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Towards Transparency in Sampling for Plant Xylem Water Isotope Studies: Current Practices and New Perspectives

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ABSTRACT

Xylem water isotope values are widely used to trace plant water sources. However, the sampling decisions that precede extraction are rarely fully reported in isotope studies, limiting reproducibility and cross comparisons. A survey of 42 researchers across five continents, complemented by a virtual community workshop, revealed substantial methodological diversity: only 51% maintain standard operating procedures, with variation in organ selection among respondents (branches 73.8%, stems 64.3%, roots 35.7%), sapwood-heartwood separation (63.2% separate), bark removal (59% always) and transport conditions (56% ice packs vs. 39% ambient). From the surveyed researchers, 77.5% adapt sampling strategies to their extraction system, but these adaptations are rarely documented. This means that reported methods systematically underrepresent the process chain influencing isotopic outcomes. Given that varying circumstances and research objectives might preclude universal standardization, we introduce a Xylem Sampling Form:

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a structured metadata tool that makes sampling details explicit and comparable across studies without prescribing specific protocols, laying groundwork for future data-driven evaluation of how upstream sampling choices propagate into isotopic uncertainty.

1 | Introduction

Plant xylem water sampling (see Table 1 for various terms and definitions) for isotopic analysis of hydrogen and oxygen in water began nearly a century ago (Washburn and Smith 1934) and remains widely useful in studies of plant water sources (e.g., Dawson and Ehleringer 1991), hydrological niche segregation (e.g., Brum et al. 2019) within-tree water movement (e.g., Nehemy, Benettin, et al. 2022) and transit times of transpiration fluxes (e.g., Gaines et al. 2016) among other applications. Despite the widespread use of xylem sampling in ecological and hydrological research, clear documentation of sampling and processing steps is often omitted from studies. Sampling details related to stem and branch characteristics (e.g., branch position, thickness), sample processing, transport and storage are often incompletely reported. However, methodological details can be critical because methods can guide or even constrain interpretations of physical and biological processes. Such practices have evolved with little formal consensus on field sampling protocols and limited detail on how they are conducted (Penna et al. 2018; Ceperley et al. 2024). Better reporting is warranted because the choice of sampling approach can influence the isotopic composition of xylem water (Dawson and Ehleringer 1993; Fischer et al. 2019; Treydte et al. 2021) and data interpretation (Herbstritt et al. 2024). Lack of clear documentation also challenges newcomers, reduces accessibility and impedes cross-study comparisons.

To understand current xylem water sampling practices, we conducted an anonymous online survey and subsequent discussions with members of the scientific community. Our goals were to: (i) document current sampling practices across ecohydrology research groups; (ii) identify common and distinct practices in sampling, transport and storage and (iii) discuss methodological variations with respondents towards understanding why distinct approaches are used. While this paper provides details that can support the development of community-wide SOPs (standard operating procedures), the diversity of sampling practices must be considered in the context of studies with different goals, resources and applications.

2 | Methods

2.1 | Online Survey

We designed an online survey consisting of 27 multiple-choice and open-ended questions (Q5 and Q17; see Supplementary Information) to investigate xylem water sample collection, field handling, storage and transport. We developed survey questions through literature review of xylem water studies to identify commonly reported and omitted methodological details. We conducted the anonymous survey via the Qualtrics platform, targeting researchers actively collecting field samples for isotope analysis.

We used a multi-pronged approach to solicit participation, including targeted emails to researchers with expertise in isotope ecohydrology and public announcements at the American Geophysical Union (2022), Canadian Geophysical Union (2023) and European Geosciences Union conferences (2023). These communications specified that we sought responses from researchers who conducted the sampling rather than the principal investigator or senior scientist. After gathering a list of interested contributors, we distributed the survey to each respondent on 29 January 2023. As additional research groups became aware of the survey, we invited more potential respondents on 26 February, 2 March, 5 May and 9 June 2023. We never posted the survey link publicly to ensure interested respondents met the criteria before receiving the link. This approach also allowed us to track who participated without linking respondents to their responses, ensuring anonymity. We also asked respondents via email to express interest in further community discussions. In total, 107 individuals from six continents were invited to participate in the survey, including early career researchers (e.g., graduate students, postdoctoral researchers, assistant professors) and senior researchers.

2.2 | Virtual Workshop Discussion Following Survey Results

Following the survey, we conducted a virtual workshop with interested survey respondents in October 2024. We provided respondents with the survey results and a document highlighting key points requiring discussion. The workshop consisted of a brief results presentation, followed by open discussion and small-group breakout sessions on specific topics. Each group shared main points with the entire group at the end. We asked groups to submit written summaries, which informed this article's discussion section and supplementary sampling guide.

Survey responses were analysed as follows. For closed-ended questions, responses were tabulated and reported as percentages of the total number of respondents who answered each question, which varied due to the optional nature of some questions and survey design. Open-ended responses were coded thematically using Claude Sonnet (claude.ai, Anthropic), which identified recurring practices across all answers and assigned each response a binary indicator (0/1) for each identified theme, with multiple themes permitted per response. The coded results were independently verified by the authors.

3 | Results

Of the 107 invitations, there were 42 respondents who completed the survey. Respondents were from different research groups, universities and continents, with the majority from North America (49%), followed by Europe (32%), South America (12%), Asia (5%) and Oceania (2%). Among respondents, 49% of research groups had no SOP, while 51% had either general SOPs (37%) or scenario-specific SOPs (14%) (Q1). A question about the study organism was asked to further understand the respondents' research focus; most respondents (70%) investigate

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TABLE 1 | Relevant definitions used in this work.

Term	Definition	References
Cavitron (centrifugal force extraction)	Cavitron is a custom-made rotor fitted to a centrifuge that can be used to extract xylem water from branch samples. This system extracts xylem water by spinning intact stem segments at high rotational speeds, generating centrifugal forces that overcome the capillary forces retaining water within the xylem conduits.	Barbeta et al. (2020); Duvert et al. (2024)
Centrifugation	A centrifuge-based extraction method in which cut or fragmented plant tissue is packed into a container (e.g., a mesh-bottomed insert within a 50-mL conical tube) and spun in a standard laboratory centrifuge, forcing water out of the tissue and into a collection vessel; unlike the Cavitron, this method does not require intact stem segments or a specialized rotor.	Sánchez-Murillo et al. (2023); Almance et al. (2025)
Cryogenic vacuum distillation	A laboratory apparatus that isolates water from plant tissue by simultaneously applying heat to the sample and cold (typically liquid nitrogen) to a collection vessel under vacuum, driving water vapour from the tissue and trapping it as ice.	Koeniger et al. (2011); Orlowski et al. (2013); Millar et al. (2018)
Evaporative fractionation	Isotopic enrichment of residual water caused by the preferential loss of lighter isotopologues during evaporation; a key source of error introduced when samples are exposed to ambient air during collection, processing or storage.	Craig and Gordon (1965)
Heartwood	The central region of secondary xylem in which parenchyma cells have undergone cell death and cell lumina are occluded by the deposition of secondary metabolites (e.g., phenolic compounds, resins and tannins); it tends to be hydraulically inactive, but can contribute to sapwood water transport under drought conditions in certain species.	
Increment borer	A hollow drill used to extract cylindrical wood cores from tree stems for sampling; used to access sapwood and heartwood.	
Inner bark (phloem)	A collective term for the living tissues located between the vascular cambium and the outer bark (periderm), comprising the phloem and associated parenchyma; serves dual functions of photosynthate transport via sieve elements and radial water storage and movement via parenchyma cells.	Evert and Eichhorn (2006); Pfautsch, Hölttä, and Mencuccini (2015)
Isotopic fractionation	The preferential transport or transformation of stable isotopes of an element between substances or phases due to small mass-dependent differences in their physical and chemical properties.	Kendall and Caldwell (1998)
Péclet effect	The general Péclet effect describes the balance between advective water flow and back-diffusion of isotopically enriched water at evaporation sites within leaves; in the context of xylem sampling, it is most relevant as the mechanism by which this enriched leaf water can diffuse backward into stem tissue, potentially altering the isotopic composition of xylem water samples collected near the leaves.	Farquhar and Lloyd (1993); Farquhar and Cernusak (2005)
Pith	The parenchymatous ground tissue (i.e., 'spongy' tissue) occupying the central axis of young stems and branches, interior to the primary xylem; it can be metabolically inactive in mature tissue.	
Radial transport	The lateral movement of water between, e.g., xylem and inner bark and the movement of water within the sapwood, mediated by ray parenchyma cells running radially across the stem.	Pfautsch, Hölttä, and Mencuccini (2015); Pfautsch, Renard, et al. (2015); Treydte et al. (2021)

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TABLE 1 | (Continued)

Term	Definition	References
Sapwood	The outer, hydraulically active xylem tissue that transports water, physically distinct from inner heartwood.	Evert and Eichhorn (2006)
Standard operating procedure (SOP)	In the scope of this study, a documented, step-by-step protocol established by a research group to standardize plant xylem sampling, processing and storage practices across field campaigns and personnel.	
Periderm (suberized bark)	The protective outer tissue of stems that replaces the epidermis during secondary growth, composed of the phellogen (cork cambium), phellem (cork) and phelloderm; the phellem cell walls are impregnated with suberin, a waxy biopolymer that renders this layer largely impermeable to water and gases. Typically removed to isolate underlying xylem tissue.	Evert and Eichhorn (2006)
Vascular cambium	A thin meristematic layer between the xylem and phloem responsible for secondary growth; often removed together with bark to isolate xylem tissue.	Evert and Eichhorn (2006)
Water source partitioning	The use of isotopic mixing models to apportion plant water uptake among potential sources (e.g., groundwater, soil water at different depths).	Rothfuss and Javaux (2017); Fu et al. (2026)
Xylem	A complex vascular tissue comprising lignified tracheary elements (tracheid and vessel members), xylem fibres and parenchyma cells; water moves passively through the dead, hollow conduits of the tracheary elements along a water potential gradient from soil to atmosphere, with living parenchyma cells mediating radial water exchange and storage within the tissue.	Evert and Eichhorn (2006)
Xylem water	Water extracted from xylem tissue, typically assumed to represent source water absorbed by roots without isotopic modification during uptake or transport; widely used to trace plant water sources.	Dawson (1993); Bachofen et al. (2024)

xylem in woody plants (i.e., trees, shrubs or both), whereas a small group focuses on herbaceous or other plants (i.e., succulents) (Q2). Although some respondents distinguished between agricultural crops and herbaceous plants, we consolidated these categories in our analysis. However, we treated cacti and lianas as distinct categories because of their different metabolisms and resource uptake strategies.

3.1 | Sampled Plant Tissue

Respondents provided information about the portion of the plant that they usually sample and could select multiple options for this question (stem, branches, roots, leaves; Q3). Most answers included sampling branches (73.8%) or stems/cores (64.3%), whereas less than half sampled roots (35.7%) and a minority sampled leaves (21.4%). A few respondents selected 'other (please specify)' and answers to questions that were misinterpreted or misread were removed; for example, one selected 'other' and specified 'basal meristem for grasses' (which we defined as stems) or specified 'in situ'.

Respondents were also asked to provide a specific description of their sampling approach (Q5; Figure 1). The most frequently mentioned practice was bark and phloem removal prior to storage (Figure 1). Roughly one-third of respondents explicitly noted the importance of avoiding green or photosynthetic tissue and minimizing evaporation during collection. Approximately one-quarter mentioned chopping or cutting samples into smaller pieces, storing samples cool or cold and sealing vials with Parafilm. Less commonly mentioned practices included targeting sapwood specifically, sample size or volume considerations, specifying sampling height and collecting only suberized branches. A minority of respondents described maintaining a minimum distance between the sampling point and the nearest leaves, with some specifying a target of at least 15–20 cm. A small number also noted the use of glass wool in vials, oblique coring to maximize xylem sampling, slow coring to avoid frictional heating and tool cleaning between samples. Notably, one respondent reported intentionally retaining bark to minimize evaporative losses, the opposite of the dominant practice. These percentages in Q5 reflect only practices explicitly mentioned and the absence of a theme should not be interpreted.

In woody plants, not all xylem is active, so subsampling may be needed for isolating specific parts of the plant. When using cores sampled by an increment borer (Q6), 63.2% separate sapwood from heartwood (before storage of the sample). Of those that separated sap- and heart wood, they primarily used visual detection (Figure 2); others assumed species-specific sapwood depth

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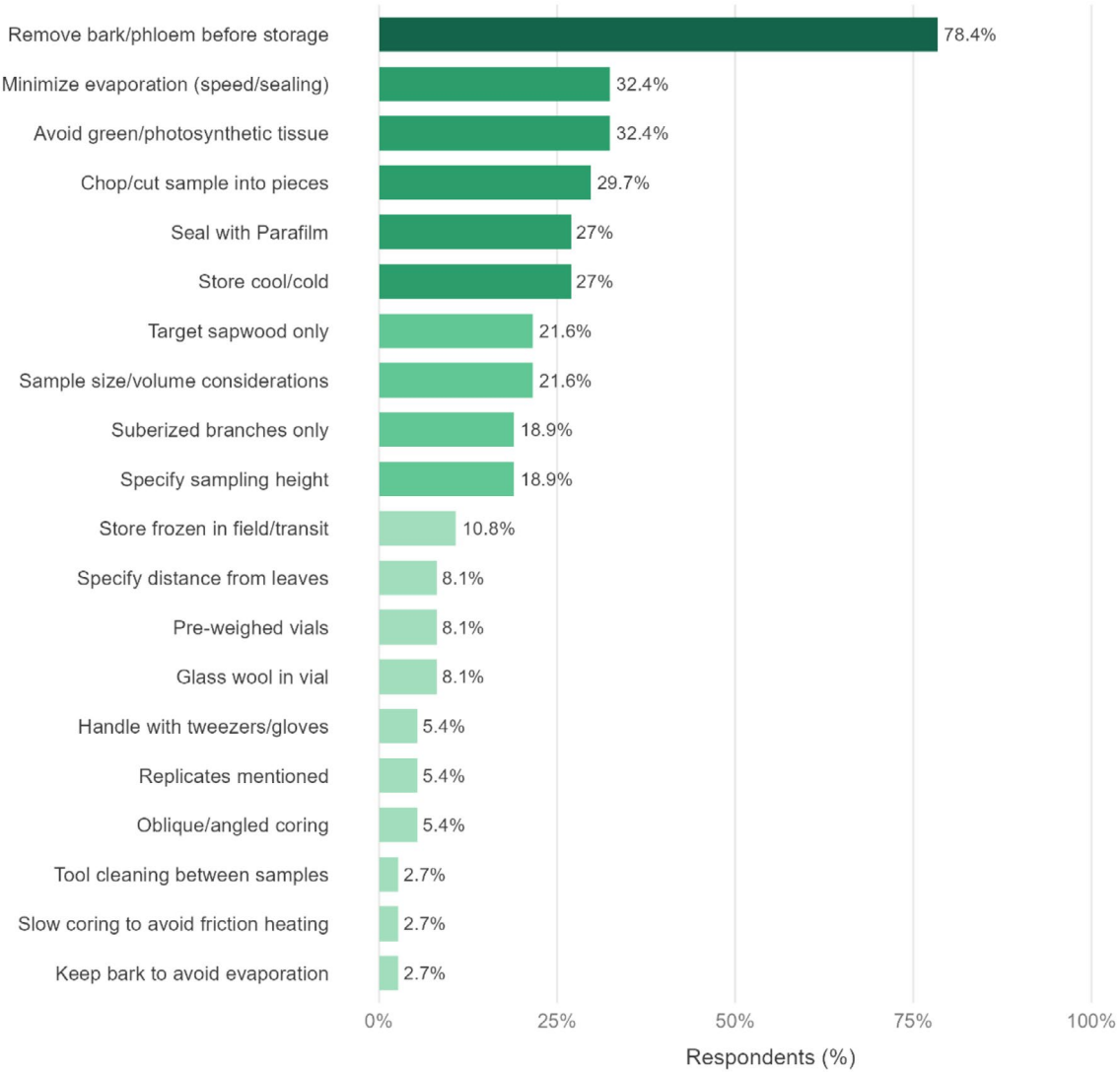


FIGURE 1 | Frequency of practices reported in free-text descriptions of xylem sampling approaches (Q5). Bars represent the percentage of respondents who mentioned each theme out of 37 respondents who answered this optional question. Multiple themes could be identified within a single response; therefore, percentages do not sum to 100%. The absence of a theme indicates that the practice was not explicitly mentioned and should not be interpreted as evidence that the opposite practice was followed. Colour intensity reflects frequency: dark teal (≥50%), medium teal (25%–49%), light-medium teal (15%–24%) and light teal (<15%).

(e.g., 2–3 cm) when visual detection was unclear or used thermal imaging (e.g., Gerchow et al. 2023). The remaining 36.8% did not separate sapwood from heartwood, such that extracted samples may contain water from both (Figure 2). For branch sampling (Q7), most (76.9%) did not separate active xylem from pith before storage; others would visually identify the pith to remove it, others would occasionally separate the pith and some would do this only for thick branches (Figure 2).

For branch thickness (Q8), 23.7% had no diameter target, while the majority aimed for specific diameters (Figure 2). For those that reported specific diameters, those varied substantially: some sampled branches > 0.5 cm (17.0%), others < 0.5 cm (17.0%), many determined diameters by vial size (28.0%) and some required clear suberized bark without specifying exact diameter (14.0%). For root diameter (Q9), most respondents who sampled roots had no specific diameter target (68.0%), while 32.0% did.

Bark tissue removal (including outer, inner and vascular cambium) from sampled material (Q10) varied (Figure 2): 59.5% always removed bark, 14.3% usually did, 14.3% sometimes did, 4.8% rarely did and 7.1% never did. Regarding timing of bark removal (Q11; multiple selections permitted), most (86.8%) removed bark in the field during sampling, while others removed it in the laboratory either frozen (10.5%) or at room temperature (5.3%). Some indicated 'other' (7.9%), noting uncertainty about typical removal amounts or removing bark when samples were near freezing (i.e., cooled at lower temperatures) but not frozen.

3.2 | Sampling Strategy and Sample Processing

To minimize evaporation, sampling should be done quickly. Therefore, we investigated how long collection actually takes in practice and what is considered a 'quick' acceptable amount of time across respondents (Q12). Time from collection (i.e.,

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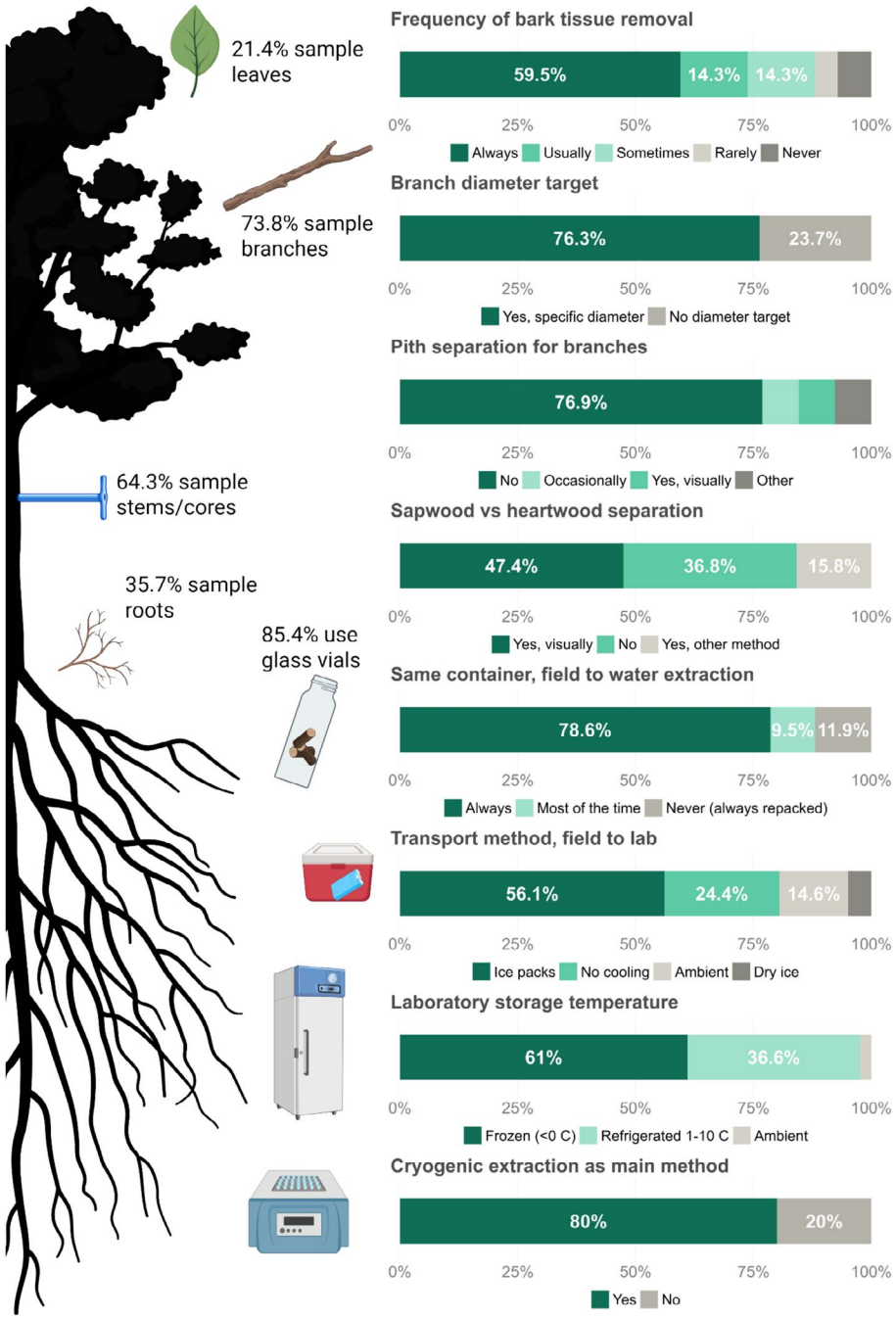


FIGURE 2 | Key xylem water sampling decisions reported by survey respondents ($N = 42$). The left panel illustrates the proportion of respondents sampling each plant tissue type. The right panel shows stacked bar charts summarizing responses to closed-ended questions on bark tissue removal frequency (Q10), branch diameter target (Q8), pith separation for branches (Q7), sapwood versus heartwood separation for cores (Q6), use of the same container from field to water extraction (Q15), transport method from field to laboratory (Q21), laboratory storage temperature (Q22) and use of cryogenic vacuum extraction as the primary extraction method (Q25). Percentage labels are omitted for response categories < 8% to avoid overcrowding the bars; the corresponding segment remains visible in each panel.

sampling cores, clipping branches, excavating roots) to sealed storage, including field processing (e.g., cutting samples into smaller pieces) if applicable, varied: < 1 min (37.8%), < 5 min (29.7%), 5–10 min (27.0%) and 11–15 min (5.4%). No respondent reported collection times exceeding 15 min.

To avoid cross-contamination among samples, sampling tools (i.e., increment borer, pruner) may be cleaned (Q14). We found that 38.1% of those respondents always clean the tools between sampling, 47.6% only when there are visible residues from previous sampling (e.g., water) and 11.9% do not clean. Others (2.4%) indicated that they clean tools between coring (i.e., increment borer) but not between clipping (i.e., collecting branches).

To increase sample volumes, fit samples into vials and/or improve water extraction efficiency, samples are sometimes cut into smaller pieces (e.g., < 1 cm) before storage (Q13). Approaches varied: 38.1% cut woody material into smaller pieces, 35.7% did

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not cut samples and 26.2% cut all sample types. Regarding laboratory re-processing (e.g., cutting into finer pieces, removing bark, subsampling) (Q18), most (67.5%) did not re-process samples. However, 10.0% re-processed at ambient temperature, 5.0% re-processed frozen samples only and 17.5% re-processed occasionally as needed. Among those that re-processed as needed, 57.0% re-processed frozen samples and 43.0% re-processed room-temperature samples. Reasons for re-processing (Q20) included reducing sample size before extraction, removing bark, reducing vial volume/weight for centrifugation or removing material with colour change or fungi/mould.

3.3 | Storage and Sample Transport

We asked whether respondents store samples in the field in the same container (vial or bag) used for extraction, such that the sample does not leave that container at any point (Q15; Figure 2). Most respondents always used the same container, 9.5% did so most of the time and 11.9% always repacked samples into different containers (Figure 2). For those who did not always use the same container (Q16), reasons were evenly distributed: changing extraction methods (33.3%), increasing field throughput (33.3%) (i.e., the vial opening is small) or extraction system incompatibility with the sampling container (33.3%).

Respondents were asked about the containers (e.g., vial, bag) used to sample xylem and provided open-ended answers to this question (Figure 3). Many respondents reported using 12 mL Exetainer vials (Labco, UK), some noting double-wadded caps. Other reported vials included 15–50 mL Falcon tubes (Corning Inc., USA). A smaller number of respondents reported plastic vials or other container types, including Vacutainer-style tubes. No other single glass vial type was commonly reported, though glass vials were used broadly; examples included 8 mL glass vials with cone-liner caps, scintillation vials with plug-style stoppers (DWK Life Sciences, Germany), Pyrex tubes (Corning Inc., USA) and 10 mL borosilicate vials with PTFE/silicone-lined caps. A small number of respondents reported using bags rather than vials for specific extraction methods: a bag used to enclose plant material for the bag-equilibration method (respondents' term: 'Weber bags') and foil-laminate bags (respondents' term: 'coffee bags') for the same method and resealable plastic bags (respondents' term: 'Ziploc bags') for Scholander pressure chamber extractions. Regarding sealing practices, most respondents reported sealing vials with laboratory sealing film (Parafilm),

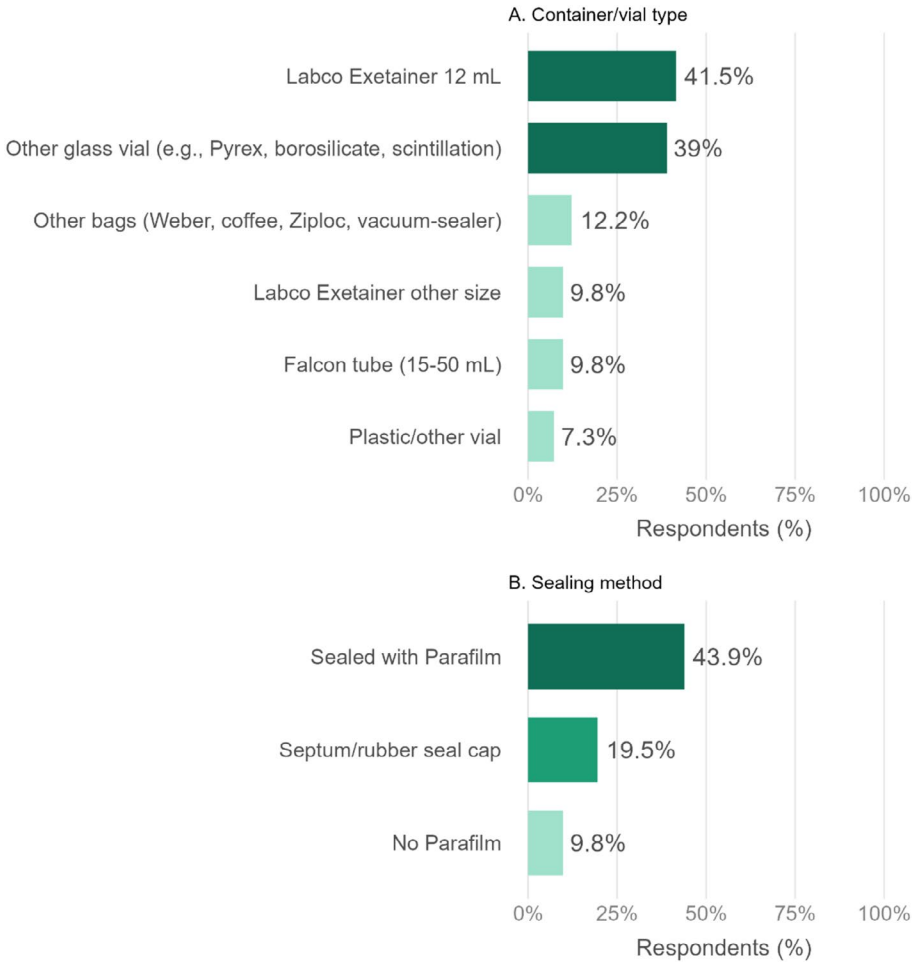


FIGURE 3 | Frequency of container/vial types (A) and sealing practices (B) reported in free-text descriptions of sample storage containers (Q17; $N = 41$ respondents). Bars represent the percentage of respondents who mentioned each theme; percentages do not sum to 100% as responses could be coded into multiple categories. Colour intensity reflects frequency: dark teal (≥ 30%), medium teal (15%–29%) and light teal (< 15%). 'Other glass vial' includes Pyrex, borosilicate and scintillation vials; 'Other bags' includes Weber bags, foil-laminate bags (respondents' term: 'coffee bags'), Ziploc/plastic bags and vacuum-sealer bags.

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a smaller group specifically reported using septum or rubber-seal caps and others explicitly reported not sealing samples with Parafilm.

Transport methods from field to laboratory varied (Q21; Figure 2): most used insulated containers with ice packs (56.1%), some used thermally insulated containers without cooling (24.4%), a small percentage used dry ice (4.9%) and the remaining transported at ambient temperature (14.6%). Laboratory storage temperature before extraction (Q22; Figure 2) also varied: most froze samples (61.0%), while others were refrigerated between 1°C and 10°C (36.6%) and a small percentage kept samples at ambient temperature (2.4%). When asked if procedures changed when freezing conditions were unavailable (Q23), most (57.9%) reported no change in sample handling, while 28.9% adopted an approach in which collected samples would be placed directly into vials such that they could remain unopened until they were directly connected to the extraction lines. Some indicated 'other', but misinterpreted the question.

3.4 | Processing After Sampling

When asked if they discard samples if there is evidence of potential issues, respondents indicated that they would do so when they identified loose vial caps, broken vials or leaky bags (92.5%), observed the presence of fungi or other biological activity in the samples (45%) or identified unwanted bark or cambium material (22.5%). In this question, respondents could select multiple options. Therefore, the percentage represents the number of respondents who selected that option in relation to the total number of respondents who answered this question. Respondents who selected 'other' specified cases, such as 'If samples are contaminated, we process them, carefully look at the data and decide then if the sample can be kept' or based on incomplete water extractions, for example 'if extraction is not complete (based on weight comparison)'. However, a group of respondents said that they usually do not discard samples (17.5%).

We found that most respondents use cryogenic vacuum distillation as the main method for xylem water extraction (80%) (yes/no) (Q25; Figure 2). Respondents were asked whether the choice of their plant water extraction system affects how they collect samples in the field. A small percentage (22.5%) indicated it would not change, whereas most indicated it would change (77.5%). Respondents who indicated 'yes' provided an additional explanation: changes are related to the required sample size, type of vial (i.e., container opening) and storage history.

The final question invited respondents to share additional field sampling and sample storage practices that were not explicitly addressed in the survey. Several important themes emerged and are discussed further below, including:

- Challenges associated with working in highly diverse ecosystems and targeting only specific species;
- Canopy position and sampling height, including the distance between branch sampling locations and leaves;
- Leaf sampling approaches (e.g., intact versus partial samples);
- The timing of sampling (e.g., midday versus other periods);
- Potential isotopic effects of frictional heating when using increment borers;
- The number of replicates collected per tree or sampling location;
- Logistical constraints related to field crew size and task allocation (e.g., pruning, processing and storage);
- Sample volume per vial, including whether headspace is left;
- Sample transport duration and conditions;
- The use of consistent or distinct extraction methods for soil and plant water (e.g., cryogenic vacuum distillation for both soil and xylem);
- The storage time after extraction and before isotope analysis.

Several respondents also noted the difficulty of locating practical sampling guidance in the literature, emphasizing that much of this information is confined to early foundational texts that are now difficult to access. Additional concerns were raised regarding extraction efficiency and water volume recovery; these important topics are beyond the scope of the present study as they were not captured by this survey, so we direct readers to these relevant prior studies related to plant water extraction and isotopic analysis (Araguás-Araguás et al. 1995; Schultz et al. 2011; Diao et al. 2022; Ceperley et al. 2024; Herbstritt et al. 2024; Wang et al. 2024; Steger et al. 2026).

4 | Discussion

4.1 | Historical and Conceptual Context for This Study: Xylem Water Sampling

A growing body of evidence suggests that differences in sampling, storage and extraction protocols can introduce systematic isotopic offsets (Spangenberg and Vennemann 2008; Fischer et al. 2019; Böttcher and Schmiedinger 2021; Millar et al. 2022). While substantial work has focused on quantifying extraction-induced isotopic offsets (e.g., Barbeta et al. 2020; Chen et al. 2020; Wang et al. 2024; Steger et al. 2026; Yang et al. 2026), the steps that precede extraction, including field sampling decisions, handling procedures and sample preparation, often receive limited attention. As a result, isotopic discrepancies in reported values are sometimes attributed solely to extraction methods, even though multiple upstream decisions may have contributed to the observed outcome. These methodological nuances, particularly those occurring during field sampling and early-stage processing, remain underreported.

Brunel et al. (1995) conducted one of the first field validations of stable-isotope methods for plant water sourcing, identifying five core assumptions underlying isotope-based interpretations: (1) root water uptake does not fractionate hydrogen or oxygen

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isotopes, (2) sampling, extraction and analysis introduce no significant errors, (3) sap water shows no significant intra-plant variability, (4) soil isotopic composition is laterally homogeneous and (5) sampling timing and isotope transport delays within trees are unimportant. They estimated combined methodological and environmental uncertainties at approximately ±5‰ for δ^2H and ±1‰ for $\delta^{18}O$.

Subsequent and prior studies have demonstrated that few of these five assumptions hold universally and that errors and natural variability can exceed the magnitude of uncertainty described by Brunel et al. (1995). Plants can exhibit isotopic fractionation during water uptake or within stem tissues under water stress (e.g., Dole 1936). They may also display significant internal transit-time lags depending on size, sampling location and dynamic root water uptake (e.g., De Deurwaerder et al. 2020; Knighton et al. 2020; Seeger and Weiler 2021; Nehemy, Maillet, et al. 2022; Shrestha and Sánchez-Murillo 2026) and soil water isotopic composition is not always laterally homogeneous (e.g., Goldsmith et al. 2019). Because many of these sources of variability are inherent to natural systems and cannot be controlled, they must be explicitly assessed through increased sampling, complementary measurements or targeted experimental design (Beyer and Penna 2021; Allen and Kirchner 2022). However, among Brunel et al.'s (1995) assertions, sampling and analytical errors (Assumption 2) are ones that likely can be mitigated.

Recent syntheses call for unified frameworks linking sampling, extraction and analysis (Ceperley et al. 2024; Lehmann et al. 2025) and have provided practical recommendations to better characterize variability and uncertainty (von Freyberg et al. 2020). Ceperley et al. (2024) recently provided guidelines for sampling plant (and soil) material, methods for water extraction and measurements of the water isotopic composition. Their review particularly highlighted the heterogeneous nature of plant (and soil) matrices, which needs to be carefully accounted for during experimental design, sample collection, water extraction and isotope analyses. Building on this work, our survey provides the first quantitative assessment of how xylem water sampling methods are implemented across laboratories worldwide. The survey showed substantial methodological diversity across all stages of sampling, from the presence of SOPs to handling and storage practices and demonstrated logistical and contextual constraints that continue to limit standardization (Figure 2). Our results also captured operational practices rarely addressed in previous studies, including sampling duration, tool cleaning, criteria for sample rejection and decisions around reprocessing, across a global pool of respondents.

4.2 | The Challenges in Building a Common Methodological Framework: Discrepancy in Sampling Approaches and Evidence in the Literature

Our survey provides empirical support for the idea of treating sampling and extraction as an integrated process chain (Ceperley et al. 2024): 77.5% of respondents reported adapting their field sampling strategy to the requirements of their extraction system—modifying vial size, opening diameter or required sample mass. This indicates that process-chain thinking is already widespread in practice, even if rarely documented in papers. The question is whether this alignment to accommodate the extraction approach (e.g., vial choice, sample size) optimally represents the natural variability of xylem isotopic data.

Survey findings reveal substantial methodological diversity, demonstrated by nearly even splits between those that have and do not have internal SOPs. Or as another example, there was a near even split between those that separate sapwood and those that do not. There are papers evidencing that these tissues can differ isotopically (White et al. 1985; Fabiani et al. 2022); however, contrasting evidence also exists (Thorburn et al. 1993). Similar ambiguities seem to surround the question of the timing of the sampling, which was not a topic included in the survey, but did arise during discussions. Optimal sampling time remains debated, with conflicting evidence regarding whether time of day affects xylem isotopic composition and midday sampling may only reflect same-day uptake dynamics in short trees, at the base of the tree or in trees with rapid transit times. For example, some studies have found that time of day might have an impact on inferences of plant water sources, for example, morning versus afternoon sampling (Dawson and Ehleringer 1993; Cernusak et al. 2005; Fabiani et al. 2022; Tierney et al. 2025), whereas findings by others might indicate otherwise (Martín-Gómez et al. 2015; De Deurwaerder et al. 2020; Magh et al. 2020; Landgraf et al. 2022; Seeger and Weiler 2021; Nehemy, Benettin, et al. 2022; Bernhard et al. 2024; Ring et al. 2024). Note that not all of these previous studies strictly compare midday and predawn values.

Sometimes methodological choices have known isotopic implications. While respondents differed substantially in the branch size that they target, it is known that smaller branches (which are closer to leaves) can have poorly suberized bark or potentially be influenced by the Péclet effect, whereby enriched leaf water diffuses back into branches and stems, potentially introducing an evaporative fractionation signal into the xylem (Farquhar and Lloyd 1993; Lehmann et al. 2018; Wang et al. 2021). Dawson and Ehleringer (1993) showed that isotopic fractionation is more common in small twigs than in larger branches, recommending sampling larger branches and at predawn to minimize fractionation. However, even with clear cases of potential effects, there are challenges to creating clear protocols when some plants have leaves along branches, including larger ones (e.g., spruces).

Bark removal was another polarizing topic (59% always remove bark, whereas 41% did not always do so). Separating inner bark from xylem may be desirable because these water pools can differ isotopically (Treydte et al. 2021; Nehemy, Benettin, et al. 2022) and sampling inner bark separately might help quantify potential fractionation and isotopic differences between xylem and phloem, especially during water stress periods (Nehemy, Benettin, et al. 2022). While removing bark is isotopically justified, the trade-off with sampling speed must be considered. Depending on species and field conditions, bark removal can substantially increase the time between processing the sample and sealing, potentially introducing evaporative fractionation that offsets the benefit of bark exclusion. Ultimately, the optimal approach likely depends on the research question and empirical testing across species and environments is needed to determine when the isotopic benefit of bark removal outweighs the risk of evaporative fractionation during processing.

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Storage and transport practices were similarly diverse: 56% use ice packs, 5% use dry ice and 39% store samples at ambient temperature in the field. During discussions, some noted that field cooling is unnecessary when sealed containers (e.g., glass vials) are used directly for extraction systems without re-exposure to ambient air, as with the Koeniger cryogenic system (Koeniger et al. 2011). While these vials might theoretically provide adequate seals for ambient storage, we also discussed that cooling is likely to reduce risk of evaporative fractionation if there are minor leaks from samples and thus cooling samples is likely worthwhile if it is feasible.

Extraction method shapes sampling strategy. Our results provide empirical evidence that cryogenic extraction remains the dominant method across laboratories (80% of respondents), shaping upstream sampling decisions accordingly. The remaining 20% of respondents used other methods, though our survey did not ask them to specify which one. Nonetheless, newer extraction approaches such as the cavitron (Barbeta et al. 2020) or centrifugation (Sánchez-Murillo et al. 2023) or the Scholander pressure chamber (Zuecco et al. 2022) are used and require fundamentally different field strategies than cryogenic systems (Koeniger et al. 2011; Orlowski et al. 2013). Cavitron sampling often demands straight and long branches for rotor size fit and sufficient sample volume, which can substantially reduce usable samples (Duvert et al. 2024). For example, Duvert et al. (2024) noted that of 40 branches collected in the field, only 18 were used for analysis. Straight, but shorter branches compared to cavitron are needed for the Scholander pressure chamber; however, the extraction of a sufficient sample volume is often challenging during dry conditions (Zuecco et al. 2022). Centrifuge-based approaches also differ in container requirements, using 50-mL polypropylene conical centrifuge tubes, rather than the vials used in cryogenic extraction (Sánchez-Murillo et al. 2023; Almance et al. 2025). These examples illustrate that the entire chain of steps leading up to extraction can influence isotopic outcomes, not only the extraction method itself.

Collectively, our results show that xylem sampling practices vary widely among research groups, indicating that a 'one-size-fits-all' SOP may not capture ecological diversity or differing research objectives, equipment availability and resource-dependent decisions. Instead, site-specific, question-driven approaches are often more appropriate. Respondents emphasized the need for small, targeted experiments to evaluate specific sampling and handling choices and for transparent reporting to support cumulative learning. It is worth noting, however, that while our survey captured responses from five continents, representation was uneven, with North America (49%) and Europe (32%) dominating. Full anonymization of the responses prevented linking individual answers to specific locations, precluding regional comparisons of sampling practices. It remains an open question whether the methodological diversity reported here reflects global variations or is partly an artefact of geographic sampling bias and regional analysis of practices might represent a valuable direction for future work.

4.3 | Improving Cross-Study Comparability in Xylem Water Isotope Research Through Standardized Methodological Reporting

During discussions of the survey results, respondents raised several practical avenues to guide the field toward stronger cross-comparisons of xylem isotope data, greater universality of results and a more actionable approach to documenting the heterogeneity of sampling and handling methods. One approach proposed was to develop a decision tree on xylem sampling to help researchers evaluate which factors most strongly influence sampling outcomes. Another was the creation of a tiered quality level for sampling protocols, similar to practices in hydrological modelling (e.g., classification of model structural complexity or calibration rigour, with implications for how outputs should be used and interpreted) (Moriasi et al. 2007; Knoben et al. 2019). No consensus emerged because of concerns on how these effects can be quantitatively or qualitatively evaluated, we do not yet know the impact of these decisions on uncertainty sizes, but we agreed that any such framework must remain flexible. The best practices assumed today may not hold as new insights accumulate. Evaluation criteria, decision trees and quality metrics should therefore be designed to evolve alongside the science, rather than impose fixed standards on a rapidly developing area of research.

Given the methodological diversity revealed by our survey and the heterogeneity of natural systems, we propose a pragmatic alternative: a standardized *Xylem Sampling Form* that researchers can include in publications or alongside datasets. This form summarizes key aspects of sample collection, handling and extraction that are often omitted from papers, without prescribing specific protocols. As the field increasingly recognizes the need for transparent and openly accessible data (Stagge et al. 2019; Hall et al. 2022), such a tool addresses the technical and logistical challenges that currently limit reproducibility and cross-study comparisons. The form enables investigation of methodological effects while acknowledging that sampling approaches must vary across species, environments and research contexts. We envision this form as a first step towards a more structured framework, one that can evolve as the field matures, ultimately supporting the development of tiered quality standards grounded in empirical evidence rather than imposed a priori.

The form serves dual purposes: as a pre-field planning aid for guiding methodological decisions and as a post-field assessment tool for evaluating how sampling choices may have influenced results. In this sense, it acts as a decision tree while leaving researchers free to challenge or accept existing assumptions. We emphasize, however, that the *Xylem Sampling Form* should not be interpreted as a list of best practices, but rather as a framework for critical reflection that enhances reproducibility, standardizes reporting of sampling practices, enables cross-study evaluation and prompts future data-driven discussions. Researchers should continue to challenge established assumptions and test them empirically, recognizing that no single approach applies universally across species, environments or sampling conditions. To support this reflective practice, we propose five guiding questions (Box 1) that researchers can ask before, during and after sampling to help explicitly link sampling decisions, potential sources of uncertainty and the precision and accuracy needed for their research question.

Testing all methodological assumptions is not always feasible; in such cases, we believe transparent reporting and adequate sample sizes represent the strongest pathway forward.

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BOX 1 | Five questions to ask before, during and after xylem sampling.

1. What collection method is best suited to your target tissue, field conditions and available laboratory resources?
2. What can you do to minimize the amount of time your samples are exposed to fractionating conditions?
3. If ideal sampling conditions cannot be met, what potential sources of error (affecting accuracy) or uncertainty (affecting precision) are introduced by necessary adaptations in the field and laboratory?
4. Are the precision requirements of your research question met by your chosen sampling approach? … And, how do you know?
5. Have you recorded sufficient information about your sampling and handling procedures to allow others to reproduce your results or compare them with their own?

The *Xylem Sampling Form* is available in an open repository (Nehemy et al. 2026) and will be updated as community knowledge evolves. We envision voluntary submission alongside datasets or as Supplementary Material in publications and anticipate that transparent reporting of sampling details will increasingly be expected by reviewers and editors. We designed the form to document methodological choices and constraints rather than to prescribe specific protocols, recognizing that research groups operate under vastly different logistical and financial conditions. Transparent reporting is a universally valid expectation, regardless of the scenarios that might preclude following rigid standard operating procedures (e.g., as may occur when working with limited resources and/or remote ecosystems). We invite the community, journals and funding agencies to embrace transparent methodological reporting as a baseline expectation and we will continue to update the form as community knowledge and best practices evolve.

5 | Conclusion

This work provides the first global overview of xylem water sampling practices for isotopic analysis in plant studies. Our survey and workshop discussions revealed methodological diversity and highlight that many common practices remain under-documented and under-tested, showcasing diverse opportunities for future studies. That said, the variability in methods likely reflects variability in settings, logistical constraints and goals. Thus, we propose a *Xylem Sampling Form* as a standardized transparency tool to document methodological choices without prescribing a single, universal sampling standard operating procedure that may not be appropriate across species, ecosystems and research objectives. By structuring metadata across field sampling, handling and extraction, this reporting form will make process-chain adaptations explicit, enable cross-study evaluations and create a foundation for future data-driven guidance. Moving forward, we encourage the community to pair transparent reporting with targeted experiments to strengthen the interpretability, reproducibility and comparability of plant water isotope studies.

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Data Availability Statement

The Xylem Sampling Form is openly available in Zenodo (Nehemy et al. 2026) at https://zenodo.org/, reference number 10.5281/zenodo.20279054.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** hyp70748-sup-0001-Supinfo.pdf.

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