



1 **Technical note: Evaluation of a new cryogenic airtight vapor** 2 **extraction (CRAVE) method for soil and plant water**

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17 **Abstract.** Accurate extraction of soil and plant water for stable isotope analysis remains a methodological challenge in
18 ecohydrology, particularly due to isotopic biases introduced by heating or selective pore-water extraction in conventional
19 techniques. This study developed and evaluated a cryogenic airtight vapor extraction (CRAVE) method from soil and
20 vegetation samples at ambient temperature within a recirculating vapor-liquid pathway. This approach avoids heating-induced
21 non-equilibrium effects and reduces matrix-dependent artifacts and organic contamination, thereby facilitating direct
22 comparison of isotopic compositions between soil and plant water. The results demonstrate that CRAVE-derived isotopic
23 signatures align with both cryogenic vacuum distillation (CVD) and suction lysimeter (SL) benchmarks. However, systematic
24 deviations were observed based on specific matrix properties. For xylem water, the d^2H offset between CRAVE and CVD was
25 strongly modulated by gravimetric water content (dry-weight basis), with CVD exhibiting greater hydrogen isotope depletion
26 under low-moisture conditions ($< 0.8 \text{ g}\cdot\text{g}^{-1}$). For soils, the isotopic divergence between CRAVE and CVD was driven primarily
27 by soil texture, with offsets increasing as clay content and depth increased ($r = 0.82\text{--}0.94$), where CVD-extracted bulk water
28 became depleted progressively in both δ^2H and $\delta^{18}O$ relative to the mobile-capillary pool captured by SL and CRAVE. The
29 Rayleigh-based framework provides a physically grounded means to reconstruct source-water isotope values from condensate
30 measurements; its potential use for mobile–immobile partitioning should, however, be treated as a future application pending
31 targeted validation. Overall, CRAVE represents a promising ambient-temperature extraction method for tracing water
32 partitioning and source-uptake dynamics within the soil–plant–atmosphere continuum.

33 **Keywords:** Stable water isotopes; Water extraction; Cryogenic vacuum distillation; Isotopic bias; Xylem water; Ecohydrology



34 1. Introduction

35 Stable isotopes of hydrogen and oxygen (^2H , ^{18}O) have been used to trace water movement in hydrological and
36 ecohydrological processes (Tao et al., 2025; Meißner et al., 2014; West et al., 2006). By analyzing the isotopic compositions
37 of xylem water, soil water, precipitation, streamflow and groundwater, researchers can identify water sources and quantify key
38 processes such as evaporation, infiltration, and plant water uptake (Sprenger et al., 2017; Liu et al., 2025; Tao et al., 2025).
39 While liquid water can be analyzed directly for its isotopic composition using isotope ratio mass spectrometry (IRMS) or laser
40 spectroscopy (LS), water in soil and plant samples must first be extracted or measured in situ (Beyer et al., 2020), which
41 remains a technically challenging process (Kocum et al., 2025; Orłowski et al., 2016).

42 Over the past decades, numerous extraction techniques have been developed and refined, yet each has inherent limitations,
43 such as potential isotopic fractionation, incomplete water recovery, organic contamination, and matrix-dependent biases (see
44 Table 1 for a summary). For instance, while the widely used cryogenic vacuum distillation (CVD) method is considered reliable,
45 it remains equipment-demanding (Orłowski et al., 2016). In practice, extractions that involve heating, such as conventional
46 CVD, seek to mitigate fractionation by ensuring the release of tightly bound water—utilizing elevated temperatures that
47 occasionally reach as high as 200°C (Wang et al., 2024). However, such thermal conditions can introduce secondary artifacts
48 that vary by sample type. For soils, high temperatures increase the risk of releasing isotopically distinct structural water from
49 hydrous minerals (Duvert et al., 2024; Wen et al., 2023; Orłowski et al., 2018). Conversely, for plant tissues, heating has been
50 shown to activate wood-water hydrogen exchange, which can significantly bias $\delta^2\text{H}$ measurements even if total water recovery
51 is achieved (Younger et al., 2024). Furthermore, while volatile organic compounds (VOCs) can be mobilized even at lower
52 temperatures, heating promotes the release of both volatile and semi-volatile organic compounds from soil and plant matrices,
53 increasing their co-extraction and thereby enhancing spectral interference in laser-based isotope analysis, which can bias the
54 measured isotopic composition (Millar et al., 2018; Bowen et al., 2025).

55 Alternatively, suction lysimeters provide a simple in situ technique for collecting the more mobile fraction of soil water
56 under controlled tension. But these instruments are restricted to high water content soils and often fail to collect a sample
57 below ~ 100 cm H_2O water tension (Sprenger et al., 2015; Sprenger et al., 2019).

58 Another vapor-mode approach is direct vapor equilibration (DVE), including laboratory-based CO_2 or H_2 equilibration,
59 direct liquid–vapor equilibration coupled to laser spectroscopy, and in situ equilibration probes (Marshall et al., 2020; Oerter
60 et al., 2019; Wassenaar et al., 2008; Vadibeler et al., 2022; Pyschik et al., 2025). These approaches offer high throughput and,
61 in some cases, in situ monitoring capability when coupled with LS. However, bag- or vial-based DVE approaches can be
62 affected by leakage, storage effects, and organic-vapor interference in laser spectrometers (Grallher et al., 2021; Mattei et al.,
63 2019; Millar et al., 2021; Nehemy et al., 2019). In situ equilibration probes reduce sample handling and can resolve high-
64 frequency isotope dynamics, but they still require field-deployable laser systems and remain susceptible to interference from
65 plant VOCs, especially in xylem applications (Herbstritt et al., 2023). Recently, Magh et al. (2022) proposed a discrete vapor-
66 sampling technique that flushes dry air through samples to collect equilibrated vapor without requiring in situ instrumentation.
67 That approach showed good agreement with in situ probe data, particularly for $\delta^2\text{H}$, but remained sensitive to vapor storage



68 effects, especially for $\delta^{18}\text{O}$ after several days. Taken together, these limitations highlight the need for a unified vapor-mode
 69 extraction method that minimizes contamination, yields a storable liquid-water product, and can be adapted to both laboratory
 70 and field applications.

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Table 1. Comparison of different water extraction methods for soil and plant isotope analysis

Method	Principle	Sample types	Advantages	Disadvantages	References
Azeotropic distillation	To extract water from samples with the assistance of specific chemical reagents	Soil	It can extract efficiently water at relatively low temperatures with stable operation and uniform heating, avoiding high-temperature fractionation or organic matter oxidation, and achieving high recovery rates for samples with low to moderate water content.	It requires the use of organic solvents or metallic reagents, making the procedure relatively complex and potentially introducing impurity contamination.	Revesz and Woods (1990); Allison et al. (1983); Kelln et al. (2001); Figueroa-Johnson et al. (2007); Thorburn et al. (1993); Zhu et al. (2014)
Mechanical squeezing or High-speed centrifugation	To apply physical pressure or centrifugal force to the samples to expel water.	Soil / Plant	It is simple to operate, allowing rapid extraction of representative mobile water while avoiding isotopic fractionation and preserving the original structure of the samples.	It is difficult to extract all the water from the samples; in clay-rich or low-moisture soils, only the loosely bound mobile water can be expelled, while a large portion of tightly bound water remains within the matrix.	Eichler (1966); Moncur et al. (2013); Kelln et al. (2001); Millar et al. (2018); Jusserand (1980); Edmunds and Bath (1976); Nehemy et al. (2025); Adams et al. (2020); Wen et al. (2023)
Cavitron extraction	By applying controlled high-speed rotation to generate precise xylem negative pressure (approximately -0.5 to -6 MPa) within plant stem segments, it simulates the natural water potential gradient in xylem conduits, forcing water to be expelled only from the continuous vessel system.	Plant	It enables rapid extraction of highly representative and precise xylem water samples, whose isotopic compositions show excellent agreement with those of the source water.	The equipment is expensive and requires skilled operation, being applicable only to woody plants with well-developed xylem and relatively large vessels; its extraction efficiency is low for fine branches or herbaceous samples, and precise control of sample size and sealing is necessary to prevent cavitation or insufficient water yield.	Barbeta et al. (2022); Wang et al. (2024); Duvert et al. (2024); Cochard (2002); Cochard et al. (2005); He et al. (2023)



Method	Principle	Sample types	Advantages	Disadvantages	References
Microwave water extraction	Using microwave heating of the samples to rapidly release water.	Plant	It can extract efficiently water within a short time, with simple operation and minimal isotopic fractionation, making it particularly suitable for rapid water extraction from plant tissues.	Strict control of heating time and power is required to ensure complete extraction, as residual unvaporized water may cause isotopic fractionation errors. In addition, differences in sample texture or moisture content can result in uneven microwave heating, leading to variations in extraction efficiency and reproducibility.	Millar et al. (2018); Munksgaard et al. (2014); Orłowski et al. (2016)
Cryogenic vacuum distillation / extraction	Heating the samples and condensing the evaporated water under vacuum conditions.	Soil / Plant	It can extract most of the water from samples, is applicable to various sample types, and can also be used for samples with low water content.	Samples with high clay or organic matter content require higher heating temperatures to release water, which can cause oxidation of organic compounds and the release of structural water from hydrous minerals, resulting in collected water that is not purely pore water and thus distorting the isotopic results.	Millar et al. (2018); Orłowski et al. (2013); Jones et al. (2017); Koeniger et al. (2011); Wang et al. (2024); Diao et al. (2022); West et al. (2006); Chen et al. (2020); Liu et al. (2022); Nehemy et al. (2025); Cai et al. (2025); Liu et al. (2025); Wu et al. (2021); Zeng et al. (2021); Wen et al. (2021)
Equilibration methods	Determining the water isotopic composition by allowing the sample to reach isotopic equilibrium with a gas of known composition.	Soil / Plant	It does not require direct extraction of liquid water and enables non-destructive, low-interference isotope measurement in a closed system. The operation is relatively simple, allowing in situ or high-throughput analysis, and it reduces the risk of isotopic fractionation during sample heating and handling.	The equilibration process is time-consuming and requires ensuring complete vapor–water equilibrium while avoiding external contamination; for samples with low water content, equilibrium may be difficult to achieve, leading to reduced measurement accuracy.	Wassenaar et al. (2008); Rothfuss et al. (2013); Gaj and McDonnell (2019); Wang et al. (2020); Vadibeler et al. (2022); Volkman and Weiler (2014); Gralher et al. (2021); Hendry et al. (2015); Mattei et al. (2019); Vadibeler et al. (2025); Pyschik et al. (2025); Orłowski et al. (2016); Magh et al.



Method	Principle	Sample types	Advantages	Disadvantages	References
Suction / Tension lysimeter	It collects soil water by applying a controlled negative pressure (typically 20-80 kPa) through a porous sampling interface such as a ceramic, PTFE, or glass filter, which draws mobile pore water from the surrounding soil matrix into a sealed collection chamber.	Soil	This technique allows in situ collection of soil water with minimal disturbance, providing samples that represent the mobile or plant-available water fraction. It also avoids potential isotopic alteration associated with physical or chemical extraction procedures performed in the laboratory.	The method extracts only the mobile fraction of pore water rather than the total soil water pool, and its efficiency strongly depends on soil texture and moisture conditions. In clay-rich or dry soils, extraction may be difficult or even impossible, and the isotopic composition of the collected water may differ from that of bulk soil water due to heterogeneity among water pools.	(2022); Kühnhammer et al. (2022); Seeger and Weiler (2021); Sprenger et al. (2025); (Ring et al., 2024) Zhang et al. (2025); Liu et al. (2025); Sprenger et al. (2015); Kabeya et al. (2007); Li et al. (2007); Weihermüller et al. (2007); Anderson et al. (1997); Yano et al. (2006); Kosugi and Katsuyama (2004); Costanza et al. (2025); Xu et al. (2025); Floriancic et al. (2024)

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74 Here, we present a new water extraction method called cryogenic airtight vapor extraction (CRAVE), which recovers a
 75 quantified fraction of sample water by condensing equilibrated headspace vapor into liquid water for stable isotope analysis.
 76 CRAVE is related to DVE and in situ vapor-probe approaches in that it relies on isotopic equilibrium between liquid water
 77 and headspace vapor at ambient temperature (Seeger and Weiler, 2021; Volkmann and Weiler, 2014; Wassenaar et al., 2008).
 78 However, CRAVE differs from these approaches because it actively converts a measured fraction of equilibrated vapor into a
 79 storable liquid-water sample for subsequent isotope analysis:

80 (i) Direct liquid collection for isotope analysis.

81 Instead of measuring vapor directly and reconstructing liquid composition solely from equilibrium relationships, CRAVE
 82 circulates headspace vapor through a cryogenic trap to collect condensed liquid water. The condensate can then be analyzed
 83 by IRMS or laser spectroscopy. This recirculating vapor–liquid pathway, together with a desiccant-buffered pressure vent, is
 84 designed to minimize leakage and VOC interference rather than rely on a purely open vapor-measurement step (Millar et al.,
 85 2021; Herbstritt et al., 2024). By condensing vapor into liquid water within a sealed system, CRAVE avoids condensation
 86 artifacts that often affect in situ vapor equilibration probes under fluctuating temperature and humidity (Beyer et al., 2020;
 87 Bailey et al., 2015). The resulting liquid sample can be transferred to sealed glass vials for storage and repeated analysis,
 88 thereby reducing the post-sampling fractionation and storage issues associated with vapor-phase samples.

89 (ii) Rayleigh-based reconstruction constrained by mass fraction and temperature.



90 CRAVE reconstructs the initial liquid-water isotope composition from the measured condensate, the condensed mass
91 fraction relative to the initial water mass in the sample, and temperature-dependent equilibrium fractionation (Gat, 1996;
92 Majoube, 1970). This physically based reconstruction links the recovered condensate to source-water isotopic composition
93 without relying on empirical post hoc corrections, although it remains contingent on accurate mass accounting, temperature
94 control, and near-equilibrium behavior during extraction.

95 (iii) Built-in QA/QC via condensation efficiency.

96 The method provides an internal diagnostic metric (condensation efficiency) to identify leakage, incomplete circulation,
97 or ambient moisture contamination during extraction (Gralher et al., 2021; Mattei et al., 2019).

98 Importantly, CRAVE is not intended to recover all water from soil or plant tissues. Rather, it recovers a small and
99 quantified fraction so that the bulk liquid reservoir is only minimally perturbed while still allowing back-calculation of the
100 initial isotopic composition under equilibrium fractionation assumptions. Potential limitations therefore include incomplete
101 equilibration, temperature gradients, and leakage pathways, all of which must be evaluated through controlled extraction
102 conditions, mass-balance checks, and replicate extractions (Gralher et al., 2021; Mattei et al., 2019). Finally, we validate
103 CRAVE using pure water, soil, and plant samples, and compare its performance with CVD and suction lysimeters (SL) to
104 assess methodological offsets among extraction techniques.

105 2. Methods

106 2.1 The cryogenic airtight vapor extraction (CRAVE) system

107 The CRAVE system was developed to recover water from samples by continuously recirculating water vapor between a
108 sample vial and a condensation vial within a predominantly closed, low-temperature loop (Fig. 1). The system operates on the
109 principle of vapor–liquid phase change within a recirculating pathway: water in the sample evaporates into the vial headspace
110 and is transported by controlled airflow to a condensation vial immersed in liquid nitrogen. There, the vapor condenses into
111 liquid water, which is collected without sample heating, vacuum extraction, or direct exposure of the condensate to ambient
112 air.

113 The system consists of four main components: (1) a sample vial containing the soil, plant, or liquid sample; (2) a
114 condensation U-tube placed in a liquid nitrogen flask to collect condensed vapor; (3) a recycling pump (GS150, Geesiniger,
115 Germany) that drives continuous vapor circulation through the recirculating loop; and (4) a desiccant-buffered vent (drying
116 tube) that provides pressure equilibration while strongly suppressing the diffusive ingress of ambient water vapor. All tubing,
117 needles, and connections were sealed with PTFE (Teflon) septa to minimize isotopic exchange with the atmosphere. A
118 schematic of the CRAVE system and vapor-flow pathway is shown in Fig. 1.

119 To ensure isotopic integrity and minimize evaporative fractionation during sampling, the empty 60 mL glass sample vials
120 (G_{sv}) and condensation vials (G_{cv}) were pre-weighed using a high-precision balance (e.g., ± 0.001 g) in the laboratory prior to
121 the field campaign. During sampling, soil and plant materials were placed immediately into the vials upon collection and sealed
122 promptly with airtight caps. The total mass of the vial plus the fresh sample (G_{ssv}) was recorded subsequently (either in the



123 field using a portable balance or upon return to the laboratory) to determine the original sample mass ($G_s = G_{ssv} - G_{sv}$). For
124 soil samples, vials were filled typically to approximately two-thirds of their volume (30–60 g) to minimize headspace, while
125 plant xylem samples ranged from 5–20 g depending on the stem diameter and water content.

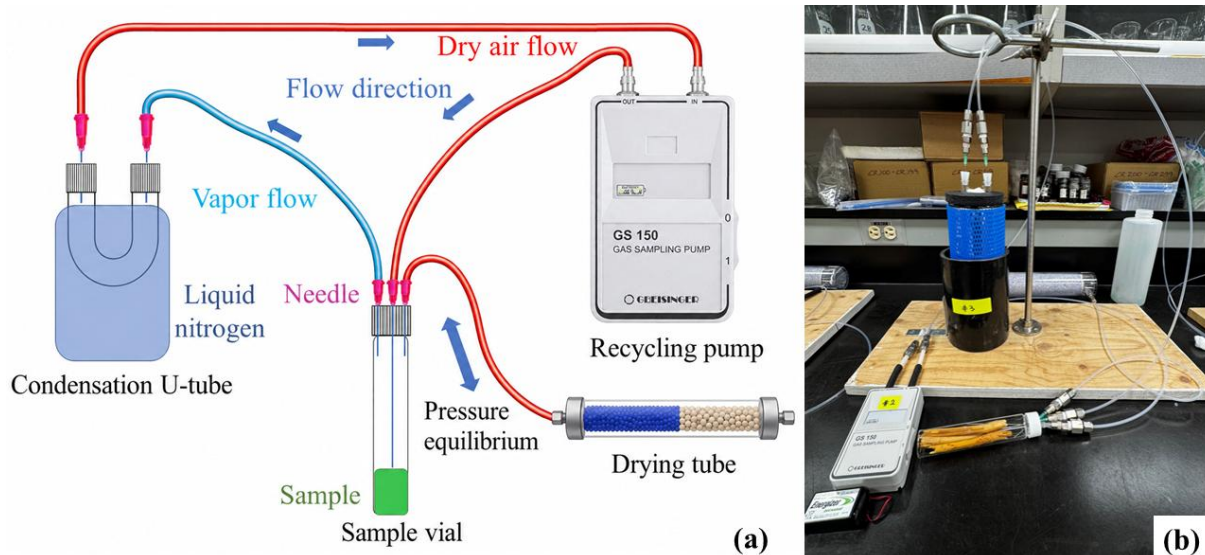
126 The sample vial was connected to the condensation tube, which was inserted into the liquid nitrogen flask so that the
127 nitrogen level covered approximately one-third of the condensation tube height. The other end of the condensation tube was
128 connected to the inlet valve of the recycling pump, and the outlet valve was connected back to the sample vial to drive vapor
129 flow through the system. A drying tube was attached to the sample vial to maintain pressure equilibrium while preventing
130 external moisture contamination.

131 Once the system was assembled, the recycling pump was activated to initiate vapor circulation and cryogenic
132 condensation. The extraction protocol was optimized for different matrices: 0.5–1 h for liquid water standards, and 0.5–3 h for
133 soil and plant samples. As an operational end point, the sample vial was placed on a high-precision electronic balance
134 throughout the process to monitor mass change in real time. Extraction was terminated when the mass of the sample vial
135 reached a stable plateau (i.e., no further measurable mass loss for at least 15–20 min), indicating that no additional readily
136 exchangeable water was being removed under the imposed extraction conditions. This real-time gravimetric monitoring
137 allowed extraction duration to be tailored to soil texture, vegetation type, and water content. All procedures were conducted at
138 room temperature (ca. 20–25 °C). To ensure precise thermal characterization, the internal temperature of each vial was
139 monitored continuously using a high-precision thermistor (ON-403-PP, Omega Engineering, USA; accuracy ± 0.1 °C). The
140 sensor was connected to a four-channel data logger (OM-CP-QUADTHERM-A1, Omega Engineering, USA) with a resolution
141 of 0.01 °C.

142 After extraction, the pump was turned off, and the condensation tube was removed from the liquid nitrogen bath and
143 allowed to warm to room temperature. The condensation vial was weighed again (G_{cw}), and the actual condensed water mass
144 (G_c) was calculated as $G_c = G_{cw} - G_{cv}$. The condensed water was then transferred into sealed glass vials and stored at 4 °C
145 until hydrogen and oxygen isotope analysis.

146 The sample vial with residual material was reweighed (G_{ssv}) to calculate the theoretical condensed water mass ($G_c' =$
147 $G_{ssv} - G_{sv}$). The condensation efficiency was expressed as $G_c/G_c' \times 100\%$. A condensation efficiency above 98% was
148 considered satisfactory; lower values indicated possible leakage or incomplete vapor circulation, whereas values exceeding
149 100% suggested contamination by ambient moisture. In this study, condensation efficiency is interpreted as a QA/QC indicator
150 of system performance rather than as proof that all physically distinct water pools were completely recovered.

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153 **Figure 1. Schematic and experimental setup of the cryogenic airtight vapor extraction (CRAVE) system: (a) schematic diagram**
 154 **illustrating the airtight circulation loop and vapor-liquid equilibrium process; (b) photograph of the laboratory-based setup for**
 155 **extracting pre-packaged soil and plant samples.**

156

157 2.2 Calculation of isotope values in the CRAVE method

158 The isotopic compositions of soil and plant water derived from the condensed vapor were reconstructed using a Rayleigh-
 159 type framework (Gat (1996):

$$160 (1 - f)^{(\alpha-1)} = \frac{\delta_R + 1000}{\delta_I + 1000} \quad (1)$$

161 where δ_I is the initial $\delta^2\text{H}$ and $\delta^{18}\text{O}$ value of sample (pure water, soil water, and plant water); δ_R is the remaining water $\delta^2\text{H}$
 162 and $\delta^{18}\text{O}$ in the sample after the vapor condensation procedure; f is the mass ratio of condensed water to initial sample, $f =$
 163 G_c/G_s ; α is the equilibrium fractionation factor between sample and vapor, which for ^{18}O and ^2H is an empirical function of
 164 the temperature (T) inside the sample vial during extraction, as described by Majoube (1970).

165 The isotopic mass balance between the initial, condensed, and remaining water can be expressed as:

$$166 \delta_R(1 - f) + \delta_c f = \delta_I \quad (2)$$

167 where δ_c is condensed water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values.

168 By substituting δ_R derived from Equation (2) in Equation (1), we obtained:

$$169 \delta_I = \frac{1000(1-f)^\alpha - 1000(1-f) - \delta_c f}{(1-f)^{\alpha-1}} \quad (3)$$

170 2.3 Samples

171 Bulk soil, rainfall, and plant xylem samples were collected from the Puding Karst Ecosystem Research Station in Guizhou,
 172 southwest China (105°42'–105°43' E, 26°14'–26°15' N).



173 Soil samples were taken from depths of 5 to 150 cm (Table A2, $n = 42$), representing the main active layer of water
174 exchange in the shallow karst profile. The soil is a loam with relatively fine texture, characterized by high clay (14–57%, mean
175 37%) and silt contents (41–65%, mean 50%) (Liu et al., 2024). Such fine-textured soils are known to amplify differences
176 among water extraction methods due to strong matrix-water interactions, making them suitable for method comparison in this
177 study (Orlowski et al., 2016). In this study, CRAVE was evaluated for whether its recovered condensate more closely reflects
178 the isotopic composition of the water sampled by suction lysimeters than that of bulk water obtained by CVD. To provide a
179 physical reference, mobile soil water was collected concurrently using suction lysimeters (SL) at the same depths and locations
180 as bulk soil sampling (Liu et al., 2025). Comparing CRAVE-derived values with SL-collected water (mobile reference) and
181 CVD-extracted water (bulk reference) allows a systematic assessment of methodological offsets among extraction mechanisms.

182 Plant xylem samples were branches (diameter 1–2 cm, $n = 37$) cut from dominant tree species at each site. Immediately
183 after sampling, all plant and soil samples were sealed in airtight containers and stored frozen ($-18\text{ }^{\circ}\text{C}$) to preserve field moisture
184 and prevent evaporative fractionation.

185 The gravimetric water content ranged from 30% to 44% for soil samples (Table A2) and from 56% to 132% for xylem
186 samples (calculated on a dry-weight basis; Table A3).

187 Rainwater samples ($n = 37$) were collected during storm events and stored at $4\text{ }^{\circ}\text{C}$ in tightly capped bottles until analysis.
188 Due to their minimal content of dissolved organic compounds and impurities compared to soil or plant tissues, these rainwater
189 samples served as a fundamental liquid-water benchmark (water-to-water test) to validate the CRAVE method's performance
190 under simplified matrix conditions.

191 **2.4 Extraction methods for soil water and plant xylem water**

192 Bulk soil and xylem samples were extracted using CVD. To ensure comparability, these subsamples were collected
193 concurrently during the same campaign as CRAVE and SL samples. A total of 79 paired soil ($n = 42$) and xylem ($n = 37$)
194 samples were extracted. The extraction was performed with a capillary-and-vial system at $200\text{ }^{\circ}\text{C}$ for 30 min for soil samples
195 and 24 min for plant xylem samples, following the protocol described by Liu et al. (2025) and Wang et al. (2024).

196 **2.5 Isotope analysis**

197 Isotopic compositions of liquid water and its condensed vapor were measured using an Off-Axis Integrated Cavity Output
198 Spectroscopy (OA-ICOS) system (Model TWIA-45EP, Los Gatos Research, San Jose, CA, USA) at the University of
199 Saskatchewan, Canada. The analytical uncertainties (2σ) for OA-ICOS measurements of liquid samples were $\pm 2\text{‰}$ and $\pm 0.8\text{‰}$
200 for $\delta^2\text{H}$, $\delta^{18}\text{O}$, respectively.

201 Water extracted from soil and plant samples was analyzed using IRMS (Delta V Advantage, Thermo Fisher Scientific,
202 Bremen, Germany). The analytical precision of IRMS was approximately $\pm 1.0\text{‰}$ for $\delta^2\text{H}$ and $\pm 0.2\text{‰}$ for $\delta^{18}\text{O}$. All isotope
203 values are reported in per mil (‰) relative to the VSMOW-SLAP scale (Millar et al., 2021).



204 2.6 Statistical analysis

205 To quantify the deviation between the isotopic values ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) obtained by the CRAVE method and those derived
206 from reference extraction methods (e.g., CVD or SL), we calculated the signed relative error (E_{ref}) for each sample:

$$207 \quad E_{ref} = \frac{\delta_{CRAVE} - \delta_{ref}}{|\delta_{ref}|} \times 100\% \quad (4)$$

208 Where δ_{CRAVE} is the isotope value extracted by the CRAVE method and δ_{ref} is the corresponding value measured by the
209 reference method. A positive E_{ref} indicates that the CRAVE method overestimates the isotope value relative to the reference,
210 while a negative E_{ref} indicates underestimation. The absolute magnitude of E_{ref} represents the percentage deviation of CRAVE-
211 derived values from the reference results.

212 The agreement between the two extraction methods was further assessed using the intraclass correlation coefficient (ICC),
213 which quantifies the degree of absolute agreement between repeated or paired measurements. We applied a two-way mixed-
214 effects model for absolute agreement and single measurements, denoted as ICC(A,1), following McGraw and Wong (1996).

215 The ICC(A,1) was computed from an analysis of variance (ANOVA) framework as follows (McGraw and Wong, 1996;
216 Koo and Li, 2016):

$$217 \quad ICC(A, 1) = \frac{MS_R - MS_E}{MS_R + (k-1)MS_E + \frac{k(MS_C - MS_E)}{n}} \quad (5)$$

218 where MS_R is the mean square between subjects (samples), MS_C is the mean square between methods (raters), MS_E is the
219 residual mean square (error), n is the number of samples, and k is the number of methods (here, $k = 2$).

220 The ICC ranges from 0 to 1, with higher values indicating greater consistency between the two methods. Following the
221 commonly accepted interpretation (Koo and Li, 2016), ICC values below 0.5 indicate poor agreement, 0.5-0.75 moderate
222 agreement, 0.75-0.9 good agreement, and above 0.9 excellent agreement.

223 To evaluate potential systematic bias, the Wilcoxon signed-rank test was performed. This non-parametric test was selected
224 to account for the potential non-normal distribution of isotopic data. The null hypothesis (H_0) for this test assumes that the
225 median of the paired differences between the CRAVE and reference values is 0; a significant result ($p_{Wilcoxon} < 0.05$)
226 therefore indicates a statistically significant systematic offset. Furthermore, the Mean Offset was calculated for each sample
227 set to represent the average arithmetic deviation between the two methods. Together, the $p_{Wilcoxon}$ value (significance of
228 bias) and the Mean Offset (magnitude of bias) provide a comprehensive evaluation of the CRAVE method's accuracy and its
229 potential for systematic enrichment or depletion across different water sources.

230 All statistical procedures were implemented in R (version 4.3.0, R Core Team).

231 3. Results

232 3.1 Validation using liquid water standards (Water-to-water test)

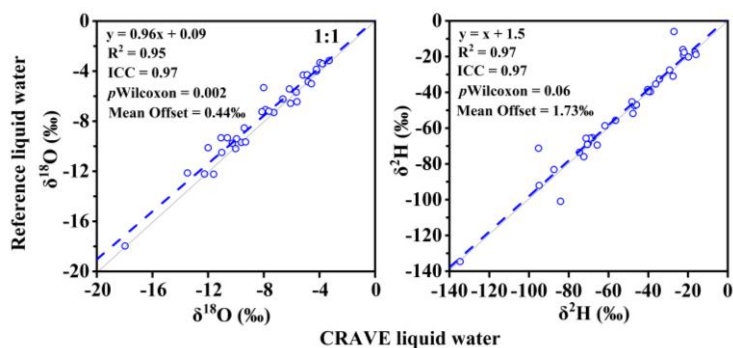
233 To evaluate the isotopic fidelity of the CRAVE method, we first conducted a water-to-water test using 33 liquid
234 precipitation samples. These samples covered a broad isotopic range, providing a fundamental benchmark to assess the
235 system's performance in the absence of complex matrix effects (Table A1). As shown in Fig. 2, the extracted isotopic



236 compositions were virtually identical to the directly measured reference values. Regression analysis yielded near-unity slopes
 237 and minimal intercepts (0.09‰ for $\delta^{18}\text{O}$ and 1.5‰ for $\delta^2\text{H}$), with exceptionally high coefficients of determination ($R^2 > 0.95$)
 238 and intraclass correlation coefficients (ICC = 0.97). These metrics indicate that the Rayleigh-based equilibrium correction (Eq.
 239 3) accurately reconstructs the source water composition by neutralizing effectively fractionation during vapor circulation and
 240 condensation.

241 Although a Wilcoxon signed-rank test revealed a statistically significant systematic offset for $\delta^{18}\text{O}$ ($p = 0.002$), the Mean
 242 Offset of 0.44‰ is physically small in the context of most ecohydrological applications. This magnitude is comparable to the
 243 analytical uncertainty of direct equilibration methods (0.2–0.5‰; Wassenaar et al. (2008)) and remains substantially lower
 244 than matrix-induced biases often exceeding 1.0‰ in conventional cryogenic vacuum distillation (Orlowski et al., 2016). In
 245 contrast, no significant systematic bias was observed for $\delta^2\text{H}$ ($p = 0.06$). Collectively, these results support the utility of
 246 CRAVE under simplified matrix conditions and show that the Rayleigh-based correction can accurately recover source-water
 247 isotope values in the absence of complex soil or plant matrices.

248



249

250 **Figure 2. Relationship between isotopic values derived from the cryogenic airtight vapor extraction (CRAVE) method and the**
 251 **reference liquid water.**

252

253 3.2 Isotopic values

254 Fig. 3 presents $\delta^2\text{H}$ - $\delta^{18}\text{O}$ dual-isotope plots for water extracted by three methods—CRAVE, CVD, and SL—across liquid,
 255 soil, and xylem water types. Detailed isotopic values and sample metadata are provided in Tables A1–A3.

256 For liquid water, the CRAVE-derived $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values mean -53.27‰ and -8.08‰ ($n = 33$, Table A1), respectively.
 257 These mean values were consistent with those from the reference liquid water (-51.54‰ and -7.63‰), showing no statistically
 258 significant bias. The regression slope for CRAVE (8.04) closely followed the reference slope (8.46), indicating that isotopic
 259 fractionation during vapor condensation was well constrained.

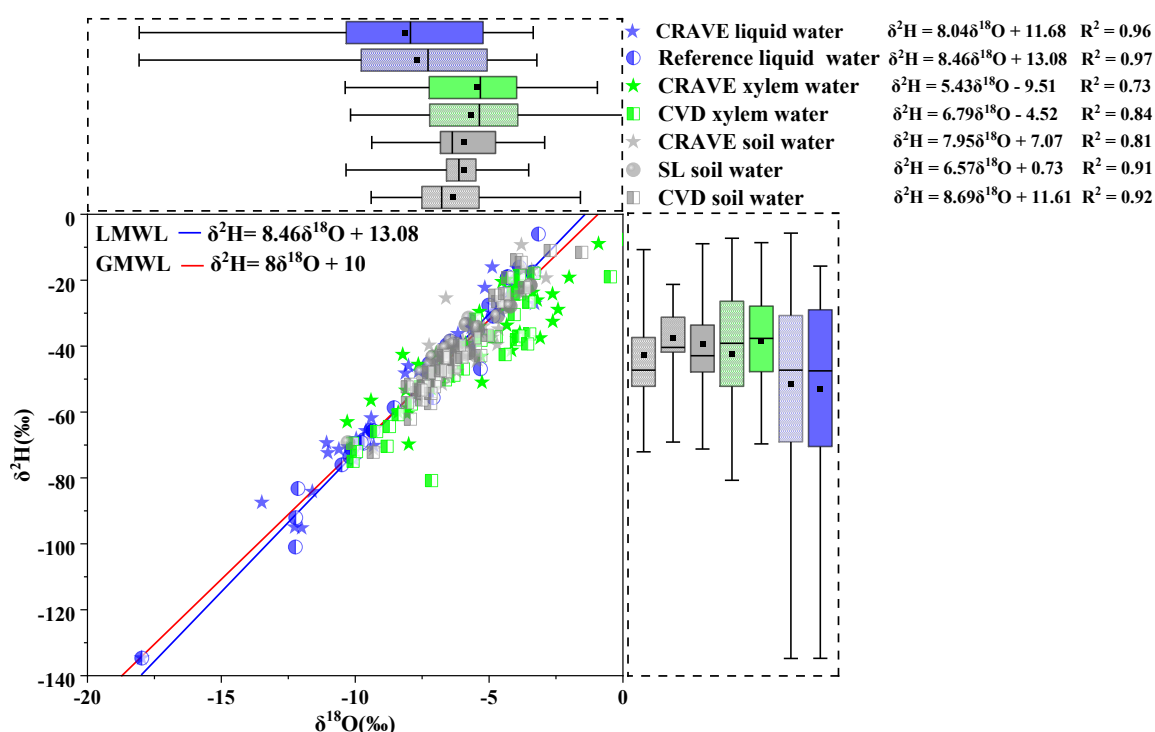
260 For soil water, CRAVE yielded mean values of -39.53‰ for $\delta^2\text{H}$ and -5.86‰ for $\delta^{18}\text{O}$, which aligned more closely with
 261 the SL reference values (-37.91‰ and -5.88‰) than with those from CVD. In contrast, the CVD method produced more
 262 depleted values (-42.93‰ and -6.27‰). The regression slope for CRAVE (7.95) fell between that of SL (6.57) and CVD



263 (8.69), suggesting that CRAVE is more consistent with the water represented by SL than with the bulk water recovered by
 264 thermal extraction.

265 For xylem water, CRAVE yielded mean $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of -38.68% and -5.37% , respectively. By comparison,
 266 CVD values were consistently more negative (-42.72% and -5.62%). The regression slope for CRAVE (5.43) was lower than
 267 that of CVD (6.79). Importantly, the xylem water extracted by CRAVE showed greater isotopic similarity to soil water
 268 collected via SL and CRAVE, while CVD-derived xylem water shifted toward the depleted signatures typical of CVD-
 269 extracted soil water.

270



271

272 **Figure 3. Relationships between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for water extracted by different methods (cryogenic airtight vapor extraction**
 273 **(CRAVE), cryogenic vacuum distillation (CVD), and suction lysimeter (SL)).**

274

275 3.3 Soil water isotope comparison

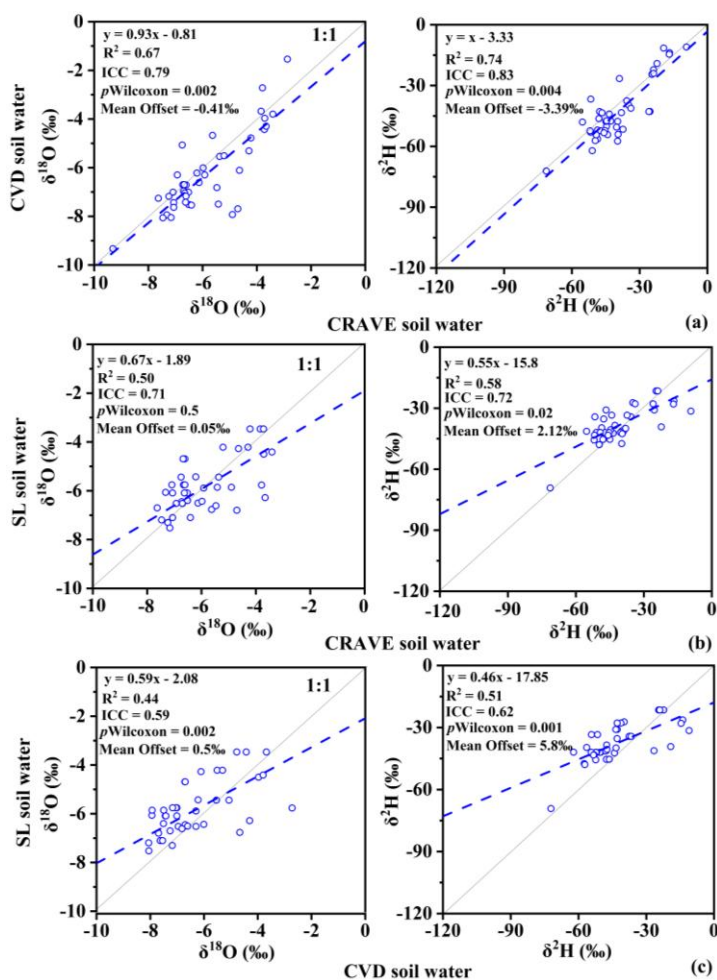
276 Fig. 4 shows the isotopic compositions of soil water obtained using CRAVE, CVD, and SL. For CRAVE and CVD, the
 277 Wilcoxon signed-rank test identified statistically significant differences for both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ (Mean Offsets of -3.39% and
 278 -0.41% , respectively; $p \leq 0.004$). However, the two methods still exhibited strong agreement, with R^2 ranging from 0.67 to
 279 0.74 and ICC of 0.79–0.83 (Fig. 4a), indicating consistent performance following Rayleigh-based correction.



280 Comparison between CRAVE and SL also showed strong consistency (Fig. 4b), with R^2 values of 0.50–0.58 and ICC
 281 values of 0.71–0.72. No significant difference was observed for $\delta^{18}\text{O}$ ($p = 0.5$), while $\delta^2\text{H}$ exhibited a small but statistically
 282 significant offset (2.12‰, $p = 0.02$).

283 In contrast, the agreement between CVD and SL was lower ($R^2 = 0.44$ –0.51; ICC = 0.59–0.62; Fig. 4c). The Wilcoxon
 284 test identified the largest systematic differences among the three comparisons (i.e., CRAVE vs. CVD, CRAVE vs. SL, and
 285 CVD vs. SL) with the CVD vs. SL comparison exhibiting the greatest offsets ($\delta^2\text{H}$: 5.8‰; $\delta^{18}\text{O}$: 0.5‰; $p \leq 0.002$). These
 286 results indicate that isotopic values from CVD tend to diverge from those derived using SL.

287



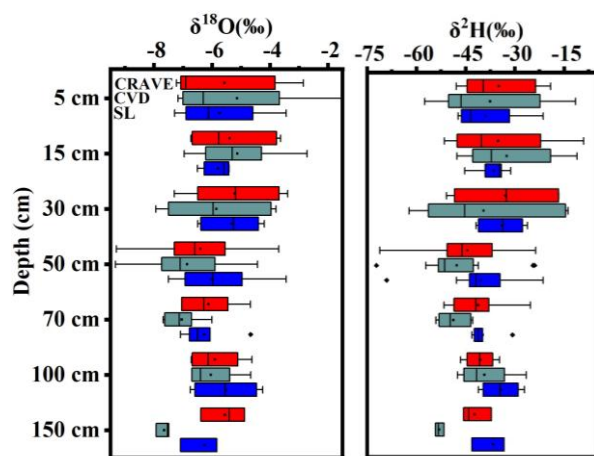
288

289 **Figure 4. Relationships between isotopic values of soil water extracted by different methods: (a) cryogenic airtight vapor**
 290 **extraction (CRAVE) vs. cryogenic vacuum distillation (CVD), (b) CRAVE vs. suction lysimeter (SL), and (c) CVD vs. SL.**



291 **3.4 Soil physical properties and isotopic deviation**

292 Fig. 5 shows the isotopic profiles ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of soil water extracted by CRAVE, CVD, and SL methods at different
 293 depths, while Fig. B1 presents the depth-dependent variation of the mean signed relative error (E_{ref}) of CRAVE relative to
 294 CVD. The three methods yielded comparable isotopic compositions in the upper soil layers, but their differences became more
 295 pronounced below 30 cm. In deeper layers, the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values obtained by CVD were systematically more depleted than
 296 those derived from CRAVE and SL, and the deviation increased with depth. This pattern is consistent with the positive
 297 correlation between E_{ref} and soil depth ($r = 0.77$ for $\delta^{18}\text{O}$, $r = 0.66$ for $\delta^2\text{H}$; Fig. B2), indicating that CRAVE-derived isotope
 298 values became enriched increasingly relative to CVD with increasing depth.
 299



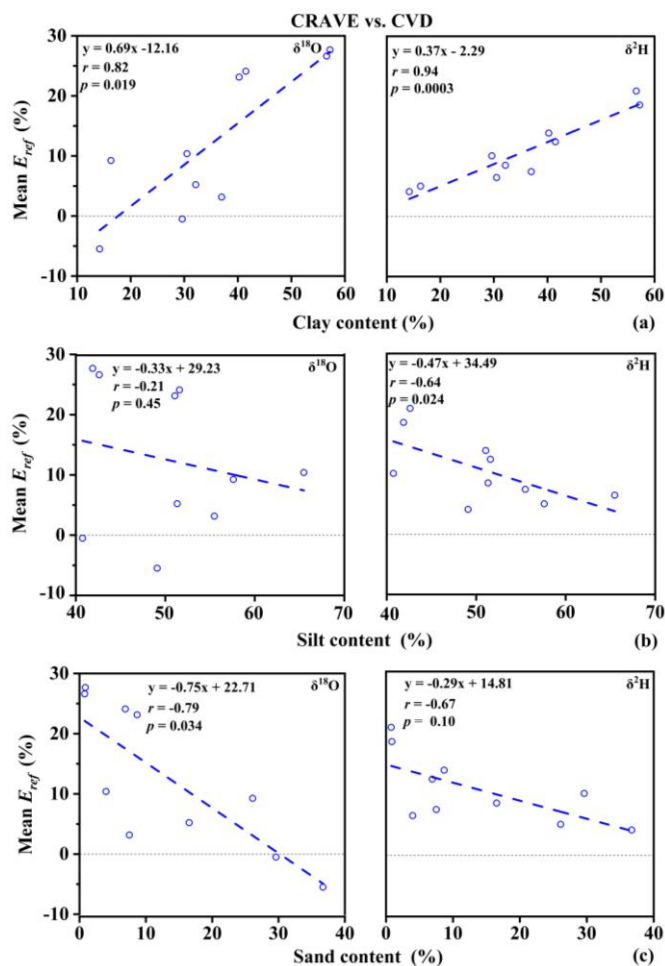
300
 301 **Figure 5. Isotopic variations ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of soil water extracted by cryogenic airtight vapor extraction (CRAVE), cryogenic**
 302 **vacuum distillation (CVD), and suction lysimeter (SL) methods at different soil depths.**

303
 304 Fig. 6 illustrates the relationship between soil particle-size fractions and the mean E_{ref} of the CRAVE method relative to
 305 CVD. Clay content exerted the strongest influence on mean E_{ref} , with highly significant positive correlations for both $\delta^{18}\text{O}$ and
 306 $\delta^2\text{H}$ ($r = 0.82\text{--}0.94$, $p = 0.0003\text{--}0.019$). Mean E_{ref} increased with clay content, indicating that the isotopic deviation between
 307 CRAVE and CVD becomes larger in fine-textured soils. In contrast, silt and sand contents were correlated negatively with
 308 mean E_{ref} ($r = -0.21$ to -0.79), reflecting the indirect influence of soil structural characteristics.

309 These observations are supported by the results in Fig. B2, which show that clay content had the strongest effect on the
 310 mean isotopic values of soil water extracted by CVD ($r = -0.74$ to -0.75 , $p = 0.013\text{--}0.015$). The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values obtained
 311 by CVD became progressively more depleted as clay content increased, whereas the isotopic compositions derived from
 312 CRAVE and SL showed little variation with texture. Furthermore, soil depth was found to correlate positively with clay content
 313 within the 0–200 cm profile ($r = 0.77$, $p = 0.001$; Liu et al. (2024)), reinforcing the interpretation that the increasing isotopic



314 deviation between CRAVE and CVD with depth (Fig. B1) is controlled largely by the higher clay fraction and stronger water
 315 retention in deeper horizons.



316
 317 **Figure 6. Relationships between soil particle size fractions and the mean signed relative error (E_{ref}) of the cryogenic airtight vapor**
 318 **extraction (CRAVE) method relative to the cryogenic vacuum distillation (CVD) method: (a) clay, (b) silt, and (c) sand contents.**
 319

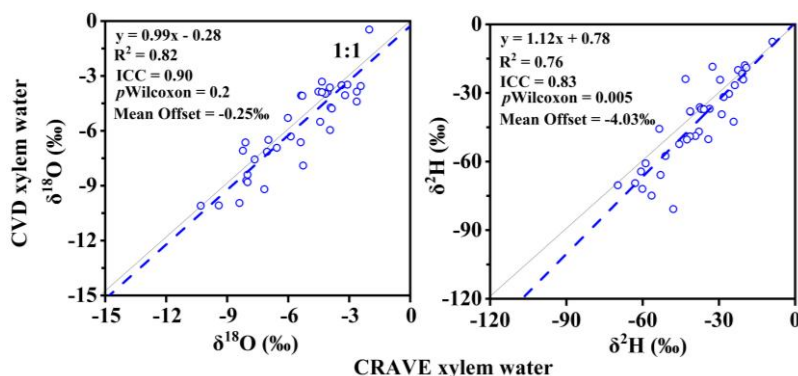
320 3.5 Xylem water isotope comparison

321 Fig. 7 compares the isotopic compositions of xylem water obtained by CRAVE and CVD. The two methods show strong
 322 consistency, with ICC = 0.83–0.90 and $R^2 = 0.76$ –0.82 for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively. The regression slopes (0.99 for $\delta^{18}\text{O}$
 323 and 1.12 for $\delta^2\text{H}$) are close to the 1:1 line. The Wilcoxon signed-rank test confirmed no significant systematic difference for
 324 $\delta^{18}\text{O}$ ($p = 0.2$). For $\delta^2\text{H}$, the test identified a statistically significant but small systematic offset ($p = 0.005$; Mean Offset = –
 325 4.03‰). While statistically detectable, this difference falls within the range of commonly reported analytical uncertainties in
 326 xylem water $\delta^2\text{H}$ measurements, especially those affected by VOC or hydrogen exchange with cell wall materials (Millar et



327 al., 2018; West et al., 2006). These results demonstrate that CRAVE-derived xylem water isotopes are highly comparable to
 328 those obtained via conventional CVD.

329

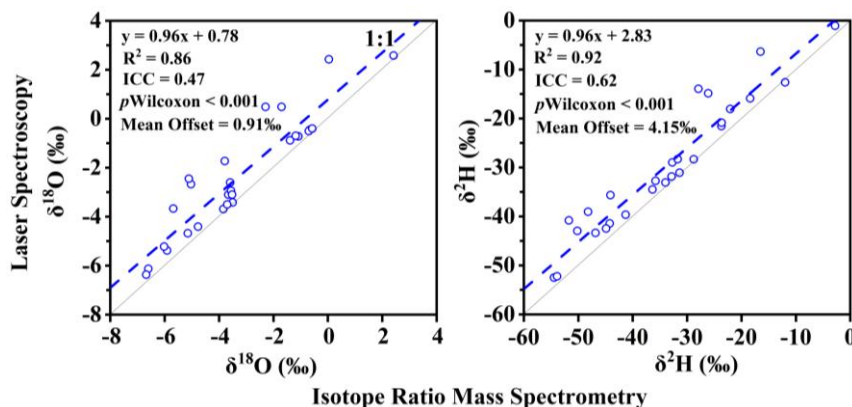


330

331 **Figure 7. Relationship between isotopic values of xylem water extracted by the cryogenic airtight vapor extraction (CRAVE) and**
 332 **cryogenic vacuum distillation (CVD) methods.**
 333

334 To further investigate the potential influence of VOCs on these measurements, a subset of xylem water samples extracted
 335 by CVD was analyzed using both LS and IRMS (Fig. 8). The results revealed highly significant systematic offsets between
 336 the two analytical platforms ($p < 0.001$ for both $\delta^{18}\text{O}$ and $\delta^2\text{H}$), where LS measurements were on average enriched by 0.91‰
 337 in $\delta^{18}\text{O}$ (-2.32‰ vs. -3.23‰ for IRMS) and 4.15‰ in $\delta^2\text{H}$ (-29.79‰ vs. -33.94‰ for IRMS). This discrepancy underscores
 338 the sensitivity of laser-based measurements to spectral interference in VOC-rich samples extracted by heating-based methods.
 339 However, because this comparison was conducted on CVD extracts rather than CRAVE condensates, it should be interpreted
 340 as supporting the general analytical rationale for CRAVE–IRMS rather than as a direct demonstration that CRAVE completely
 341 eliminates organic interference.

342



343

344 **Figure 8. Comparison of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values of xylem water extracted by cryogenic vacuum distillation and analyzed using isotope**
 345 **ratio mass spectrometry and laser spectroscopy.**

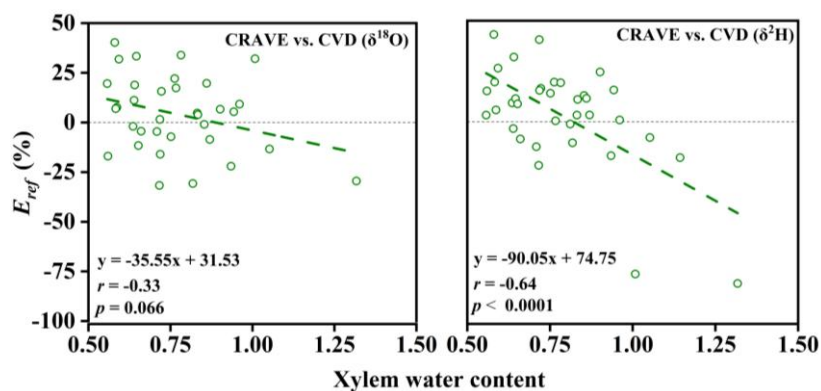


346 **3.6 Sample water content effects on isotope deviations**

347 Figures 9 and B3 show the influence of sample water content on the signed relative error (E_{ref}) of the CRAVE method
 348 relative to other extraction techniques. For soil water, the correlation between E_{ref} and water content was weak ($r = -0.11$ to
 349 0.24 , Fig. B3), probably because the gravimetric water content of the soil samples was relatively narrow (30–44%; Table A2)
 350 and did not include very dry conditions where extraction-induced fractionation could be enhanced. On average, E_{ref} of CRAVE
 351 relative to CVD was slightly positive, while E_{ref} relative to SL was slightly negative, reflecting minor differences in the
 352 dominant water pools accessed by each method.

353 For xylem water, E_{ref} for both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ exhibited negative correlations with water content, with a stronger relationship
 354 for $\delta^2\text{H}$ ($r = -0.64$, $p < 0.0001$, Fig. 9). When the xylem water content (dry-weight basis) exceeded approximately 0.8–0.9, E_{ref}
 355 shifted from positive to negative, indicating that CVD produced more depleted isotopic values under drier conditions, likely
 356 due to the heating process releasing isotopically lighter bound water. The CRAVE method, operating at ambient temperature,
 357 was less affected by this bias.

358



359

360 **Figure 9. Relationship between xylem water content (dry-weight basis) and the signed relative error (E_{ref}) of the cryogenic airtight**
 361 **vapor extraction (CRAVE) method relative to the cryogenic vacuum distillation (CVD) method.**
 362

363 **4. Discussions**

364 **4.1 Mechanistic bases for method-dependent extraction differences**

365 Recent studies have demonstrated that the extraction technique can significantly alter the measured $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of soil
 366 water, especially because different methods access tightly bound versus mobile water to different degrees (Adams et al., 2020).
 367 CVD, which operates under high temperature and vacuum, tends to extract water held at stronger matric potentials, including
 368 tightly bound or immobile water associated with fine pores and mineral surfaces. This bound fraction is commonly isotopically
 369 depleted due to prolonged residence times and mineral–water interactions (Sprenger et al., 2019; Orłowski et al., 2016;
 370 McDonnell, 2014). In contrast, SL primarily accesses the more freely draining mobile water that represents the more dynamic
 371 and often isotopically enriched part of the soil-water continuum (Sprenger et al., 2019). The CRAVE method, operating



372 isothermally and without vacuum, interacts with vapor generated from the fraction of sample water that exchanges with
373 headspace vapor under ambient conditions, thereby reducing mobilization of isotopically distinct bound water relative to CVD.

374 These differences in the water pools accessed by each technique explain the extraction patterns observed across depths
375 and soil textures. At greater depth, tightly bound water may constitute a larger proportion of total soil water as pore connectivity
376 declines and mineral associations strengthen. This causes CVD to extract increasingly depleted water relative to CRAVE and
377 SL. Similar depth-related separations between mobile and bulk soil water have been reported previously, with correlations
378 between SL- and CVD-derived isotopes weakening in deeper layers and indicating divergent source pools (Liu et al., 2025).
379 Soil texture further modulates these method-dependent differences. Clay-rich soils retain larger fractions of tightly bound
380 water and contain abundant reactive mineral particles, including clays and iron–aluminum oxides, whose surfaces can
381 exchange hydrogen and oxygen isotopes with surrounding water (Orlowski et al., 2016; Sprenger et al., 2019; Wen et al., 2021;
382 O'Neil and Kharaka, 1976). Work by Gaj et al. (2017) and Wen et al. (2021) found that higher extraction temperatures in CVD
383 release more adsorbed clay-bound water, whereas lower-temperature extractions recover less of that pool and therefore yield
384 different isotope values. These mineral–water interactions help explain why CVD-extracted water became more depleted as
385 clay content increased, whereas CRAVE and SL showed weaker texture dependence.

386 Plant xylem water also shows method-dependent differences, although the mechanisms differ from those in soils. Xylem
387 conduits contain mainly mobile water within vessel lumens (Barbeta et al., 2022), and xylem tissues, composed largely of
388 cellulose, hemicellulose, and lignin (Wang and Pollack, 2024), contain very little mineral matter, resulting in minimal isotope
389 exchange with inorganic surfaces. However, cell-wall polymers include hydroxyl functional groups that can retain small
390 amounts of structural water and weakly bound hydration water. These minor water pools have been shown to exhibit isotopic
391 compositions that differ from bulk xylem water, particularly for hydrogen because of exchange with hydroxyl groups (Zhao
392 et al., 2016; Younger et al., 2024). The bias is more pronounced under low tissue moisture (dry wood), where a greater
393 proportion of water comes from these loosely bound pools and isotopic exchange with cell-wall –OH can occur before the
394 water is liberated. For example, Younger et al. (2024) modeled and observed that cryogenic extraction often yields xylem
395 water depleted in deuterium by several per mil compared to the source water, especially when wood moisture content is low.

396 Heating may also enhance the volatilization of VOCs, which can introduce additional isotope effects, particularly for
397 hydrogen (Martín-Gómez et al., 2015; Lehmann et al., 2025; Herbstritt et al., 2024). Recent work has documented that
398 cryogenic distillation tends to co-extract such organics because it removes nearly all liquid constituents from plant tissue,
399 including soluble compounds; these co-extracted compounds can bias isotope values, sometimes making $\delta^2\text{H}$ appear
400 erroneously enriched or depleted (Herbstritt et al., 2024). To address this problem, newer methods aim to minimize VOC co-
401 extraction. Previous studies have shown that such VOC-related biases are substantially reduced in closed, ambient-temperature,
402 or otherwise low-disturbance extraction systems, including Cavitron and vapor-equilibration techniques (Bowers and Williams,
403 2022; Wen et al., 2022; Sobota et al., 2024; Barbeta et al., 2022; Kübert et al., 2023). Herbstritt et al. (2024) found that direct
404 vapor equilibration and in-situ probes had minimal need for spectral corrections, whereas CVD-extracted samples required
405 substantial post-correction for organics.



4.2 Analytical benefits of CRAVE-IRMS compared to vapor-equilibration

The CRAVE method shares several operational advantages with vapor-equilibration techniques, notably its ambient-temperature protocol and recirculating vapor pathway. By avoiding the high temperatures typically required for vacuum distillation, CRAVE minimizes heating-induced non-equilibrium effects and reduces the likelihood of wood–water hydrogen exchange and thermally enhanced release of organic compounds. However, unlike vapor-equilibration methods that rely on isotopic equilibrium between vapor and water and then infer liquid composition via LS (Wassenaar et al., 2008; Gralher et al., 2021), CRAVE collects condensed liquid water directly. This workflow enables physically based reconstruction of source-water isotope composition using Rayleigh fractionation principles. It also allows analysis of the condensate by IRMS, which is less sensitive to VOC-induced spectral interference than laser-based techniques (Millar et al., 2021; Herbstritt et al., 2024; West et al., 2010). Because IRMS converts water to gas under high-temperature analytical conditions, it effectively excludes many volatile organic compounds that might otherwise bias $\delta^2\text{H}$ measurements (Gehre et al., 2015). Our results demonstrate this advantage for CVD-derived xylem water, although they do not demonstrate that CRAVE condensates are completely free from organic interference. CRAVE–IRMS should therefore be viewed as a method that reduces, rather than automatically eliminates, the analytical risks associated with VOC-rich plant and soil samples.

4.3 The implications of this study

As shown in Tables A2 and A3, the isotopic compositions of plant and soil water extracted by the same method are generally consistent, whereas slight but systematic differences exist among different extraction techniques. Xylem water extracted by CVD shows a slightly more depleted $\delta^2\text{H}$ signal compared to that obtained via CRAVE and SL, while for soil water the CVD method produces minor depletions in both $\delta^2\text{H}$ and $\delta^{18}\text{O}$. These results indicate that small isotopic offsets introduced during extraction are method-dependent. For studies that compare isotopic compositions between plant and soil water, particularly those using mixing models for source attribution, applying the same extraction method can help minimize methodological bias and improve data comparability. However, this does not exclude the use of different methods when justified by sample properties or practical limitations. For example, the Cavitron centrifuge has been shown to extract effectively xylem water with minimal thermal and organic interference (Barbeta et al., 2022), even though it cannot be used for soil samples. In such cases, calibration procedures, correction factors, or dual-method validation may be necessary to ensure consistency between plant and soil water isotopic measurements (Chen et al., 2020; He et al., 2023; Allen and Kirchner, 2022). In contrast, the CRAVE method evaluated in this study allows for simultaneous extraction of both soil and plant water using a single, unified protocol. This feature supports internally consistent comparisons and reduces the need for cross-method correction.

Furthermore, the CRAVE system can be adapted for in situ extraction of xylem vapor under natural field conditions. Although this study confirmed strong isotopic agreement between CRAVE and CVD xylem water samples under controlled laboratory conditions, direct field deployment may introduce sampling-induced temporal bias. During the typical 0.5–3 hour extraction period, xylem water isotopic compositions can be influenced by short-term fluctuations in transpiration and



440 environmental forcing. To ensure the reliability of such in situ applications, we propose the following methodological
441 guidelines: (1) Rigorous temperature monitoring of the extraction environment is mandatory, as the stable isotopic equilibrium
442 within the CRAVE system is highly temperature-dependent; accurate thermal data are essential for the Rayleigh-based
443 correction to reconstruct precisely source water isotopes. (2) Extractions should ideally be conducted during periods of steady-
444 state transpiration (e.g., mid-morning) to avoid rapid isotopic shifts associated with peak midday transpiration. The CRAVE
445 system is compact, portable, and energy-efficient, allowing for direct coupling to plant stems or soil probes for on-site vapor
446 condensation. Unlike conventional laboratory-based CVD systems, this field-compatible setup is designed to perform reliably
447 under varying environmental conditions, making it well-suited particularly for characterizing high-frequency ecohydrological
448 dynamics in the field.

449 Beyond its methodological contribution, the CRAVE method provides new opportunities for tracing water movement and
450 partitioning within the soil-plant-atmosphere continuum. By enabling accurate and consistent isotope measurements of both
451 soil and plant water, the CRAVE approach offers a valuable experimental tool to test and refine the “two water worlds”
452 framework (Brooks et al., 2010; McDonnell, 2014; Sprenger et al., 2019; Dubbert et al., 2019), which posits the coexistence
453 of mobile and immobile water pools with distinct isotopic signatures. Specifically, the differentiation between water pools
454 held under different tensions within the soil profile and how plants extract water from these pools over time (Li et al., 2026).

455 In this context, the CRAVE method provides a physically grounded framework to relate the isotopic composition of
456 condensed vapor to that of the original water pool using Rayleigh-based correction principles (Gat, 1996). The parameter f is
457 defined here as the ratio of condensed water mass to the initial water mass in the sample ($f = G_c / G_s$), indicates the extent of
458 vapor removal during. When f is small, the collected condensate is expected to reflect the portion of sample water that
459 exchanges most readily with headspace vapor under the extraction conditions, whereas larger f values may increasingly
460 integrate signals from more tightly retained water.

461 Although this pattern aligns qualitatively with conceptual models of mobile versus immobile water pools, we caution
462 against interpreting f as a direct quantitative proxy for such partitioning without targeted validation. The representativeness of
463 the condensed vapor depends on factors such as equilibration time, soil water potential, pore structure, and temperature
464 gradients (Brooks et al., 2010; McDonnell, 2014; Sprenger et al., 2019). Controlled experiments, such as stepwise CRAVE
465 extractions under defined matric potentials or sequential collection of condensate fractions, are needed to evaluate this potential
466 rigorously. In this study, we therefore present mobile–immobile partitioning as a promising future application of the CRAVE
467 framework rather than a demonstrated quantitative capability.

468 Integrating CRAVE-derived isotope observations with isotope-enabled hydrological models, including recent mobile-
469 immobile mixing frameworks and sub-daily plant water-uptake simulations (Cain et al., 2019; Beyer et al., 2020; Fu et al.,
470 2024; Ring et al., 2025; Radolinski et al., 2021; Finkenbiner et al., 2022), would allow future studies to mechanistically resolve
471 how source-water separation evolves across diurnal to seasonal timescales.



472 **5. Conclusions**

473 This study developed and tested a new CRAVE method for extracting soil and plant water for stable isotope analysis.
474 Operating at ambient temperature within a recirculating vapor–liquid pathway, the CRAVE system minimizes heating-related
475 isotope artifacts, reduces organic contamination, and enables direct comparison of soil and vegetation water isotopic
476 compositions. Isotopic values obtained by CRAVE showed strong agreement with those derived from CVD and SL, confirming
477 the method’s potential utility and reproducibility under the tested conditions. Differences among extraction methods were
478 primarily controlled by xylem water content, soil clay fraction, and sampling depth. Xylem water content had a pronounced
479 effect on the isotopic deviation between CRAVE and CVD, with stronger hydrogen-isotope depletion in CVD under low-
480 moisture conditions, whereas for soils the isotopic differences between methods increased with clay content and depth as CVD
481 produced progressively more depleted $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values. Across these gradients, CRAVE remained comparatively stable
482 and was generally more consistent with SL than with CVD. By integrating Rayleigh-based isotope correction, the CRAVE
483 approach provides a physically grounded means to reconstruct source-water isotope values from condensate measurements.
484 Its potential application to mobile–immobile soil-water partitioning and to field monitoring is promising, but both require
485 additional targeted validation. Owing to its simplicity and no-heating extraction design, CRAVE offers a useful new tool for
486 laboratory ecohydrological studies of water partitioning and source dynamics within the soil–plant–atmosphere continuum.



487 **Appendix A**

488 **Table A1. Sample information and isotope values ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of liquid water and cryogenic airtight vapor extraction (CRAVE)**
 489 **samples.**

No.	Sample mass	Condensed water mass (g)	Liquid water sample values		Initial condensed water values (δ_c)		CRAVE values (δ_l)	
			$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$
W1	6.81	0.27	-19.11	-4.32	-96.80	-14.59	-22.24	-5.16
W2	13.14	0.15	-38.44	-6.45	-113.88	-15.10	-40.32	-5.61
W3	7.88	0.63	-30.95	-4.85	-99.23	-13.92	-27.53	-4.81
W4	10.96	0.35	-20.20	-4.00	-94.64	-13.67	-19.81	-4.21
W5	6.79	0.37	-17.53	-3.33	-89.89	-13.21	-16.44	-3.96
W6	7.08	0.23	-27.56	-5.01	-103.07	-14.00	-29.23	-4.57
W7	10.02	0.22	-16.05	-3.87	-96.41	-13.57	-22.58	-4.22
W8	8.25	0.80	-76.03	-10.50	-141.56	-20.17	-72.40	-11.02
W9	8.43	0.68	-18.88	-4.31	-90.19	-14.19	-15.99	-4.88
W10	11.68	0.50	-47.45	-7.30	-119.17	-16.63	-46.81	-7.27
W11	16.16	0.40	-39.63	-6.58	-113.14	-15.62	-38.89	-6.07
W12	16.04	0.49	-46.91	-5.32	-119.36	-17.49	-45.99	-8.01
W13	12.66	0.30	-65.40	-9.41	-139.40	-19.39	-68.09	-9.96
W14	11.56	0.16	-73.56	-10.20	-145.72	-19.50	-74.61	-10.02
W15	15.46	0.15	-6.01	-3.16	-101.81	-12.83	-27.09	-3.30
W16	18.57	0.43	-100.94	-12.24	-154.10	-21.01	-84.12	-11.60
W17	20.21	0.58	-58.68	-8.55	-133.41	-18.82	-61.79	-9.40
W18	20.60	0.40	-83.22	-12.14	-157.51	-22.93	-87.43	-13.49
W19	22.04	0.18	-17.65	-3.42	-96.98	-13.30	-21.95	-3.78
W20	25.97	0.40	-69.46	-9.70	-138.16	-19.16	-65.69	-9.60
W21	24.03	0.54	-39.44	-6.23	-114.06	-16.17	-39.92	-6.63
W22	24.02	0.42	-32.59	-5.68	-109.01	-15.22	-34.32	-5.66
W23	20.20	0.21	-92.06	-12.22	-165.38	-21.82	-94.90	-12.26
W24	18.30	0.16	-68.35	-9.66	-142.27	-18.86	-70.17	-9.31
W25	18.86	0.54	-35.35	-5.43	-110.09	-15.62	-36.23	-6.16
W26	12.21	0.44	-65.71	-9.34	-140.73	-20.49	-69.37	-11.07
W27	18.58	0.49	-69.19	-9.78	-142.17	-19.74	-70.59	-10.26
W28	19.50	0.13	-71.29	-10.13	-165.31	-21.52	-95.18	-12.00
W29	23.03	0.25	-55.64	-7.09	-129.64	-17.43	-56.41	-7.87
W30	17.93	0.36	-51.83	-7.21	-121.38	-17.17	-47.69	-7.63
W31	18.70	0.15	-45.28	-7.24	-122.24	-17.72	-48.21	-8.13
W32	17.83	0.39	-65.71	-9.34	-143.08	-20.11	-71.31	-10.61
W33	20.18	0.22	-134.67	-17.97	-201.85	-27.46	-134.63	-17.97
Mean	15.87	0.36	-51.54	-7.63	-125.81	-17.53	-53.27	-8.08



490 **Table A2. Sample information and isotope values ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of soil water extracted by different methods: cryogenic vacuum**
 491 **distillation (CVD), suction lysimeter (SL), and cryogenic airtight vapor extraction (CRAVE).**

No.	Depth (cm)	Gravimetric water content		Sample mass (g)	Condensed water mass (g)	CVD values		SL values		Initial condensed water values (δ_c)		CRAVE values (δ_i)	
		%				$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$
S1	5	30		45.32	0.32	-22.27	-3.68	-21.54	-3.47	-99.50	-13.45	-23.85	-3.84
S2	5	38		36.97	0.33	-11.57	-1.54			-94.52	-12.41	-19.33	-2.87
S3	5	32		35.65	0.40	-50.18	-7.01	-41.98	-5.76	-118.61	-16.65	-44.70	-7.09
S4	5	39		40.54	0.48	-46.36	-6.30	-45.37	-6.52	-121.52	-16.47	-48.04	-6.93
S5	5	44		36.83	0.18	-57.44	-7.18	-47.45	-7.30	-113.53	-16.76	-39.76	-7.24
S6	15	33		41.44	0.14	-36.71	-5.07	-34.29	-5.44	-125.27	-16.35	-51.61	-6.75
S7	15	32		45.34	0.53	-19.19	-4.30	-39.23	-6.29	-97.36	-13.20	-22.28	-3.66
S8	15	32		42.09	0.32	-11.02	-2.72	-31.49	-5.77	-86.04	-13.40	-9.25	-3.79
S9	15	33		33.04	0.36	-42.90	-6.22	-35.35	-5.43	-121.42	-15.78	-47.71	-6.21
S10	15	35		37.32	0.42	-37.59	-5.54	-34.29	-5.44	-110.28	-14.94	-35.67	-5.37
S11	15	33		41.74	0.17	-47.68	-6.96	-45.37	-6.52	-119.11	-16.30	-44.97	-6.70
S12	30	30		45.40	0.61	-42.86	-5.31	-27.87	-4.22	-101.69	-13.91	-25.90	-4.29
S13	30	34		34.99	0.32	-47.72	-6.61	-41.22	-6.51	-113.92	-15.69	-39.72	-6.13
S14	30	32		48.77	0.29	-13.83	-3.97	-26.33	-4.50	-92.06	-13.24	-16.76	-3.71
S15	30	39		49.15	0.18	-62.18	-7.94	-41.94	-6.07	-124.46	-16.90	-50.95	-7.32
S16	30	33		50.41	0.35	-14.58	-3.80	-27.99	-4.42	-93.62	-13.07	-16.90	-3.41
S17	30	34		51.82	0.14	-56.36	-7.51	-39.66	-6.40	-121.97	-16.07	-48.61	-6.51
S18	50	34		48.00	0.16	-41.22	-5.51	-27.87	-4.22	-108.31	-14.77	-33.85	-5.21
S19	50	30		37.66	0.17	-48.03	-7.26	-41.39	-6.70	-127.88	-17.15	-55.28	-7.63
S20	50	33		42.72	0.15	-50.54	-7.01	-42.03	-6.09	-106.64	-11.46	-40.23	-6.52
S21	50	34		54.27	0.15	-54.36	-7.17	-41.98	-5.76	-123.53	-16.26	-49.20	-6.61
S22	50	38		37.46	0.21	-24.51	-4.78	-21.54	-3.47	-99.66	-13.78	-24.55	-4.22
S23	50	31		44.51	0.56	-52.37	-8.06	-45.65	-7.20	-125.74	-17.06	-51.95	-7.47
S24	50	30		39.37	0.15	-24.04	-4.43	-21.54	-3.47	-99.02	-13.29	-23.83	-3.72
S25	50	34		36.36	0.15	-57.18	-8.05	-47.94	-7.53	-123.90	-16.80	-49.65	-7.16
S26	50	31		38.41	0.18	-44.07	-6.30	-42.34	-5.89	-117.53	-15.51	-43.48	-5.92
S27	50	33		52.79	0.20	-52.32	-7.04	-41.98	-5.76	-120.66	-16.21	-47.23	-6.66
S28	50	35		42.14	0.23	-52.23	-7.42	-42.03	-6.09	-118.85	-16.17	-45.34	-6.63
S29	50	30		32.82	0.13	-72.19	-9.33	-69.23	-10.27	-142.71	-18.82	-71.33	-9.31
S30	70	31		38.51	0.34	-42.85	-6.71	-30.91	-4.69	-100.14	-16.12	-25.40	-6.61
S31	70	32		41.40	0.36	-52.00	-7.44	-42.03	-6.09	-121.63	-16.57	-48.67	-7.07
S32	70	32		40.88	0.18	-53.06	-7.63	-43.32	-7.10	-124.49	-16.56	-51.76	-7.06
S33	70	33		47.13	0.17	-47.41	-6.82	-40.61	-6.62	-118.22	-15.03	-44.61	-5.47
S34	70	34		40.80	0.16	-54.13	-7.69	-42.57	-6.80	-113.36	-14.25	-39.53	-4.70
S35	70	32		42.78	0.23	-43.40	-6.01	-39.93	-6.44	-112.12	-15.53	-38.04	-5.98
S36	100	30		35.09	0.37	-26.57	-4.67	-41.22	-6.77	-113.12	-15.19	-38.91	-5.63
S37	100	33		45.14	0.23	-43.48	-6.70	-30.91	-4.69	-120.10	-16.22	-46.68	-6.68
S38	100	35		30.02	0.53	-47.55	-6.70	-38.47	-6.45	-116.55	-16.26	-42.75	-6.73
S39	100	34		38.54	0.30	-39.77	-6.11	-27.30	-4.27	-108.88	-14.17	-34.82	-4.64
S40	150	33		45.29	0.12	-53.35	-7.54	-43.32	-7.10	-120.36	-16.06	-45.76	-6.41
S41	150	31		41.30	0.10	-51.58	-7.93	-33.43	-5.86	-112.16	-14.52	-37.37	-4.90
S42	150	33		31.80	0.31	-54.23	-7.50	-33.43	-5.86	-117.44	-14.94	-44.17	-5.42
Mean	52	33		41.48	0.27	-42.93	-6.27	-37.91	-5.88	-113.52	-15.32	-39.53	-5.86



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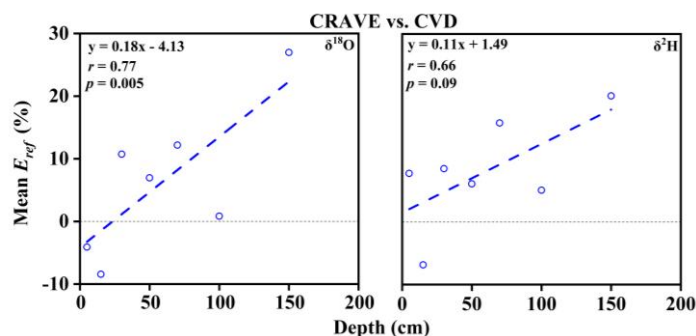
Table A3. Sample information and isotope values ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of plant xylem water extracted by different methods: cryogenic vacuum distillation (CVD) and cryogenic airtight vapor extraction (CRAVE).

No.	Gravimetric water content	Sample mass	Condensed water mass	CVD values		Initial condensed water values (δ_c)		CRAVE values (δ_i)	
	%			(g)	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$
P1	96	9.68	0.12	-70.39	-8.81	-141.75	-17.55	-69.76	-8.00
P2	72	10.32	0.31	-80.80	-7.15	-120.96	-16.51	-47.93	-7.04
P3	64	12.67	0.37	-69.46	-10.10	-134.93	-19.75	-62.97	-10.30
P4	59	15.96	0.31	-64.37	-8.72	-133.04	-17.57	-60.62	-8.06
P5	83	13.04	0.13	-60.81	-8.40	-131.68	-17.56	-58.80	-8.00
P6	76	14.53	0.24	-65.89	-9.20	-125.34	-16.64	-52.95	-7.17
P7	65	15.47	0.20	-57.58	-7.90	-124.03	-14.80	-50.99	-5.26
P8	105	12.72	0.22	-38.25	-5.30	-115.22	-15.55	-41.23	-6.01
P9	72	10.80	0.22	-24.30	-4.07	-104.29	-14.89	-29.54	-5.36
P10	71	9.01	0.18	-20.00	-3.87	-97.77	-13.59	-22.47	-4.05
P11	87	12.78	0.21	-21.60	-3.64	-96.41	-13.51	-20.88	-3.95
P12	82	13.49	0.35	-17.91	-3.32	-95.05	-13.85	-19.77	-4.34
P13	132	11.59	0.14	-23.95	-4.09	-116.98	-14.85	-43.11	-5.30
P14	101	11.43	0.24	-18.54	-3.86	-107.04	-12.18	-32.51	-2.62
P15	56	8.96	0.36	-24.11	-3.86	-95.21	-13.96	-20.44	-4.51
P16	93	12.06	0.21	-45.74	-6.64	-126.21	-17.59	-53.43	-8.10
P17	90	5.74	0.31	-74.93	-10.08	-128.06	-18.75	-56.46	-9.41
P18	72	14.23	0.14	-72.00	-9.95	-132.59	-17.92	-60.11	-8.39
P19	81	14.92	0.19	-18.96	-0.47	-94.71	-11.58	-19.17	-2.01
P20	75	12.27	0.14	-49.16	-6.50	-116.03	-16.50	-42.23	-6.97
P21	85	13.69	0.23	-52.39	-7.57	-118.92	-17.14	-45.58	-7.64
P22	94	9.39	0.12	-49.05	-6.93	-115.20	-16.09	-41.35	-6.56
P23	72	14.02	0.38	-50.42	-7.09	-116.57	-17.74	-42.58	-8.23
P24	64	14.17	0.19	-50.25	-6.63	-108.51	-14.92	-34.15	-5.38
P25	83	13.87	0.14	-26.65	-3.51	-98.94	-12.94	-23.71	-3.37
P26	56	15.02	0.24	-37.14	-4.79	-110.02	-13.39	-35.91	-3.85
P27	128	13.19	0.10	-30.41	-4.06	-100.66	-12.73	-26.03	-3.20
P28	58	20.00	0.13	-48.84	-6.31	-113.46	-15.44	-39.25	-5.87
P29	58	17.52	0.12	-42.57	-4.40	-99.59	-12.23	-24.19	-2.63
P30	77	19.79	0.23	-37.10	-4.73	-111.13	-13.47	-36.93	-3.91
P31	78	15.49	0.26	-46.94	-5.96	-111.33	-13.43	-37.86	-3.94
P32	86	14.94	0.24	-31.79	-5.51	-102.84	-13.96	-28.11	-4.43
P33	114	15.44	0.25	-7.58	0.21	-84.69	-10.44	-8.92	-0.91
P34	66	14.96	0.08	-38.05	-3.98	-115.60	-13.76	-41.30	-4.16
P35	64	18.27	0.28	-36.29	-3.48	-111.84	-12.67	-37.52	-3.09
P36	59	14.44	0.13	-39.34	-3.56	-103.79	-12.01	-28.89	-2.43
P37	65	13.06	0.11	-36.94	-3.89	-107.99	-13.88	-33.66	-4.34
Mean	79	13.48	0.21	-42.72	-5.62	-112.66	-14.90	-38.68	-5.37

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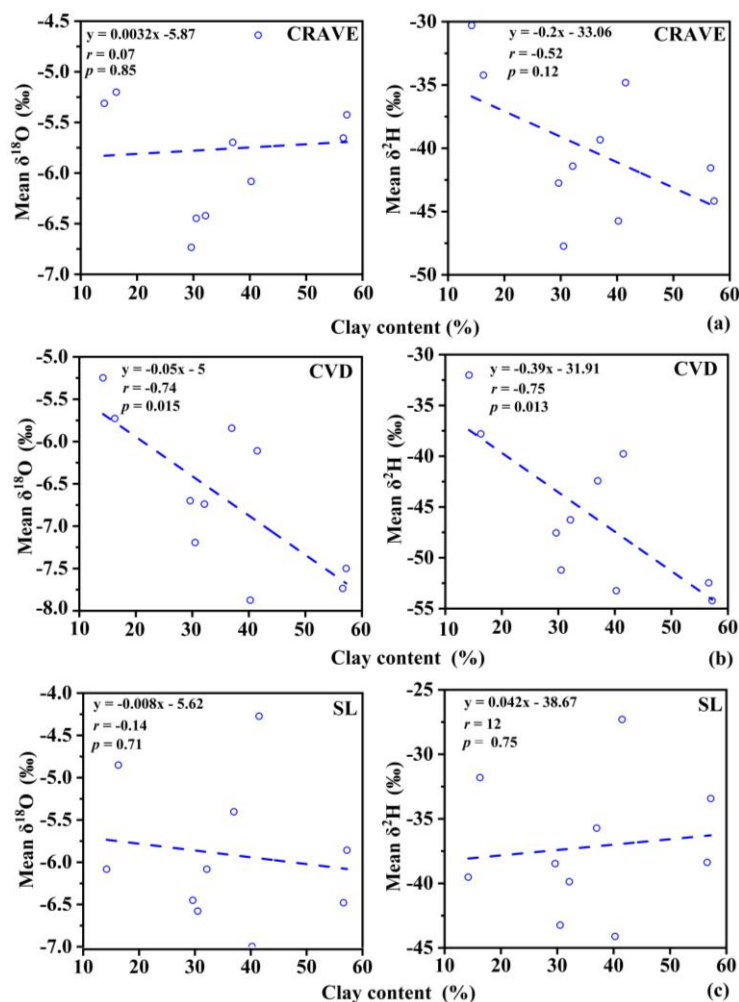


496 **Appendix B**



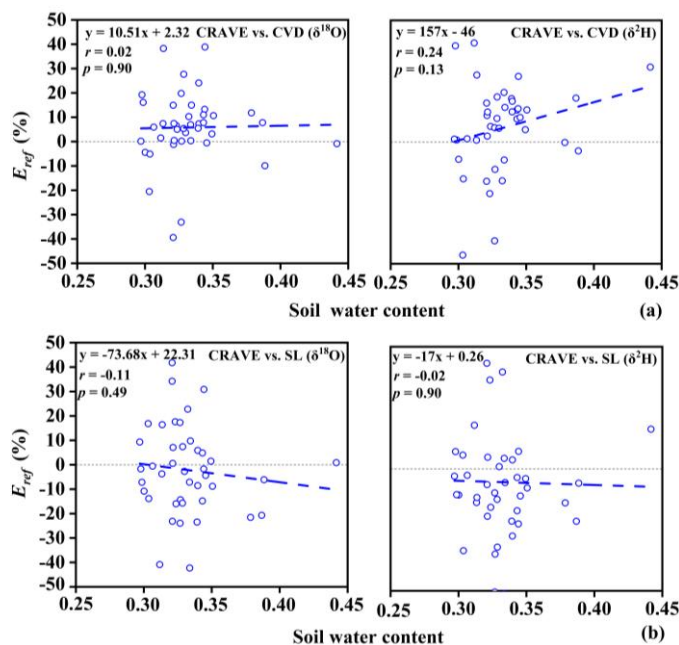
497

498 **Figure B1.** Relationships between soil depth and mean signed relative error (E_{ref}) of the cryogenic airtight vapor extraction
 499 (CRAVE) method relative to the cryogenic vacuum distillation (CVD) method.
 500



501

502 **Figure B2.** Influence of clay content on the mean $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values of soil water extracted by different methods: (a) cryogenic
 503 airtight vapor extraction (CRAVE), (b) cryogenic vacuum distillation (CVD), and (c) suction lysimeter (SL).



504

505 **Figure B3. Relationships between soil water content and mean signed relative error (E_{ref}) of the cryogenic airtight vapor extraction**
506 **(CRAVE) method relative to different extraction methods: (a) CRAVE vs. cryogenic vacuum distillation (CVD) and (b) CRAVE**
507 **vs. suction lysimeter (SL).**

508

509 Data Availability Statement

510 All data generated or analyzed during this study are included in this published article and its Appendix A

511 Declaration of Interests

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

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