

Lawrence Berkeley National Laboratory

LBL Publications

Title

Quantitative contribution of cryogenic vacuum extraction and radial water transport to xylem-source water deuterium offsets

Permalink

<https://escholarship.org/uc/item/4kt2c5hp>

Authors

Li, Yue

Song, Xianfang

Wang, Lixin

et al.

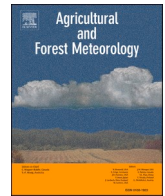
Publication Date

2024-02-01

DOI

10.1016/j.agrformet.2023.109837

Peer reviewed



Quantitative contribution of cryogenic vacuum extraction and radial water transport to xylem-source water deuterium offsets

Yue Li^{a,b,c}, Xianfang Song^{a,b}, Lixin Wang^c, Matthias Sprenger^d, Ying Ma^{a,b,*}

^a Key Laboratory of Water Cycle and Related Land Surface Processes, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

^b University of Chinese Academy of Sciences, Beijing 100049, China

^c Department of Earth Sciences, Indiana University Indianapolis, Indianapolis, IN 46202, United States

^d Earth and Environmental Sciences, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States

ARTICLE INFO

Keywords:

Cryogenic vacuum extraction (CVE)
 Ecohydrology
 Modified potential water source line (MPWL)
 Radial water transport (RWT)
 $\delta^2\text{H}$ offsets

ABSTRACT

The positions and magnitudes of deuterium offsets between bulk xylem and corresponding source waters are under debate and quantifying them is essential for isotope-based ecohydrological investigations. In this study, stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$), iteration method, and rehydration experiments were combined to quantitatively determine the magnitude of cryogenic vacuum extraction (CVE)- and radial water transport (RWT)-induced deuterium offsets using one riparian tree species *Salix babylonica* L. A modified potential water source line (MPWL) was proposed to identify the total $\delta^2\text{H}$ offsets between bulk xylem and source waters. The relationships between $\delta^2\text{H}$ offsets induced by CVE or RWT and plant water content, leaf $\delta^{13}\text{C}$ values, soil water content (SWC), and the depth to the water table (WTD) were investigated. Results showed that the bulk xylem waters in different tissue positions of *S. babylonica* showed -7.0‰ to -4.0‰ deuterium depletion relative to MPWL at four different sites ($p < 0.01$). The isotopic compositions of sap water coincided well with MPWL on the dual-isotope plot at the four sites. The CVE- and RWT-induced $\delta^2\text{H}$ offsets accounted for 75.1% and 24.9% of the total $\delta^2\text{H}$ offsets, respectively. The CVE-induced $\delta^2\text{H}$ offsets were significantly negatively correlated with plant water content. In comparison, the RWT-induced $\delta^2\text{H}$ offsets were negatively related to plant leaf $\delta^{13}\text{C}$ values, trunk water content, and SWC, but positively correlated with WTD. This study provides a quantitative contribution of two major sources of deuterium offsets. The results provide critical insights into isotope-based plant water source identification and evapotranspiration partitioning.

1. Introduction

Stable isotopes (i.e., $\delta^2\text{H}$ and $\delta^{18}\text{O}$) are powerful tracing water movements in the groundwater-soil-plant-atmosphere continuum (e.g., plant water use and evapotranspiration partitioning) (Brooks et al., 2010; Chen et al., 2020; Evaristo et al., 2015; Wang et al., 2018). They have been widely used for terrestrial water flux estimation and forest management (Rothfuss and Javaux, 2017; Sprenger et al., 2017). An essential prerequisite for isotope-based ecohydrological research is that the isotope fractionation does not occur during root water uptake (RWU), water transport within plants, and water extraction processes (Barbeta et al., 2022; Chen et al., 2020). However, multiple studies reported that the bulk xylem water had different deuterium ($\delta^2\text{H}$) values from source water (with the highest deuterium depletion of -29‰) in

naturally grown plants (Barbeta et al., 2019; Wang et al., 2017; Zhao et al., 2016), soil-potted plants (Barbeta et al., 2022; Chen et al., 2020; Duvert et al., 2022; Vargas et al., 2017), and hydroponic plants (Baan et al., 2023; Lin and Sternberg, 1993; Roden and Ehleringer, 1999, 2000). Therefore, quantifying the magnitudes and potential causes of deuterium offsets (i.e., the $\delta^2\text{H}$ difference between xylem and source waters) plays a critical role in understanding terrestrial ecohydrological processes using isotopes.

There has been an ongoing debate on which process(es) causing the plant xylem deuterium offsets in recent years. The RWU process was initially thought to be the most possible position of deuterium offsets for halophytes and xerophytes (Dawson and Ehleringer, 1993; Ellsworth and Williams, 2007). Many previous studies attributed these RWU-induced deuterium offsets to the aquaporins in the Casparian strip,

* Corresponding author.

E-mail address: maying@igsnr.ac.cn (Y. Ma).

<https://doi.org/10.1016/j.agrformet.2023.109837>

Received 21 July 2023; Received in revised form 28 October 2023; Accepted 27 November 2023

Available online 2 December 2023

0168-1923/© 2023 Elsevier B.V. All rights reserved.

which could help halophytes and xerophytes prevent water loss or exclude salts from roots (Ellsworth and Williams, 2007; Lin and Sternberg, 1993). The hydrogen bonds of soil water isotopologues must be broken down to get through the cell membranes of halophytes and xerophytes (i.e., symplastic water transport pathways), which consequently led to deuterium depletion (Lin and Sternberg, 1993). However, the deuterium offsets between bulk xylem and source waters were also observed in non-halophytes and non-xerophytes (Evaristo et al., 2017; Li et al., 2021; Vargas et al., 2017; Zhao et al., 2016). These plants primarily relied on unfractionated apoplastic water transport pathways to take up water from the soil water reservoir (Lin and Sternberg, 1993). In addition, more and more studies have demonstrated that the $\delta^2\text{H}$ of sap water in conductive xylem conduits was consistent with the corresponding source waters (Barbeta et al., 2022; Kuebert et al., 2023; Zhao et al., 2016; Zuecco et al., 2022). Therefore, deuterium fractionation is not necessarily caused by RWU, especially for those non-halophytes and non-xerophytes (Barbeta et al., 2022; Zhao et al., 2016).

Various methods have been developed to separate within-plant sap water in conductive xylem conduits, uncondusive tissue waters (i.e., water held in fibers and living parenchyma cells surrounding the conduits), and steady-state leaf transpired vapor (Barbeta et al., 2022; Chen et al., 2020; Kuebert et al., 2023; Wang et al., 2012; Zhao et al., 2016; Zuecco et al., 2022). In addition to sap water in xylem conduits, the steady-state leaf transpired vapor has also been confirmed to match well with source waters in deuterium (Chen et al., 2020; Kuebert et al., 2023; Wang et al., 2010). This demonstrated unambiguously that there was no deuterium fractionation during apoplastic pathway-dependent vertical water transport in conductive xylem conduits from roots and transpired sites (i.e., leaves) (Barbeta et al., 2022). Nevertheless, the xylem non-conductive tissue water showed a significant deuterium depletion (ranging from -3.3‰ to -25.4‰) relative to sap water (Barbeta et al., 2022; Wen et al., 2022; Zhao et al., 2016; Zuecco et al., 2022). This $\delta^2\text{H}$ -depleted tissue water has further been reported to make up a large proportion of the bulk xylem water ($78 \pm 5.6\%$) (Barbeta et al., 2022). Therefore, the radial sap- and tissue-water hydrogen heterogeneity can be a possible reason for the widespread deuterium depletion of bulk xylem water (Barbeta et al., 2022; Wen et al., 2022; Zhao et al., 2016; Zuecco et al., 2022). The empirical plant water potential threshold was uniquely used to separate xylem sap water in conductive xylem conduits and non-conductive tissue waters. However, it is challenging to accurately determine this threshold for different plant species due to various plant physiological structures and disparate sap- and tissue-water exchange processes under different plant habitats. As a consequence, how to accurately quantify radial water transport (RWT)-induced $\delta^2\text{H}$ offsets between sap- and tissue-waters remains unknown.

More recently, cryogenic vacuum extraction (CVE) has also been suggested to be a potential cause for $\delta^2\text{H}$ offsets between bulk xylem and source waters. Chen et al. (2020) eliminated all the deuterium heterogeneity within the xylem and determined the CVE-induced $\delta^2\text{H}$ offsets based on a rehydration experiment. They concluded that the CVE method is the root cause for the observed deuterium offsets due to $\delta^2\text{H}$ exchanges between environment water and exchangeable deuterium in organic matter (O-bound and/or N-bound hydrogen). However, Barbeta et al. (2022) and Zhao et al. (2016) reported much higher (i.e., more negative) $\delta^2\text{H}$ offsets (with the highest value of -26.4‰) between bulk xylem and source waters than the average CVE-induced $\delta^2\text{H}$ offsets (-9.2‰) in Chen et al. (2020). As such, the total $\delta^2\text{H}$ offsets between bulk xylem and potential source waters cannot be attributed solely to the CVE methodology artifact (De la Casa et al., 2022; Wen et al., 2022; Zuecco et al., 2022). In particular, the radial deuterium heterogeneity should also contribute to the overall $\delta^2\text{H}$ offsets (De la Casa et al., 2022; Ellsworth and Williams, 2007; Lin and Sternberg, 1993). Wen et al. (2022) further separated the CVE- and RWT-induced $\delta^2\text{H}$ offsets and found CVE-induced $\delta^2\text{H}$ offsets ($-8.5 \pm 0.9\text{‰}$) between rehydrated bulk xylem water and certain reference water of known isotopic compositions were significantly greater than RWT-induced $\delta^2\text{H}$ offsets ($-3.3 \pm 0.8\text{‰}$)

between sap and tissue waters. However, Wen et al. (2022) did not account for the total $\delta^2\text{H}$ offsets between cryogenically extracted bulk xylem water and source waters since no isotopic signature in potential source waters was obtained in their study. It is thus uncertain whether the sum of CVE- and RWT-induced $\delta^2\text{H}$ offsets can completely account for the total $\delta^2\text{H}$ offsets between bulk xylem and source waters.

Although the quantification of the total $\delta^2\text{H}$ offsets between bulk xylem and source waters is essential to understand deuterium fractionation, there is no standardized approach to determining total $\delta^2\text{H}$ offsets. Previous studies usually applied the direct comparison method to identify the total $\delta^2\text{H}$ offsets between bulk xylem and source waters for those soil- or hydroponic-potted plant species in the controlled experiment (Barbeta et al., 2022, 2020; Ellsworth and Williams, 2007; Vargas et al., 2017). However, the direct comparison could lead to inaccurate total $\delta^2\text{H}$ offsets when plants took up water from more than two sources with different proportions, especially under natural conditions. Barbeta et al. (2019) and De la Casa et al. (2022) used the soil water line (SWL) to quantify $\delta^2\text{H}$ offsets between bulk xylem water and corresponding soil water sources. Furthermore, the potential water source line (PWL) with regard to isotopic compositions of all the potential water sources was modified for the quantification of $\delta^2\text{H}$ offsets between bulk xylem water and PWL (Canham et al., 2021; Li et al., 2021). Nevertheless, both the SWL and the PWL methods did not take the proportional contributions of different water sources to plants into account. How to accurately identify the total $\delta^2\text{H}$ offsets between bulk xylem and source waters in consideration of plant water source contributions is an urgent need.

The aim of this study was to quantitatively identify the effects of both CVE and RWT on the total $\delta^2\text{H}$ offsets between bulk xylem and corresponding source waters. Focusing a widespread riparian deep-rooted *Salix Babylonica* L. along the Jian and Chaobai Rivers in China, the objectives of this study were to: (1) propose a modified potential water source line (MPWL) to quantify the total $\delta^2\text{H}$ offsets between bulk xylem and source waters; (2) quantify the CVE-induced $\delta^2\text{H}$ offset via the rehydration experiment and its relationship with relative water content; (3) identify the RWT-induced $\delta^2\text{H}$ offset using the total $\delta^2\text{H}$ offsets minus the CVE-induced $\delta^2\text{H}$ offsets and its relationship with soil water availability and leaf $\delta^{13}\text{C}$.

2. Materials and methods

2.1. Study area and field measurements

The field observations were conducted alongside the Jian and Chaobai Rivers in Shunyi District, Beijing, China ($40^{\circ}07'30''\text{N}$, $116^{\circ}40'37''\text{E}$) (Fig. 1). The study area has a temperate continental monsoonal climate, with the annual mean temperature of $11.5\text{ }^{\circ}\text{C}$ and average annual precipitation of 532 mm from 1961 to 2021. The *S. babylonica* tree is one of the most widely planted riparian species nearby rivers. Four representative sites (A, B, C, and D) with various depths to the water table (WTD), SWC, and soil textures were selected for field measurements (Fig. S1 and Table S1). The average WTD was 20.5 m, 2.8 m, 1.3 m, and 3.9 m at Sites A, B, C, and D, respectively (Li et al., 2022). At Sites A, B, and C, four plots planted with *S. babylonica* in similar diameter at breast height ($23.8 \pm 3.6\text{ cm}$) were selected at distances of 5 m, 20 m, 45 m, and 100 m away from the riverbank for sample collection (Fig. 1). Due to the tree availability, three plots (5 m, 20 m, and 45 m away from the riverbank) planted with *S. babylonica* were selected at Site D (Fig. 1).

2.2. Sample collections and isotopic analysis

We collected plant, soil, and groundwater samples in each plot at the four representative sites in late May 2021 (during the dry season). Precipitation samples were taken on event basis using a homemade sampler made up of a funnel, a polyethylene bottle, and a ping-pong ball. We collected 67 precipitation samples in 2021 to establish the

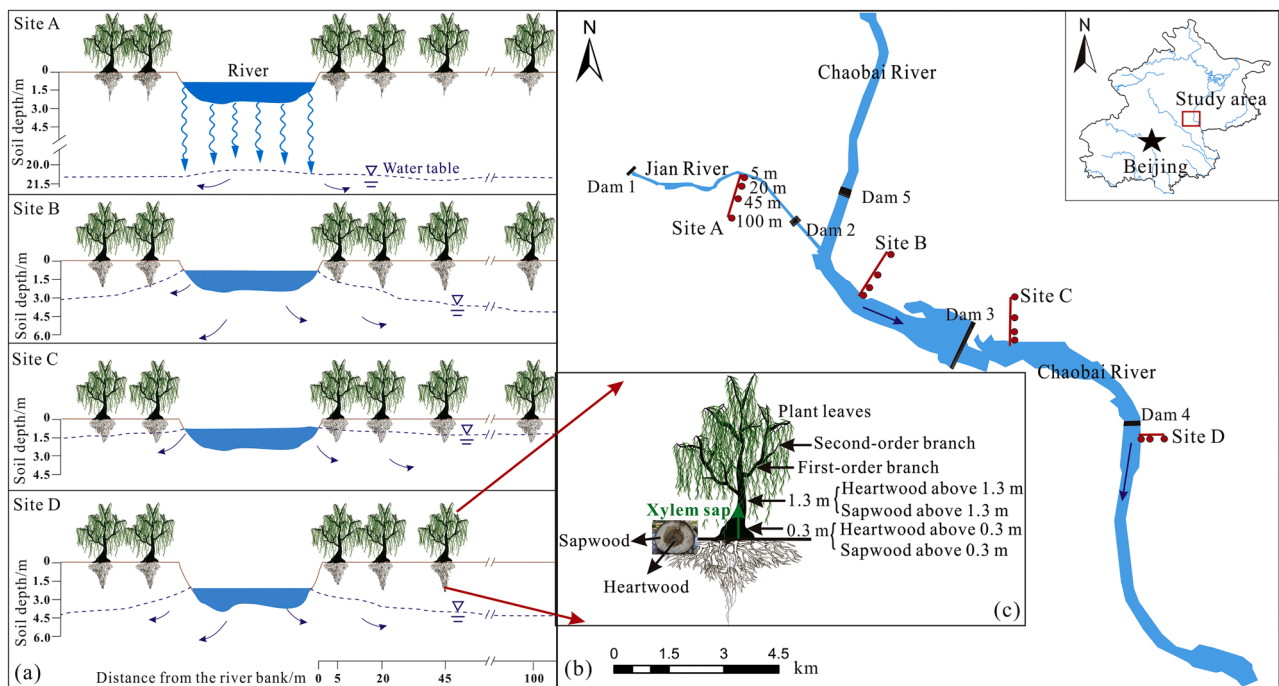


Fig. 1. Schematic diagram of (a) four sites (A, B, C, and D) in the study area, (b) plots at different distances (5 m, 20 m, 45 m, and 100 m) away from the riverbank in each site, and (c) plant sampling positions (sap water in xylem conduits and bulk xylem samples including the first-order branches, second-order branches, trunk sapwoods above 0.3 m and 1.3 m, trunk heartwoods above 0.3 m and 1.3 m).

local meteoric water line (LMWL). Bulk xylem tissue samples (trunk heartwoods above 0.3 m and 1.3 m, trunk sapwoods above 0.3 m and 1.3 m, first-order branches, second-order branches), xylem sap water, and plant leaves were collected from one *S. babylonica* tree in each plot (Fig. 1c).

2.2.1. Bulk xylem tissue samples of trunks and branches

The heartwoods and sapwoods of the *S. babylonica* trunk above 0.3 m and 1.3 m were sampled via a tree coring (Haglof, Eco Tech Co Ltd., Beijing, China) in each plot at four sites. The mature and suberized first-order branches as well as the second-order branches of the same tree in each plot were sampled using an averruncator. Both the bark and phloem of the collected branches were removed, leaving only the xylem samples. The heartwoods and sapwoods of trunks and bulk xylem tissues of branches were put into glass vials and sealed with parafilm, then stored at -4°C in a refrigerator until water cryogenic extraction and isotopic analysis.

2.2.2. Xylem sap water in trunk samples

In order to determine whether deuterium offsets occurred during vertical xylem sap water transport and radial water transport, the xylem sap water in sapwoods of *S. babylonica* was sampled by a Scholander pressure chamber (Millar et al., 2022; Zuecco et al., 2022). It is generally believed that the mobile sap water (i.e., xylem capillary water and water from large xylem conduits) would be preferentially released when the sapwood water potential ranged between -0.5 and -3 MPa (Magh et al., 2022; Nakada et al., 2019; Wen et al., 2022; Zuecco et al., 2022). In this study, the pressure in the chamber was controlled between -0.5 and -2 MPa in order to avoid extracting xylem tissue waters held in fibers, living parenchyma cells, or cell walls (Barbeta et al., 2022). The xylem sap water samples were collected from *S. babylonica* growing in the farthest and driest plot with the lowest water table at each site for isotopic analysis. This is because several previous studies have reported that deuterium offsets between bulk xylem and source waters were smaller in dry conditions compared to wet conditions (De la Casa et al., 2022; Zhao et al., 2016). If there were significant deuterium offsets

between sap water and bulk xylem water in the most water-deficit plot at each site, we can speculate those deuterium offsets also occurred in other plots. The 10 cm-length sapwood samples above 1.3 m of *S. babylonica* were clamped into the pressure chamber to collect the sap water in xylem conduits. In this study, a steady pressure (between -0.5 and -2 MPa) was added in the chamber until a drop of xylem sap water yielded at the exposed end of sapwood samples. The needle with a 15-cm syringe was tightly put over the exposed end to collect the xylem sap water. Then the pressure inside the chamber was increased slowly until a total of 0.3 ml xylem sap water was obtained for sap water isotopic analysis.

2.2.3. Plant leaf samples

The mature leaves (approximately 50 pieces) in the second-order branches were sampled in each plot at the four sites. The leaf samples were oven-dried at 65°C for 72 h to reach constant weight, grounded, and sieved via a 0.15-mm mesh screen for leaf $\delta^{13}\text{C}$ analysis.

2.2.4. Soil samples

One soil core within 1 m distance of each selected *S. babylonica* tree was sampled using a soil auger. Soil samples at depths of 0–5 cm, 5–10 cm, 10–20 cm, 20–30 cm, 40–60 cm, 90–110 cm, 150–170 cm, 190–210 cm, 250–270 cm, and 280–300 cm were collected in each plot at each site. Additional soils at depths of 350–370 cm and 380–400 cm were sampled at Site D. One part of each soil sample was put into a glass vial, then sealed with parafilm and stored at -4°C in a refrigerator for water cryogenic extraction and isotopic analysis. Another part was used for gravimetric SWC measurements based on the oven-dry method (Li et al., 2022; Wang et al., 2020). The rest of each soil sample was air-dried, ground, and passed through a 2-mm sieve to measure soil texture using a laser particle size analyzer (Master-size-2000, Malvern Instruments Ltd., UK).

2.2.5. Groundwater samples

The groundwater in each plot at the four sites was sampled from the monitoring well using a sucking pump. They were stored at -4°C in a

refrigerator until isotopic analysis.

2.2.6. Isotopic analysis

The bulk water in the *S. babylonica* xylem and soil samples was extracted using an automatic cryogenic vacuum distillation system (LI-2100, LICA, Beijing, China). The water extraction process for bulk xylem and soil samples lasted for three hours. The samples were weighed before extraction and after extraction and oven-dried processes. All the extraction efficiencies reached up to 99 %. The isotopic ratio infrared spectroscopy (IRIS) system (DLT-100, Los Gatos Research, Mountain View, USA) was used to measure the water isotopic compositions in soil water, groundwater, and precipitation. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the bulk xylem water, xylem sap water, and the $\delta^{13}\text{C}$ values of plant leaves were analyzed by the Isotope Ratio Mass Spectrometry (IRMS) system (MAT253, Thermo Fisher Scientific, Bremen, Germany) in order to prevent isotopic measurements from plant organic contamination (Zhao et al., 2011). The measuring precisions were $\pm 1 \text{ ‰}$ for $\delta^2\text{H}$ and $\pm 0.1 \text{ ‰}$ for $\delta^{18}\text{O}$ of different water samples, and $\pm 0.15 \text{ ‰}$ for $\delta^{13}\text{C}$ in plant leaf samples. The measured $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values were calibrated with the Vienna Standard Mean Ocean Water (VSMOW), while the measured $\delta^{13}\text{C}$ values of plant leaves were calibrated with the Vienna Pee Dee Belemnite (V-PDB) (Sun et al., 2019; Zhao, 2021).

2.3. Determination of total $\delta^2\text{H}$ offsets using a modified potential water source line (MPWL)

In order to accurately estimate the total $\delta^2\text{H}$ offsets between the bulk xylem and potential source waters, we need to obtain the isotopic signatures of all water sources. Moreover, the contribution of each water source to the plant transpiration water should be quantified, because the plant transpiration water was a mixture of its potential water sources in unequal proportions. Our previous study (Li et al., 2021) proposed a PWL to quantify $\delta^2\text{H}$ offsets (PW-excess) between the bulk xylem water and PWL based on the LMWL (lc-excess) (Landwehr and Coplen, 2006) and the soil water line (SW-excess) (Barbeta et al., 2019). However, the PWL was directly fitted with all the water isotopic data of potential water sources in dual-isotope plots and did not take into account different water source contributions to plant transpiration as weighting factors. In this case, the PWL was obtained under the premise that different potential water sources contributed to plant transpiration equally, which could lead to biases on $\delta^2\text{H}$ offset estimations between the bulk xylem water and PWL.

In this study, the RWU patterns were identified as the weighting factors to fit a MPWL using the weighted least squares regression and multiple iteration methods (Fig. S2). Firstly, the RWU patterns of *S. babylonica* trees were determined initially by the Bayesian MixSIAR mixing model v3.1 (Stock and Semmens, 2016) accompanied by PWL-corrected $\delta^2\text{H}$ and raw $\delta^{18}\text{O}$. Secondly, the initially calculated water source contributions to *S. babylonica* trees were characterized as weighting factors to fit MPWL, and the first-iteration $\delta^2\text{H}$ offsets between the bulk xylem water and MPWL were quantified. Both weighting factors and stable isotopic compositions of all the source waters were put into the weighted least square linear regression model to fit the MPWL. The weighting factor of each source water sample (W_s) can be calculated by the following Eq. (1):

$$W_s = \frac{P_c}{N_s \times N_p} \quad (1)$$

where P_c represents proportional contributions of each water source to *S. babylonica* trees; N_s represents the number of water samples for each water source at each plot; N_p represents the number of plots at each site.

Thirdly, the first-iteration water source proportions of *S. babylonica* trees can be identified through the MixSIAR model coupled with MPWL-corrected $\delta^2\text{H}$ in the first iteration and raw $\delta^{18}\text{O}$. And this first-iteration water source proportions of *S. babylonica* trees were further regarded as

weighting factors to fit second-iteration MWPL and the second-iteration $\delta^2\text{H}$ offsets between bulk xylem water and MPWL were quantified. The iterations would not stop until the difference between the $\delta^2\text{H}$ offsets in the last iteration and the penultimate iteration was less than 0.2 ‰ (Fig. S2). This multiple iteration method can help us more accurately estimate the MPWL, RWU patterns, and the total deuterium offsets between bulk xylem and source waters.

2.4. Quantification of cryogenic vacuum extraction (CVE)-induced $\delta^2\text{H}$ offsets and radial water transport (RWT)-induced $\delta^2\text{H}$ offsets

In this study, the rehydration experiment was conducted in order to determine the CVE-induced $\delta^2\text{H}$ offsets. Before the rehydration experiment, all the cryogenically extracted bulk xylem samples and soil samples were oven-dried at 105 °C for 24 h to completely remove the residual moisture and eliminate the isotopic heterogeneity within the plant xylem and soil samples. Each completely dried bulk xylem sample (a total of 55 samples) was rehydrated separately in a 100-ml brown glass bottle which was filled with reference water of known isotopic compositions (-56.7 ‰ for $\delta^2\text{H}$ and -7.0 ‰ for $\delta^{18}\text{O}$) at 25 °C for 48 h. After rehydration, the surface moisture on each bulk xylem sample was wiped off. Then they were put into a 12-ml brown glass bottle and stored at -4 °C in the refrigerator until cryogenic water re-extraction and isotopic analysis. A total of 60 oven-dried soil samples with various soil water content and textures were rehydrated for $\delta^2\text{H}$ offsets analysis. Each soil sample (with a range of oven-dried soil range from 6 g to 11 g) was injected with 5 ml reference water with known isotopic compositions (-56.7 ‰ for $\delta^2\text{H}$ and -7.0 ‰ for $\delta^{18}\text{O}$), sealed with parafilm, and stored at 25 °C for 48 h until cryogenic water re-extraction and isotopic measurement. The IRIS and IRMS systems were used to measure $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of re-extracted water from the rehydrated soil and xylem samples, respectively.

Assuming that there was no deuterium fractionation during RWU and vertical xylem water transport processes, the RWT-induced $\delta^2\text{H}$ offsets can be determined by subtracting the CVE-induced $\delta^2\text{H}$ offsets from the total $\delta^2\text{H}$ offsets between bulk xylem and source waters.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) incorporating Kolmogorov-Smirnov, Levene's and posthoc Tukey's tests ($p < 0.05$) were applied to compare the differences in the isotopic compositions between different water bodies, total $\delta^2\text{H}$ offsets between the xylem and source waters, and CVE-induced $\delta^2\text{H}$ offsets as well as RWT-induced $\delta^2\text{H}$ offsets across four sites. All the statistical analyses were performed using Microsoft Excel (v2016), SPSS (24.0, Inc., Chicago, IL, USA), and RStudio (v1.4).

3. Results

3.1. Plant water stable isotopes at different tissue positions and site locations

There was no significant difference in the isotopic compositions of first-order branches and second-order branches at the four sites ($p > 0.05$) (Fig. 2a). The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the trunks of *S. babylonica* were significantly lower than those in branches at Site D. However, little difference was observed in the water isotopic values of trunks and branches at Sites A, B, and C (Fig. 2b). The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the trunk-heartwoods of *S. babylonica* were not statistically different to those in the trunk-sapwoods at the four sites (Fig. 2c). As shown in Fig. 2d and 2e, there was no significant difference in the isotopic compositions between the trunk-heartwoods/sapwoods above 0.3 m and 1.3 m at Sites A, B, and C. Although no significant difference in $\delta^2\text{H}$ values between the heartwoods/sapwoods above 0.3 m and 1.3 m were observed at Site D, the $\delta^{18}\text{O}$ values in the heartwoods above 0.3 m were

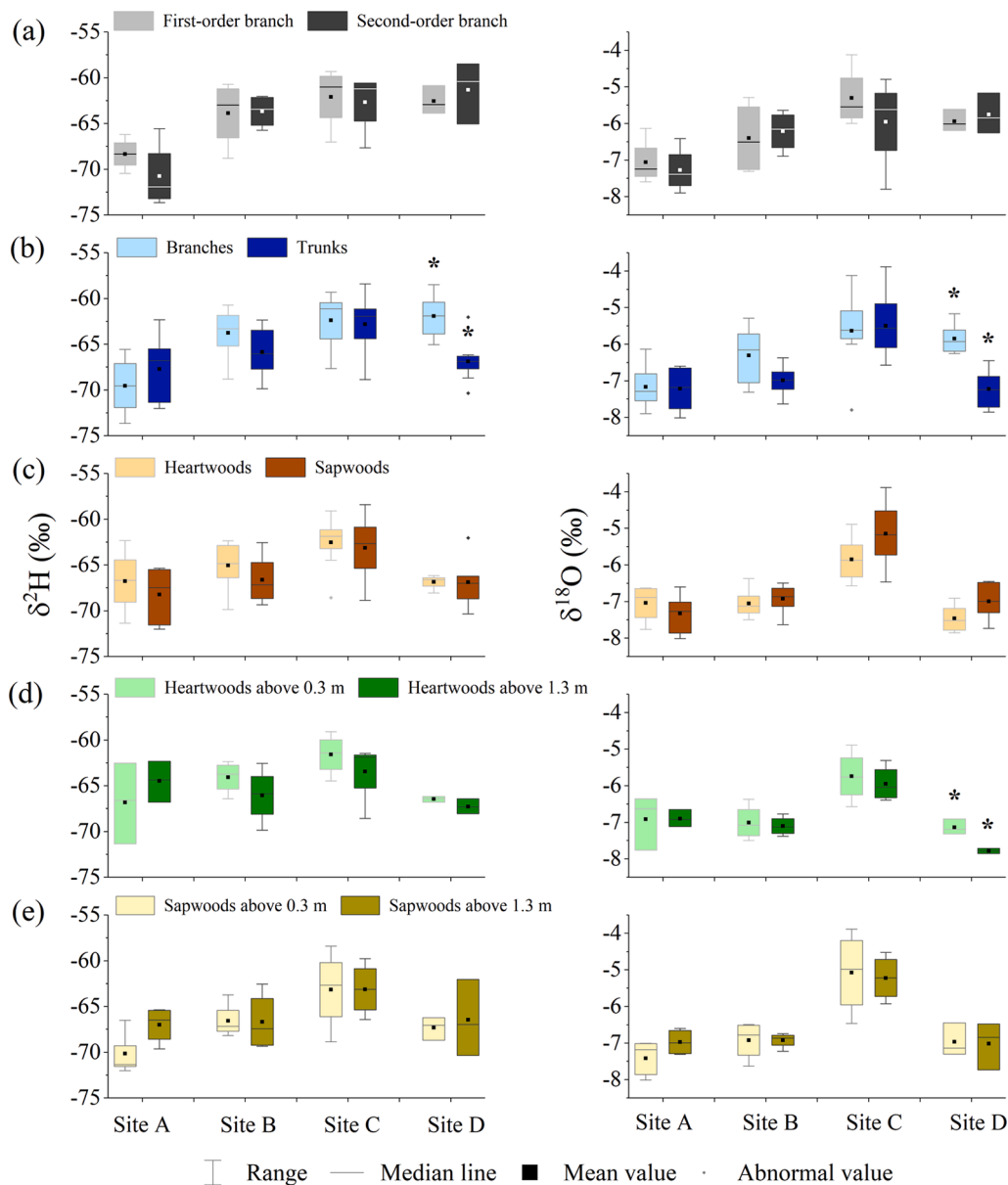


Fig. 2. Comparison of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of plant water between different tissues of *S. babylonica*, including (a) first-order branch vs. second-order branch, (b) branch vs. trunks, (c) trunk heartwood vs. trunk sapwood samples, (d) heartwoods above 0.3 m vs. heartwoods above 1.3 m, and (e) sapwoods above 0.3 m vs. sapwoods above 1.3 m at Sites A, B, C, and D. The letters “*” represent significant differences in isotopic compositions between different plant tissue waters ($p < 0.05$).

significantly higher than that above 1.3 m ($p < 0.05$). These results suggested that the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the cryogenically extracted bulk xylem water were statistically consistent between different tissue positions of *S. babylonica*.

Compared with xylem sap water, the bulk xylem water of *S. babylonica* growing the farthest from the riverbank showed a significant deuterium depletion of -7.4‰ , -8.8‰ , -10.5‰ , and -4.5‰ at Sites A, B, C, and D, respectively ($p < 0.01$) (Fig. 3). However, the $\delta^{18}\text{O}$ values of bulk xylem water barely deviated from xylem sap water (-0.1‰ , -0.5‰ , -0.4‰ , and -0.1‰ at Sites A, B, C, and D, respectively) ($p > 0.05$) (Fig. 3). This indicated that there were significant deuterium offsets but no oxygen discrepancies between sap water and bulk xylem waters of *S. babylonica* ($p < 0.01$). In particular, the deuterium offsets between the CVE-extracted bulk sapwood water and pressure chamber-extracted mobile xylem sap water from sapwoods were observed to be statistically different from zero at the four sites (-6.5‰ at Site A, -9.7‰ at Site B, -9.8‰ at Site C, and -5.6‰ at Site D) ($p < 0.01$) (Fig. 3).

The CVE-extracted bulk heartwood water also showed a significant deuterium offset (depletion) of -7.4‰ , -9.8‰ , -11.0‰ , and -5.7‰ compared to pressure chamber-extracted mobile xylem sap water in sapwoods of *S. babylonica* at Sites A, B, C, and D, respectively ($p < 0.01$) (Fig. 3).

3.2. Variations of total $\delta^2\text{H}$ offsets inferred by the modified potential water source line (MPWL)

The LMWL ($\delta^2\text{H} = 7.8\delta^{18}\text{O} + 10.6$, $R^2 = 0.96$, $p < 0.01$) established using the precipitation isotopic data for the whole year of 2021 had a significantly ($p < 0.01$) higher slope than the MPWL (mean of 3.1) (Fig. 4). This suggests that the potential water sources for *S. babylonica* have experienced strong evaporation. The PWL was found to be similar to the MPWL at Sites C and D, whereas the slope of PWL (with the PWL slope of 3.4 at Site A and 3.7 at Site B) was relatively higher than MPWL (with the MPWL slope of 3.1 at Site A and 3.0 at Site B) at Sites A and B

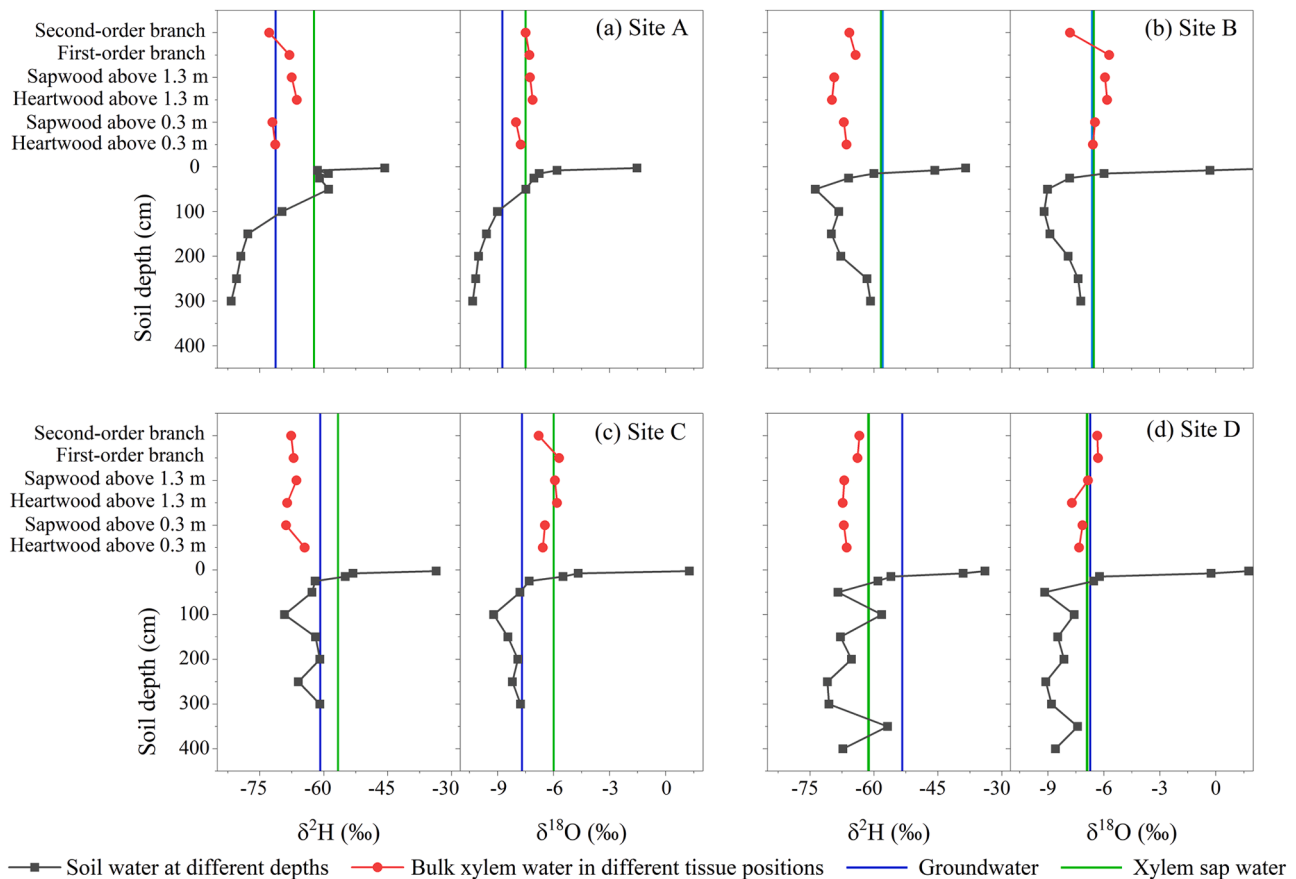


Fig. 3. The spatial variations of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of groundwater, soil water in different layers, xylem sap water, and bulk xylem water in different tissue positions of *S. babylonica* at Sites (a) A, (b) B, (c) C, and (d) D.

(Fig. 4). The differences between PWL and MPWL can be attributed to the different proportional contributions of water sources in different layers to riparian *S. babylonica* (Fig. S3).

The $\delta^2\text{H}$ values of bulk xylem water of *S. babylonica* were more depleted than in potential water sources and thus, the bulk xylem samples fell to the lower right of the MPWL in the dual-isotope plots at all four sites (Fig. 4). The average $\delta^2\text{H}$ offset between bulk xylem and source waters was -7.0 ± 2.2 ‰, -5.7 ± 2.2 ‰, -6.9 ± 2.4 ‰, and -4.0 ± 1.5 ‰ at Sites A, B, C, and D, respectively (Fig. 5). The $\delta^2\text{H}$ offsets between bulk xylem and source waters at Site D were significantly lower (more positive) compared to Sites A and C ($p < 0.01$) (Fig. 5). No significant difference in the $\delta^2\text{H}$ offsets was observed between different tissue positions of *S. babylonica* across the three Sites B, C, and D ($p > 0.05$) (Fig. 5). Nevertheless, the $\delta^2\text{H}$ offsets of bulk trunk water above 1.3 m (-4.8 ± 2.1 ‰) were significantly higher (more negative) than those of bulk second-order branch water (-9.2 ± 1.5 ‰) at Site A ($p < 0.05$) (Fig. 5). The isotopic compositions of sap water in the trunk xylem conduits of *S. babylonica* were consistent with those of corresponding potential source waters and fell on the MPWL in the dual-isotope plots at all four sites (Fig. 4). This suggested that there was no $\delta^2\text{H}$ offset of sap water in the xylem conduits of *S. babylonica* across the four sites.

3.3. Deuterium offsets induced by cryogenic vacuum extraction (CVE) and radial water transport (RWT)

The CVE-induced $\delta^2\text{H}$ offsets between the rehydrated bulk xylem water and reference water were -5.4 ± 1.7 ‰, -5.5 ± 1.3 ‰, -5.7 ± 1.6 ‰, and -4.3 ± 0.4 ‰ at Sites A, B, C, and D, respectively (Fig. 6). No significant difference in the $\delta^2\text{H}$ offsets of rehydrated bulk xylem waters

from the reference water was observed between the four sites ($p > 0.05$). The CVE-induced $\delta^2\text{H}$ offsets accounted for 74.9 %, 79.7 %, 70.4 %, and 75.4 % of the total $\delta^2\text{H}$ offsets at Sites A, B, C, and D, respectively (Fig. 7). There was no significant difference in the percentage of CVE-induced $\delta^2\text{H}$ offset to total $\delta^2\text{H}$ offsets among four sites ($p > 0.05$) (Fig. 7). No significant difference in the CVE-induced $\delta^2\text{H}$ offsets was observed between trunks and branches at four sites ($p > 0.05$). The RWT process between sap and tissue waters contributed less to $\delta^2\text{H}$ offset compared to the CVE process, with the mean value of 25.1 %, 20.3 %, 29.6 %, and 24.6 % at Sites A, B, C, and D, respectively (Fig. 7).

3.4. Influence of multiple factors on CVE- and RWT-induced $\delta^2\text{H}$ offsets

As shown in Fig. 8, the CVE-induced $\delta^2\text{H}$ offset of bulk xylem water was significantly positively correlated with the plant water content ($y = 12.6x - 9.7$, $R^2 = 0.41$, $p < 0.05$). Specifically, both the branch water content ($y = 19.5x - 12.2$, $R^2 = 0.36$, $p < 0.05$) and trunk water content ($y = 7.6x - 7.5$, $R^2 = 0.23$, $p < 0.05$) were observed to be in a linear correlation with corresponding CVE-induced $\delta^2\text{H}$ offsets, respectively (Fig. 8). Nevertheless, the CVE-induced $\delta^2\text{H}$ offsets of bulk branch xylem water showed a more significant increase with the decreasing branch water content compared to bulk trunk xylem water (Fig. 8). The $\delta^2\text{H}$ offsets between the rehydrated bulk soil water and reference water showed significantly negative correlations with soil clay content ($p < 0.05$) and soil silt content ($p < 0.05$) (Fig. 9a and b). In contrast, a significantly positive correlation was found between the CVE-induced $\delta^2\text{H}$ offsets and soil sand content ($p < 0.05$) (Fig. 9c). There was no significant relationship between the CVE-induced $\delta^2\text{H}$ offsets and soil water content ($p > 0.05$) (Fig. 9d).

There were significantly negative relationships between the RWT-

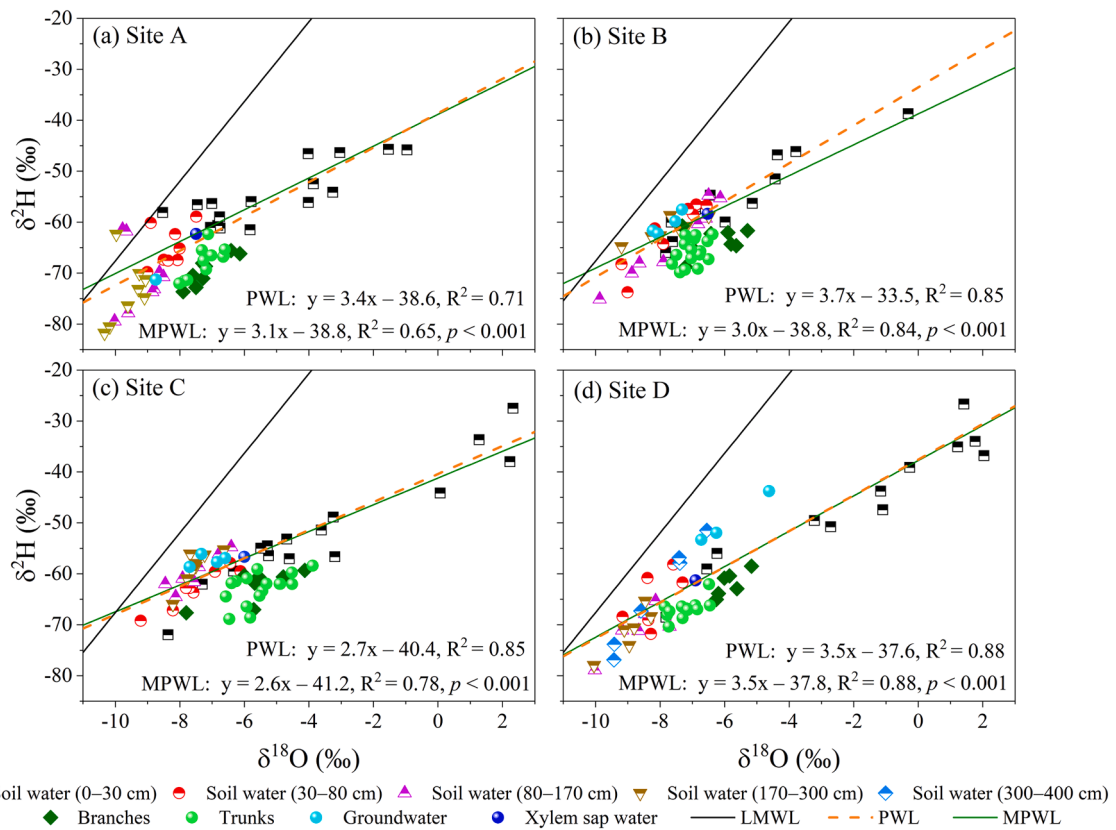


Fig. 4. Dual-isotope ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) biplots of different water bodies at Sites (a) A, (b) B, (c) C, and (d) D. The Local Meteoric Water Line (LMWL) was fitted by the precipitation isotopic data for the whole year of 2021 (LMWL: $\delta^2\text{H} = 7.88\delta^{18}\text{O} + 10.6, R^2 = 0.96, p < 0.01$). The modified potential water source line (MPWL) was fitted by the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of all potential source waters for riparian *S. babylonica* considering the proportional water sources contributions as weight factors. The water isotopic compositions of different water sources with the same proportions were used to establish the potential water source line (PWL).

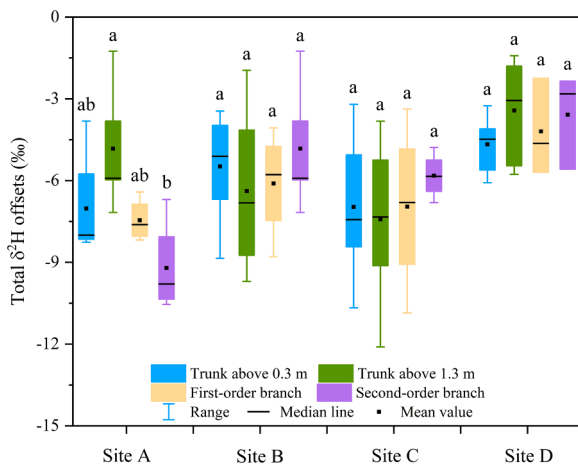


Fig. 5. The total $\delta^2\text{H}$ offsets between the bulk xylem water and modified potential water source line (MPWL) at Sites A, B, C, and D. The letters (a and b) represent significant differences in $\delta^2\text{H}$ offsets between different bulk xylem waters of different tissue positions of *S. babylonica* ($p < 0.05$).

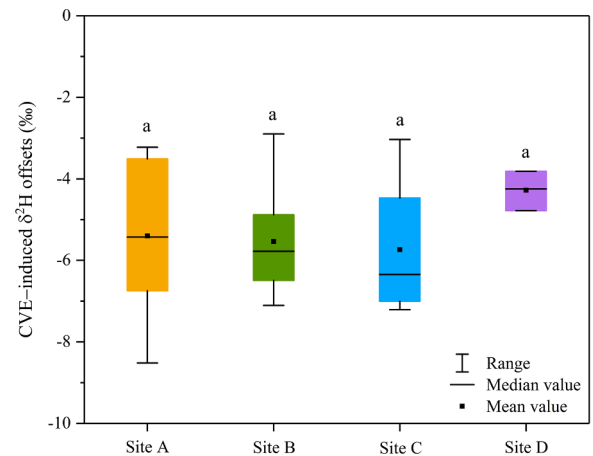


Fig. 6. The cryogenic vacuum extraction (CVE)-induced $\delta^2\text{H}$ offsets between the rehydrated bulk xylem water and reference water. The letters (a and b) represent significant differences in CVE-induced $\delta^2\text{H}$ offsets for bulk xylem water at four representative sites ($p < 0.05$).

induced $\delta^2\text{H}$ offsets and leaf $\delta^{13}\text{C}$ values ($y = -2.2x + 65.9, R^2 = 0.64, p < 0.05$) (Fig. 10a) and trunk water content ($y = -20.6x + 2.6, R^2 = 0.38, p < 0.05$) (Fig. 10b). The RWT-induced $\delta^2\text{H}$ offset was also found to be negatively ($y = -27x + 1.9, R^2 = 0.39, p < 0.05$) and positively ($y = x - 4.1, R^2 = 0.48, p < 0.05$) correlated with the SWC and WTD in linear function, respectively (Fig. 10c and d). This result indicated that the wetter environment could lead to more negative (larger) RWT-induced $\delta^2\text{H}$ offsets. In addition, the SWC positively affected the leaf $\delta^{13}\text{C}$

values ($y = 5.9x - 30.0, R^2 = 0.40, p < 0.05$) (Fig. S4a) and trunk water content ($y = 0.9x + 0.3, R^2 = 0.48, p < 0.05$) (Fig. S4b).

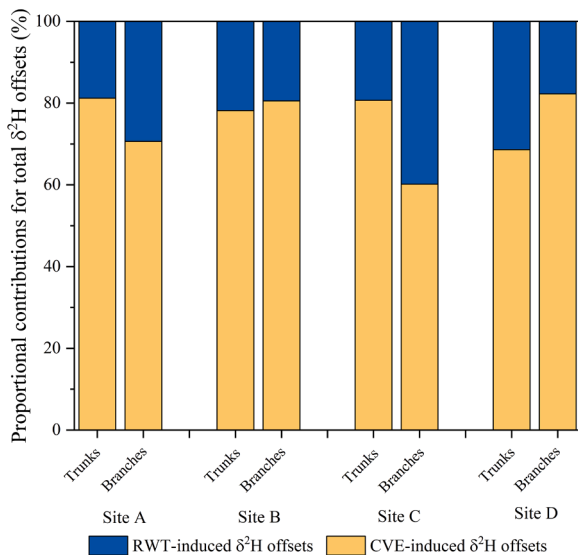


Fig. 7. The percentages of cryogenic vacuum extraction (CVE)- and radial water transport (RWT)-induced $\delta^2\text{H}$ offsets to total $\delta^2\text{H}$ offsets between the bulk xylem water and modified potential water source line (MPWL) at Sites A, B, C, and D.

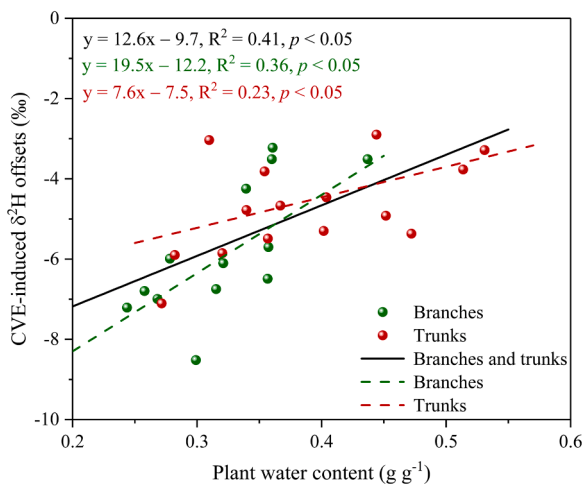


Fig. 8. Relationship between the cryogenic vacuum extraction (CVE)-induced $\delta^2\text{H}$ offsets of rehydrated plant water and plant water content.

4. Discussion

4.1. Identification of $\delta^2\text{H}$ fractionation positions and corresponding $\delta^2\text{H}$ offsets

Our results revealed that deuterium was fractionated in the sap-tissue RWT process, while riparian tree RWU and vertical sap water transport from roots to leaves did not affect the deuterium ratio of xylem water (Fig. 11). This can be supported by the following three lines of evidence. Firstly, the $\delta^2\text{H}$ of xylem sap water at 1.3 m of *S. babylonica* sampled with a Scholander pressure chamber matched well with MPWL and corresponding source waters (Figs. 3 and 4). Our results indicated that non-fractionated sap water reflecting the isotopic information of potential source water did exist in conductive xylem conduits at 1.3 m of riparian *S. babylonica*. This is consistent with some previous studies reporting that no deuterium fractionation occurred during water uptake from soil water to root xylem conduits (Barbeta et al., 2022; Chen et al., 2020), and during vertical sap water transport from roots to the transpired sites (Dawson et al., 2002; Treydte et al., 2021; Zhao et al., 2016).

Thus, we excluded both RWU and vertical sap water transport as potential causes for deuterium fractionations. Secondly, we observed deuterium offsets between sap water and bulk xylem water in different positions of *S. babylonica* at the four sites (Fig. 3). Such deuterium fractionation might be attributed to the radial isotopic exchanges between sap water in conductive xylem conduits and tissue waters in non-conductive xylem (Barbeta et al., 2022; Nehemy et al., 2022; Wen et al., 2022; Zhao et al., 2016). Interestingly, the deuterium of bulk xylem water remained statistically consistent along the bottom (trunks at 0.3 m), middle (trunks at 1.3 m), and upper positions (the first- and second-order branches) of riparian *S. babylonica* at Sites A, B, and C (Fig. 2). This is likely because the magnitude of RWT-induced deuterium offsets in different positions of *S. babylonica* is consistent, highlighting the negligible influence of sampling positions on the deuterium fractionation estimation. Thirdly, a large number of immature or discontinuous aquaporins attaching to the roots and conductive xylem conduits could provide many unfractionated apoplastic pathways for RWU and vertical sap water transport, especially for those non-halophytes and non-xerophytes (Barbeta et al., 2022; Ellsworth and Williams, 2007; Lin and Sternberg, 1993; Nehemy et al., 2022). However, the mature aquaporin-mediated RWT process may preferentially drive lighter hydrogen isotopologues in sap water to go through tissue waters and lead to highly $\delta^2\text{H}$ -depleted tissue waters (Nehemy et al., 2022).

Except for the sap-tissue RWT process, the RWT process between sapwood- and heartwood-water has been suggested to be a possible reason for RWT-induced $\delta^2\text{H}$ offsets. For example, White et al. (1985) and Treydte et al. (2021) found that the $\delta^2\text{H}$ values of bulk sapwood water were significantly higher compared to heartwood water. They explained that the radial symplastic water movement from “conductive sapwood” to “non-conductive heartwood” resulted in $\delta^2\text{H}$ fractionation and consequent $\delta^2\text{H}$ -depleted heartwood water. In contrast, no significant difference in $\delta^2\text{H}$ values between bulk sapwood and heartwood water was found here (Fig. 2) and also in previous studies (Thorburn et al., 1993; Fabiani et al., 2022). The bulk sapwood water and bulk heartwood water of *S. babylonica* showed statistically consistent deuterium offsets from sap water in xylem conduits (Fig. 3). Thus, our results excluded the possibility that deuterium fractionation occurs during the RWT process between sapwood- and heartwood-water. According to Jupa et al. (2016), plant sapwood typically contained conductive conduits and non-conductive tissues including fibers and parenchyma. Furthermore, Barbeta et al. (2022) reported that the ratio of sap water to bulk xylem water content in potted *F. sylvatica* ranged from 8 % to 30 %. The relatively $\delta^2\text{H}$ -enriched sap water could have a minor effect on the deuterium of bulk sapwood water if the sap water reservoir in xylem conduits is much smaller than the tissue water reservoir. Consequently, the sapwood water was observed to be consistent with heartwood water in deuterium in our study. In comparison, when the ratio of sap water to bulk xylem water content reached up to 30 %, the ratio of sap water to tissue water in sapwood could be approximately 50 %. This large enriched sap water reservoir in sapwood could be a possible reason for the $\delta^2\text{H}$ -enriched sapwood water in White et al. (1985) and Treydte et al. (2021). As such, we ruled out sapwood-heartwood water exchange as a potential reason for $\delta^2\text{H}$ fractionation during the RWT process of riparian *S. babylonica*.

The $\delta^2\text{H}$ offsets between bulk xylem and source waters have also been attributed to the water isotopic heterogeneity within the micro soil pores (Barbeta et al., 2022; Zhao et al., 2022). We estimated the isotopic composition of unbound soil water based on the empirical formulation proposed by Chen et al. (2016) and Barbeta et al. (2022) (Eqs. S1 and S2, Fig. S5). The results showed that $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in unbound soil water were not statistically distinguished from those in bulk soil water at the four sites (Fig. S5), which consequently indicated an isotopic homogeneity among different soil pools (i.e., bound soil water and unbound soil water). In contrast, Barbeta et al. (2022) and Duvert et al. (2022) emphasized the relative enrichment of heavy isotopes in unbound soil

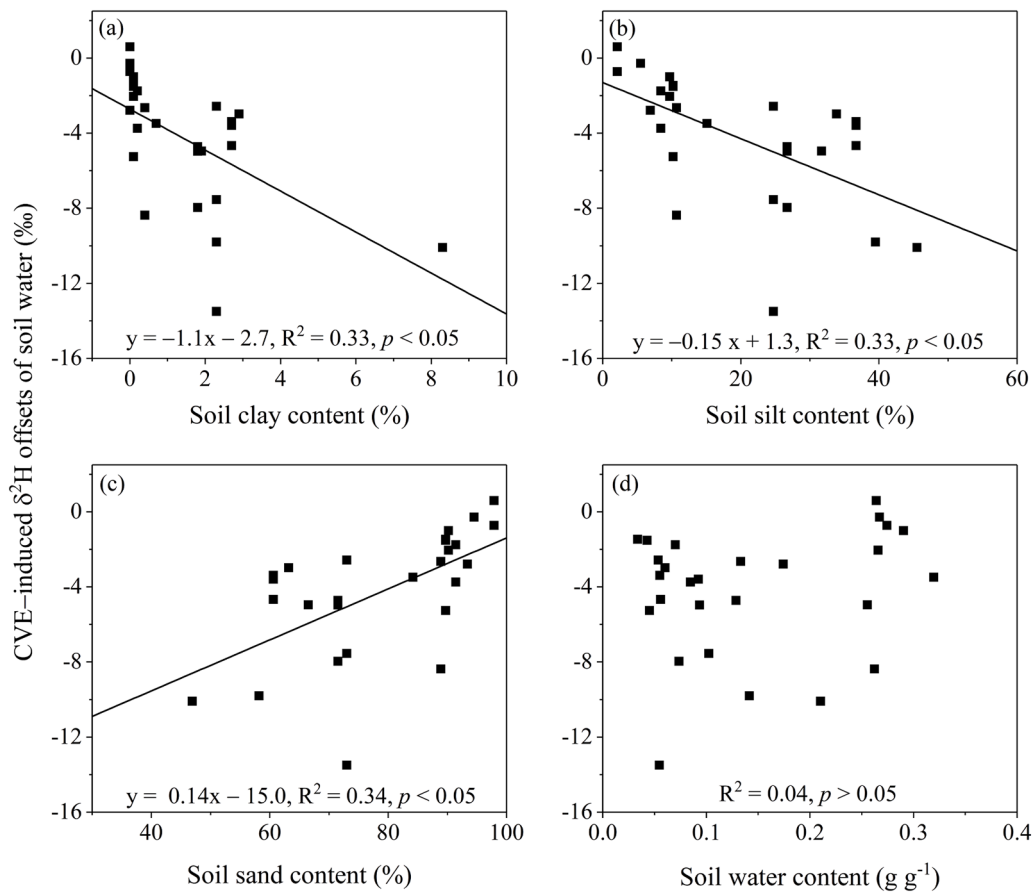


Fig. 9. Relationships between the cryogenic vacuum extraction (CVE)-induced $\delta^2\text{H}$ offsets of rehydrated soil water and (a) soil clay content, (b) soil silt content, (c) soil sand content, and (d) soil water content.

water compared to bulk soil water deriving from the empirical formulation. This soil water isotopic heterogeneity could attribute to high soil organic content in the riparian karstic canyon zone (Barbeta et al., 2022) and streamside Ranger Uranium Mine (Duvert et al., 2022). Chen et al. (2021) suggested that the organic content was the primary reason for isotopic fractionation and soil water isotopic heterogeneity. Several previous studies also reported a positive linear correlation between soil clay and organic content due to the close association of soil clay particles and organic matter (Johannes et al., 2017; Prout et al., 2021). As a consequence, the soil water isotopic homogeneity could derive from low soil organic content under low soil clay content conditions (0–9.4%) in this study (Table S1). Therefore, we elucidated that the xylem-source deuterium offsets cannot be attributed to the micrometer-scale isotopic heterogeneity in the soil water within micro-pores.

It was suggested that the CVE process was a primary potential cause of the total $\delta^2\text{H}$ offsets between bulk xylem and source waters, which can be inferred from the higher proportion of CVE-induced $\delta^2\text{H}$ offsets (75.1%) compared to the RWT-induced $\delta^2\text{H}$ offsets (24.9%) (Figs. 7 and 11). Wen et al. (2022) also demonstrated that the RWT- and CVE-induced $\delta^2\text{H}$ offsets explained 28.1% and 71.9% of the $\delta^2\text{H}$ offsets between sap water and tissue waters. The significantly higher CVE-induced $\delta^2\text{H}$ offsets than RWT-induced $\delta^2\text{H}$ offsets could be explained that the original structures or compositions of all the xylem water molecules have been altered during the CVE process. In comparison, most of the immobile tissue water remains its original state during the RWT process. It was worth noting that the magnitude of RWT-induced $\delta^2\text{H}$ offsets in this study (mean of -1.8‰) was much smaller than those previously reported in dryland riparian *Populus Euphratica* species (up to -26‰) (Zhao et al., 2016). This could be because Zhao et al. (2016) attributed the $\delta^2\text{H}$ offsets between bulk xylem and source waters (groundwater)

entirely to the radial water transport from sap water in conductive xylem conduits to non-conductive tissue waters, without separating CVE-induced $\delta^2\text{H}$ offsets.

4.2. Possible causes for $\delta^2\text{H}$ offsets resulting from cryogenic vacuum extraction (CVE) methodology artifact

The CVE-induced $\delta^2\text{H}$ offsets can be attributed to the deuterium exchange between exchangeable hydrogen atoms in xylem organic matter (O-bound and/or N-bound aromatic hydrogen) (hereafter as $\delta^2\text{H}_{\text{exOM}}$) and environmental water (hereafter as $\delta^2\text{H}_{\text{water}}$) during the CVE processes proposed by Chen et al. (2020). According to wood anatomy, the exchangeable hydrogen bound to oxygen in hydroxyl groups accounts for 30% of the hydrogen atoms of cellulose, whereas 70% is bound to carbon and isotopically non-exchangeable (Epstein et al., 1976; Feng et al., 1993; Filot et al., 2006). Moreover, isotopic fractionations existed during such deuterium exchange process between $\delta^2\text{H}_{\text{exOM}}$ and $\delta^2\text{H}_{\text{water}}$ and eventually reached a complete equilibration (i.e., $\delta^2\text{H}_{\text{exOM}} = \delta^2\text{H}_{\text{water}} + \epsilon$, where ϵ is a negative fractionation factor) within minutes at temperatures above 100 °C and within days at a temperature of 0 °C (Yakir and Deniro, 1990; Feng et al., 1993; Lehmann et al., 2022). In this regard, it is reasonable to infer that the dynamic equilibrium of deuterium exchange between $\delta^2\text{H}_{\text{exOM}}$ and $\delta^2\text{H}_{\text{water}}$ can be achieved at a temperature of 150 °C during the CVE process in our study, consequently causing that $\delta^2\text{H}_{\text{exOM}}$ values always equal to $\delta^2\text{H}_{\text{water}}$ values plus ϵ . Furthermore, the progressive evaporation enrichment of xylem residual water could constantly lead to the deuterium enrichment in plant organic matter due to the presence of dynamic deuterium exchange during water distillation (Chen et al., 2020). With an isotope mass balance system, the resultant enriched

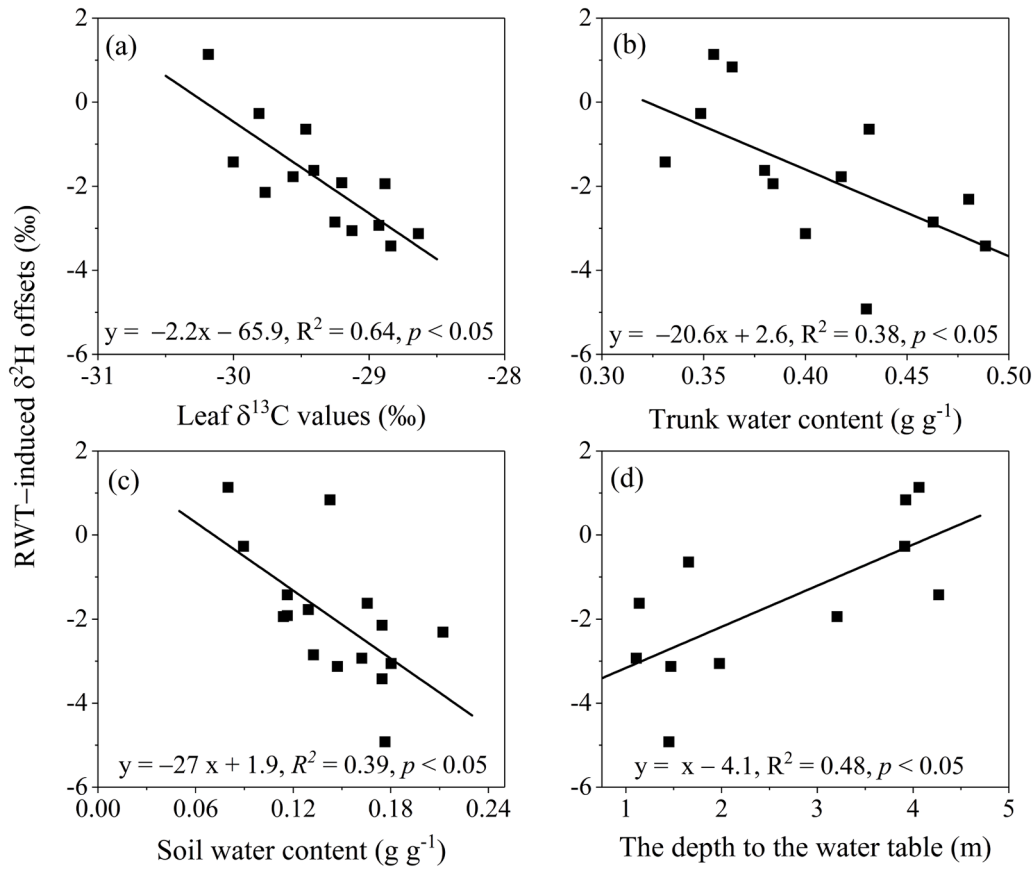


Fig. 10. Relationships between the radial water transport (RWT)-induced $\delta^2\text{H}$ offsets and (a) leaf $\delta^{13}\text{C}$ values, (b) trunk water content, (c) soil water content, and (d) the depth to the water table.

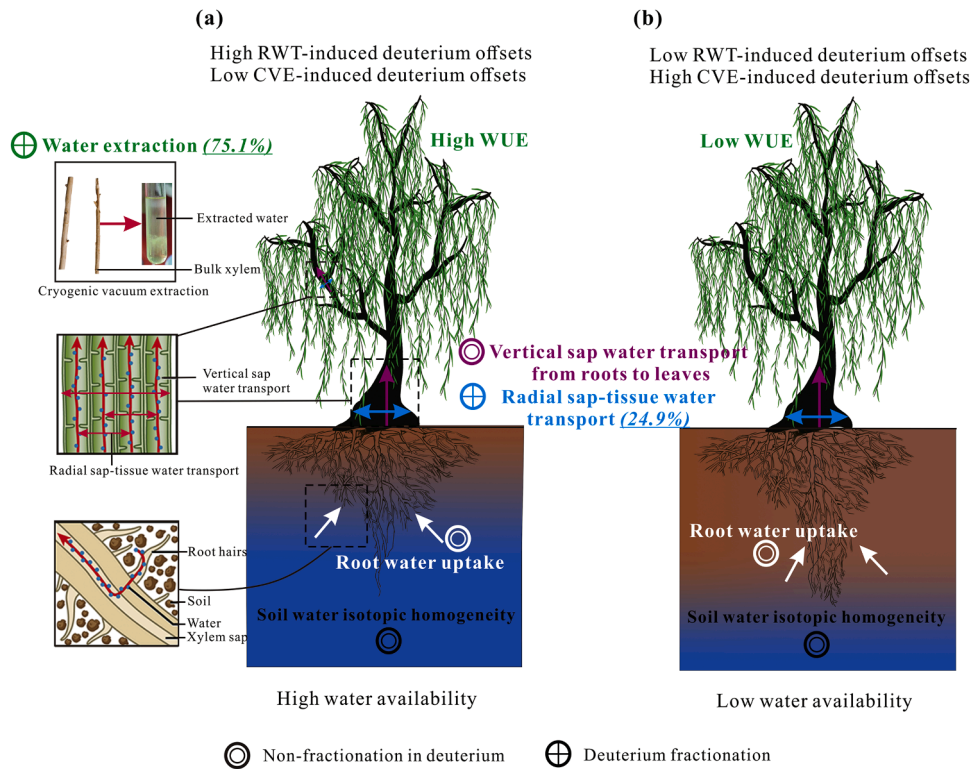


Fig. 11. Schematic diagram of the positions and magnitudes of deuterium fractionation of riparian *S. babylonica* during (a) high and (b) low water availability conditions. RWT represents radial water transport, while CVE represents cryogenic vacuum extraction.

deuterium in plant organic matter can likely cause the isotopically depleted $\delta^2\text{H}$ values of cryogenically extracted bulk xylem water. This interpretation can be further supported by the positively linear correlations between CVE-induced $\delta^2\text{H}$ offsets and plant water content observed in our study (Fig. 7) and Chen et al. (2020). Because given the isotope mass balance, the increasing plant water content could enlarge the water reservoir surrounding organic matter and buffer the effect of deuterium exchanges between $\delta^2\text{H}_{\text{exOM}}$ and $\delta^2\text{H}_{\text{water}}$ on CVE-induced $\delta^2\text{H}$ offsets (Chen et al., 2020; Wen et al., 2022). In addition to water content, the observed negative linear relationship between CVE-induced $\delta^2\text{H}$ offsets and soil clay content suggested that deuterium exchange-derived $\delta^2\text{H}$ offsets could be enhanced (more negative) when soil clay content is high due to a close association between soil clay- and organic content (Fig. 9a and b).

However, a completely different explanation of CVE-induced $\delta^2\text{H}$ offsets was proposed by Diao et al. (2022), reporting that the combined effect of deuterium exchange between $\delta^2\text{H}_{\text{exOM}}$ and $\delta^2\text{H}_{\text{water}}$ during rehydration process and evaporation- or sublimation-derived isotopic enrichments during CVE process resulted in CVE-derived $\delta^2\text{H}$ offsets. We have reasons to believe this explanation likely does not apply to our case. First, we used a large 100-ml brown glass bottle for rehydrating each oven-dried bulk xylem sample, which contained enough reference water in order to eliminate the effect of deuterium exchange between xylem organic matter and reference water on isotopic changes of reference water during the 24 h rehydration process. Our results found that there was no significant difference between isotopic values of reference water before and after rehydration process ($p > 0.05$). Thus, the effect of deuterium exchange between $\delta^2\text{H}_{\text{exOM}}$ and $\delta^2\text{H}_{\text{water}}$ during rehydration process on CVE-induced $\delta^2\text{H}$ offsets can be negligible. Second, according to Diao et al. (2022), the effect of evaporation- or sublimation-derived isotopic enrichment on the CVE-derived $\delta^2\text{H}$ offsets during CVE process was particularly low when the absolute amount of extracted water exceeded 0.6 ml. In our study, all the absolute amount of extracted water of rehydrated samples is more than 1 ml, which led us to rule out the evaporation- or sublimation-derived isotopic enrichment during CVE process as the potential cause of CVE-derived $\delta^2\text{H}$ offsets.

4.3. Possible causes for $\delta^2\text{H}$ offsets resulting from radial water transport (RWT)

Our results suggested that higher (more negative) RWT-induced $\delta^2\text{H}$ offsets between sap and tissue waters were accompanied by higher trunk water content and higher water availability (i.e., higher SWC and higher water table) (Fig. 10b–d). Moreover, both greenhouse-controlled (Barbeta et al., 2020) and natural field experiments (De la Casa et al., 2022; Tetzlaff et al., 2021) reached the consistent conclusions that the $\delta^2\text{H}$ offsets were higher (more negative) during wetter conditions. Our previous study found that riparian *S. babylonica* tree species would increase transpiration rate and show a water use strategy without water limitations under higher soil water availability conditions (Li et al., 2022). This evidence supported previous findings stressing that the higher transpiration rate of plant species could significantly aggravate $\delta^2\text{H}$ fractionation during radial water transport (Lin and Sternberg, 1993; Nehemy et al., 2022; Steppe et al., 2012). In this regard, a compelling explanation was provided by Nehemy et al. (2022) and Treydte et al. (2021), suggesting that the aquaporins attached to the cell membrane provided the main pathway for radial water movement when the plant transpiration rate was high, which in turn enhanced $\delta^2\text{H}$ fractionations. It is likely because the cell-to-cell pathways modulated by aquaporins broke down large water isotopologues and allowed lighter isotopologues in sap water to preferentially pass into parenchyma tissue waters (Aroca et al., 2005; Cochard et al., 2006; Kozono et al., 2002; Lin and Sternberg, 1993). In comparison, the radial water movement between sap water in xylem conduits and tissue waters might primarily rely on a non-fractionated apoplasmic pathway when the transpiration rate and hydraulic demand of plant species were lower in water deficit conditions

(Nehemy et al., 2022; Steppe et al., 2012).

We also observed the higher (more negative) RWT-induced $\delta^2\text{H}$ offsets between sap and tissue waters when the leaf $\delta^{13}\text{C}$ values were higher (more positive) at the moister environment (Figs. 10a and S4a). Farquhar et al. (1989) characterized leaf $\delta^{13}\text{C}$ as a proxy of plant WUE (i.e., the ratio of photosynthetic rate to transpiration rate). The positive linear relationships between plant trunk water content and soil water content revealed that plant transpiration flux increased with the increasing soil water availability (Fig. 10b). Moreover, the increasing soil water availability could induce a stronger increase in photosynthetic rate than transpiration rate, which can be inferred from the positive relationship between the plant WUE and soil water content in this study (Fig. S4a). The increasing photosynthetic rate of riparian *S. babylonica* could produce more exchangeable O- or N-bound ^2H in organic matter and eventually enhance the deuterium exchange between parenchyma cell organics and environmental water (Barbeta et al., 2022; Chen et al., 2016; Nehemy et al., 2022; Treydte et al., 2021). It is uncertain whether the RWT-induced $\delta^2\text{H}$ offsets were attributed to the high transpiration-induced breakdown of hydrogen bonds through the symplastic pathway, or high photosynthetic-induced $\delta^2\text{H}$ -exchange between exchangeable O- or N-bound ^2H in organic matter and ^2H in environmental water. Nevertheless, it is clear from these previous studies that the RWT-induced $\delta^2\text{H}$ offsets between sap and tissue waters could be affected by both soil water availabilities and plant physiological characteristics.

4.4. Implications and further directions

This study quantified the total, CVE-induced, and RWT-induced $\delta^2\text{H}$ offsets between bulk xylem and corresponding source waters in field observations based on the MPWL, iteration method, and rehydration experiments. The CVE- and RWT-induced $\delta^2\text{H}$ offsets must be considered in isotope-based ecohydrological research since they accounted for 75.1 % and 24.6 % of the total $\delta^2\text{H}$ offsets, respectively. The deuterium exchange between exchangeable $\delta^2\text{H}$ in organic matter and $\delta^2\text{H}$ in environmental water is the root cause of $\delta^2\text{H}$ fractionation during the CVE process, which generally weakens with the increasing plant water content. Regarding various plant species and extraction instruments in different studies, rehydration experiments should be conducted to determine CVE-induced $\delta^2\text{H}$ offsets between bulk xylem and source waters.

Until now, multiple methods (e.g., cavitron flow-rotor, self-made flow through leaf chamber, and direct syringe collection from positive-pressure xylem sap) have been used to extract a part of sap water in xylem conduits from the bulk xylem water (Barbeta et al., 2022; Kuebert et al., 2023; Millar et al., 2018; Wen et al., 2022; Zhao et al., 2016). However, it is challenging to completely separate xylem sap water and tissue water due to their constant water exchanges. Our results also suggest that both water availabilities and plant physiological characteristics have a significant effect on the RWT-induced $\delta^2\text{H}$ offsets between sap and tissue waters. As such, it is quite difficult to directly quantify RWT-induced $\delta^2\text{H}$ offsets between xylem sap and tissue waters. This study provides an alternative method to indirectly determine RWT-induced $\delta^2\text{H}$ offsets using the total $\delta^2\text{H}$ offsets minus the CVE-induced $\delta^2\text{H}$ offsets, which can be extended to other isotope-based ecohydrological research.

There are several issues that required further investigation. Firstly, only one riparian tree species (deep-rooted *S. babylonica*) with distinct WUE under different WTDs were investigated in this study, and further investigations should be conducted for various plant species in different habitats. Secondly, the water isotopic heterogeneity within the micro soil pores could play an important role in the $\delta^2\text{H}$ offsets between bulk xylem and source waters (Barbeta et al., 2022; Zhao et al., 2022). It is necessary to quantify the soil water isotopic heterogeneity-induced $\delta^2\text{H}$ offsets under relatively high soil organic content conditions in future research.

5. Conclusions

In this study, the magnitude of the total $\delta^2\text{H}$ offsets between bulk xylem and source waters through CVE or RWT pathways were quantified via the stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$) and rehydration experiments. Our results provide support to the common assumption that no $\delta^2\text{H}$ fractionation occurred during root water uptake and vertical sap water transport from roots to leaves. The soil water isotopic homogeneity between bound soil water and unbound soil water in this study suggested the $\delta^2\text{H}$ offsets cannot be attributed to the micrometer-scale isotopic heterogeneity in the soil water within micro-pores. The CVE- and RWT-induced $\delta^2\text{H}$ offsets accounted for 75.1 % and 24.9 % of total $\delta^2\text{H}$ offsets, respectively. The CVE-induced $\delta^2\text{H}$ offsets result from the deuterium exchange between exchangeable hydrogen atoms in xylem organic matter and environmental water, which decreased with increasing plant water content. The higher plant transpiration rate or photosynthetic rate in wetter environments could significantly aggravate RWT-induced $\delta^2\text{H}$ offsets between sap and tissue waters. Our results suggested that both water availability (i.e., SWC and WTD) and plant physiological characteristics had a significant effect on the RWT-induced $\delta^2\text{H}$ offsets between sap and tissue waters. This study provides critical and comprehensive insights into potential causes of deuterium offsets and helps to accurately determine plant water uptake patterns and evapotranspiration partitioning.

CRedit authorship contribution statement

Yue Li: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Xianfang Song:** Supervision, Writing – review & editing, Project administration. **Lixin Wang:** Supervision, Writing – review & editing, Project administration. **Matthias Sprenger:** Writing – review & editing. **Ying Ma:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2021YFC3201203) and the National Natural Science Foundation of China (42371036; 41730749). LW acknowledges partial support from the Division of Earth Sciences of the National Science Foundation (EAR-1554894). Sincere thanks go to Yiran Li, Lihu Yang, and Binghua Li for their assistance in experiments. We thank Editor Tim J Griffiths and the anonymous reviewer for their professional comments which help us greatly improve the overall quality of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.agrformet.2023.109837](https://doi.org/10.1016/j.agrformet.2023.109837).

References

- Aroca, R., Amodeo, G., Fernández-Illescas, S., Herman, E.M., Chaumont, F.o., Chrispeels, M.J., 2005. The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. *Plant Physiol.* 137 (1), 341–353. <https://doi.org/10.1104/pp.104.051045>.
- Baan, J., Holloway-Phillips, M., Nelson, D.B., Kahmen, A., 2023. The metabolic sensitivity of hydrogen isotope fractionation differs between plant compounds. *Phytochemistry* 207, 113563. <https://doi.org/10.1016/j.phytochem.2022.113563>.
- Barbeta, A., Burrell, R., Martin-Gomez, P., Frejaville, B., Devert, N., Wingate, L., et al., 2022. Evidence for distinct isotopic compositions of sap and tissue water in tree stems: consequences for plant water source identification. *New Phytol.* 233 (3), 1121–1132. <https://doi.org/10.1111/nph.17857>.
- Barbeta, A., Gimeno, T.E., Clave, L., Frejaville, B., Jones, S.P., Delvigne, C., et al., 2020. An explanation for the isotopic offset between soil and stem water in a temperate tree species. *New Phytol.* 227 (3), 766–779. <https://doi.org/10.1111/nph.16564>.
- Barbeta, A., Jones, S.P., Clave, L., Wingate, L., Gimeno, T.E., Frejaville, B., et al., 2019. Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrol. Earth Syst. Sci.* 23 (4), 2129–2146. <https://doi.org/10.5194/hess-23-2129-2019>.
- Brooks, J.R., Barnard, H.R., Coulombe, R., McDonnell, J.J., 2010. Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nat. Geosci.* 3 (2), 100–104. <https://doi.org/10.1038/ngeo722>.
- Canham, C.A., Duvert, C., Beesley, L.S., Douglas, M.M., Setterfield, S.A., Freestone, F.L., et al., 2021. The use of regional and alluvial groundwater by riparian trees in the wet-dry tropics of northern Australia. *Hydrol. Process* 35 (5). <https://doi.org/10.1002/hyp.14180>.
- Chen, G., Auerswald, K., Schnyder, H., 2016. H-2 and O-18 depletion of water close to organic surfaces. *Biogeosciences* 13 (10), 3175–3186. <https://doi.org/10.5194/bg-13-3175-2016>.
- Chen, G., Li, X., Qin, W., Lei, N., Sun, L.Z., Cao, L., Tang, X., 2021. Isotopic fractionation induced by a surface effect influences the estimation of the hydrological process of topsoil. *Hydrol. Process* 35 (1), e14019. <https://doi.org/10.1002/hyp.14019>.
- Chen, Y.L., Helliker, B.R., Tang, X.H., Li, F., Zhou, Y.P., Song, X., 2020. Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water. *Proc. Natl. Acad. Sci.* 117 (52), 33345–33350. <https://doi.org/10.1073/pnas.2014422117>.
- Cochard, H., Venisse, J.-S.p., Barigah, T.T.S.v.r., Brunel, N., Herbet, S.p., Guilliot, A.s., et al., 2006. Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. *Plant Physiol.* 143 (1), 122–133. <https://doi.org/10.1104/pp.106.090092>.
- Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P., 2002. Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst.* 33, 507–559. <https://doi.org/10.1146/annurev.ecolsys.33.020602.095451>.
- De la Casa, J., Barbeta, A., Rodriguez-Una, A., Wingate, L., Ogee, J., Gimeno, T.E., 2022. Isotopic offsets between bulk plant water and its sources are larger in cool and wet environments. *Hydrol. Earth Syst. Sci.* 26 (15), 4125–4146. <https://doi.org/10.5194/hess-26-4125-2022>.
- Diao, H., Schuler, P., Goldsmith, G.R., Siegwolf, R.T.W., Saurer, M., Lehmann, M.M., 2022. Technical note: on uncertainties in plant water isotopic composition following extraction by cryogenic vacuum distillation. *Hydrol. Earth Syst. Sci.* 26, 5835–5847. <https://doi.org/10.5194/hess-26-5835-2022>.
- Duvert, C., Canham, C.A., Barbeta, A., Alvarez Cortes, D., Chandler, L., Harford, A.J., et al., 2022. Deuterium depletion in xylem water and soil isotopic effects complicate the assessment of riparian tree water sources in the seasonal tropics. *Ecohydrology* 15 (6). <https://doi.org/10.1002/eco.2383>.
- Ellsworth, P.Z., Williams, D.G., 2007. Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant Soil* 291 (1–2), 93–107. <https://doi.org/10.1007/s11104-006-9177-1>.
- Epstein, S., Yapp, C.J., Hall, J.H., 1976. The determination of the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth Planet. Sci. Lett.* 30 (2), 241–251. [https://doi.org/10.1016/0012-821X\(76\)90251-X](https://doi.org/10.1016/0012-821X(76)90251-X).
- Evaristo, J., Jasechko, S., McDonnell, J.J., 2015. Global separation of plant transpiration from groundwater and streamflow. *Nature* 525 (7567), 91–107. <https://doi.org/10.1038/nature14983>.
- Evaristo, J., McDonnell, J.J., Clemens, J., 2017. Plant source water apportionment using stable isotopes: a comparison of simple linear, two-compartment mixing model approaches. *Hydrol. Process* 31 (21), 3750–3758. <https://doi.org/10.1002/hyp.11233>.
- Fabiani, G., Penna, D., Barbeta, A., Klaus, J., 2022. Sapwood and heartwood are not isolated compartments: consequences for isotope ecohydrology. *Ecohydrology* 15 (8). <https://doi.org/10.1002/eco.2478>.
- Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 503–537. <https://doi.org/10.1146/annurev.arplant.40.1.503>.
- Feng, X., Krishnamurthy, R., Epstein, S., 1993. Determination of D/H ratios of nonexchangeable hydrogen in cellulose: a method based on the cellulose-water exchange reaction. *Geochim. Cosmochim. Acta* 57 (17), 4249–4256. [https://doi.org/10.1016/0016-7037\(93\)90320-V](https://doi.org/10.1016/0016-7037(93)90320-V).
- Filot, M.S., Leuenberger, M., Pazdur, A., Boettger, T., 2006. Rapid online equilibration method to determine the D/H ratios of non-exchangeable hydrogen in cellulose. *Rapid Commun. Mass Spectrom.* 20 (22), 3337–3344. <https://doi.org/10.1002/rcm.2743>.
- Johannes, A., Matter, A., Schulin, R., Weisskopf, P., Baveye, P.C., Boivin, P., 2017. Optimal organic carbon values for soil structure quality of arable soils. *Does clay*

- content matter? *Geoderma* 302, 14–21. <https://doi.org/10.1016/j.geoderma.2017.04.021>.
- Jupa, R., Plavcová, L., Gloser, V., Jansen, S., 2016. Linking xylem water storage with anatomical parameters in five temperate tree species. *Tree Physiol.* 36 (6), 756–769. <https://doi.org/10.1093/treephys/tpw020>.
- Kozono, D., Yasui, M., King, L.S., Agre, P., 2002. Aquaporin water channels: atomic structure molecular dynamics meet clinical medicine. *J. Clin. Invest.* 109 (11), 1395–1399. <https://doi.org/10.1172/JCI15851>.
- Kuebert, A., Dubbert, M., Bamberger, I., Kuehnhammer, K., Beyer, M., van Haren, J., et al., 2023. Tracing plant source water dynamics during drought by continuous transpiration measurements: an in-situ stable isotope approach. *Plant Cell Environ.* 46 (1), 133–149. <https://doi.org/10.1111/pce.14475>.
- Landwehr, J.M., Coplen, T.B., 2006. 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*. Vienna. IAEA, pp. 132–135. ISSN: 1562-4153.
- Lehmann, M.M., Schuler, P., Cormier, M.A., Allen, S.T., Leuenberger, M., Voelker, S., 2022. The stable hydrogen isotopic signature: from source water to tree rings. In: Siegwolf, R.T.W., Brooks, J.R., Roden, J., Saurer, M. (Eds.), *Stable Isotopes in Tree Rings: Inferring Physiological, Climatic and Environmental Responses*. Springer International Publishing, Cham, pp. 331–359. https://doi.org/10.1007/978-3-030-92698-4_11.
- Li, Y., Ma, Y., Song, X.F., Wang, L.X., Han, D.M., 2021. A delta H-2 offset correction method for quantifying root water uptake of riparian trees. *J. Hydrol. (Amst.)* 593. <https://doi.org/10.1016/j.jhydrol.2020.125811>.
- Li, Y., Ma, Y., Song, X.F., Wang, L.X., Yang, L.H., Li, X.Y., Li, B.H., 2022. Contrasting water use characteristics of riparian trees under different water tables along a losing river. *J. Hydrol. (Amst.)* 611, 128017. <https://doi.org/10.1016/j.jhydrol.2022.128017>.
- Lin, G.H., Sternberg, L.D.L., 1993. Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. *Stab. Isotop. Plant Carbon-Water Relat.* 497–510. <https://doi.org/10.1016/B978-0-08-091801-3.50041-6>.
- Magh, R.-K., Gralher, B., Herbstritt, B., Kubert, A., Lim, H., Lundmark, T., Marshall, J., 2022. Technical note: conservative storage of water vapour-practical in situ sampling of stable isotopes in tree stems. *Hydrol. Earth Syst. Sci.* 26 (13), 3573–3587. <https://doi.org/10.5194/hess-26-3573-2022>.
- Millar, C., Janzen, K., Nehemy, M.F., Koehler, G., Herve-Fernandez, P., Wang, H., et al., 2022. On the urgent need for standardization in isotope-based ecohydrological investigations. *Hydrol. Earth Syst. Sci.* 26 (10). <https://doi.org/10.1002/hyp.14698>.
- Millar, C., Pratt, D., Schneider, D.J., McDonnell, J.J., 2018. A comparison of extraction systems for plant water stable isotope analysis. *Rapid Commun. Mass Spectrom.* 32 (13), 1031–1044. <https://doi.org/10.1002/rcm.8136>.
- Nakada, R., Okada, N., Nakai, T., Kuroda, K., Nagai, S., 2019. Water potential gradient between sapwood and heartwood as a driving force in water accumulation in wetwood in conifers. *Wood Sci. Technol.* 53 (2), 407–424. <https://doi.org/10.1007/s00226-019-01081-4>.
- Nehemy, M.F., Benettin, P., Allen, S.T., Steppe, K., Rinaldo, A., Lehmann, M.M., McDonnell, J.J., 2022. Phloem water isotopically different to xylem water: potential causes and implications for ecohydrological tracing. *Ecohydrology* 15 (3). <https://doi.org/10.1002/eco.2417>.
- Prout, J.M., Shepherd, K.D., McGrath, S.P., Kirk, G.J.D., Haefele, S.M., 2021. What is a good level of soil organic matter? An index based on organic carbon to clay ratio. *Eur. J. Soil Sci.* 72 (6), 2493–2503. <https://doi.org/10.1111/ejss.13012>.
- Roden, J.S., Ehleringer, J.R., 1999. Hydrogen and oxygen isotope ratios of tree-ring cellulose for riparian trees grown long-term under hydroponically controlled environments. *Oecologia* 121 (4), 467–477. <https://doi.org/10.1007/s004420050953>.
- Roden, J.S., Ehleringer, J.R., 2000. There is no temperature dependence of net biochemical fractionation of hydrogen and oxygen isotopes in tree-ring cellulose. *Isotop. Environ. Health Stud.* 36 (3), 303–317. <https://doi.org/10.1080/10256010008036389>.
- Rothfuss, Y., Javaux, M., 2017. Reviews and syntheses: isotopic approaches to quantify root water uptake: a review and comparison of methods. *Biogeosciences* 14 (8), 2199–2224. <https://doi.org/10.5194/bg-14-2199-2017>.
- Sprenger, M., Tetzlaff, D., Soulsby, C., 2017. Soil water stable isotopes reveal evaporation dynamics at the soil-plant-atmosphere interface of the critical zone. *Hydrol. Earth Syst. Sci.* 21 (7), 3839–3858. <https://doi.org/10.5194/hess-21-3839-2017>.
- Steppe, K., Cochard, H., Lacoite, A., Ameglio, T., 2012. Could rapid diameter changes be facilitated by a variable hydraulic conductance? *Plant Cell Environ.* 35 (1), 150–157. <https://doi.org/10.1111/j.1365-3040.2011.02424.x>.
- Stock, B.C., Semmens, B.X., 2016. MixSIAR GUI User Manual. <https://doi.org/10.5281/zenodo.1209993> version 3.1. <https://github.com/brianstock/MixSIAR>.
- Sun, H.G., Gu, X.F., Zhu, J.T., Yu, Z.B., Zhang, Y., 2019. Fractal nature of groundwater level fluctuations affected by riparian zone vegetation water use and river stage variations. *Sci. Rep.* 9. <https://doi.org/10.1038/s41598-019-51657-0>.
- Tetzlaff, D., Buttle, J., Carey, S.K., Kohn, M.J., Laudon, H., McNamara, J.P., et al., 2021. Stable isotopes of water reveal differences in plant-soil water relationships across northern environments. *Hydrol. Earth Syst. Sci.* 25 (1). <https://doi.org/10.1002/hyp.14023>.
- Thorburn, P.J., Walker, G.R., Brunel, J.-P., 1993. Extraction of water from Eucalyptus trees for analysis of deuterium and oxygen-18: laboratory and field techniques. *Plant Cell Environ.* 16 (3), 269–277. <https://doi.org/10.1111/j.1365-3040.1993.tb00869.x>.
- Treydt, K., Lehmann, M.M., Wyczaszny, T., Pfautsch, S., 2021. Radial and axial water movement in adult trees recorded by stable isotope tracing. *Tree Physiol.* 41 (12), 2248–2261. <https://doi.org/10.1093/treephys/tpab080>.
- Vargas, A.I., Schaffer, B., Li, Y.H., Sternberg, L.D.L., 2017. Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. *New Phytol.* 215 (2), 582–594. <https://doi.org/10.1111/nph.14616>.
- Wang, J., Fu, B.J., Lu, N., Zhang, L., 2017. Seasonal variation in water uptake patterns of three plant species based on stable isotopes in the semi-arid Loess Plateau. *Sci. Total Environ.* 609, 27–37. <https://doi.org/10.1016/j.scitotenv.2017.07.133>.
- Wang, J., Fu, B.J., Wang, L.X., Lu, N., Li, J.Y., 2020. Water use characteristics of the common tree species in different plantation types in the Loess Plateau of China. *Agric. For. Meteorol.* 288–289, 108020. <https://doi.org/10.1016/j.agrformet.2020.108020>.
- Wang, L.X., Caylor, K.K., Villegas, J.C., Barron-Gafford, G.A., Breshears, D.D., Huxman, T.E., 2010. Partitioning evapotranspiration across gradients of woody plant cover: assessment of a stable isotope technique. *Geophys. Res. Lett.* 37, 7. <https://doi.org/10.1029/2010gl043228>.
- Wang, L.X., Good, S.P., Caylor, K.K., Cernusak, L.A., 2012. Direct quantification of leaf transpiration isotopic composition. *Agric. For. Meteorol.* 154, 127–135. <https://doi.org/10.1016/j.agrformet.2011.10.018>.
- Wang, P., Li, X.Y., Wang, L.X., Wu, X.C., Hu, X., Fan, Y., Tong, Y.Q., 2018. Divergent evapotranspiration partition dynamics between shrubs and grasses in a shrub-encroached steppe ecosystem. *New Phytol.* 219 (4), 1325–1337. <https://doi.org/10.1111/nph.15237>.
- Wen, M.Y., He, D., Li, M., Ren, R.Q., Jin, J.J., Si, B.C., 2022. Causes and factors of cryogenic extraction biases on isotopes of xylem water. *Water Resour. Res.* 58 (8). <https://doi.org/10.1029/2022wr032182>.
- White, J.W.C., Cook, E.R., Lawrence, J.R., Broecker, W.S., 1985. The DH ratios of sap in trees—Implications for water sources and tree-ring DH ratios. *Geochim. Cosmochim. Acta* 49 (1), 237–246. [https://doi.org/10.1016/0016-7037\(85\)90207-8](https://doi.org/10.1016/0016-7037(85)90207-8).
- Yakir, D., DeNiro, M.J., 1990. Oxygen and hydrogen isotope fractionation during cellulose metabolism in lemna gibba L. 1. *Plant Physiol.* 93 (1), 325–332. <https://doi.org/10.1104/pp.93.1.325>.
- Zhao, L.J., Wang, L.X., Cernusak, L.A., Liu, X.H., Xiao, H.L., Zhou, M.X., Zhang, S.Q., 2016. Significant difference in hydrogen isotope composition between xylem and tissue water in populus euphratica. *Plant Cell Environ.* 39 (8), 1848–1857. <https://doi.org/10.1111/pce.12753>.
- Zhao, L.J., Xiao, H.L., Zhou, J., Wang, L.X., Cheng, G.D., Zhou, M.X., et al., 2011. Detailed assessment of isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. *Rapid Commun. Mass Spectrom.* 25 (20), 3071–3082. <https://doi.org/10.1002/rcm.5204>.
- Zhao, Y., 2021. An incorrect wetness-based correction method for deuterium offset. *Proc. Natl. Acad. Sci.* 118 (15). <https://doi.org/10.1073/pnas.2026641118>.
- Zhao, Y., Dai, J., Tang, Y., Wang, L., 2022. Illuminating isotopic offset between bulk soil water and xylem water under different soil water conditions. *Agric. For. Meteorol.* 325. <https://doi.org/10.1016/j.agrformet.2022.109150>.
- Zuecco, G., Amin, A., Frentress, J., Engel, M., Marchina, C., Anfodillo, T., et al., 2022. A comparative study of plant water extraction methods for isotopic analyses: scholander-type pressure chamber vs. cryogenic vacuum distillation. *Hydrol. Earth Syst. Sci.* 26 (13), 3673–3689. <https://doi.org/10.5194/hess-26-3673-2022>.