Ecohydrology

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RESEARCH ARTICLE OPEN ACCESS

Xylem and Soil Water Extraction via Centrifugation in a Subtropical Urban Green Space

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Received: 31 May 2024 | **Revised:** 23 April 2025 | **Accepted:** 25 June 2025

Funding: This work was supported by the University of Texas at Arlington (314075) and the University of Texas System (AR911486).

Keywords: centrifugation extraction | isotopic bias | soil and xylem water isotopes | urban tree species

ABSTRACT

Extracting water from discrete xylem and soil samples for $\delta^{18}O$ and $\delta^{2}H$ analysis remains a central challenge in ecohydrology. This study evaluates the applicability of the centrifugation method in extracting (a) mobile water with (b) no induced fractionation and (c) low organic contamination. Three common urban tree species (elderberry, *Sambucus canadensis*; cherry laurel, *Prunus caroliniana*; and boxelder maple, *Acer negundo*) were studied for 1 year in the Fort Worth Botanic Garden (north-central Texas, United States). Xylem ($N = 110$) isotope values were compared alongside throughfall ($N = 33$), soil water ($N = 79$) and lysimeter water ($N = 42$) at different depths (0–38 cm). Xylem and soil water extraction volumes ranged from 0.1 to 7.5 mL and 0.1 to 10.5 mL, respectively. Extracted water volumes exhibited no strong correlations with isotopic values. Water was extracted from soil and xylem samples with gravimetric water contents as low as 10.6% and 17.8%, respectively. Although elderberry and cherry laurel exhibited narrowband and broadband values (indicators for organic contamination) similar to throughfall, soil water and lysimeter water, boxelder maple reported higher values in summer and fall. Annual $\delta^{18}O$ mean values in soil water (−3.2‰ ± 1.7‰) and lysimeter water (−3.3‰ ± 1.1‰) corresponded with the throughfall input (−3.1‰ ± 2.0‰). Xylem $\delta^{18}O$ exhibited higher values in winter, summer and fall than throughfall, soil water or lysimeter water. However, during spring, xylem mean $\delta^{18}O$ values were less variable and close to soil and lysimeter $\delta^{18}O$ values: elderberry (−3.4‰ ± 0.5‰), cherry laurel (2.9‰ ± 0.5‰) and boxelder (−2.3‰ ± 0.4‰). Our results highlight the applicability of the centrifugation method in extracting mobile water, unbiased by low extraction volumes, temperature-induced fractionation or high organic contamination. Low xylem and soil water contents limit the extraction capacity of centrifugation and increase the concentration of organics, particularly during summer and fall seasons.

1 | Introduction

For decades, cryogenic vacuum extraction (CVE) has been used as the reference method to obtain soil and xylem water for $\delta^{2}H$ and $\delta^{18}O$ analysis in ecohydrological studies (Dalton 1989; Brooks et al. 2010; Koeniger et al. 2011; Orlowski et al. 2013). Although CVE yields high water extraction efficiencies when compared to other methods (Wen et al. 2022), the cryo-extracted water is a combination (of unknown proportions) of chemically bound water (i.e., immobile mineralogical or biological/enzymatic water, often hydraulically isolated from active transpiration transport) and soil/xylem mobile water (i.e., the analyte of interest involved in the transpiration process) (Gaj et al. 2017; von Freyberg et al. 2020; Millar et al. 2021). In addition, CVE

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potentially enhances isotope exchange between xylem water and noncrystalline hydroxyl groups of wood cellulose and hemicellulose (Younger et al. 2024).

Despite the extended use of CVE in the isotope ecohydrology community, this analytical procedure presents a significant challenge in the form of complex temperature-induced fractionation effects (Wen et al. 2022; Sobota et al. 2024; Haberstroh et al. 2024; Wang et al. 2024), often accompanied by poor assessments on the impacts of low extraction volumes in the isotope values. As evidence of these challenges, a growing body of scientific literature points towards substantial analytical biases in soil and xylem CVE-δ^2H ratios (Orlowski et al. 2016; Chen et al. 2020; Allen and Kirchner 2022; Song et al. 2021; Wen et al. 2022; Zuecco et al. 2022; Yang et al. 2023; Wen et al. 2023; Younger et al. 2024; Duvert et al. 2024; Ceperley et al. 2024). For example, a recent study of plant xylem water across northeastern US trees (Sobota et al. 2024) demonstrated that CVE-δ^2H biases can result in a significantly different understanding of water uptake and transpiration and recommended that xylem δ^2H values via CVE should not be used in ecohydrological studies. Laboratory experiments have also shown that labelled water used in 'spike tests' could not be reliably recovered due to the inherent complexity of wetting and drying hysteresis processes in soils and plant samples (Thielemann et al. 2019; Wen et al. 2021). These analytical artefacts result in unexpected and sometimes enigmatic high and more commonly low isotopes values (Duvert et al. 2022; Nehemy et al. 2022; Jiang et al. 2022).

The advent of laser spectroscopy has motivated the introduction of new in-house and field extraction techniques, such as vapour equilibration, centrifugation, cavitron, microwave in-line distillation, Scholander pressure chamber, high-pressure mechanical squeezing, passive lysimeters and hygroscopic salt of a high water absorbance capacity ($CaCl_2$) (Fischer et al. 2019; Millar et al. 2022; Zuecco et al. 2022; He et al. 2023; Duvert et al. 2024; El-Shenawy et al. 2024). Although these new extraction techniques offer a large potential in decreasing the analytical bias in xylem and soil isotope values, there is still a lack of standard operating protocols (SOP) to conduct robust intercomparisons, as clearly pointed out in recent reviews by Millar et al. (2022) and Ceperley et al. (2024).

In soils, water exists in various physicochemical states, ranging from loosely bound mobile water, which actively participates in plant water uptake and hydrological processes, to immobile water, which includes tightly bound water adsorbed onto mineral surfaces or trapped in micropores. CVE extracts all water regardless of its mobility. As a result, it can include immobile water with distinct isotopic compositions that are not accessible to plants, potentially introducing biases (Orlowski et al. 2016; Gaj et al. 2017; Sprenger et al. 2019). Similarly, in xylem tissues, physical methods may extract water from functional (transpiration-contributing) and nonfunctional (e.g., parenchyma or embolized conduits) compartments. These compartments may contain immobile water with isotopic signatures not representative of the transpiration stream, especially when water is extracted from deeper tissues or during periods of low water content (Barbeta et al. 2020; Poca et al. 2019). Recent studies advocate for methods that selectively extract mobile water, such as centrifugation under controlled negative pressure, to address these issues. This method has shown promise in isolating plant-accessible water with minimal isotopic fractionation and organic contamination (Chen et al. 2020; Wen et al. 2022).

Constraining the negative pressure applied to extract the water offers the best physically based parameter to discriminate water extraction types. Clearly, CVE combines residual moisture at higher pressures or beyond the xylem limits and mobile water versus extracting water in the plant-available region via centrifugation. We present a detailed analytical centrifugation procedure to study a subtropical urban green space in north-central Texas (United States) from January to December 2023. The main objectives are to (a) evaluate the applicability of the centrifugation extraction method in a water-stressed setting and (b) understand seasonal isotopic variability from three common urban tree species (elderberry, *Sambucus canadensis*; cherry laurel, *Prunus caroliniana*; and boxelder maple, *Acer negundo*), during an unprecedented warm period under the influence of a strong El Niño event (Jiang et al. 2024). During a hydrological year, monthly xylem samples are compared with throughfall, soil water and lysimeter water (at different depths, 0–38 cm). We hypothesize that centrifugation can extract (a) mainly mobile water with (b) no induced fractionation and (c) low organic contamination. Our findings contribute to the ongoing global discussion on analytical water extraction techniques by highlighting the applicability and limitations of the centrifugation method in obtaining isotopically representative water for stable isotope analysis in xylem and soil samples. The improved SOP presented in this study, previously validated in the wet/dry tropics of Central America (Sánchez-Murillo et al. 2023), was evaluated under drier conditions within a subtropical humid climate.

2 | Experimental Ecohydrological Site

The Fort Worth Botanic Garden is a 120-acre (0.48 km^2) temperate evergreen and deciduous forest in Fort Worth, Texas, within the Cross Timbers ecoregion (Griffith et al. 2004). The Botanic Garden lies within the Trinity River watershed, specifically the Clear Fork tributary. Historically, the site was within the Trinity River floodplain, but channelization and levee projects have altered the river's natural hydrologic flowpaths and affected riparian vegetation (Best 2021). The predominant soil textures are loam and clay loam. The site has a humid subtropical climate with hot, humid summers and mild winters. Based on a 30-year normal period, the mean annual (high-low) temperature varies between 24.9°C and 13.6°C, respectively (US Department of Commerce, NOAA 2024). The city experiences high temperatures in the summer, often exceeding 32.2°C and sometimes reaching over 37.8°C. Average annual precipitation is 940 mm, with wet spring and fall seasons and drier summer and winter seasons (US Department of Commerce, NOAA 2024). Most annual rainfall in the region comes from frontal storms in spring and fall and convection during summer (TWDB 2012). The Gulf of Mexico breeze and subtropical disturbances generate summer thunderstorms. During spring and fall, warm, dry air from northern

Mexico collides with humid air from the Gulf of Mexico due to the forcing of the jet stream and the Bermuda High System. This collision leads to severe thunderstorms and tornadoes. Thunderstorms are common, especially in the spring and summer months. Most of the annual rainfall in Texas originates from storms like these, with flashy episodes of heavy precipitation inputs. On average, however, potential evapotranspiration exceeds precipitation. The average annual potential evapotranspiration in Fort Worth is 1419 mm (Texas A&M AgriLife Extension Service 2024: https://texaset.tamu.edu/). El Niño Southern Oscillation (ENSO) has long-term influences on Texas' precipitation, with above-average precipitation occurring during El Niño and below-average precipitation and drought occurring during La Niña conditions.

3 | Methods and Materials

3.1 | Throughfall Collection

Weekly throughfall samples ($N = 33$) were collected from a 3L RS1 Palmex passive collector (Palmex Ltd., Croatia; Gröning et al. 2012) installed beneath the canopy 1 m above the ground. Precipitation volume was measured using a 1000-mL graduated cylinder and converted to mm using the funnel area. The water was then poured to fill a 30-mL HDPE bottle labelled with the location, time, date and volume of precipitation. Samples were capped (no headspace), sealed with Parafilm and transported to the lab in a cooler with ice gel packs at approximately 5°C. Fort Worth weather data were collected from the NOAA Climate Data Online website from station USW00013911 (latitude/longitude: 32.77°, −97.45°; elevation: 185.3 masl) (NCEI 2024). All weather data are available in Table S1.

3.2 | Cup Suction Lysimeters Installation, Soil Water Collection and Soil Water Content and Temperature Monitoring

Slim tube ceramic cup suction lysimeters (Model 1905L, SoilMoisture Equipment Corporation, United States) were installed at depths of 13, 25 and 38 cm, each at an angle of 45° to the ground. In addition, a bentonite layer was also applied at the surface to avoid preferential flow paths (Product #0922W050, SoilMoisture Equipment Co., United States). Cup samplers were placed 20 cm apart in a horizontal line about 1 m from the throughfall sampler, away from major roots or large trees. A suction of 80 cbar (80 kPa) was applied to each cup sampler every week using a vacuum hand pump (Model 2005G2, SoilMoisture Equipment Co., United States). Lysimeter water samples ($N = 42$) were extracted with a 1-L polypropylene Erlenmeyer flask, nylon tubing and stopper assembly. Suction was applied by hand pump until all the water in the cup sampler was drained into the flask. Samples were capped (no headspace), sealed with Parafilm and transported to the lab in a cooler with ice gel packs at approximately 5°C. Soil conditions were monitored using a solar-powered HOBOlink Micro RX wireless data logger station (1-h transmission interval) connected to three wireless HOBOnet T12 Soil Moisture (m^3/m^3) and temperature (°C) sensors, each installed at 0-, 18- and 38-cm soil depth. Sensors were installed on 28 June 2023; these data only cover the summer and fall seasons. All soil sensor data is available in Table S1.

3.3 | Soil Texture Analysis

Four soil samples were collected from the surface to a depth of 40 cm in agreement with the typical shallow root systems of the three species selected (Section 3.5). Below this soil depth, clayey restrictive layers can be found (USDA 2024). Each sample was divided into two 1-L-size plastic bags and mailed to the Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory for texture analysis (sand, silt and clay) and organic matter content. All analyses were reported on a dry soil basis (Texas A&M AgriLife Extension 2012). Upon arrival at the Texas A&M soil testing laboratory, samples were transferred to an aluminium pan and oven-dried at 65°C (± 2°C) in a forced air oven for 16 h or until fully dry. Samples were pulverized using an open mesh bottom hammer-style soil pulverizer and screened to remove particles larger than 2 mm. A weight loss-on-ignition procedure determined organic carbon content (Schulte and Hopkins 1996) after being ground to pass through a No. 80 mesh. Soil texture fractions were determined via a classical hydrometer procedure (Day 1965).

3.4 | Plant Species Selection

The aim of our selection of plants for this study was to include woody plant species common to the urban green space of the Dallas-Fort Worth metroplex in north-central Texas. The following trees were selected for sampling: elderberry (*S. canadensis*), cherry laurel (*P. caroliniana*) and boxelder maple (*A. negundo*). Elderberry is a deciduous tree or shrub native to North America with an aggressive root system. The individual sampled was approximately 2.5 m tall, and it had thin (up to 1-cm diameter) fibrous roots and a deeper taproot. Cherry laurel is an evergreen tree native to the southern United States with a shallow but complex root system, making it drought-tolerant when established. The sampled individual was approximately 9 m tall and had roots within 10 to 30 cm of soil depth, about 6 cm in diameter. Boxelder maple is a relatively short-lived (about 60 years; USDA 2024), brittle-wooded, deciduous tree native to North America with shallow, strong lateral roots. The sampled individual was about 20 m tall and had roots up to 5 cm in diameter at about 12-cm depth. Voucher specimens for all three species were deposited in the herbarium at the Botanical Research Institute of Texas (BRIT) (*A. negundo*, M.B. Byerley 715; *P. caroliniana*, M.B. Byerley 716; and *S. canadensis*, M.B. Byerley 747).

3.5 | Stem and Soil Sample Collection

Stem ($N = 160$) and soil ($N = 105$) samples were collected during monthly campaigns throughout 2023. Stem samples were collected by cutting and removing woody stems. Twigs, leaves and knots were removed. The branch was then trimmed and divided into six subsamples. Subsamples were transferred to 50-mL polypropylene conical centrifuge tubes (Falcon No. 352070). For elderberry, the branch lengths ranged from 72 cm to 1 m, with an average diameter of 0.55 ± 0.17 cm. Boxelder branches ranged

from 1.0 cm to 1.5 m, with an average diameter of 1.55 ± 0.79 cm. Cherry laurel branches ranged from 1.0 cm to 2.0 m, with an average diameter of 1.69 ± 0.47 cm. Each centrifuge tube was filled on average with ~30 g (stems) or ~77 g (soils). Because our study aims to analyse the mobile water stored in the tree, the bark, phloem and cambium layers of the stems were scraped off using a pocketknife, leaving only the xylem wood for extraction. Destructive soil samples were triplicated using 50-mL polypropylene conical centrifuge tubes (Falcon No. 352070). A new trench was excavated each time across the study area. These samples were collected using a soil auger or garden shovel, extracting samples from specific depth intervals. Samples were taken typically at defined layers: 0, 13, 25 and 38 cm. All centrifuge tubes were immediately covered with Parafilm, stored in a cooler with ice gel packs (5°C) and transported to the laboratory for water extractions. In the lab, samples were stored at approximately 5°C, and extractions were conducted within 1–2 days after collection.

3.6 | Xylem and Soil Water Extraction and Gravimetric Analysis

The total water content (TWC, %) and the proportion of water extracted (%) (both calculated by gravimetric analysis) were used to evaluate the potential impacts of low extraction volumes on xylem and soil isotope values. Before centrifugation, all samples were weighed (wet mass) (± 0.01 g) in the laboratory. Xylem or soil material was removed to balance the mass in the centrifugation tubes. Following the methodology proposed by Sánchez-Murillo et al. (2023), water was extracted from xylem and soil samples at 11,000 rpm and 5°C for 1.5 h (Eppendorf 5810R centrifuge). After centrifugation, the extracted water (EW) was transferred immediately with a 1000 μL micropipettor (Four E's Scientific, United States) to a 2-mL glass vial and sealed with a septum cap. A 300-μL glass insert was used when the EW volume was below 1.0 mL. The remnant stem and soil samples were weighed (wet mass after centrifugation), transferred to an oven-safe aluminium pan and oven-dried for 48 h at 105°C. The final sample mass (oven-dried) was also recorded. Gravimetric analysis results are provided in Table S2.

3.7 | Isotopic Analysis

Isotopic analysis was conducted by the Tracer Hydrology Group in the Department of Earth and Environmental Sciences at the University of Texas-Arlington, using a GLA431-TLWIA triple water isotope analyser. Three calibrated secondary standards 5D ($\delta^2H = -10.5‰$ and $\delta^{18}O = -3.00‰$), 4D ($\delta^2H = -48.7‰$ and $\delta^{18}O = -7.63‰$) and 1D ($\delta^2H = -161.3‰$ and $\delta^{18}O = -20.72‰$) were used to normalize the results using a block standardization procedure (i.e., LGR LWIA Post Analysis Software Version 4.5.0.6). An additional standard was used as an internal control, IC ($\delta^2H = -78.0‰$ and $\delta^{18}O = -10.76‰$). This internal control was injected three times per analytical batch. The total number of injections per sample or standard comprised two preparations and eight measurement injections. The $^{18}O/^{16}O$ and $^2H/^1H$ ratios are presented in delta notation δ (‰), relative to the VSMOW2-SLAP2 scale. The analytical uncertainty was ± 0.33‰ for δ^2H and ± 0.08‰ for $\delta^{18}O$. The deuterium excess (hereafter *d*-excess; Dansgaard 1964) was calculated as $d\text{-}excess = \delta^2H\text{-}8{\cdot}\delta^{18}O$. The LC-excess (Landwehr and Coplen 2006) was computed as $LC\text{-}excess = \delta^2H - a{\cdot}\delta^{18}O\text{-}b$, where a and b are the slope and intercept of the meteoric water line, respectively. The narrowband (e.g., methanol) and broadband (e.g., ethanol) (unitless) were recorded and used as indicators of organic contamination (Leen et al. 2012). Elderberry and cherry laurel exhibited narrowband and broadband values similar to throughfall, soil water and lysimeter water, whereas boxelder maple reported higher values in summer and fall (see Figure S1). In general, narrowband values were in the reported range of methanol concentrations below 25 ppm_v, corresponding to potential isotope measurement errors below 0.2% in $\delta^{18}O$ and δ^2H (Leen et al. 2012), whereas broadband values also suggested minimal influence of large organic molecules with -OH groups (Leen et al. 2012). Isotope results are provided in Table S2.

4 | Results

4.1 | Dynamics in Hydroclimate and Soil Characteristics

The total precipitation during the study period was 661.0 mm. During summer, precipitation contributed only 5.8%, whereas spring and fall precipitation corresponded with 72.3% (Figure 1A). The largest daily precipitation was 125.5 mm during fall. Rainy days averaged 9.7 ± 18.0 mm. The mean annual air temperature was 21.1°C, with mean summer and winter temperatures of 31.9°C and 11.8°C, respectively. The minimum winter temperature was −3.3°C, and the maximum summer temperature was 35.8°C. Mean atmospheric temperatures were 22.4°C and 17.5°C in spring and fall. Summer 2023 was characterized by 69 days with a mean daily temperature above 30°C and only four rainfall events (Figure 1A).

Soil water temperature followed the atmospheric trend during summer and fall. During the summer, mean soil surface temperature was 29.3°C ± 2.1°C, with a maximum temperature of 32.1°C by the end of August. Mean soil temperature was cooler by 1.2°C (18 cm) and 1.9°C (38 cm) with depth. Maximum soil temperatures at 18 and 38 cm were 30.3°C and 29.4°C, respectively (Figure 1B). Cooler soil conditions were observed during fall, with a mean soil surface temperature of 17.4°C ± 4.9°C. Similarly, lower mean soil temperatures were reported at 18 cm (18.2°C ± 4.4°C) and 38 cm (18.8°C ± 4.0°C).

During summer, the lowest mean volumetric water content was recorded at the surface (0.14 ± 0.03 m^3/m^3). The higher clay content with depth (Figure 1D) contributed to greater mean volumetric water content at 18 cm (0.19 ± 0.01 m^3/m^3) and 38 cm (0.18 ± 0.03 m^3/m^3). Although the volumetric water content showed a decreasing trend in the summer, more steady conditions were observed with depth. In the fall, precipitation events increased the soil volumetric water content, with similar mean values at the surface (0.28 ± 0.03 m^3/m^3) and at 18 cm (0.28 ± 0.04 m^3/m^3) and 38 cm (0.27 ± 0.05 m^3/m^3) (Figure 1C).

The textural class of the soils ranged from loam at the surface (0 cm) to clay loam at all other depths (13, 25 and 38 cm), with increasing clay content with depth up to 34% (Figure 1D). Organic

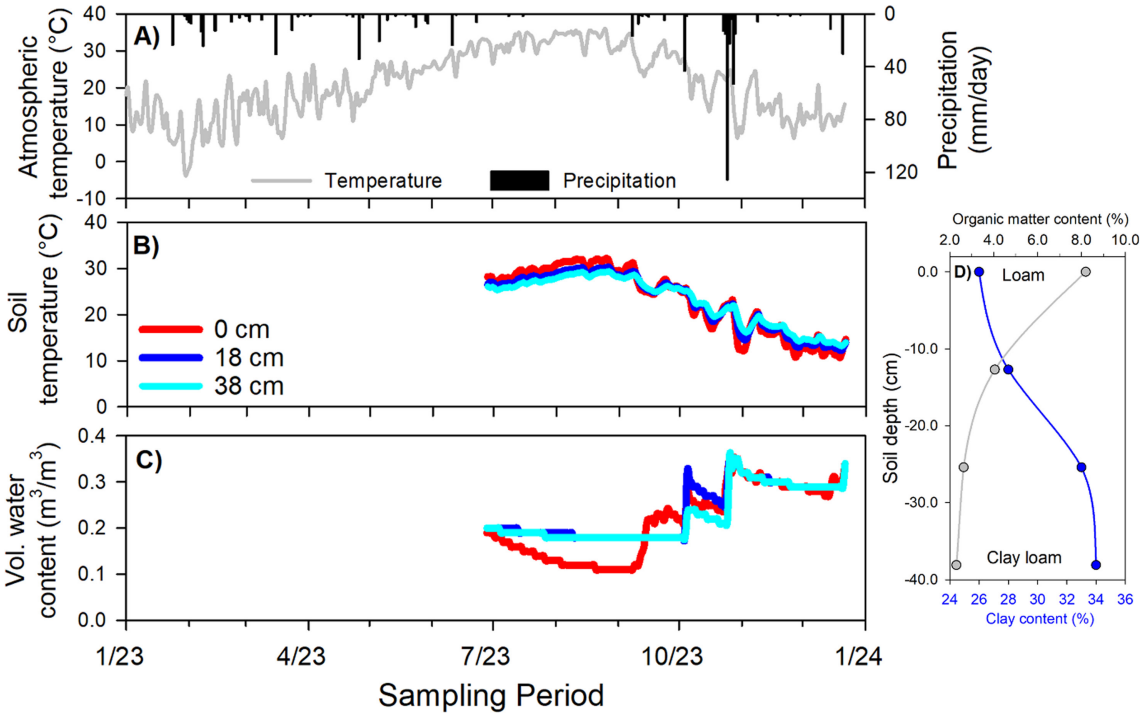


FIGURE 1 | (A) Daily precipitation (mm/day) (black bars) and mean daily atmospheric temperature (°C) (grey line) during 2023. Data obtained from the Fort Worth weather station USW00013911 (lat/long: 32.77°, −97.45°; elevation: 185.3 masl). (B) Mean daily soil temperature (°C) at three depths (0, 18 and 38 cm). (C) Mean daily volumetric water content (m^3/m^3) at three depths (0, 18 and 38 cm). Soil sensor data were collected from 28 June to 22 December 2023. Soil depths (0, 18 and 38 cm) are colour-coded. (D) Organic matter (%) and clay content (%) with depth. Soil texture varies from loam at the surface (0–10 cm) to clay loam (13–38 cm).

matter content decreased with depth as follows: 8.2% (0 cm), 4.1% (13 cm), 2.6% (25 cm) and 2.3% (38 cm) (Figure 1D).

4.2 | Xylem and Soil Gravimetric Total Water Content and Extracted Water

TWC and EW varied across endmembers and seasons. Among vegetation types, elderberry exhibited the highest median TWC (60.0%). Boxelder maple and cherry laurel followed with median TWC values of 47.4% and 40.3%, respectively (Figure 2A). Across seasons, median TWC peaked in spring for elderberry (72.0%) with a progressive decrease in summer and fall. In contrast, median TWC values were fairly consistent for boxelder maple across all seasons, with the lowest TWC values in late spring (21.6%) and summer (25.2%). Cherry laurel exhibited similar median TWC values across all seasons, with more variability during the fall season.

The selectivity of the centrifugation method for assessing mobile water is reflected in the proportion of EW relative to the TWC available in the xylem. Boxelder maple resulted in the highest median EW (7.5%), with values ranging from 24.0% (spring) to 1.2% (summer) (Figure 2B). For cherry laurel, the median EW was 2.5%, with values ranging from 28.1% (fall) to 0.9% (spring). One outlier EW value (76.5%) was also reported in the fall for cherry laurel. The highest and lowest EW values in elderberry were reported in the spring (17.6%) and fall (1.6%). Elderberry exhibited a median EW value of 5.4%, with a consistent decreasing trend towards the fall (Figure 2B).

Soil samples displayed a clear depth-dependent gradient. Surface soil (0 and 13 cm) had median TWC values around 24%, with EW medians ranging from 9.0% to 10.8% (Figure 2A,B). In contrast, deeper soil layers (25 and 38 cm) showed slightly lower TWC values (22.4% and 19.5%, respectively). Interestingly, the 25-cm layer had the highest EW among all soils (18.4%), whereas the deeper soil layer had the lowest (5.9%). Generally, the lowest soil TWC values were reported in the summer, whereas the highest TWC values were recorded in the spring and fall seasons.

4.3 | Influence of Extracted Water Volume on Xylem and Soil Isotope Values

The potential influence of EW volume on xylem and soil isotope values has been poorly assessed in the literature. In this study, all adjusted R^2 values indicate relatively poor or noisy relationships between EW volume and $\delta^{18}O$ and *d*-excess values (Figure 3A,B). However, larger extracted volumes were associated with more stable or less variable $\delta^{18}O$ and *d*-excess values, particularly in shallow soil layers. In contrast, smaller extracted volumes were linked to greater isotopic variability. Among the plant species, boxelder maple exhibited the highest and significant adjusted R^2 values for $\delta^{18}O$ (0.19, $p = 0.005$) and *d*-excess (0.14, $p = 0.01$). Cherry laurel and elderberry adjusted R^2 indicated a relatively poor influence of the EW volume on the isotope values. Across the soil profile, the highest adjusted R^2 values for $\delta^{18}O$ (0.12; $p = 0.03$) and *d*-excess (0.12) were reported at 25 cm, whereas adjusted R^2 values in surface soil layers (0–13 cm) suggested a weak effect on the isotope values. The limited number

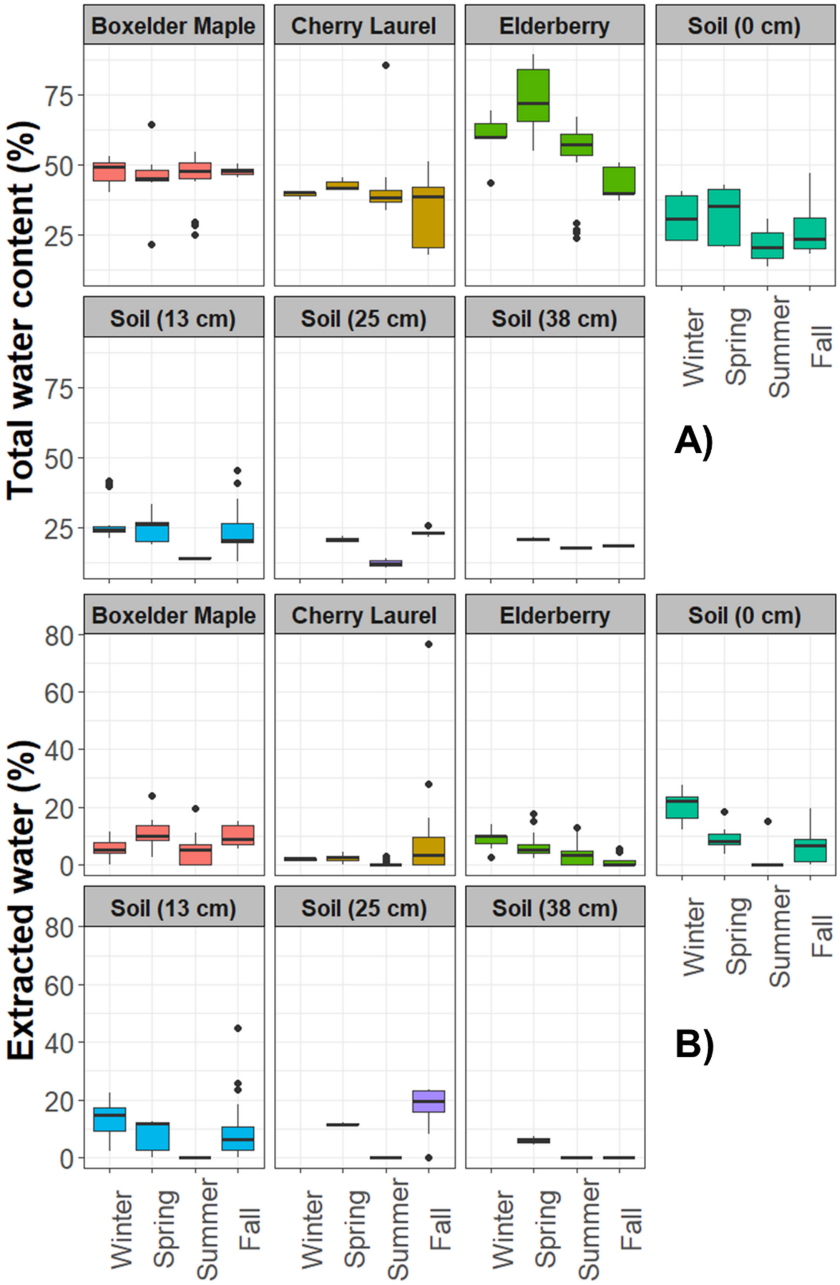


FIGURE 2 | (A) Seasonal box plots showing gravimetric total water content (%) in three selected urban plant species and soils at different depths (0, 13, 25 and 38 cm). (B) Seasonal box plots showing gravimetric extracted water (%) via centrifugation in three selected urban plant species and soils at different depths (0, 13, 25 and 38 cm).

of observations at 38 cm prevented calculating an adjusted R^2 value. In several cases, the adjusted R^2 was low or even negative. Low adjusted R^2 values indicate a weak relationship between the volume of EW and $\delta^{18}O$ or *d*-excess. Negative adjusted R^2 values occur when the model fits the data worse than a horizontal line (i.e., worse than using the mean as a predictor), highlighting a lack of explanatory power.

4.4 | Throughfall, Soil Water, Lysimeter Water and Xylem Seasonal/Annual Isotope Variability

Throughfall exhibited variable $\delta^{18}O$ values, with a higher median value in summer (−1.2‰) than winter (−2.6‰), spring (−2.6‰) and fall (−5.0‰). Higher median *d*-excess was recorded in winter (12.1‰) and fall (15.8‰), with spring (9.7‰) and summer (9.3‰) closer to the global *d*-excess value (Figure 4A,B). Lysimeter water showed a clear decreasing trend in median $\delta^{18}O$ values with depth: 0 cm (−1.9‰), 13 cm (−2.9‰), 25 cm (−3.8‰) and 38 cm (−4.3‰). In contrast, *d*-excess was characterized by an increasing trend with depth: 0 cm (9.4‰), 13 cm (10.4‰), 25 cm (12.3‰) and 38 cm (15.9‰). Soil water median $\delta^{18}O$ values at the surface (−3.3‰) were lower than lysimeter water. Lysimeter water was collected weekly, whereas destructive soil samples were often collected after or within precipitation events. Soil water median $\delta^{18}O$ values showed a similar decreasing trend with depth: 13 cm (−3.1‰), 25 cm (−3.8‰) and 38 cm (−4.0‰). Similarly, soil

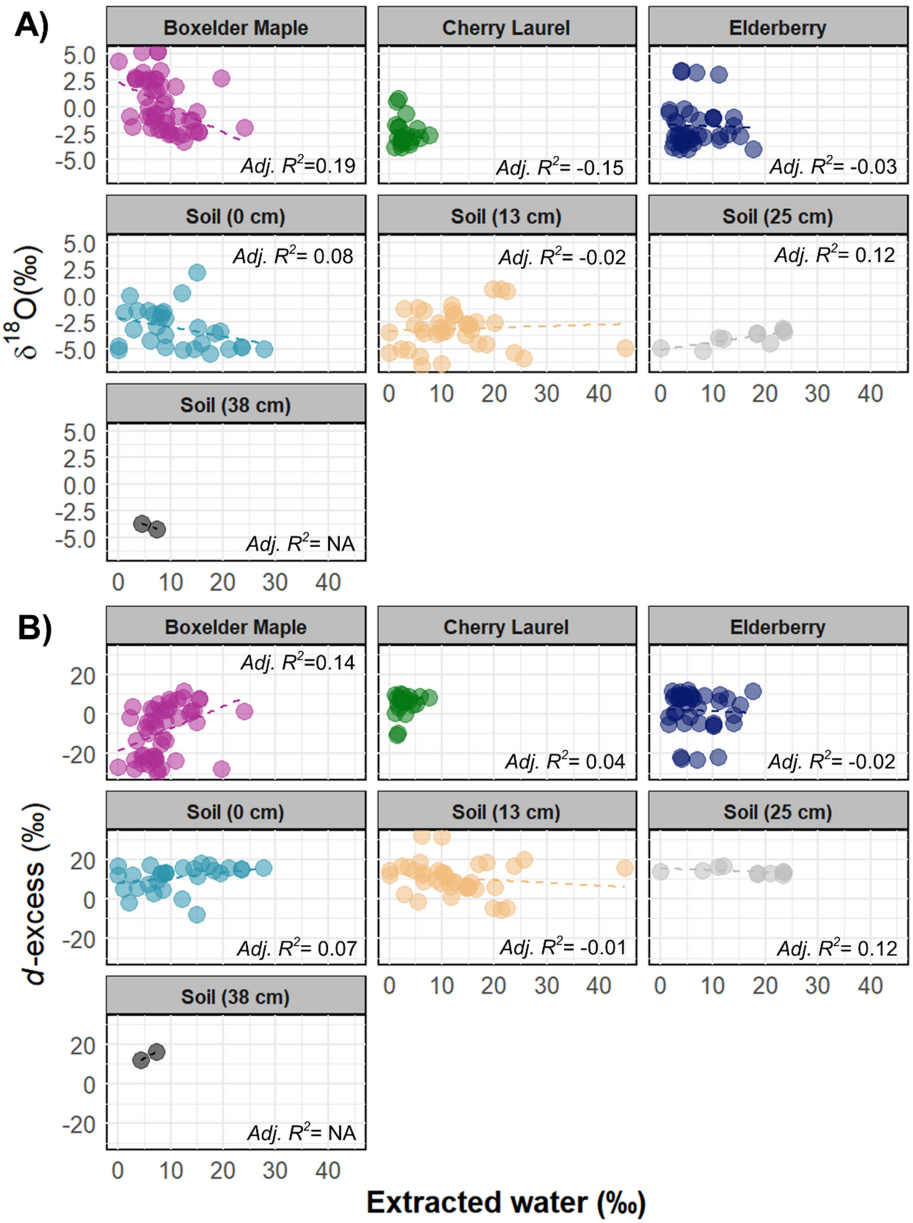


FIGURE 3 | Linear regressions between extracted water (%) via centrifugation and $\delta^{18}O$ (‰) and *d*-excess (‰) values for soils and xylems (Bolxelder Maple, Cherry Laurel and Elderberry). Adjusted R^2 values denote the negligible influence of the extracted water amount on the isotopic values.

water samples showed high *d*-excess across the depth profile. Both lysimeter water and soil water *d*-excess agree with throughfall (Figure 4A,B).

Boxelder maple exhibited relatively high $\delta^{18}O$ values across seasons, with lower median values in spring (−2.3‰) and fall (−0.5‰) than in winter (3.2‰) and summer (2.5‰). The highest *d*-excess values were recorded in spring (5.0‰), with low values in fall (−4.0‰), winter (−21.4‰) and summer (−22.2‰) (Figure 4A,B). Cherry laurel showed lower $\delta^{18}O$ values compared to boxelder maple, particularly in spring and winter (−3.0‰ and −3.8‰, respectively), followed by fall (−2.4‰) and summer (−1.2‰). The *d*-excess pattern revealed a strong seasonal contrast, with a low median value of 1.7‰ in summer and high values in winter (9.7‰), spring (6.8‰) and fall (6.7‰). Elderberry presented the strongest connectivity with throughfall and soil/lysimeter median $\delta^{18}O$ values in the spring (−3.4‰) and summer (−2.0‰). Higher median $\delta^{18}O$ values were observed in elderberry in winter (−1.2‰) and fall (−0.4‰) than in the growing season. Similarly, median *d*-excess values were higher in spring (8.7‰) and summer (4.3‰) than in winter (−4.5‰) and fall (−3.2‰) (Figure 4A,B).

On an annual basis, median $\delta^{18}O$ and *d*-excess values in soil (−3.42‰ and 12.8‰) and lysimeter (−3.33‰ and 12.2‰) align with throughfall (−3.28‰ and 12.2‰). Plant species exhibited higher annual median $\delta^{18}O$ and *d*-excess values than throughfall: cherry laurel (−2.89‰ and 6.8‰), elderberry (−2.63‰ and 6.2‰) and boxelder maple (−0.64‰ and −4.38‰). During the growing spring season, all tree species showed a strong connectivity to the soil available moisture, which was depicted in similar $\delta^{18}O$ and *d*-excess: cherry laurel (−3.0‰ and 6.8‰),

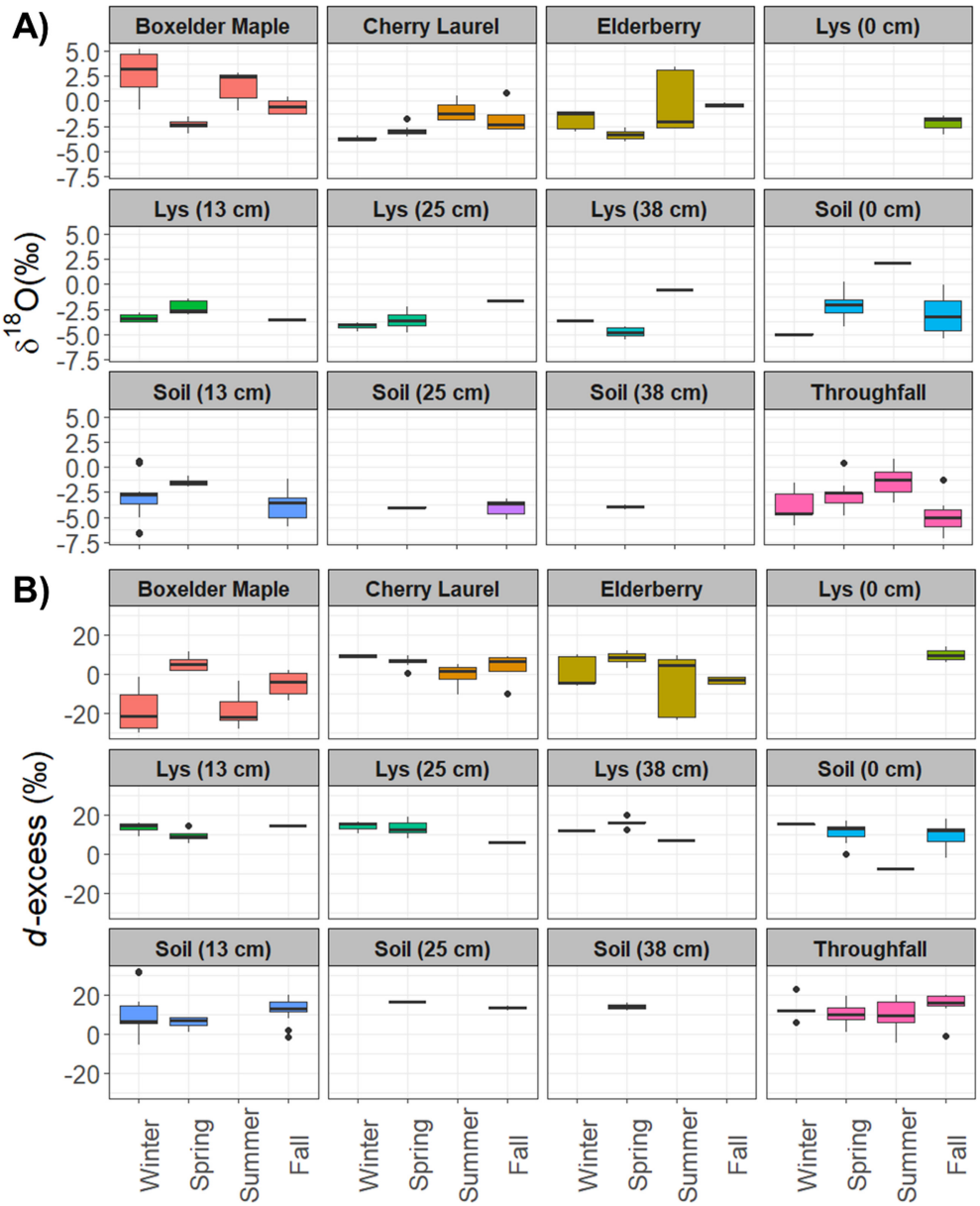


FIGURE 4 | Seasonal box plots showing (A) $\delta^{18}O$ (‰) and (B) *d*-excess (‰) variability of throughfall, soil water (lysimeters and destructive soil samples at 0, 13, 25 and 38 cm) and xylem water (Boxelder Maple, Cherry Laurel and Elderberry).

elderberry (−3.4‰ and 8.7‰) and boxelder maple (−2.3‰ and −5.0‰).

4.5 | Dual-Isotope Space and Deviation From Meteoric Conditions

Figure 5 shows the dual δ^2H-$\delta^{18}O$ space for throughfall, soil water, lysimeter water and xylem water. The throughfall meteoric water line is described as $\delta^2H = 5.44 \cdot \delta^{18}O + 3.66$ (adj. $R^2 = 0.85$), with $\delta^{18}O$ and δ^2H values ranging from 1.25‰ to −7.5‰ and from 10‰ to −40‰, respectively. Soil and lysimeter water isotope values are distributed across the throughfall domain and share similar *d*-excess and LC-excess median values. LC-excess values in soil water denote a clear evapoconcentration effect compared to lysimeter water. Soil water and lysimeter linear regressions are described as $\delta^2H = 4.71 \cdot \delta^{18}O + 0.15$ (adj. $R^2 = 0.89$) and $\delta^2H = 5.02 \cdot \delta^{18}O + 2.62$ (adj. $R^2 = 0.89$). Although the lysimeter regression closely aligns with meteoric conditions, the lower slope and intercept of the soil water regression highlight the influence of secondary evaporation.

Xylem samples show a clear meteoric origin in agreement with throughfall, soil water and lysimeter water median $\delta^{18}O$ values. Deviations from meteoric conditions occurred mainly in boxelder and elderberry during summer and fall. These deviations also corresponded with a sporadic increase in organic contamination (see Figure S1). The latter is reflected in boxelder and elderberry regression lines, $\delta^2H = 2.87 \cdot \delta^{18}O - 7.56$ (adj. $R^2 = 0.79$) and $\delta^2H = 3.15 \cdot \delta^{18}O - 7.10$ (adj. $R^2 = 0.30$). Cherry laurel exhibited fewer deviations from meteoric conditions, corresponding to a low organic presence in EW.

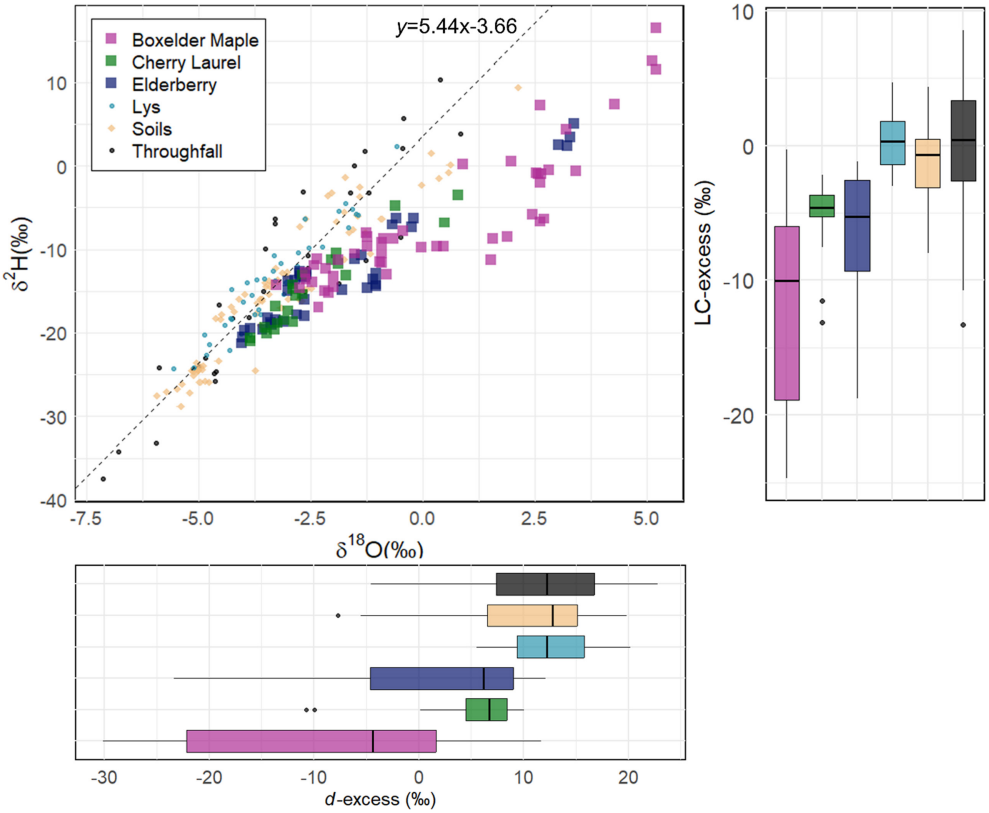


FIGURE 5 | Dual isotope plot ($\delta^{18}O$ and $\delta^{2}H$) and marginal boxplots (*d*-excess and LC-excess) including throughfall, soil water, lysimeter water and xylem water (boxelder maple, cherry laurel and elderberry). The throughfall local meteoric water line (grey dashed line) is included for reference.

The regression line for cherry laurel can be described as $\delta^2H = 3.78 \cdot \delta^{18}O - 5.49$ (adj. $R^2 = 0.87$). Consequently, the lower xylem *d*-excess and LC-excess median values were reported in boxelder and elderberry.

5 | Discussion

Reducing the variability in water extraction techniques remains a critical challenge for improving the accuracy and comparability of stable isotope measurements in ecohydrological studies. Despite the widespread use of CVE, inconsistencies mainly related to extraction temperature and mixing of mobile versus immobile water can introduce significant isotopic biases, particularly in δ^2H values. These methodological differences complicate cross-study comparisons and may obscure real hydrological patterns. Standardization of extraction protocols, including controlled temperature and heating profiles, defined sample preparation procedures, coextract organic identification and clear reporting of analytical metadata, is essential to enhance reproducibility and interpretation. Moreover, as emerging methods like the Cavitron (Barbeta et al. 2022) and direct vapour equilibration (Birkel et al. 2025) aim to isolate mobile water fractions more reliably, their comparative performance under varied environmental conditions must be rigorously evaluated. Addressing parameter variability across techniques is thus not only a methodological concern but a foundational step towards refining ecohydrological inference and advancing the field's capacity to trace water movement through complex systems.

5.1 | Extracting Mobile Water

The improved centrifugation method presented in this study, previously tested in the wet/dry tropics of Costa Rica across five different ecosystems (Sánchez-Murillo et al. 2023), offers unique analytical advantages. First, the negative pressure applied (~2.4 MPa) via centrifugation at 11,000 rpm allows mobile water extraction. This is demonstrated by the proportion of EW relative to the TWC. EW ranged between 2.5% and 7.5% among the plant species and from 5.9% to 18.4% across the soil profile. In addition, reporting extraction volumes as part of the analytical metadata and evaluating the potential influence of the EW volume on the isotope values has been poorly reported in the literature across extraction techniques. Our extractions resulted in volumes ranging from 0.1 to 7.5 mL and from 0.1 to 10.5 mL in plant and soil samples, respectively. The extracted volumes were sufficient for the isotopic analysis and showed no strong influence on the isotope values.

5.2 | Experimental Fractionation Effects

Second, in the analytical procedure presented, sample temperature can be controlled from sampling until extraction at ~5°C, which significantly decreases the potential for secondary evaporation and reduces the risk of exchange reactions between water and plant organics during the entire process, from sampling to isotopic analysis. Although CVE is also temperature-controlled, typically ranging from 105°C to 205°C (Koeniger et al. 2011; Zuecco et al. 2022), it has been recently recognized that high

temperatures enhance the hydrogen exchange between the hydroxyl groups in plant organics and the surrounding water molecules (Wen et al. 2022), as well as the coextraction of volatile organic compounds and thermal decomposition metabolites (Schmidt et al. 2012). CVE extracts nearly 100% of the sample water content at a higher temperature. This high extraction efficiency can be seen as an analytical advantage when compared to centrifugation–EW values. However, this high extraction efficiency results in low δ^2H values, widely reported due to enhanced exchange reactions between water and plant organic materials, which are typically highly depleted compared to water sources (Wen et al. 2022, and references therein).

5.3 | Influence of Organic Contamination

Third, extracting mainly mobile water reduces the influence of organic contamination, which is traceable by comparing narrow and broadband metrics of soils and xylem water with other non-contaminated endmembers (e.g., throughfall or pore water via cup lysimeters). In this study, plant species and soil showed narrowband and broadband values largely in agreement with throughfall and lysimeter water values (Figure S1). However, seasonal changes were observed mainly in the deciduous trees (boxelder maple and elderberry). During late summer, the combination of high ambient temperature and limited soil water availability reduced the xylem water content and active water transport, resulting in higher organic concentration in the EW (i.e., dilution-type effect). In the fall, as transpiration decreases, spikes in narrowband and broadband values were also observed. This may also suggest the existence of hydraulically isolated water in the stems and a 'legacy-type' effect from the warm and water-limited summer season towards the beginning of a quasidormant period in fall and winter. Elderberry also experiences one high narrowband value during the spring, which can be only explained by its flowering, involving higher production of sugars and organic compounds (Veberic et al. 2009). In contrast, cherry laurel (evergreen tree) consistently showed low narrowband and broadband values. Overall, during the active spring season, when the median $\delta^{18}O$ and *d*-excess values in all plant species closely align with throughfall and soil water, low or negligible organic interferences were reported via centrifugation. The latter highlights the applicability of the centrifugation method for extracting isotopically unbiased xylem and soil water during the active growing season in subtropical tree species. Although sampling outside the growing season may be irrelevant in temperate climates, understanding seasonal effects is highly important in regions where water connectivity between soils-plants-atmosphere exists throughout the entire hydrological year.

5.4 | Centrifugation Limitations and Future Work

The negative pressure applied via centrifugation limits water extraction under drier conditions. Other techniques, such as CVE, outperformed centrifugation in extracting xylem and water held at lower tensions. Our results showed a methodological threshold of about 10.6% (soils) and 17.8% (xylem) in gravimetric TWCs. Below these limits, no water was extracted in 31% and 27% of the xylem and soil samples. In some cases, water extraction was below 0.1 mL, making it difficult for a micropipette to recover. The number of samples analysed per day may also be considered a limitation. Our procedure involves collecting three and six replicates (50-mL centrifugation tubes) for soils and xylem samples. Common centrifugation rotors for 50-mL tubes are only equipped with six sample locations; therefore, based on the centrifugation time (1.5 h), only 36 subsamples can be extracted during a regular lab day. Other techniques may be able to adapt extraction lines to process more samples per day.

Future work in the tuning up of this centrifugation procedure for discrete xylem and soil samples should include the evaluation of (a) extraction performance versus similar emergent techniques such as Cavitron (Barbeta et al. 2022), (b) seasonal specificity in organic contamination (narrow and broadband values) between deciduous and evergreen species across multiple climates (e.g., tropical, subtropical, temperate and cold) and growing stages, (c) the potential organic coextract (i.e., concentration and compound specific determination), (d) the negative pressure needed on different soil textures and water contents and (e) parallel evaluation of isotope values between laser spectroscopy and mass spectrometry.

6 | Conclusions

This study provides a detailed methodology and evidence of the applicability of using the centrifugation method for water extraction from discrete xylem and soils in a humid subtropical urban green space. Our centrifugation procedure extracted (a) mainly mobile water with (b) no induced fractionation and (c) low organic contamination. Our results identified a methodological threshold of 10.6% for soils and 17.8% for xylem gravimetric TWC. Below these limits, water recovery was minimal (< 0.1 mL) or failed in 31% of xylem and 27% of soil samples. During the active growing season, xylem median isotope values resembled the throughfall and soil isotope values. This denotes the nonfractionating nature of transpiration (Jasechko et al. 2013; Barbeta and Peñuelas 2017) and highlights the usefulness of the centrifugation technique for ecohydrological studies focusing on water partitioning during active water use seasons. Organic contamination in deciduous species was identified during water-limited periods (summer) or less active transpiration stages (fall). Cherry laurel, an evergreen species, showed minimal influence of organic contamination throughout the hydrological year. Studies during water-limited conditions should also consider the effects of increasing organic concentrations beyond active transpiration seasons.

Acknowledgements

The authors would like to thank the Fort Worth Botanic Garden and the Botanical Research Institute of Texas for providing access to an experimental urban plot within the south woods of the botanic gardens, assistance with plant identification and root profiles, and enthusiastic support of our project. The authors also recognize the support from the UT System STARs Program (No. AR911486) and the Office of the Provost funds at the University of Texas-Arlington (No. 314075).

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information.

References

Allen, S. T., and J. W. Kirchner. 2022. "Potential Effects of Cryogenic Extraction Biases on Plant Water Source Partitioning Inferred From Xylem-Water Isotope Ratios." *Hydrological Processes* 36, no. 2: e14483. https://doi.org/10.1002/hyp.14483.

Barbeta, A., R. Burlett, P. Martín-Gómez, et al. 2022. "Evidence for Distinct Isotopic Compositions of Sap and Tissue Water in Tree Stems: Consequences for Plant Water Source Identification." *New Phytologist* 233, no. 3: 1121–1132. https://doi.org/10.1111/nph.17857.

Barbeta, A., T. E. Gimeno, L. Clavé, et al. 2020. "An Explanation for the Isotopic Offset Between Soil and Stem Water in a Temperate Tree Species." *New Phytologist* 227, no. 3: 766–779.

Barbeta, A., and J. Peñuelas. 2017. "Relative Contribution of Groundwater to Plant Transpiration Estimated With Stable Isotopes." *Scientific Reports* 7: 10580. https://doi.org/10.1038/s41598-017-09643-x.

Best, B. 2021. *The BRIT Prairie*. Fort Worth Botanic Garden. https://fwbg.org/research-projects/biodiversity-and-floristics/the-brit-prairie/.

Birkel, C., D. Tetzlaff, A. M. Ring, and C. Soulsby. 2025. "Does High Resolution In Situ Xylem and Atmospheric Vapor Isotope Data Help Improve Modeled Estimates of Ecohydrological Partitioning?" *Agricultural and Forest Meteorology* 365: 110467. https://doi.org/10.1016/j.agrformet.2025.110467.

Brooks, R. J., H. R. Barnard, R. Coulombe, and J. J. McDonnell. 2010. "Ecohydrologic Separation of Water Between Trees and Streams in a Mediterranean Climate." *Nature Geoscience* 3, no. 2: 100–104. https://doi.org/10.1038/ngeo722.

Ceperley, N., T. E. Gimeno, S. R. Jacobs, et al. 2024. "Toward a Common Methodological Framework for the Sampling, Extraction, and Isotopic Analysis of Water in the Critical Zone to Study Vegetation Water Use." *WIREs Water* 11: e1727. https://doi.org/10.1002/wat2.1727.

Chen, Y., B. R. Helliker, X. Tang, F. Li, Y. Zhou, and X. Song. 2020. "Stem Water Cryogenic Extraction Biases Estimation in Deuterium Isotope Composition of Plant Source Water." *Proceedings of the National Academy of Sciences of the United States of America* 117, no. 52: 33345–33350. https://doi.org/10.1073/pnas.2014422117.

Dalton, F. N. 1989. "Plant Root Water Extraction Studies Using Stable Isotopes." In *Structural and Functional Aspects of Transport in Roots Developments in Plant and Soil Sciences*, edited by B. C. Loughamn, O. Gašparíková, and J. Kolek, 151–155. Springer. https://doi.org/10.1007/978-94-009-0891-8_29.

Dansgaard, W. 1964. "Stable Isotopes in Precipitation." *Tellus* 16, no. 4: 436–468. https://doi.org/10.3402/tellusa.v16i4.8993.

Day, P. R. 1965. "Particle Fractionation and Particle-Size Analysis." In *Methods of Soil Analysis: Part 1. Agronomy Monographs*, edited by C. A. Black, 545–567. ASA and SSSA. https://doi.org/10.2134/agronmonogr9.1.c43.

Duvert, C., A. Barbeta, L. B. Hutley, L. Rodriguez, D. J. Irvine, and A. R. Taylor. 2024. "Cavitron Extraction of Xylem Water Suggests Cryogenic Extraction Biases Vary Across Species but Are Independent of Tree Water Stress." *Hydrological Processes* 38, no. 2: e15099. https://doi.org/10.1002/hyp.15099.

Duvert, C., C. A. Canham, A. Barbeta, 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, no. 6: e2383. https://doi.org/10.1002/eco.2383.

El-Shenawy, M. I., D. Herwartz, and M. Staubwasser. 2024. "A Passive Method for Sampling Water in the Soil–Plant–Atmosphere Continuum for Stable Hydrogen and Oxygen Isotope Analyses." *Rapid Communications in Mass Spectrometry* 38, no. 2: e9646. https://doi.org/10.1002/rcm.9646.

Fischer, B., J. Frentress, S. Manzoni, et al. 2019. "Mojito, Anyone? An Exploration of Low-Tech Plant Water Extraction Methods for Isotopic Analysis Using Locally-Sourced Materials." *Frontiers in Earth Science* 7: 150. https://doi.org/10.3389/feart.2019.00150.

Gaj, M., S. Kaufhold, P. Koeniger, M. Beyer, M. Weiler, and T. Himmelsbach. 2017. "Mineral Mediated Isotope Fractionation of Soil Water." *Rapid Communications in Mass Spectrometry* 31: 269–280. https://doi.org/10.1002/rcm.7787.

Griffith, G. E., S. A. Bryce, J. M. Omernik, et al. 2004. "Ecoregions of Texas (Color Poster With Map, Descriptive Text, and Photographs): Reston, Virginia, U.S. Geological Survey (Map Scale 1:2,500,000)." https://texashistory.unt.edu/ark:/67531/metapth1662409/.

Gröning, M., H. O. Lutz, Z. Roller-Lutz, M. Kralik, L. Gourcy, and L. Pöltenstein. 2012. "A Simple Rain Collector Preventing Water Re-Evaporation Dedicated for Δ18O and Δ2H Analysis of Cumulative Precipitation Samples." *Journal of Hydrology* 448: 195–200. https://doi.org/10.1016/j.jhydrol.2012.04.041.

Haberstroh, S., A. Kübert, and C. Werner. 2024. "Two Common Pitfalls in the Analysis of Water-Stable Isotopologues With Cryogenic Vacuum Extraction and Cavity Ring-Down Spectroscopy." *Analytical Science Advances* 5: 2300053. https://doi.org/10.1002/ansa.202300053.

He, D., M. Wen, Y. Wang, et al. 2023. "Xylem Water Cryogenic Vacuum Extraction: Testing Correction Methods With CaviTron-Based Apple Twig Sampling." *Journal of Hydrology* 621: 129572. https://doi.org/10.1016/j.jhydrol.2023.129572.

Jasechko, S., Z. Sharp, J. Gibson, et al. 2013. "Terrestrial Water Fluxes Dominated by Transpiration." *Nature* 496: 347–350. https://doi.org/10.1038/nature11983.

Jiang, H., H. Gu, H. Chen, H. Sun, X. Zhang, and X. Liu. 2022. "Comparative Cryogenic Extraction Rehydration Experiments Reveal Isotope Fractionation During Root Water Uptake in Gramineae." *New Phytologist* 236: 1267–1280. https://doi.org/10.1111/nph.18423.

Jiang, N., C. Zhu, Z. Z. Hu, et al. 2024. "Enhanced Risk of Record-Breaking Regional Temperatures During the 2023–24 El Niño." *Scientific Reports* 14: 2521. https://doi.org/10.1038/s41598-024-52846-2.

Koeniger, P., J. D. Marshall, T. Link, and A. Mulch. 2011. "An Inexpensive, Fast, and Reliable Method for Vacuum Extraction of Soil and Plant Water for Stable Isotope Analyses by Mass Spectrometry." *Rapid Communications in Mass Spectrometry* 25, no. 20: 3041–3048. https://doi.org/10.1002/rcm.5198.

Landwehr, J. M., and T. B. Coplen. 2006. "Line-Conditioned Excess: A New Method for Characterizing Stable Hydrogen and Oxygen Isotope Ratios in Hydrologic Systems." In *Isotopes in Environmental Studies*, 1st ed., IAEA-CN-118/56, 132–135. IAEA. https://inis.iaea.org/search/search.aspx?orig_q=RN:37043527.

Leen, B. J., E. S. F. Berman, L. Liebson, and M. Gupta. 2012. "Spectral Contaminant Identifier for Off-Axis Integrated Cavity Output Spectroscopy Measurements of Liquid Water Isotopes." *Review of Scientific Instruments* 83, no. 4: 044305. https://doi.org/10.1063/1.4704843.

Millar, C., K. Janzen, M. F. Nehemy, et al. 2022. "On the Urgent Need for Standardization in Isotope-Based Ecohydrological Investigations." *Hydrological Processes* 36, no. 10: e14698. https://doi.org/10.1002/hyp.14698.

Millar, C., K. Janzen, M. F. Nehemy, G. Koehler, P. Hervé-Fernandez, and J. J. McDonnell. 2021. "Organic Contamination Detection for Isotopic Analysis of Water by Laser Spectroscopy." *Rapid Communications in Mass Spectrometry* 35, no. 15: e9118. https://doi.org/10.1002/rcm.9118.

National Centers for Environmental Information (NCEI). 2024. *Daily Summaries Station Details*. Daily Summaries Station Details: Fort Worth, TX US, GHCND: USW00013911, Climate Data Online (CDO). National Climatic Data Center (NCDC). https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USW00013911/detail.

Nehemy, M. F., P. Benettin, S. T. Allen, et al. 2022. "Phloem Water Isotopically Different to Xylem Water: Potential Causes and Implications for Ecohydrological Tracing." *Ecohydrology* 15, no. 3: e2417. https://doi.org/10.1002/eco.2417.

Orlowski, N., L. Breuer, and J. J. McDonnell. 2016. "Critical Issues With Cryogenic Extraction of Soil Water for Stable Isotope Analysis." *Ecohydrology* 9, no. 1: 1–5. https://doi.org/10.1002/eco.1722.

Orlowski, N., H. G. Frede, N. Brüggemann, and L. Breuer. 2013. "Validation and Application of a Cryogenic Vacuum Extraction System for Soil and Plant Water Extraction for Isotope Analysis." *Journal of Sensors and Sensor Systems* 2, no. 2: 179–193. https://doi.org/10.5194/jsss-2-179-2013.

Poca, M., O. Coomans, C. Urcelay, et al. 2019. "Isotope Fractionation During Root Water Uptake by Acacia Caven is Enhanced by Arbuscular Mycorrhizas." *Plant and Soil* 441, no. 1: 485–497.

Sánchez-Murillo, R., D. Todini-Zicavo, M. Poca, et al. 2023. "Dry Season Plant Water Sourcing in Contrasting Tropical Ecosystems of Costa Rica." *Ecohydrology* 16, no. 5: e2541. https://doi.org/10.1002/eco.2541.

Schmidt, M., K. Maseyk, C. Lett, et al. 2012. "Reducing and Correcting for Contamination of Ecosystem Water Stable Isotopes Measured by Isotope Ratio Infrared Spectroscopy." *Rapid Communications in Mass Spectrometry* 26: 141–153. https://doi.org/10.1002/rcm.5317.

Schulte, E. E., and B. G. Hopkins. 1996. "Estimation of Soil Organic Matter by Weight Loss-On-Ignition." In *Soil Organic Matter: Analysis and Interpretation*, edited by F. R. Magdoff, M. A. Tabatabai and E. A. Hanlon Jr., 21–23. Special publication No. 46. Soil Science Society of America. Soil Science Society of America. https://doi.org/10.2136/sssaspecpub46.c3.

Sobota, M., K. Li, M. Hren, and J. Knighton. 2024. "Evidence for Variations in Cryogenic Extraction Deuterium Biases of Plant Xylem Water Across Foundational Northeastern US Trees." *Hydrological Processes* 38, no. 2: e15079. https://doi.org/10.1002/hyp.15079.

Song, X., Y. Chen, B. R. Helliker, X. Tang, F. Li, and Y. Zhou. 2021. "Reply to Evaristo et al.: Strong Evidence for the Need of Correcting Extraction Bias in an Early Study of Ecohydrological Separation." *Proceedings of the National Academy of Sciences of the United States of America* 118, no. 17: e2103604118. https://doi.org/10.1073/pnas.2103604118.

Sprenger, M., P. Llorens, C. Cayuela, F. Gallart, and J. Latron. 2019. "Mechanisms of Consistently Disjunct Soil Water Pools Over (Pore) Space and Time." *Hydrology and Earth System Sciences* 23, no. 6: 2751–2762. https://doi.org/10.5194/hess-23-2751-2019.

Texas A&M AgriLife Extension. 2012. *Soil, Water and Forage Testing Laboratory Methods*. Soil Testing Lab. https://soiltesting.tamu.edu/laboratory-methods/.

Texas A&M AgriLife Extension. 2024. *Historic ETo Reference*. TexasET network. https://texaset.tamu.edu/DataSummary/Daily/83.

Texas Water Development Board. 2012. "Chapter 4: Climate of Texas. 145147." In *Water for Texas 2012 State Water Plan*. Texas Water Development Board. https://www.twdb.texas.gov/publications/state_water_plan/2012/04.pdf.

Thielemann, L., R. Gerjets, and J. Dyckmans. 2019. "Effects of Soil-Bound Water Exchange on the Recovery of Spike Water by Cryogenic Water Extraction." *Rapid Communications in Mass Spectrometry* 33: 405–410. https://doi.org/10.1002/rcm.8348.

US Department of Agriculture. 2024. "Web Soil Survey." https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx.

US Department of Agriculture NRCS National Plant Data Center & the Biota of North America Program. 2024. *Plant Guide: Boxelder (*Acer negundo *L.)*. USDA Natural Resources Conservation Service. https://plants.usda.gov/DocumentLibrary/plantguide/pdf/pg_acne2.pdf.

US Department of Commerce, NOAA. 2024. *DFW—Normals, Means, and Extremes*. National Weather Service. https://www.weather.gov/fwd/dfw_records_normals.

Veberic, R., J. Jakopic, F. Stampar, and V. Schmitzer. 2009. "European Elderberry (*Sambucus nigra* L.) Rich in Sugars, Organic Acids, Anthocyanins and Selected Polyphenols." *Food Chemistry* 114, no. 2: 511–515. https://doi.org/10.1016/j.foodchem.2008.09.080.

von Freyberg, J., S. T. Allen, C. Grossiord, and T. E. Dawson. 2020. "Plant and Root-Zone Water Isotopes Are Difficult to Measure, Explain, and Predict: Some Practical Recommendations for Determining Plant Water Sources." *Methods in Ecology and Evolution* 11: 1352–1367. https://doi.org/10.1111/2041-210X.13461.

Wang, H., H. Yu, D. He, et al. 2024. "Cryogenic Vacuum Distillation vs Cavitron Methods in Ecohydrology: Extraction Protocol Effects on Plant Water Isotopic Values." *Journal of Hydrology* 642: 131853. https://doi.org/10.1016/j.jhydrol.2024.131853.

Wen, M., D. He, M. Li, R. Ren, J. Jin, and B. Si. 2022. "Causes and Factors of Cryogenic Extraction Biases on Isotopes of Xylem Water." *Water Resources Research* 58, no. 8: e2022WR032182. https://doi.org/10.1029/2022WR032182.

Wen, M., B. Si, Y. Lu, and H. Wang. 2021. "Water Recovery Rate and Isotopic Signature of Cryogenic Vacuum Extracted Spiked Soil Water Following Oven-Drying at Different Temperatures." *Hydrological Processes* 35, no. 6: e14248. https://doi.org/10.1002/hyp.14248.

Wen, M., X. Zhao, B. Si, et al. 2023. "Inter-Comparison of Extraction Methods for Plant Water Isotope Analysis and Its Indicative Significance." *Journal of Hydrology* 625: 130015. https://doi.org/10.1016/j.jhydrol.2023.130015.

Yang, B., G. G. Dossa, Y. Hu, et al. 2023. "Uncorrected Soil Water Isotopes Through Cryogenic Vacuum Distillation May Lead to a False Estimation on Plant Water Sources." *Methods in Ecology and Evolution* 14, no. 6: 1443–1456. https://doi.org/10.1111/2041-210x.14107.

Younger, S. E., J. Blake, C. R. Jackson, and D. P. Aubrey. 2024. "δ2H Isotopic Offsets in Xylem Water Measurements Under Cryogenic Vacuum Distillation: Quantifying and Correcting Wood-Water Hydrogen Exchange Influences." *Ecohydrology* 17, no. 3: e2640. https://doi.org/10.1002/eco.2640.

Zuecco, G., A. Amin, J. Frentress, et al. 2022. "A Comparative Study of Plant Water Extraction Methods for Isotopic Analyses: Scholander-Type Pressure Chamber vs. Cryogenic Vacuum Distillation." *Hydrology and Earth System Sciences* 26, no. 13: 3673–3689. https://doi.org/10.5194/hess-26-3673-2022.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.