sources

To test the TWW hypothesis, we calculated the line-conditioned excess (lc-excess) values of bulk soil water, mobile water, groundwater and xylem 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:

\[
lc - \text{excess} = \delta^2H_s - a\delta^{18}O_s - b
\]

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 \(\delta^2H\) 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 estimated \(\delta^2H\) and \(\delta^{18}O\) values of tightly bound water to test the TWW hypothesis. 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:

\[
\delta_{\text{LMW}} = \frac{\delta_{\text{BW}} \cdot \theta_{\text{BW}} - \delta_{\text{MW}} \cdot \theta_{\text{MW}}}{\theta_{\text{LMW}}}
\]

Here, \(\delta\) and \(\theta\) represent the isotopic composition and GWC of samples, respectively, while, the subscripts ‘TW’, ‘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. matsu 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 (\(\delta^2H\) 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 \(\delta^2H\) 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
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 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

#### 3.1 Dual-isotope plots

Plots of the stable isotopic composition (δ²H and δ¹⁸O) 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, δ²H = 7.67 δ¹⁸O + 5.91, R² = 0.96) were lower than those of the global meteoric water line (GMWL, δ²H = 8 δ¹⁸O + 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), at 20, 30 and 50 cm depths (δ²H = 6.70 δ¹⁸O – 2.10, R² = 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 (δ²H = 5.91 δ¹⁸O – 13.05, R² = 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 (δ²H = 7.25 δ¹⁸O – 4.35, R² = 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 overlapped with root water and trunk water (Fig. 3a and d). Root water (δ²H = 5.47 δ¹⁸O – 21.46, R² = 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 (δ²H = 3.69 δ¹⁸O – 38.20, R² = 0.31, N = 61, p < 0.001) was only-isotopically similar to root water at 100-160 cm depths (Fig. 2a and e-f).
3.2 The lc-excess of mobile water and bulk soil water and less mobile water

Fig. 34

As shown in Fig. 34 and Table 2, the mean lc-excess values were $-1.27 \pm 2.10$, $-2.35 \pm 0.62$, $-6.72 \pm 1.24$ and $-13.82 \pm 1.01$ for mobile water, groundwater, bulk soil water and xylem water, respectively. 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 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 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, $y$
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.

**3.3 Comparison between root water and bulk soil water isotopes** at different depths

As shown in Fig. 5b-5d and d, there were no significant differences (p > 0.05) in isotopic composition (δ²H and δ¹⁸O) of either root water or bulk soil water between 40 cm and 80 cm horizontal distances 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 δ²H and δ¹⁸O 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 δ²H at 80-140 cm depths (p < 0.05) and δ¹⁸O at 60-160 cm depths (p < 0.01) (Fig. 5a, c). The maximum differences between bulk soil water and root water were −8.6 and −1.8‰ for Δ²H and Δ¹⁸O, respectively. Although δ²H and δ¹⁸O values of root water and bulk soil water behaved differently, a strong correlation was observed between Δ¹⁸O (Δ¹⁸O = δ¹⁸Osoil − δ¹⁸Oroot) and Δ²H (Δ²H = δ²Hsoil − δ²Hroot) for soil-root offset (Fig. 6a) at 0-160 cm depths (bulk soil water-root water: y = 3.83x + 0.99, R² = 0.69, N = 24, p < 0.001). Similarly, a strong correlation was observed between Δ¹⁸O (Δ¹⁸O = δ¹⁸Osoil − δ¹⁸Oxylemtrunk) and Δ²H (Δ²H = δ²Hsoil − δ²Hxylemtrunk) soil-xylemtrunk offsets during August 4 to September 15 (bulk soil water-xylemtrunk water: y = 6.80x + 6.52, R² = 0.83, N = 42, p < 0.001; mobile water-xylemtrunk water: y = 5.93x + 10.87, R² = 0.81, N = 42, p < 0.001) (Fig. 6b). These results 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.

**3.4 Contributions**

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, 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

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 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 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).

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
to the seasonal variation in precipitation, winter and summer precipitation have different isotope signals (Bowen et al., 2019), resulting in distinct differences in isotopic patterns between mobile and bulk soil water. 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, at our study site. Such small amounts of winter precipitation might not be able to fill the small pores. However, our finding that mobile and bulk soil water maintained distinct isotopic signals indicates that this separation may be caused by other factors, and not necessarily by seasonal variation in precipitation.

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. The effect of soil evaporation on bulk soil water gradually weakens with increasing soil depths. Thus, the enriched isotopic signals formed by evaporation in bulk soil water gradually decline or even disappear. In addition, mobile water in deep layers is more likely to be recharged by both preferential and matrix flows (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. Mobile water mixes with tightly bound water (Sprenger et al., 2016; Kurbert et al., 2020). Although the mixing of mobile and tightly bound water conflicts with the original hypothesis of Brooks et al. (2010), evidence of this phenomenon has been provided by Vargas et al. (2017), who found that 75 to 95% of tightly bound less mobile water is 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 soil water isotopic 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 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

We compared the isotopic composition of root water and bulk soil water at the same depth (Fig. 6). Contrary to expectations, the root water and bulk soil water at 0-60 cm depths showed enriched isotopic signals (δ²H and δ¹⁸O values) that were consistent with bulk soil water δ²H and δ¹⁸O 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, δ²H and δ¹⁸O values of root water deviated significantly from those of bulk soil water and mobile water, especially δ²H values. These results showed that the isotopic 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
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 cm³ 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 δ²H and δ¹⁸O of soil, trunk and root water from potted *Fagus sylvatica* saplings under control and drought treatments by Barbeta et al. (2020) showed that the δ²H of trunk water consistently matched the δ²H of root water, and deviated significantly from the δ²H of soil water under both treatments.

**Fig. 8** 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, 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 boundless mobile water that is was strongly affected by evaporative effects in 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 δ²H depletion in trunk water than in bulk soil water. Similarly, Poca et al. (2019) found that trunk water was significantly more depleted in δ²H 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 δ²H 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 (Fig. 2 and 5). However, our results showed that bulk soil water did not match root water isotopes at soil depths greater than
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 Δ²H and Δ¹⁸O, respectively: lower than the differences between bulk soil water and xylem water reported by Barbeta et al. (2020) (ca. −11 and −2‰ for Δ²H and Δ¹⁸O, respectively) and Poca et al. (2019) (−24.6 and −2.9‰ for Δ²H and Δ¹⁸O, 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 (Martín Gomez et al., 2017) and plant species (Dubbert et al., 2019). We also detected a positive linear relationship between Δ¹⁸O and Δ²H 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 isotopic composition

We found that xylem trunk water mainly overlapped isotopically with root water at 100-160 cm depths (Fig. 23), while the isotopically enriched root water at 0-80 cm depths was not reflected in the xylem trunk water isotopic composition. As the time required for isotopic tracer (D₂O) 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 δ²H and δ¹⁸O 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 δ²H and δ¹⁸O 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.
2019, and found no significant differences (p > 0.05) (Fig. S53). 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, 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, 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 isotope signals 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 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 ($D_2$O) 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 $\delta^2H$ and $\delta^{18}O$ 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.
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. 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 δ^2H and δ^18O 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 and 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 δ^18O between bulk soil water and root water were −8.6 and −1.8‰, respectively. Overall, the δ^2H and δ^18O 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 δ^18O, 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.

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.

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References


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 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.
Figure 2-3 (a) $\delta^{18}$O and $\delta^2$H isotope values of soil water (BW), mobile water (MW), root water (RW), xylem-trunk water (XW-TW), less mobile water (LMW), and groundwater (GW). (b) $\delta^{18}$O and $\delta^2$H isotope values 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 (ef) **XW-TW** collected from different tree heights. The red line represents the 2016-2019 local meteoric water line (LMWL, $\delta^2H = 5.91 + 7.67 \delta^{18}O$, $R^2 = 0.96$) 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.
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), \textit{xylem-trunk} water (XTW), 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=64176) 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=33), 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).
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 isotope composition ($\delta^2$H 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 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).
Figure 6.7 (a) Relationship of hydrogen isotope offset ($\Delta^{2}H$, $\Delta^{2}H = \delta^{2}H_{soil} - \delta^{2}H_{root}$) 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 ($\Delta^{2}H$, $\Delta^{2}H = \delta^{2}H_{soil} - \delta^{2}H_{xylem}$) and oxygen isotope offset ($\Delta^{18}O$, $\Delta^{18}O = \delta^{18}O_{soil} - \delta^{18}O_{xylem}$) 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).
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.
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 brown 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

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<th>Soil depth (cm)</th>
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Table 2 Water stable isotopes (see Fig. 3) and lc-excess values (Fig. 4) for all water samples. Range values show min, max (mean)

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