Technical note: Water vapor sampling for stable isotope analysis: systematic testing of sampling conditions and development of a robust protocol

Alberto Iraheta[1], Elise Malsch-Fröhlich[1], Malkin Gerchow[1] and Matthias Beyer[1]

[1]Institute of Geoecology, Technical University of Braunschweig, Braunschweig, 38106, Germany

Correspondence to: Alberto Iraheta (a.iraheta@tu-braunschweig.de)

Abstract. Ecohydrological water isotope studies often rely on destructive sampling of soils and plants. Recently, techniques for collecting equilibrated water vapor were developed as alternative. Here, we present a systematic evaluation of water vapor sampling methods with the goal of identifying how key parameters influence $\delta^{18}O$ and $\delta^{2}H$ values. In controlled laboratory experiments we first tested the isotopic stability of three container types: 250 mL infusion glass bottles, 1 L FlexFoil sample bags, and 500 mL Aluminum-zip bags. The most suitable container was subsequently tested for different storage times (6 hours to 7 days), storage temperatures (4 to 40 °C) and sampling flow rates (35 to 125 mL min^{-1}). The 250 mL infusion glass bottles showed the best performance, with deviations to reference standards of ±0.5 ‰ for $\delta^{18}O$ and ±1 ‰ for $\delta^{2}H$ followed by FlexFoil sample bags (deviations of ±1 ‰ for $\delta^{18}O$ and −3 to +2 ‰ for $\delta^{2}H$). Aluminum bags showed the largest deviations (−2.5 to −3 ‰ for $\delta^{18}O$ and −12 to −25 ‰ for $\delta^{2}H$). Further extensive testing of the most suitable container type - the infusion glass bottles – revealed that the Mean Absolute Error (MAE) for $\delta^{18}O$ remained stable under all tested conditions (<0.6 ‰). For $\delta^{2}H$, best results were obtained using flow rates of 100–125 mL min^{-1}, and storage times of ≤1 day under ambient conditions (20–25 °C) (MAE = ±0.5 for $\delta^{18}O$ and ±1.5 ‰ for $\delta^{2}H$); hence, our recommendation is using these settings. Although vapor sampling cannot match the analytical precision of conventional methods, it offers a practical, inexpensive framework. The protocol enables reliable water vapor isotope measurements and is especially advantageous in remote areas or locations with limited infrastructure. Therefore, it has great potential for investigating ecohydrological processes in a customizable spatiotemporal resolution without the need of repeated destructive sampling or full *in situ* setups.

1 Introduction

The water stable isotopes oxygen-18 ($\delta^{18}O$) and deuterium ($\delta^{2}H$) are fundamental to advances in modern ecohydrology (Sprenger et al., 2016), as they allow the tracing of water through precipitation, soil, plants, surface water and groundwater in different environments (Zhang et al., 2022, Tetzlaff et al., 2023). Therefore, they were heavily employed as tools to understand hydrological processes in forested and agricultural systems (Haberstroh et al., 2024), studies of exchanges between water, plants, and the atmosphere (Dubbert and Werner, 2019; Allen and Kirchner, 2022; Liebhard, 2022), tracing of water sources and their redistribution in ecosystems (Beyer et al., 2020; Rothfuss et al., 2021, Penna et al., 2018).

The need to generate continuous data sets has been identified repeatedly, as high-resolution time series can capture the natural dynamics of ecohydrological and physiological processes (Simonin et al., 2013; Gaj et al., 2016; Seeger and Weiler, 2023). Established methods provide limited temporal information and often rely on destructive sampling of soil and plant xylem, where water needs to be extracted with methods such as e.g., cryogenic vacuum extraction or centrifugation (Orlowski et al., 2018; Kübert et al., 2020). Extraction techniques for water isotope analysis have recently been increasingly questioned due to its potential impact on the isotope composition of soil and plant water, particularly for deuterium (Chen et al., 2020; Allen and

Kirchner, 2022; Wen et al., 2022; Ceperley et al., 2024). Despite these limitations, extraction techniques continue to be widely used in studies to monitor hydrological processes in ecosystems (Barbeta et al., 2022; Koeniger et al., 2011).

To obtain continuous data, the scientific community developed *in situ* approaches using laser spectroscopy to enable direct measurements of water stable isotopes in soils and plants under field conditions (Kühnhammer et al., 2022; Mennekes et al., 2021). Although highly valuable for ecohydrological research, this approach involves logistical, technical, and financial challenges constraining its widespread deployment (Rothfuss and Javaux, 2017; Volkmann et al., 2016). In this context, field-equilibrated water vapor sampling emerges as a valuable alternative for obtaining temporally higher-resolution water isotope data without the need for destructive sampling (Magh et al., 2022; Gralher et al., 2021; Kübert et al., 2020; Galewsky et al., 2016). Water vapor sampling refers to the collection of water vapor that is in isotopic equilibrium with the media to be analyzed (i.e., plant xylem, soil, or liquid water) and subsequent analysis in the laboratory. In simple terms, instead of using a true *in situ* water isotope setup, the water vapor is stored in suitable sampling vessels in place of being sent to the laser spectrometer directly. Water vapor sampling is more flexible (not limited to a small number of trees compared to true *in situ*), cheaper (no isotope analyzer required in the field), and better suited to remote sites (less equipment required); it allows repeated sampling of soils and trees at the desired frequency without destructive sampling, avoids liquid water extraction in the laboratory, and does not require an isotope analyzer in the field (Havranek et al., 2020). Recent studies have explored different methods for water vapor sampling and developed strategies that allow the collection and storage of water vapor while conserving its isotopic signature (Diekmann et al., 2025). Magh et al. (2022) developed the Vapor Storage Vial System (VSVS) The system was evaluated by storing samples between 0 to 14 days with variations of 0.6 to 4.4 ‰ for δ^2H and 0.6 to 0.8 ‰ for $\delta^{18}O$ after storage. Havranek et al. (2023) implemented and evaluated an automated method, the Soil Water Isotope Storage System (SWISS), which combines several components such as permeable probes, glass flasks, stainless steel tubes and switching valves. This system allows automated sampling and storage of equilibrated vapor for later laboratory analysis. The authors reported a precision of ±0.9 ‰ for $\delta^{18}O$ and ±3.7 ‰ for δ^2H after up to 14 days of storage. Herbstritt et al., (2023) developed a sampling technique based on inflatable bags with diffusion-tight seals and found variations of 0.4 ‰ for $\delta^{18}O$ and 1.9 ‰ for δ^2H. Dahlmann et al., (2025) established system for water vapor extraction using permeable membranes and special gas bags (Multi-Layer Foil Bags with stainless steel fitting, Sense Trading B.V., Netherlands) for storage. They also tested the possibility of reusing containers and reported relatively small variations after 24 hours of storage, with values between 0.7 and ±2.3 ‰ for δ^2H and 0.2 to 0.9 ‰ for $\delta^{18}O$.

Although these studies represent important methodological advances, none of them provides a technical, practical, and comprehensive sampling protocol identifying ideal sampling materials and procedures as well as storage and analysis conditions. For instance, Dahlmann et al. (2025) and Herbstritt et al. (2023) use bags for collecting water vapor; Magh (2022) and Havranek (2023) utilize glass vials. The differences in the employment, reusability and performance under equal conditions have never been studied; neither the practical implications have.

In this study, we carry out a systematic test of sampling vessels for water vapor storage and analysis as well as an investigation of crucial parameters of the entire sampling-to-analysis workflow (e.g., sampling flow rate to ensure isotopic equilibrium, storage temperatures, maximum storage times).

The objectives of this research are therefore:

i.) To carry out a systematic laboratory experiments to evaluate the effects of storage time, temperature, and container type on the stability water vapor isotope values.

ii.) To develop and test a practical, minimally invasive, and efficient method for collecting water vapor samples for application in various environments.

iii.) To identify optimal conditions for water vapor sampling, storage, and analysis.

The novelty of this contribution lies in the integrated evaluation of factors that affect its isotopic composition. The systematic analysis provided herein will enable a wider, more unified, and tailor-made application of water vapor sampling methods to the specific research environment and objectives.

2 Materials and methods

This section describes the experimental procedures and the tests carried out. The detailed list of materials, instruments and equipment used in the experiments can be found in Text S1.

2.1 Experimental design

To determine the optimal sampling method for analyzing isotopes in water vapor, several experiments were conducted in the laboratory, as shown in Fig. 1. The study consisted of two consecutive phases. In phase 1, we tested different storage containers in two experiments (referred to as experiments 1 and 2). We evaluated three sample storage containers: 250 mL infusion glass bottles, 500 mL Aluminum-zip bags, and 1 L FlexFoil sample bags. In experiment 1, we filled these containers with dry air and stored them at three temperatures (4, 20–25, and 40 °C) for three storage times before measurement (6 hours, 1 day, and 3 days) to evaluate hermeticity (the leak-tightness of the containers). In experiment 2, we used isotopic reference standards and filled the containers with equilibrated water vapor, then repeated the procedure from experiment 1 to assess isotopic stability. For the final selection of the most suitable sampling container for water vapor analysis, we defined six decision criteria (Fig. 1).

Using the most suitable container, we conducted two additional experiments (experiments 3 and 4) in Phase 2. In these, we expanded the parameters to include 7-day storage, tested two syringe sizes, and evaluated four different flow rates (35, 75, 100 and 125 mL min^{-1}) using three isotopic standards. Experiment 3 examined whether sealing integrity was affected by puncture diameter or septum properties, while experiment 4 determined the optimal flow rate for maximizing isotopic precision and ensuring of isotopic equilibrium. The technical details are provided in the following sections.

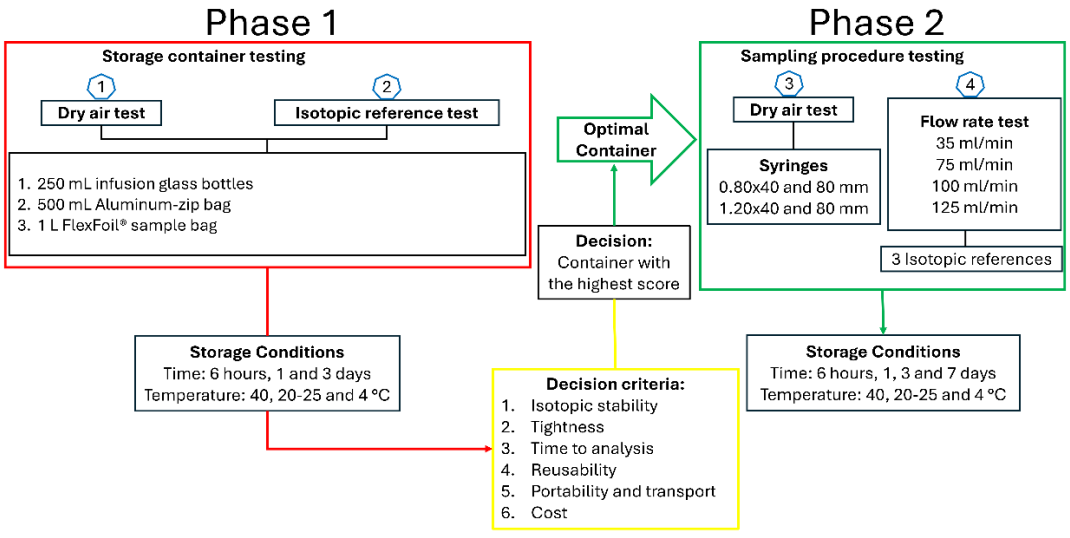


Figure 1. Illustration of the experimental design for water vapor testing sampling and storage and. Phase 1 (red box) was dedicated to the testing of storage containers, and Phase 2 (green box), focused on optimizing the sampling procedure. Blue hexagons indicate the four experiments carried out: [1] dry air airtightness test; [2] isotopic stability test for container selection; [3] dry air leakage

test for syringe-septum interaction; and [4] flow rate performance test using three isotopic references. The decision on the ideal container was made by a multi-criteria decision analysis (yellow box).

The methodological framework outlined in Fig. 1 was implemented using setups specifically designed for water vapor sampling and analysis with a CRDS isotope analyzer. The technical implementation of the experiments is detailed in Fig. 2.

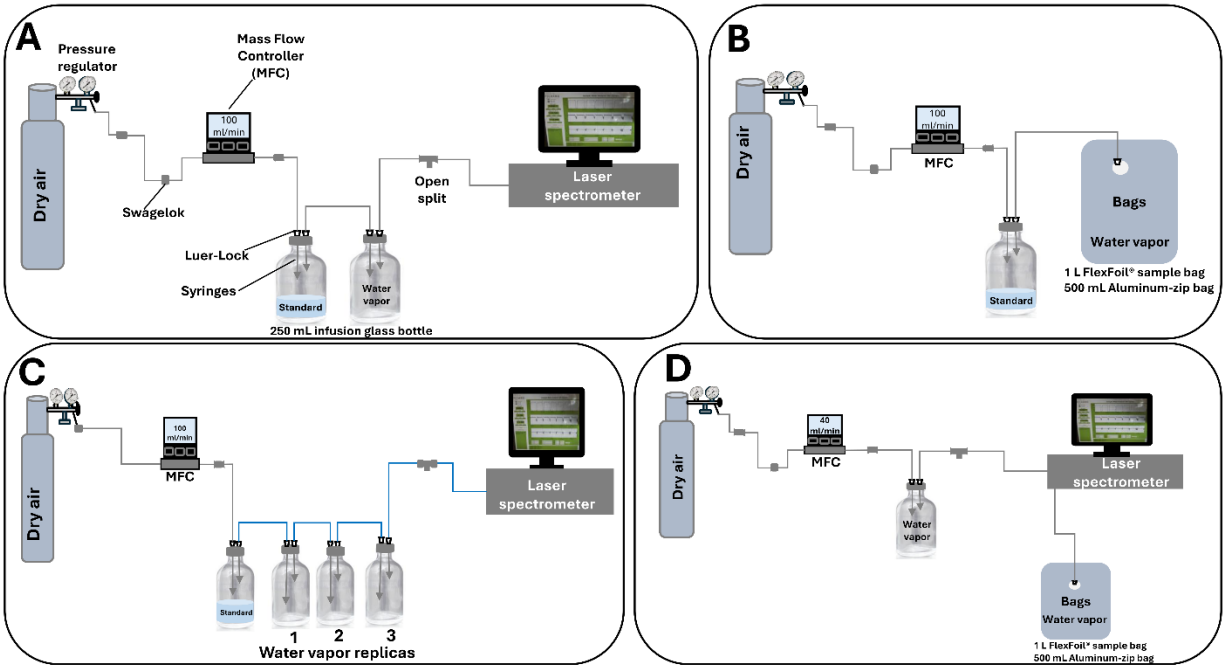


Figure 2. Technical implementation of the experiments. The schemes show the components used throughout the experiments: (A) setup for continuous generation of reference water vapor from a liquid isotopic standard and direct analysis via laser spectroscopy (Picarro L2130-i); (B) configuration for filling 1 L FlexFoil sample bags and 500 mL Aluminum-zip storage bags with reference vapor; (C) system for simultaneous filling of three 250 mL infusion glass bottle replicates to ensure consistent water vapor isotopic composition for flow rate experiments; and (D) measurement configurations for stored samples, including a dry air push system with a T-junction open split for 250 mL infusion glass bottles and bags (left), and a direct inlet connection for gas bag analysis (right). In all configurations, gas flow rates were precisely controlled using a pressure regulator and an electronic mass flow controller (MFC).

2.2 Storage container testing

The first phase of the experiments focused on evaluating different storage containers to assess their tightness and the isotopic stability of water vapor (Fig. 1). To test this the storage containers were filled with dry air (experiment 1) and with a reference with known isotope values (experiment 2). The dry air and water vapor samples were stored under three temperature conditions. Two of these were controlled: a fridge at 4°C and an oven at 40°C. The third condition were ambient conditions in our laboratory where the temperature fluctuated between 20 and 25 °C and was uncontrolled. Three storage times were tested for each temperature condition: 6 hours, 1 day, and 3 days.

2.2.1 Experiment 1 - Tightness of the containers

Before the experiment several preparatory measures were taken. The 250 mL infusion glass bottles were dried overnight in an oven at 60 °C to remove residues, then sealed with a butyl stopper and an aluminum cap secured with a 32 mm aluminum cap closure clip. The glass bottles were flushed with dry air for 5 minutes before sampling. After measuring the samples, the bottles were cleaned and dried again for 24 hours at 60-80 °C for reuse. For the 500 mL Aluminum-zip bags with zippers (these bags were not reused because reusing sampling bags is impractical; it requires thorough and repeated preparation to remove isotopic

memory effects and maintain accurate measurements, a process that is too labor-intensive for routine field or laboratory use (Herbstritt et al., 2023)), silicone was applied as a septum for the syringes and dried at room temperature for three days. The bags were then heated overnight at 50 °C to remove potential organic residues. The bags were tightly sealed by folding the top opening and securing it with folding clips, without using heat sealing, to prevent the emission of any compounds potentially affecting the isotope values. The 1 L FlexFoil sample bags were purged three times with dry air through the valves prior to sampling to minimize memory effects and allow reuse.

After the preparatory measures, the containers were filled with dry air from a cylinder regulated by a pressure regulator connected to a mass flow controller. The air was passed through PTFE tubing (outer diameter: 1/8") connected to a syringe using a Luer adapter. Fine syringes (0.80 x 40 mm) were used for sampling to minimize the size of the perforations in the septa. Consequently, five 250 mL infusion glass bottles were purged with the sample for 12 minutes at a flow rate of 100 mL min^{-1}, with equilibrium between the inlet and outlet of dry air established by an additional connection to the CRDS isotope analyzer. An open split was used to avoid overpressure in the analyzer. Five 500 mL Aluminum-zip bags were filled in six minutes under the same flow conditions by inserting the syringe through the silicone septum into the bag. For the 1 L FlexFoil sample bags, a 1/4" PTFE tube was attached. This tube was connected via a Swagelok connector to a second 1/8" tube, which carried the dry air until the bag was filled.

2.2.2 Experiment 2 -Isotopic reference test

The sampling configuration and container types were identical to those described above. However, this experiment differed by storing saturated water vapor of a known isotopic composition within the containers. Each container type (250 mL infusion glass bottle, 1 L FlexFoil sample bag and 500 mL Aluminum-zip bag) was prepared in five replicates and stored for 6 hours, 1 day, and 3 days at different temperatures: Fridge, Room, and Oven (4, 20-25, and 40 °C).

An initial measurement was performed by directly measuring the isotopic water vapor with the CRDS analyzer; these values were used as comparison values for the evaluation. The vapor flow rate was chosen at 100 mL min^{-1} for all containers. Based on preliminary testing, a 12 minutes sampling time was selected for glass bottles to achieve optimal isotopic stability and minimize memory effects. Conversely, a shorter sampling time 6 minutes was sufficient for the bags to prevent overpressurization and subsequent leakage. The vapor was generated in a closed system (headspace) where dry air flowed through a glass bottle containing the known isotopic reference. This system included connections with syringes and PTFE tubing regulated by an electronic mass flow controller, and an open T-shaped split before entering the analyzer to prevent overpressure (Fig. 2 A and B)

2.2.3 Decision criteria for the selection of the optimal container

Experiments 1 and 2 were essential for selecting the optimal container. Six evaluation criteria were defined (Fig. 1) to ensure objective selection process. These were divided into critical (criteria 1-3) and non-critical (criteria 4-6) categories. Critical decision criteria were: (1) isotopic stability; the capability of the sampling container to maintain the isotopic signature without significant changes; (2) tightness; the prevention of exchange with atmospheric air; and (3) storage time; the storage time of a particular water vapor sample. Non-critical criteria were: (4) reusability of the storage container; a measure to sustainability and long-term cost reduction; (5) portability and transport resistance; a handling criteria including weight, volume, and risk of damage or loss of tightness during transport; and (6) cost, based on supplier prices in the European market. Each criterion was rated on a scale from 1 to 5 (1 = poor, 5 = excellent) and assigned a relative importance which we defined as follows: isotope stability (70%), tightness (10%), storage time (5%), reusability (5%), portability and transport (5%), and cost (5%). The sum

of the weighted scores allowed for an objective comparison among the evaluated containers. The container with the highest total score from the combined decision criteria was considered the optimal choice and used in the detailed testing in phase 2.

2.3 Sampling procedure testing

In the second phase of the experiment, we only used the best container from phase 1. The main objective was to optimize the water vapor sampling process and define optimal conditions and procedures by evaluating key factors such as container, temperature, storage time, and flow rate to ensure high-quality water isotope data. Additionally, this phase tested a longer storage period, with samples kept for up to 7 days after sampling. The use of different storage intervals (6 hours, 1 day, 3 and 7 days) allowed a more accurate assessment of whether the isotopic variation remained similar to that observed after 3 days or increased with longer storage times. Three replicates were prepared for each interval, all were collected simultaneously but measured only at their respective storage times.

Experiment 3 was conducted with dry air and two syringe sizes, with samples stored at 40 °C, 20–25 °C, and 4 °C (Oven, Room, and Fridge temperatures, respectively) and four storage times: 6 hours, 1 day, 3 and 7 days. In experiment 4, four sampling flow rates (35, 75, 100 and 125 mL min^{-1}) were tested with three isotope references (Table 1) for water vapor sampling. The minimum flow rate was 35 mL min^{-1}, which corresponds roughly to the operating flow rate of our CDRS isotope analyzer.

2.3.1 Experiment 3 - Influence of syringe size on the tightness of the optimal container

As both sampling of water vapor and measurement on the laser spectroscope require puncturing of the septum with a syringe, we were interested in the effect of the syringe sizes responsible for the puncture - in particular the syringe diameter. Though theoretically sealing, use of a thick syringe creates a substantial hole in the septum which might cause intrusion of atmospheric air and effect isotopic stability. On the other hand, a syringe to thin might not allow for the desired flow rates. Despite being a very detailed aspect, we considered it worth testing. Two syringe sizes (0.80 x 40 mm, 80 mm, and 1.20 x 40 mm, 80 mm) were used to assess whether the puncture in the septum affected container tightness isotope values. Tests were conducted using 250 mL infusion glass bottles filled with dry air. After sampling with both syringes, the bottles containing air samples were stored under different temperatures and storage times. This evaluation identified whether syringe puncture had a negative effect on tightness or whether tightness depended mainly on the closure system (see Fig. 1). It also determined the most suitable syringe size for water vapor sampling.

2.3.2 Experiment 4 – Testing the flow rate of the sampling in the optimal container

The precision of the water vapor sampling method was evaluated at different sampling flow rates using only the containers previously identified as the most suitable. Three reference waters with known isotopic compositions were used in this experiment, namely ALBI1 (enriched in deuterium oxide), BS (intermediate isotopic composition), and KEI (light isotopic composition). Their isotope values are summarized in table 1.

Table 1. Liquid isotope concentrations of the reference standards.

Standards	$\delta^{18} O_{liquid}$ [‰]	$\delta^{2} H_{liquid}$ [‰]
ALBI1	-8.39	129.2
BS	-8.5	-57.71
KEI	-21.1	-158.08

The water vapor samples were generated under controlled laboratory conditions (Fig. 1). Sampling occurred at four different flow rates: 35, 75, 100, and 125 mL min^{-1}. Each combination of reference water and flow rate was repeated three times. This experimental design allowed us to assess the uncertainty of the sampling method and to identify whether specific flow rates significantly affect the accuracy of the isotope values measured in water vapor. Before sampling, the 250 mL infusion glass bottles were cleaned by placing them in an oven at 60-80 °C for 24 hours. After drying, the warm bottles were transferred to a dry, insulated aluminum box and allowed to cool under protected conditions to minimize exposure to ambient humidity and prevent condensation caused by rapid temperature changes. Before use, each bottle was flushed with a strong flow of dry air for 1 to 2 minutes to remove any residual from the interior. The bottles were then sealed by placing a butyl stopper on the opening, and sealing it with and aluminum crimp cap and crimping pliers to ensure a secure and gas-tight closure. During water vapor sampling, the temperature was monitored using a sensor (Sensirion AG, Sht4x Smart Gadget). Approximately 100 mL of the reference standard was placed in a 250 mL infusion glass bottle. During the sampling period, a dynamic equilibrium was established between the liquid water and the headspace vapor, allowing isotope exchange to approach the temperature-dependent equilibrium fractionation factor.

The sampling system was designed to pass a controlled stream of dry air through the bottle containing the reference water, generating water vapor that had equilibrated isotopically with the liquid water before flushing the sampling bottles. A synthetic dry air cylinder (20.5 Vol. % O2, Rest N2, KW-free) with a pressure regulator was connected to an electronic mass flow controller (PN 35828, Analyt MTC, Müllheim, Germany), allowing the sampling flow rate be precisely regulated. Connections were made with 1/8" PTFE tubing. The end connected to the mass flow controller used Swagelok connectors, while the other end had a Luer-lock adapter with a long needle inserted through the septum into the glass bottle. A second, shorter needle was inserted through the septum to transfer the generated water vapor to the sampling bottles.

The three replicate sampling bottles were connected in series in this setup, vapor generated from the reference water first passed through the first replicate bottle, then the second, and finally the third. The sampling was conducted for 35 minutes. The outlet of the third bottle was connected to the CRDS to monitor the vapor signal and obtain an initial reference measurement. Before connecting to the CRDS, an open-split configuration was used to prevent overpressure in the analytical system. The sampling system configuration is shown in Fig. 2C.

For each of the three reference waters, samples were prepared at each of the four sampling flow rates: 35, 75, 100, and 125 mL min^{-1}. Each reference-water and flow-rate combination was replicated three times, resulting in 36 samples per storage time (3 reference waters × 4 flow rates × 3 replicates). Across the four storage times, this resulted in 144 samples per temperature condition and 432 samples for the entire experiment.

2.4 Measurement of the water vapor samples

Before measurement, the water vapor samples were kept at Room temperature for one hour, ensuring that this temperature was higher than the temperature during sampling to achieve a homogeneous temperature inside the bottles and to prevent condensation.

The measurements were carried out with a Picarro L2130-i isotope analyzer, which determines the isotope-ratios of $\delta^{18}O$ and $\delta^{2}H$ using Cavity Ring-Down Spectroscopy (CRDS). The samples in the 500 mL Aluminum-zip bags were connected directly to the isotope analyzer using a Swagelok connector and 1/8" diameter PTFE tubing. At the other end, a Luer lock adapter was attached to a syringe, which was inserted through the silicone septum into the bag, allowing the isotope analyzer to extract the

water vapor at a flow rate of approximately 35 mL min^{-1}. For the 1 L FlexFoil sample bags with a gas valve, the measurement was made through the valve connected to a 1/4" PTFE tube. This tube was connected via a Swagelok connector to a second 1/8" tube that directed the vapor directly to the CRDS (see Fig. 2D).

The 250 mL infusion glass bottles were connected to the isotope analyzer using PTFE tube attached to a Luer lock adapter with a 0.80 x 40 mm syringe inserted into the bottle. The other end of the tubing was connected via a Swagelok connector to a stainless-steel T-piece and from there to the isotope analyzer. In contrast to the bags, the bottles were connected to a second tube with a 1.20 x 40 mm syringe, which introduced a constant flow of dry air into the bottle at a rate of 40 mL min^{-1}, controlled by a mass flow controller. This strategy was used because the applied flow exceeded the analyzer demand, maintaining positive pressure and minimizing atmospheric air intrusion. A T-piece vented excess flow (open split), preventing overpressure, and ensuring continuous sample delivery. An electronic rotameter confirmed excess venting at 5 - 7 mL min^{-1}, indicating that the analyzer received only sample (Fig. 2D). Each sample was measured for 6 minutes. Between measurement, the isotope analyzer was purged with dry air for 2 minutes to clean the measurement system and minimize the risk of memory effects (see Fig. S2).

2.5 Data processing

Water vapor isotope values are presented in delta notation (δ) relative to the international standard VSMOW (Vienna Standard Mean Ocean Water) according to equation 1 (Craig, (1961); Coplen, (2011)):

$$\delta sample = \left(\frac{R_{sample}}{R_{VSMOW}} - 1\right) * 1000\ [‰] \quad (1)$$

Where: R_{sample} is the isotopic ratio ($^{18}O/^{16}O$ or $^{2}H/^{1}H$) of the sample, and R_{VSMOW} is the ratio of the international standard VSMOW.

The first and the last 2 minutes of each 6-minute measurement were excluded to minimize potential memory effects in the system and water vapor concentration-dependent isotope effects. This was necessary because during measurements of stored samples, the water vapor concentration decreased over time, which could potentially affect the isotope values. Therefore, mean values for each replicate were calculated from the remaining central 2 minutes of the measurement.

From these data, the average values per replicate were calculated, and the isotopic values corresponding to the liquid phase of each sample were determined. For this purpose, the temperatures recorded during the sampling period were used and the equations for isotope fractionation in equilibrium between vapor and liquid were applied. These equations (2, 3, and 4) are based on the temperature-dependent fractionation factor in Kelvin [K] (Horita et al., 2008).

Equation 2; equilibrium fractionation ratio (α) for $\delta^{18}O$

$$10^3\ ln_{L/V}(^{18}O/^{16}O) = -2.0667 - 0.4156\left(\frac{10^3}{T}\right) + 1.137\left(\frac{10^6}{T^2}\right) \quad (2)$$

Equation 3; fractionation ratio at equilibrium (α) for δ^2 H

$$10^3\ ln_{L/V}(^{2}H/^{1}H) = 52.612 - 76.248\left(\frac{10^3}{T}\right) + 24.844\left(\frac{10^6}{T^2}\right) \quad (3)$$

Equation 4; liquid phase isotopic value

$$\delta_l = (\delta_v + 1000) * \alpha - 1000 \quad (4)$$

where:δ_l is the liquid isotopic value,δ_v is the vapor isotopic value, and α is the fractionation factor for ^{18} O and ^{2} H obtained from equations 2 and 3.

Based on obtained liquid values, a linear regression was calculated between the measured and known values. This regression was applied to correct and standardize all samples so that the final isotopic values were determined in accordance with the international VSMOW scale.

As part of the data processing, a quality check of the replicates was performed by comparing them with the known isotopic signatures of the standards. To ensure the precision and reliability of the standardization, a quality filter was applied in Python, excluding replicates that showed significant deviations from the expected value. All possible subsets of 2 and 3 replicates were evaluated for each combination of experimental conditions (standard type, temperature, storage time and flow rate). For each subset, the mean and standard deviation of $\delta^{18}O$ and $\delta^{2}H$, as well as their distance from the target value, were calculated. The subgroup with the lowest sum of standard deviations was selected, favoring subgroups with the highest number of replicates. This procedure allowed the determination of the most representative and reliable replicates for each experimental condition, maximizing both the accuracy and precision of the measurements.

2.6 Data analysis

The data was analyzed using the Python programming language (version 3.13.1) with libraries such as Pandas, NumPy, Matplotlib, Seaborn and SciPy. Graphical and statistical comparisons were conducted to evaluate the effects of storage temperature and container type on the stability of isotope values. Differences between groups were visualized with boxplots and statistically evaluated using non-parametric Kruskal-Wallis test, as the data did not follow a normal distribution.

To validate the precision of the isotope values, the Mean Absolute Error (MAE) between the measured values and the known values of the reference standards was calculated. This metric represents the average deviation from the reference value, providing an objective measure of the quality of the fit.

3 Results

3.1 Storage container testing

3.1.1 Tightness of storage containers.

Figure 3 shows the results for the tightness of the various containers. It indicates a clear increase in H_2O concentration (ppm) over time (= intrusion of atmospheric water vapor) in the dry air samples, depending on the type of container, storage time, temperature, and volume.

The 250 mL infusion glass bottles showed a substantial increase in H_2O concentration after 3 days of storage, reaching approximately ~2200 ppm at 40 °C and about ~1250 ppm under ambient conditions (20–25 °C), while values at 4 °C remained lower at around ~480 ppm. The 500 mL Aluminum-zip bags showed a similar pattern, but with the strongest increase under ambient conditions (~1750 ppm). Under extreme temperature conditions, such as the Oven (40 °C) and Fridge (4 °C), they showed comparable concentrations of ~1000 ppm after 3 days. The 1 L FlexFoil sample bags showed the least variability, with values of approximately ~100 and ~600 ppm across all storage conditions and times, with no significant increase.

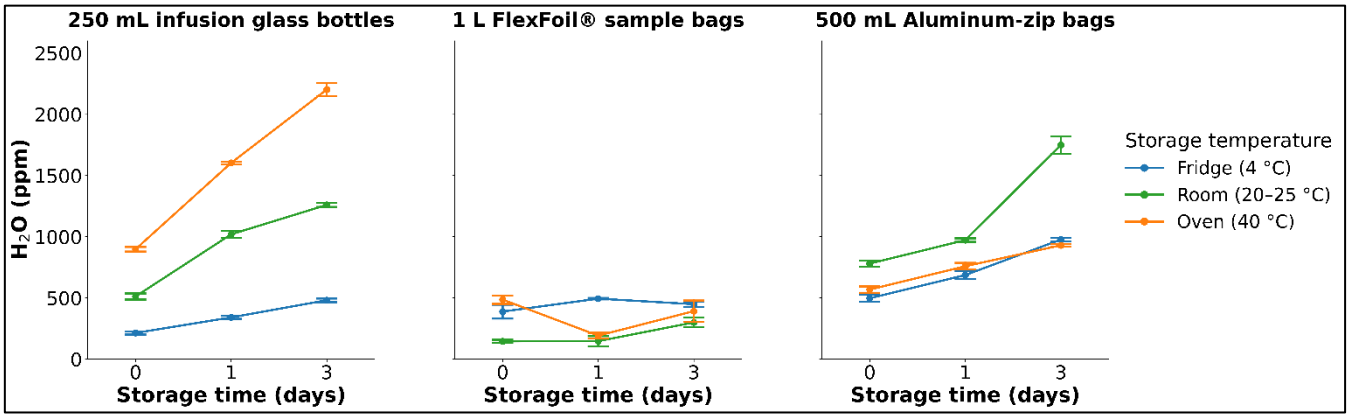


Figure 3. The graph shows the changes in water vapor concentration (H_2O, in ppm) in dry air samples after different storage times (0, 1, and 3 days) in three types of containers (250 mL infusion glass bottles, 1 L FlexFoil sample bags and 500 mL Aluminum-zip bags) and under three temperature conditions (Fridge, Room and Oven).

3.1.2 The efficiency of containers in preserving the isotopic composition of water vapor

An isotopic reference was used to assess whether the isotopic composition remained stable after sampling in three types of containers and during storage at different temperatures and times. The results show a varying degree isotopic deviation of the measured values from the known values of the isotopic reference source. This deviation strongly depends on temperature, storage time and container type, and generally increases with longer storage time. In the 250 mL infusion glass bottles, the isotopic deviations in $\delta^{18}O$ remained stable within a range of ±0.9 ‰ across all three storage times (0, 1, and 3 days) under all temperature conditions. Slight variations in $\delta^{2}H$ were observed, particularly for samples stored in the Oven (+5 ‰) and Fridge (−5 ‰) after three days, while samples stored at Room temperature showed a stable deviation of ±0.5 ‰ at all storage times. Overall, the results show that analyzing of the samples until 24 hours after sampling provides reliable values without deviations of -1 ‰ for $\delta^{18}O$ and $\delta^{2}H$ (+2.3 ‰ was observed exclusively in the Oven for $\delta^{2}H$; see Fig. 4).

In contrast, the 1 L FlexFoil sample bags showed $\delta^{18}O$ deviations within ±1 ‰ for samples stored under Fridge and Oven conditions on days 0, 1, and 3. However, samples stored at Room temperature showed deviations of −1.35 ‰ on the first day and a +1.08 ‰ enrichment after three days. The deviations in $\delta^{2}H$ were more pronounced from 0 to 1 day, with Room temperature showing deviations of -3 ‰, and values reaching up to ±8 ‰ after three days under Room and Fridge conditions. In the Oven, deviations remained close to +2 ‰, indicating a clear trend toward isotopic enrichment under these conditions. These results show that deviations are expected in this type of container from the first day of storage and tend to increase over time. Additionally, the fact that the deviations varied depending on the temperature conditions indicates that this material reacts differently to temperature changes, which significantly affects the isotopic signature of the water vapor (Fig. 4).

Finally, the 500 mL Aluminum-zip bags showed the largest increase in isotopic deviations for $\delta^{18}O$, reaching −3 ‰ under Fridge and Room conditions. In the Oven, the samples had values of −2.5 ‰ on days 0 and 1, followed by a slight enrichment to +1 ‰ on the third day. For $\delta^{2}H$, isotopic fractionation toward depletion was observed under all three temperature conditions, with deviations of up to −25 ‰ in the Room, −16 ‰ in the Fridge and −12 ‰ in the Oven (Fig. 4).

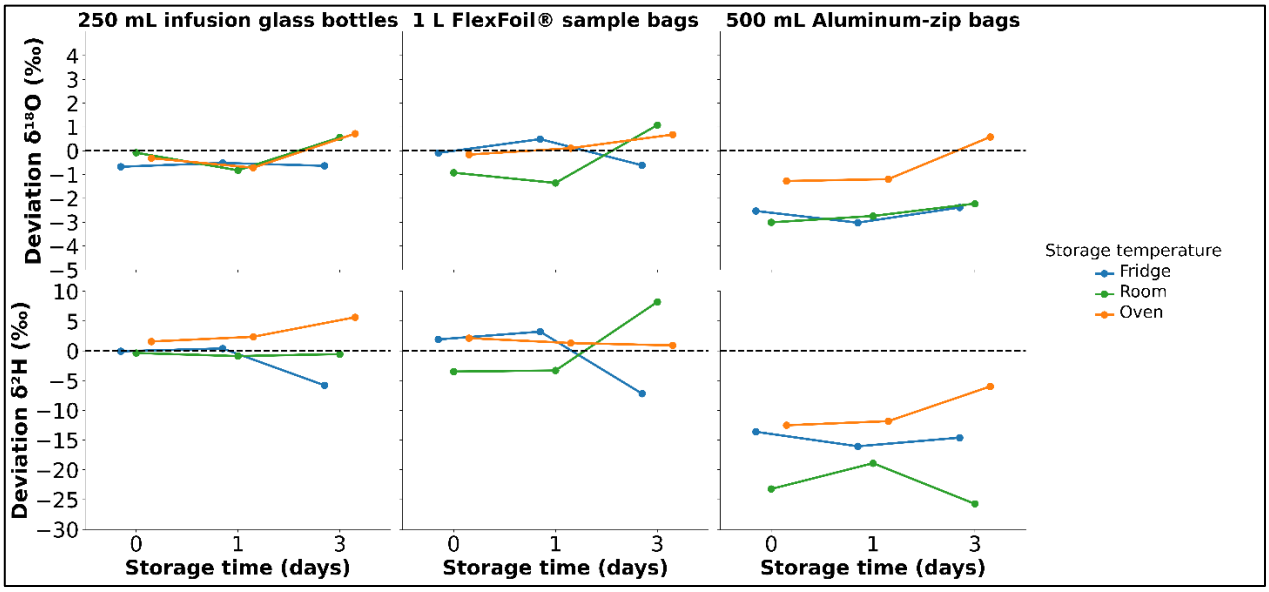
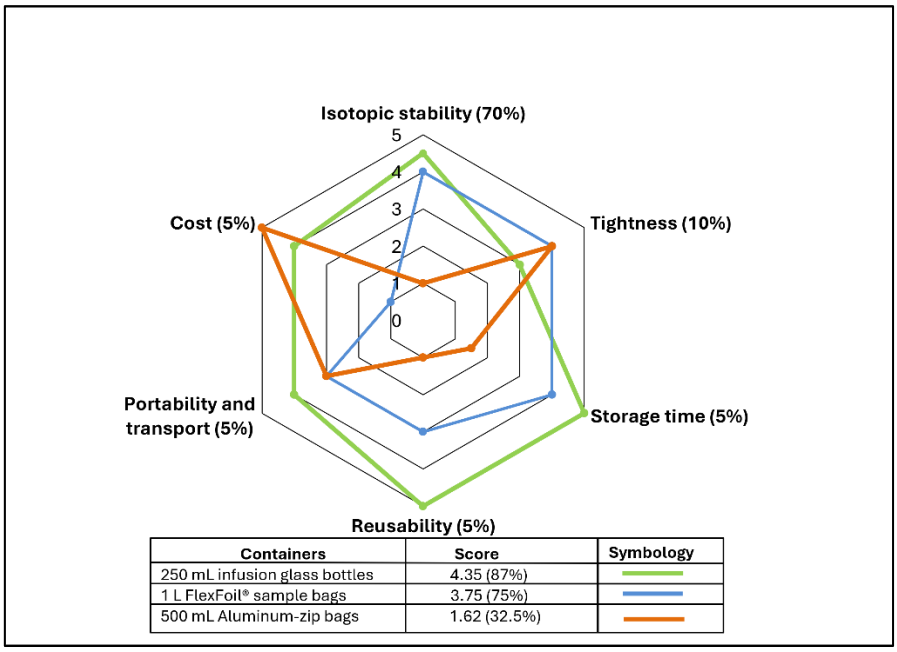


Figure 4. Isotopic deviations ($\delta^{18}O$ and $\delta^{2}H$) in water vapor samples stored in different containers (250 mL infusion glass bottles, 1 L FlexFoil sample bags, and 500 mL Aluminum-zip bags) at three storage temperatures (Fridge, Room, Oven) for 0, 1 and 3 days. The dashed line represents the reference value (0 ‰), indicating no isotope deviation.

3.1.3 Selection of the optimal container

Figure 5 shows the results of the multicriteria analysis performed to determine the optimal container.



Containers	Score	Symbology
250 mL infusion glass bottles	4.35 (87%)	
1 L FlexFoil® sample bags	3.75 (75%)	
500 mL Aluminum-zip bags	1.62 (32.5%)	

Figure 5. Evaluation and scoring of decision criteria (1 = poor; 5 = excellent) for the optimal container for water vapor isotope measurements. Critical criteria: isotopic stability (70%), tightness (10%), and storage time (5%). Non-critical criteria: reusability (5%), portability and transport (5%) and cost (5%).

The 250 mL infusion glass bottles achieved the highest overall score of 4.35 (87%) and were therefore the optimal container for water vapor sampling and "winner" of the testing. They were characterized by isotopic stability during the first days of storage (±1 ‰ deviation for both $\delta^{18}O$ and $\delta^{2}H$; Fig. 4). Although they showed a greater increase in H_2O compared to the other containers (Fig. 3), their reusability, low cost in the European market, and reasonable transport stability make them a practical and cost-effective option for field sampling campaigns.

As a second option, the 1 L FlexFoil sample bag performed well in terms of tightness with a score of 3.75 (75%) (Fig. 5), although it showed greater isotopic deviations than the glass bottles, especially under Room temperature conditions (Fig. 4). Its reusability is limited to a maximum of three cycles, and it is the most expensive container on the European market. Its main disadvantage is its portability, as the occupied volume increases when filled, making it difficult to transport and store large numbers of samples and increasing the risk of damage during handling.

Finally, the 500 mL Aluminum-zip bags received the lowest score of 1.62 (32.5%). This poor performance was mainly due to their limited isotopic stability (Fig. 4) and the increase in H_2O observed during the tightness tests, especially after 3 days of storage. Although they offer the advantage of low cost, their reusability is practically limited to a single use. In addition, their portability and resistance to transportation are unfavorable, as the occupied volume makes handling multiple samples difficult and the material is prone to punctures.

Overall, glass bottles offer advantages that make them the preferable container. The criteria supporting this choice are particularly related to their ability to maintain a reliable isotopic signature during the first day of storage under various temperature conditions. Additional information and the rationale for each assessed criterion are provided in Table S2.

3.2 Results of sampling procedure testing with 250 mL infusion glass bottles

3.2.1 Effect of syringe size on the tightness of 250 mL infusion glass bottles

The values obtained include the initial H_2O concentration, which was 200 to 350 ppm. The results show that the H_2O content in ppm increases progressively with storage time and is higher at higher temperatures. Samples stored at 40 °C reached the highest concentrations after 7 days, around ~4000 ppm, followed by samples stored at 20-25 °C at approximately ~2000 ppm, and finally samples stored at 4 °C, which had the lowest H_2O concentrations of about ~700 ppm after 7 days.

The trends in H_2O concentration in the dry air samples in the 250 mL infusion glass bottles were consistent between the two syringe sizes, indicating that syringe size had no significant effect on the increase in H_2O concentration as a function of storage time and temperature, as shown in Fig. 6.

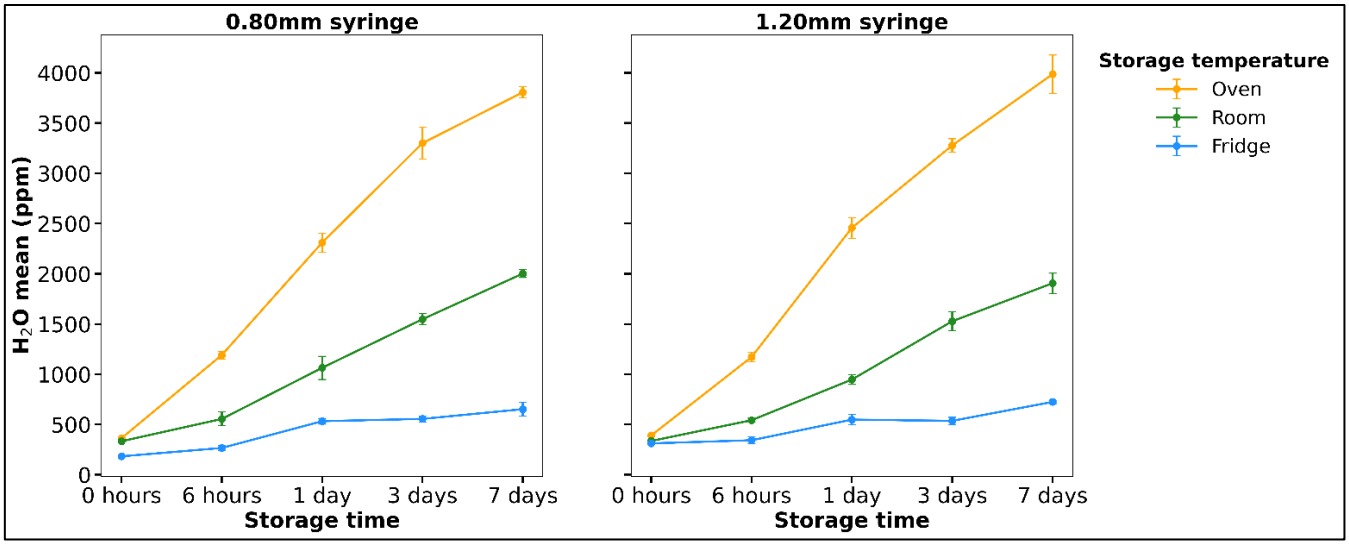


Figure 6. Absolute H_2O concentration (ppm) in synthetic dry air samples stored in 250 mL infusion glass bottles and analyzed with small and large syringes at different temperatures and times. The initial H_2O at 0 hours was approximately 200–300 ppm; the reported values represent the total measured concentration, including any increase during storage.

3.2.2 Effect of sampling flow rate on water vapor stored in 250 mL infusion glass bottles

Water vapor sampling was conducted at four flow rates (35, 75, 100, and 125 mL min^{-1}). The H_2O concentration (ppm)measured during sampling with the CRDS, reached a plateau after approximately 25 minutes at 100 and 125 mL min^{-1} and after 31 minutes at 75 mL min^{-1}, whereas, it did not fully stabilize within 35 minutes at 35 mL min^{-1}. A stable plateau was observed at 100 and 125 mL min^{-1}, but at 35 and 75 mL min^{-1}, the isotopic plateau developed more gradually over the sampling period, particularly for δ^2H (Fig. S1).

3.2.2.1 Impact of sampling flow rate on $\delta^{18}O$ and δ^2H isotopic composition

The stability of the water vapor samples was highly dependent on flow rate, with δ^2H showing much larger fluctuations than $\delta^{18}O$. The 35 mL min^{-1} rate showed the greatest variability, with δ^2H interquartile range (IQR) of ±5.38 ‰ and outliers reaching ±15 ‰, this was mirrored in the $\delta^{18}O$ by a noticeable shift toward negative deviations (±1 ‰). Optimal precision was found between 75 to 125 mL min^{-1}, where 75% of δ^2H values remained below ±1.5 ‰. In this range, the 75 mL min^{-1} rate achieved the lowest median deviation (±0.18 ‰), suggesting that higher flows stabilize the vapor stream against ambient interference (Fig. 7A). However, isotopic integrity degraded proportionally with time, a trend visible in both isotopes but amplified in the δ^2H signal. At 1 day, the system reached its peak stability with a δ^2H IQR of, only ±2.04 ‰ and median near zero (±0.22 ‰). However, by day 7, the median deviation climbed to ±0.76 ‰ with a dispersion of ±3.76 ‰. Notably, at this 7-day mark, $\delta^{18}O$ displayed significant negative outliers, confirming that long-term storage triggers a coupled fractionation process-likely due to vapor escape-that affects both isotopic signatures (Fig. 7B).

Contrary to our expectations, Room temperature storage yielded the highest precision (median: ±0.14 ‰; IQR: ±2.72 ‰ for δ^2H). While the Oven samples showed slightly more scatter, the Fridge treatment was surprisingly the least stable. It produced the highest δ^2H median deviation ±0.59 ‰ and the widest IQR ±3.38 ‰, with extreme outliers of ±15 ‰. This increased variability in the Fridge, visible in both δ^2H and $\delta^{18}O$, suggests that cold conditions might interface with the internal equilibrium of the 250 mL glass bottle (fig. 7C). Finally, the deviations for δ^2H were strongly influenced by the isotopic weight of the standards. KEI was the most consistent (IQR: ±1.83 ‰), whereas HB showed the highest deviation values with ±2.03 ‰ and an IQR of ±3.68 ‰, indicating greater dispersion and lower stability compared to the other standards. However, ALBI1 had a larger spread with an IQR of ±3.16 ‰ and outliers of ±15 ‰. $\delta^{18}O$ fluctuations up to ±1 ‰ indicating that heavier isotopes are more susceptible to memory effects (Fig. 7D).

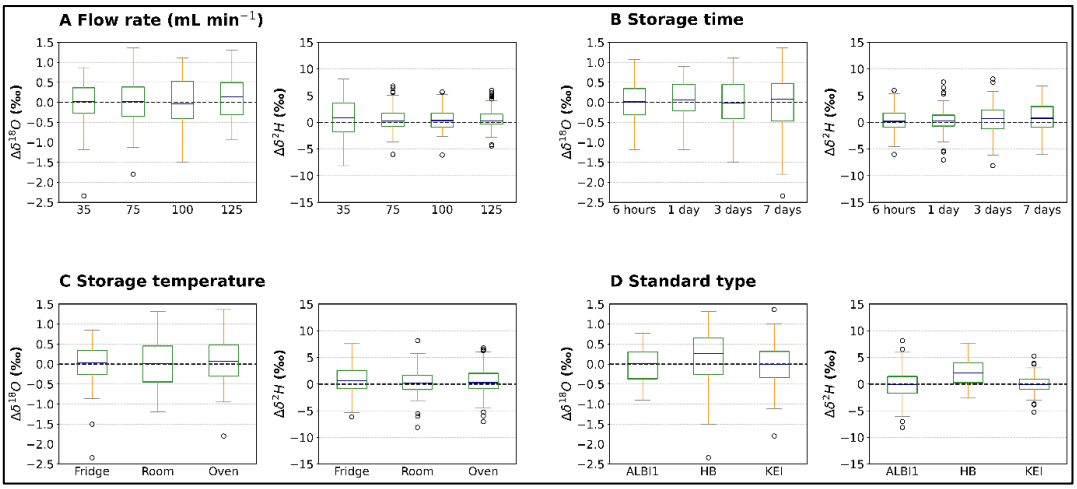


Figure 7. Isotopic deviations ($\Delta\delta^{18}O$ and $\Delta\delta^2H$) of water vapor samples under different factors: A) Flow rates (mL min^{-1}), B) Storage time, C) Storage temperature, and D) Standard type. Boxplots represents the median (blueline), the interquartile range (green box)

and the whiskers extend to the most extreme data points not considered outliers. Circles represent individual outliers. The dashed horizontal line indicates zero deviation from the reference value (Total sample set 432).

3.2.3 Mean Absolute Error (MAE) and relative error of the results obtained under optimal sampling conditions

The Mean Absolute Error analysis confirmed that the experimental conditions significantly affect the precision of water vapor isotope measurements, especially for δ^2H. The smallest deviations between measured and target values occurred when flow rates of 75, 100, or 125 mL min^{-1} were used for water vapor sampling and samples were stored for 6 hours to 1 day at ambient conditions with temperatures between 20 and 25 °C. Under these conditions the best isotope values are obtained, with deviations of ±1.52 to ±1.89 ‰. However, at a flow rate of 35 mL min^{-1}, the deviations increase to 3.55 ‰, and if samples are stored for more than 1 day (e.g., 3 or 7 days), the deviations also increase. Similarly, very low (4 °C) or high (40 °C) temperatures result in larger deviations. For $\delta^{18}O$, the MAE indicates that deviations are small, with a precision of ±0.6 ‰ under all evaluated conditions, indicating that only δ^2H is directly affected by variations in temperature, air flow, storage time and isotope concentration (Standard), as shown in Table. 2.

Table 2. Mean Absolute Error of $\delta^{18}O$ (A) and δ^2H measurements under different experimental factors and conditions, including flow rate, storage time, temperature, and standard type.

Experimental factor	Condition	MAE $\delta^{18}O$ (‰)	MAE δ^2H (‰)
Flow rate	35 mL min^{-1}	0.39	3.55
	75 mL min^{-1}	0.41	1.89
	100 mL min^{-1}	0.50	1.62
	125 mL min^{-1}	0.44	1.52
Storage time	6 hours	0.39	1.56
	1 day	0.37	1.73
	2 days	0.45	2.44
	7 days	0.53	2.72
Storage temperature	Fridge	0.37	2.45
	Oven	0.44	2.10
	Room	0.48	1.78
Standard type	ALBI1	0.37	2.50
	BS	0.56	2.65
	KEI	0.38	1.19

The analysis of optimal conditions for water vapor extraction indicates that more than one flow rate may be suitable, depending on measurement duration and temperature conditions. For samples stored for 6 hours, both 100 and 125 mL min^{-1} produced small and consistent errors for δ^2H (< ±1.3 ‰) and $\delta^{18}O$ (< ±0.5 ‰), while 75 mL min^{-1} was acceptable for $\delta^{18}O$ but showed variability up to ±3 ‰ for δ^2H and therefore can only be considered a partial alternative. After 1 day, the flow rate of 125 mL min^{-1} was most optimal, yielding errors below ±0.5 ‰ for $\delta^{18}O$ and ±0.3 – 1.5 ‰ for δ^2H. However, 100 mL min^{-1} may still be acceptable under certain conditions, provided the vapor samples are not exposed to extreme temperatures, as this could otherwise result in δ^2H errors exceeding ±2 ‰. During prolonged storage (3 and 7 days), only the flow rate of 125 mL min^{-1} remained within the range of ±1.5 to ±4 ‰, while the other flow rates showed greater variability, resulting in lower reliability. Therefore, if samples are analyzed within a short time (6 hours), 100 mL min^{-1} is the optimal flow rate, while 125 mL min^{-1} provides greater stability under storage conditions of one day or longer (>1 day) (see Table 3).

Table 3. Recommended optimal flow rates for water vapor samples as a function of time and storage conditions in 250 mL infusion glass bottles.

Storage time	Flow rate (mL min^{-1})	Optimal conditions	Expected MAE δ^2H (‰)	Expected MAE $\delta^{18}O$ (‰)
≤ 6 h	100, 125 and (75 alternative option)	4 °C and 20-25 °C	< 1.3 (75 mL min^{-1} to 3‰)	<0.5
24 h (1 day)	125 (optimal) and 100 (acceptable)	20-25 °C and 40 °C	0.3 – 1.5	0.2 – 0.5
>24 h (72 and 168 hours)	125	20-25 or 40 °C	>1.5	0.4 – 0.6

Relative errors were calculated for the sampling flow rates considered optimal (75 to 125 mL min^{-1}), with data categorized into three storage duration groups (6 hours, 1 day, and >1 day). Figure 8 shows that isotopic concentrations remained relatively stable for up to 24 hours after sampling, with values generally below 4.5% for $\delta^{18}O$ and 2.5% for δ^2H across all analyzed flow rates. However, a distinct increase in relative error occurred when storage duration exceeded 1 day; this trend was particularly pronounced for $\delta^{18}O$, which reached a maximum relative error of 5.6% at a flow rate of 100 mL min^{-1}. In contrast, δ^2H showed greater stability over time, with relative errors remaining below 3%. Although sampling flow rates influenced the magnitude of the error, storage duration was the primary factor determining isotopic variability.

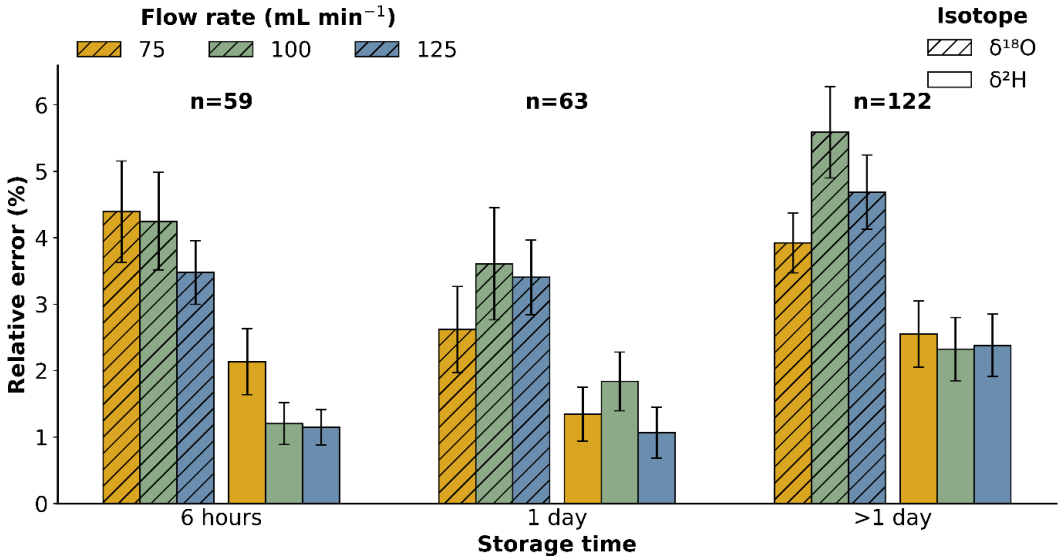


Figure 8. Relative error (%) for $\delta^{18}O$ (hatched bars) and δ^2H (solid bars) as a function of storage time (6 hours, 1 day, and >1 day) and sampling flow rates (75, 100, and 125 mL min^{-1}). The number of observations (*n*) for each storage category is shown above the bars. Error bars represent the standard deviation.

4 Discussion

4.1 Evaluation of containers and criteria for water vapor storage

Our results show that vapor exchange with dry air depended on container type as well as storage temperature and duration. The 250 mL infusion glass bottles exhibited the highest H_2O concentration (ppm) among the tested containers. The most likely reason for this is the puncturing of the septum with the syringe needle during sampling. Similar low-level increases during dry-air storage were reported by Magh et al., (2022), indicating that even high-quality vial systems are not completely diffusion-tight when punctured. However, this aspect did not seem to effect water vapor isotope values substantially because in the stability test the 250 mL infusion glass bottles performed best. The 500 mL Aluminum-zip bags had the highest H_2O increase under ambient conditions rather than extreme temperatures, suggesting gradual exchange through zip-seal microleaks. In contrast, the 1 L FlexFoil sample bags remained the most stable system, with low variability and no significant increase across storage treatments.

To evaluate whether this minor diffusive exchange could affect isotope values, we tested the reproducibility of a reference water with a known isotopic composition. The 250 mL infusion glass bottles yielded the most stable isotope values, particularly for $\delta^{18}O$, with noticeable $\delta^{2}H$ deviations occurring only after 3 days of storage. Although FlexFoil sample bags effectively limited exchange, deviations from the reference water, especially for $\delta^{2}H$, were detectable after 1 day, indicating that low vapor leakage does not necessarily ensure isotopic stability. In contrast, the 500 mL Aluminum-zip bags showed the largest isotopic deviations under the procedures used in this study. These patterns indicate that $\delta^{2}H$ was more sensitive than $\delta^{18}O$ to storage-related effects, consistent with previous storage assessments (e.g., Magh et al., 2022). Temperature changes promoting condensation, evaporation, and incomplete internal equilibration, together with potential isotope exchange with the bag material (Herbstritt et al., 2023), likely contributed to the greater variability observed in the bag systems.

The combined evaluation of container tightness, vapor storage performance, and practical criteria such as analysis time, reusability, cost, portability, and transport, provided a robust basis for selecting the most suitable container for water vapor sampling. Although secondary criteria were less decisive (see Fig. 5), they helped support the final methodological recommendation. Overall, the 250 mL infusion glass bottles offered best compromise between isotopic preservation and practical applicability, particularly when analyzed shortly after sampling. They can be reused after drying at 60–80 °C and are suitable for field campaigns because large numbers can be transported in insulated cases. For longer storage periods, for instance when sampling at a remote site, we recommend sealing the needle punctures after sampling. Although this procedure was not tested in the present study, it would likely further reduce diffusive exchange and improve the storage performance of glass bottles.

Preservation of the original isotopic signature was the most important criterion. The performance of the 250 mL glass bottles suggests that moderate changes in internal H_2O vapor concentration do not necessarily compromise isotope stability when the stored vapor originates from a highly saturated source. This interpretation is consistent with previous observations that limited diffusive exchange may have only minor effects on isotopic composition under high water vapor concentrations (Magh et al., 2022). In contrast, bag systems appeared more susceptible to storage-related isotopic variability under the filling and storage procedures used in this study. These findings indicate that rigid glass containers are more suitable for short-term vapor storage, whereas bag systems require more careful consideration of storage duration, temperature conditions, and potential material-related isotope exchange.

We offer the following possible explanations for the observed differences in isotope values between the bag-type containers and the glass bottles:

i. Differences in the sampling of water vapor.

When sampling water vapor in bottles, two syringes are inserted through the septum, serving as an inlet and an outlet for the equilibrated water vapor. Once the sampling time is reached, both syringes are removed. In contrast, both types of bags are filled with equilibrated air using a single syringe. The throughflow sampling may provide a more stable isotope signature because the throughflow duration is longer than the filling time for the bags (12 minutes per sample vs. 6 minutes per sample).

ii. Isotopic exchange with container material.

Adsorptive isotope exchange between the Aluminum bags material and the sample air may account for their poorer performance (Herbstrit et al., 2023). Thies effect is absent in glass bottles and appears less pronounced in FlexFoil sample bags. Therefore, the production of the aluminum bags might have an influence if they are not pre-treated.

Although 1 L FlexFoil sample bags ranked as the second-best option in our evaluation (Fig. 5), their suitability appears more limited than that of glass bottles. They performed well in leak-tightness tests but this did not translate into comparable isotopic stability during storage. From a practical perspective, their higher cost and larger volume may complicate transport logistics, and their durability may become a limitation when large numbers of samples are required. Reuse in another important consideration, as it requires labor-intensive cleaning to reduce memory effects. For example, Dahlmann et al., (2025) used affordable multilayer 1 L gas bags with modified valves and reported good performance when the bags were new. However, they also identified a storage–time–dependent memory effect, indicating that reuse may be problematic when samples cover broad isotopic ranges or when consecutive sampling events are involved. This is particularly relevant in tracer experiments, where cross-contamination between samples may compromise data quality.

Finally, the 500 mL Aluminum-zip bags performed least favorably among the tested systems, combining limited storage stability with only moderate leak-tightness after prolonged storage. Despite their low cost, they are effectively single-use and more vulnerable to handling damage during transport. Under the filling and storage conditions applied in this study, their use therefore appears less suitable when accurate isotope preservation is required.

The contrasting performance among the tested systems indicates that both leak-tightness and isotopic stability depend on interactions among container material, closure design, internal headspace conditions and temperature sensitivity. For short-term sampling under variable field temperatures, the 250 mL infusion glass bottles remained the most robust option due to their reusability, cost-effectiveness, practical transport capacity, and comparatively stable isotope fluctuation.

4.2 Protocol and optimal container for water vapor sampling and storage

Methods for measuring stable isotopes of water are constantly improved. Water vapor sampling has emerged a novel approach to overcoming the reported limitations and potential biases of extraction-based methods (Kübert et al., 2020; Mennekes et al., 2021). However, this technique requires proper implementation and rigorous, efficient sampling procedures. To reduce uncertainties in water vapor sampling and analysis, we propose a refined protocol that controls those factors most affecting the isotopic signature while ensuring data quality. According to our results, it is essential to consider container type, flow rate, storage time, and temperature conditions, a necessity previously underscored in the literature (Van Duren, 2004; Benetti et al., 2017; Bagheri et al., 2021; Magh et al., 2022; Herbstritt et al., 2023). Our data show that experimental conditions substantially influence the precision of water vapor isotope measurements, especially for δ^2H, whereas $\delta^{18}O$ remains relatively stable. Therefore, we emphasize maintaining controlled temperature conditions to minimize measurement errors, due to the greater

sensitivity to kinetic and diffusive processes (Horita and Wesolowski, 1994; Wen et al., 2008; Lamb et al., 2017; Wei et al., 2022; Weng et al., 2024;).

The results confirmed a progressive increase in H_2O vapor concentration, and the septum perforation diameter does not appear to be the cause, as both syringe sizes showed similar patterns. The most plausible explanation is diffusive exchange with ambient air, which initiates immediately after septum perforation during sampling and measurement, although this mechanism warrants further investigation. This pattern was consistent with stronger, δ^2H deviations after prolonged storage, highlighting that short storage times are critical to minimize isotopic drift (see Table 3).

Our results regarding the optimal time window for high-quality measurements are consistent with other studies. Dahlmann et al., (2025) and Herbstritt et al., (2023) reported acceptable results for samples stored up to 24 hours (±0.7 and ±2.3 ‰ for δ^2H and ±0.2 to ±0.9 ‰ for $\delta^{18}O$). Similarly, Magh et al., (2022) indicated that reliable measurements could be obtained up to 3 days after sampling, emphasizing short-term stability while noting an increasing bias by day 7, which closely aligns with our observations. In contrast, Havranek et al., (2023) implemented an automated soil water isotope storage system based on 650 mL flasks that maintained isotope values (±0.9 ‰ for $\delta^{18}O$ and ±3.7 ‰ for δ^2H) for substantially longer periods under both laboratory and field conditions. This extended stability may be attributed to the larger flask volume, although such setup may be less practical for rapid field sampling and handling. Regardless, sample analysis within the first 24 hours should be considered the most robust and conservative approach, though storage up to 3 days can still yield reliable results under controlled conditions. According to our findings, more prolonged storage may lead to significant isotopic deviations, particularly under temperature fluctuations (4 to 40 °C), suggesting progressive vapor exchange and subsequent fractionation over time. Consequently, the system developed by Havranek et al., (2023) represents a platform specifically optimized for autonomous, leak-resistant long-term storage.

Storing water vapor under ambient conditions can preserve the isotopic signal without refrigeration, supporting the feasibility of this method for field campaigns in remote areas or regions with limited infrastructure. Our findings further demonstrate that $\delta^{18}O$ was consistently more stable than δ^2H and can therefore be used more reliably in ecohydrological applications. Precise measurements require controlling the sampling flow rate, storage time, and temperature, with the best performance obtained under moderate-to-high flow rates, storage within 1 day, and stable conditions. Prolonged storage or extreme temperatures increased deviations, particularly for δ^2H, and may compromise data interpretation (Bagheri Dastgerdi et al., 2021; Magh et al., 2022). Minimizing isotopic memory through appropriate material selection therefore remains an important practical challenge (Weng et al., 2024).

5 Conclusion and implications

We optimized a practical sampling and storage protocol for water vapor isotope analysis by systematically evaluating multiple parameters, including container type, temperature, storage time, and sampling flow rate. Detailed experiments showed that 250 mL infusion glass bottles were the most reliable system for collecting and storing vapor samples for isotope analysis, outperforming 1 L FlexFoil sample bags and 500 mL Aluminum-zip bags. Our results demonstrate that the isotopic stability of water vapor is influenced by container type, storage temperature, storage time, and flow rate. The optimal sampling configuration was achieved at flow rates of 100 – 125 mL min^{-1} and temperatures of 20 – 25 °C. Storage for up to 3 days yields reliable results under controlled conditions. $\delta^{18}O$ remained highly stable under nearly all tested conditions, whereas δ^2H was more sensitive to storage time and temperature variation. This indicates that faster sample processing, preferably within 1 day, is the most robust strategy to minimize isotopic deviations. Overall, our systematic testing and developed protocol provide a rigorous framework for sampling water vapor for stable isotope analysis, which has great potential for ecohydrological

research. It could serve as a semi-*in situ* alternative for measuring soil and plant water isotopes when true *in situ* measurements are not feasible, and it can be further applied to sampling atmospheric water vapor (e.g., for studies on ET partitioning).

A potential source of error identified in our sampling configuration is leakage through the septum after syringe perforation, which may allow vapor loss or ambient air intrusion thereby contributing to isotopic fractionation. Increasing the number of replicates number and modestly extending the sampling time can reduce variability and improve quality control. Future applications may benefit from improved sealing systems (e.g., resistant septa, integrated valves, or secondary seals) and experimental controls to better quantify leakage effects, thereby enhancing reproducibility under variable field conditions. Specifically for sampling campaigns in remote locations, we recommend sealing the syringe puncture points immediately after collection. Although not explicitly tested in this study, this measure is expected to further enhance the long-term integrity and performance of the 250 mL infusion glass bottles by minimizing diffusive exchange.

Data availability

All data supporting the findings of this study, including raw and processed water vapor isotope measurements, calibration files, and experimental metadata, are openly available in the Zenodo repository: Iraheta, A., Malsch-Fröhlich, E., Gerchow, M., and Beyer, M. (2025). Water vapor isotope sampling dataset for the study "Technical note: Water vapor sampling for the analysis of water stable isotopes in trees and soils – optimizing sampling protocols" (Version 1.0). Zenodo. https://doi.org/10.5281/zenodo.17667032.

Author contribution

AI and EMF designed the study and conducted the laboratory experiments. AI analyzed the data, wrote the first draft of the manuscript, and implemented the revisions. MG developed Python scripts for data analysis and visualization and contributed to the manuscript review. MB supervised the study and contributed to the review and editing of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

We are grateful to the German Academic Exchange Service (DAAD) for the doctoral scholarship. This research was further supported by the German Research Foundation under the grant number 501530203. We kindly appreciate their support. We thank R. Magh and one anonymous reviewer for their constructive and encouraging feedback.

References

Allen, S. T. and Kirchner, J. W.: Potential effects of cryogenic extraction biases on plant water source partitioning inferred from xylem-water isotope ratios, Hydrological Processes, 36, e14483, https://doi.org/10.1002/hyp.14483, 2022.

Bagheri Dastgerdi, S., Behrens, M., Bonne, J.-L., Hörhold, M., Lohmann, G., Schlosser, E., and Werner, M.: Continuous monitoring of surface water vapour isotopic compositions at Neumayer Station III, East Antarctica, The Cryosphere, 15, 4745–4767, https://doi.org/10.5194/tc-15-4745-2021, 2021.

Barbeta, A., Burlett, R., Martín-Gómez, P., Fréjaville, B., Devert, N., Wingate, L., Domec, J.-C., and Ogée, J.: Evidence for distinct isotopic compositions of sap and tissue water in tree stems: consequences for plant water source identification, New Phytologist, 233, 1121–1132, https://doi.org/10.1111/nph.17857, 2022.

Benetti, M., Reverdin, G., Aloisi, G., and Sveinbjörnsdóttir, Á.: Stable isotopes in surface waters of the Atlantic Ocean: indicators of ocean–atmosphere water fluxes and oceanic mixing processes, Journal of Geophysical Research: Oceans, 122, 4723–4742, https://doi.org/10.1002/2017JC012712, 2017.

Beyer, M., Kühnhammer, K., and Dubbert, M.: In situ measurements of soil and plant water isotopes: a review of approaches, practical considerations and a vision for the future, Hydrology and Earth System Sciences, 24, 4413–4440, https://doi.org/10.5194/hess-24-4413-2020, 2020.

Ceperley, N., Gimeno, T. E., Jacobs, S. R., Beyer, M., Dubbert, M., Fischer, B., Geris, J., Holko, L., Kübert, A., Le Gall, S., Lehmann, M. M., Llorens, P., Millar, C., Penna, D., Prieto, I., Radolinski, J., Scandellari, F., Stockinger, M., Stumpp, C., … Rothfuss, Y. (2024). Toward a common methodological framework for the sampling, extraction, and isotopic analysis of water in the Critical Zone to study vegetation water use. WIREs Water, 11(4), e1727. https://doi.org/10.1002/wat2.1727

Chen, Y., Helliker, B. R., Tang, X., Li, F., Zhou, Y., and Song, X.: Stem water cryogenic extraction biases estimation in deuterium isotope composition of plant source water, Proceedings of the National Academy of Sciences, 117, 33345–33350, https://doi.org/10.1073/pnas.2014422117, 2020.

Coplen, T. B.: Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results, Rapid Communications in Mass Spectrometry, 25, 2538–2560, https://doi.org/10.1002/rcm.5129, 2011.

Craig, H.: Isotopic variations in meteoric waters, Science, 133, 1702–1703, 1961.

Dahlmann, A., Marshall, J. D., Dubbert, D., Hoffmann, M., and Dubbert, M.: Simple water vapor sampling for stable isotope analysis using affordable valves and bags, Atmospheric Measurement Techniques, 18, 2607–2618, https://doi.org/10.5194/amt-18-2607-2025, 2025.

De Deurwaerder, H. P. T., Visser, M. D., Detto, M., Boeckx, P., Meunier, F., Kuehnhammer, K., Magh, R.-K., Marshall, J. D., Wang, L., Zhao, L., and Verbeeck, H.: Causes and consequences of pronounced variation in the isotope composition of plant xylem water, Biogeosciences, 17, 4853–4870, https://doi.org/10.5194/bg-17-4853-2020, 2020.

Diekmann, C. J., Schneider, M., Knippertz, P., Trent, T., Boesch, H., Roehling, A. N., Worden, J., Ertl, B., Khosrawi, F., and Hase, F.: Water vapour isotopes over West Africa as observed from space: which processes control tropospheric H_2O/HDO pair distributions, Atmospheric Chemistry and Physics, 25, 5409–5431, https://doi.org/10.5194/acp-25-5409-2025, 2025.

Dubbert, M. and Werner, C.: Water fluxes mediated by vegetation: emerging isotopic insights at the soil and atmosphere interfaces, New Phytologist, 221, 1754–1763, https://doi.org/10.1111/nph.15547, 2019.

Gaj, M., Beyer, M., Koeniger, P., Wanke, H., Hamutoko, J., and Himmelsbach, T.: In situ unsaturated zone water stable isotope (^2H and ^{18}O) measurements in semi-arid environments: a soil water balance, Hydrology and Earth System Sciences, 20, 715–731, https://doi.org/10.5194/hess-20-715-2016, 2016.

Galewsky, J., Steen-Larsen, H. C., Field, R. D., Worden, J., Risi, C., and Schneider, M.: Stable isotopes in atmospheric water vapor and applications to the hydrologic cycle, Reviews of Geophysics, 54, 809–865, https://doi.org/10.1002/2015RG000512, 2016.

Gunther Liebhard, A., Klik, A., Stumpp, C., and Nolz, R.: Partitioning evapotranspiration using water stable isotopes and information from lysimeter experiments, Hydrological Sciences Journal, 67, 646–661, https://doi.org/10.1080/02626667.2022.2030866, 2022.

Haberstroh, S., Kübert, A., and Werner, C.: Two common pitfalls in the analysis of water-stable isotopologues with cryogenic vacuum extraction and cavity ring-down spectroscopy, Analytical Science Advances, 5, 2300053, https://doi.org/10.1002/ansa.202300053, 2024.

Havranek, R. E., Snell, K., Kopf, S., Davidheiser-Kroll, B., Morris, V., and Vaughn, B.: Technical note: lessons from and best practices for the deployment of the Soil Water Isotope Storage System, Hydrology and Earth System Sciences, 27, 2951–2971, https://doi.org/10.5194/hess-27-2951-2023, 2023.

Havranek, R. E., Snell, K. E., Davidheiser-Kroll, B., Bowen, G. J., & Vaughn, B. (2020). The Soil Water Isotope Storage System (SWISS): An integrated soil water vapor sampling and multiport storage system for stable isotope geochemistry. Rapid Communications in Mass Spectrometry, 34(12), e8783. https://doi.org/10.1002/rcm.8783

Herbstritt, B., Gralher, B., Seeger, S., Rinderer, M., and Weiler, M.: Technical note: discrete in situ vapor sampling for subsequent lab-based water stable isotope analysis, Hydrology and Earth System Sciences, 27, 3701–3718, https://doi.org/10.5194/hess-27-3701-2023, 2023.

Horita, J. and Wesolowski, D. J.: Liquid–vapor fractionation of oxygen and hydrogen isotopes of water from the freezing to the critical temperature, Geochimica et Cosmochimica Acta, 58, 3425–3437, https://doi.org/10.1016/0016-7037(94)90096-5, 1994.

Horita, J., Rozanski, K., and Cohen, S.: Isotope effects in the evaporation of water: a status report of the Craig–Gordon model, Isotopes in Environmental and Health Studies, 44, 23–49, https://doi.org/10.1080/10256010801887174, 2008.

Koeniger, P., Marshall, J. D., Link, T., and Mulch, A.: An inexpensive, fast, and reliable method for vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry, Rapid Communications in Mass Spectrometry, 20, https://doi.org/10.1002/rcm.5198, 2011.

Kübert, A., Paulus, S., Dahlmann, A., Werner, C., Rothfuss, Y., Orlowski, N., and Dubbert, M.: Water stable isotopes in ecohydrological field research: comparison between in situ and destructive monitoring methods to determine soil water isotopic signatures, Frontiers in Plant Science, 11, 387, https://doi.org/10.3389/fpls.2020.00387, 2020.

Kühnhammer, K., Dahlmann, A., Iraheta, A., Gerchow, M., Birkel, C., Marshall, J. D., and Beyer, M.: Continuous in situ measurements of water stable isotopes in soils, tree trunk and root xylem: field approval, Rapid Communications in Mass Spectrometry, 36, e9232, https://doi.org/10.1002/rcm.9232, 2022.

Lamb, M., Horita, J., and Arakawa, A.: Measurement of equilibrium fractionation factor of HDO/H_2O at temperatures between −86 and +5 °C: implications for Antarctic ice core records, Isotopes in Environmental and Health Studies, 54, 615–627, https://doi.org/10.1080/10256016.2018.1435533, 2017.

Magh, R.-K., Gralher, B., Herbstritt, B., Kübert, A., Lim, H., Lundmark, T., and Marshall, J. D.: Technical note: conservative storage of water vapour – practical in situ sampling of stable isotopes in tree stems, Hydrology and Earth System Sciences, 26, 3573–3587, https://doi.org/10.5194/hess-26-3573-2022, 2022.

Mennekes, D., Rinderer, M., Seeger, S., & Orlowski, N. (2021). Ecohydrological travel times derived from in situ stable water isotope measurements in trees during a semi-controlled pot experiment. Hydrology and Earth System Sciences, 25(8), 4513-4530. https://doi.org/10.5194/hess-25-4513-2021

Orlowski, N., Breuer, L., Angeli, N., Boeckx, P., Brumbt, C., Cook, C. S., Dubbert, M., Dyckmans, J., Gallagher, B., Gralher, B., Herbstritt, B., Hervé-Fernández, P., Hissler, C., Koeniger, P., Legout, A., Macdonald, C. J., Oyarzún, C., Redelstein, R.,

Seidler, C., … McDonnell, J. J. (2018). Inter-laboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water. Hydrology and Earth System Sciences, 22(7), 3619-3637. https://doi.org/10.5194/hess-22-3619-2018

Orlowski, N., Rinderer, M., Dubbert, M., Ceperley, N., Hrachowitz, M., Gessler, A., Rothfuss, Y., Sprenger, M., Heidbüchel, I., Kübert, A., Beyer, M., Zuecco, G., and McCarter, C.: Challenges in studying water fluxes within the soil–plant–atmosphere continuum: a tracer-based perspective on pathways to progress, Science of the Total Environment, 881, 163510, https://doi.org/10.1016/j.scitotenv.2023.163510, 2023.

Penna, D., Hopp, L., Scandellari, F., Allen, S. T., Benettin, P., Beyer, M., Geris, J., Klaus, J., Marshall, J. D., Schwendenmann, L., Volkmann, T. H. M., von Freyberg, J., Amin, A., Ceperley, N., Engel, M., Frentress, J., Giambastiani, Y., McDonnell, J. J., Zuecco, G., and Kirchner, J. W.: Ideas and perspectives: tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes – challenges and opportunities from an interdisciplinary perspective, Biogeosciences, 15, 6399–6415, https://doi.org/10.5194/bg-15-6399-2018, 2018.

Rothfuss, Y. and Javaux, M.: Reviews and syntheses: isotopic approaches to quantify root water uptake: a review and comparison of methods, Biogeosciences, 14, 2199–2224, https://doi.org/10.5194/bg-14-2199-2017, 2017.

Rothfuss, Y., Quade, M., Brüggemann, N., Graf, A., Vereecken, H., and Dubbert, M.: Reviews and syntheses: gaining insights into evapotranspiration partitioning with novel isotopic monitoring methods, Biogeosciences, 18, 3701–3732, https://doi.org/10.5194/bg-18-3732-2021, 2021.

Seeger, S., & Weiler, M. (2023). Dye-tracer-aided investigation of xylem water transport velocity distributions. Hydrology and Earth System Sciences, 27(18), 3393-3404. https://doi.org/10.5194/hess-27-3393-2023

Simonin, K. A., Roddy, A. B., Link, P., Apodaca, R., Tu, K. P., Hu, J., Dawson, T. E., and Barbour, M. M.: Isotopic composition of transpiration and rates of change in leaf water isotopologue storage in response to environmental variables, Plant, Cell and Environment, 36, 2190–2206, https://doi.org/10.1111/pce.12129, 2013.

Sprenger, M., Leistert, H., Gimbel, K., and Weiler, M.: Illuminating hydrological processes at the soil–vegetation–atmosphere interface with water stable isotopes, Reviews of Geophysics, 54, 674–704, https://doi.org/10.1002/2015RG000515, 2016.

Sturm, P. and Knohl, A.: Water vapor δ^2H and $\delta^{18}O$ measurements using off-axis integrated cavity output spectroscopy, Atmospheric Measurement Techniques, 3, 67–77, https://doi.org/10.5194/amt-3-67-2010, 2010.

Tetzlaff, D., Smith, A., Kleine, L., Daempfling, H., Freymueller, J., and Soulsby, C.: Integrated ecohydrological hydrometric and stable water isotope data of a drought-sensitive mixed land use lowland catchment, Earth System Science Data, 15, 1543–1554, https://doi.org/10.5194/essd-15-1543-2023, 2023.

Van Duren, M.: Investigation on effects of bottle material on water evaporation and isotopic changes in δ^2H and $\delta^{18}O$ during sample storage, International Symposium on Quality Assurance for Analytical Methods in Isotope Hydrology, 85–87, https://inis.iaea.org/records/36330-9kf23, 2004.

Volkmann, T. H. M., Haberer, K., Gessler, A., and Weiler, M.: High-resolution isotope measurements resolve rapid ecohydrological dynamics at the soil–plant interface, New Phytologist, 210, 839–849, https://doi.org/10.1111/nph.13868, 2016.

Wei, Z., Yoshimura, K., Okazaki, A., and Yamamoto, A.: Stability of isotopic composition in stored vapor samples: implications for field measurements, Hydrology and Earth System Sciences, 26, 3573–3584, https://doi.org/10.5194/hess-26-3573-2022, 2022.

Wen, M., He, D., Li, M., Ren, R., Jin, J., and Si, B.: Causes and factors of cryogenic extraction biases on isotopes of xylem water, Water Resources Research, 58, e2022WR032182, https://doi.org/10.1029/2022WR032182, 2022.

Wen, X., Lee, X., and Xiao, W.: Simultaneous measurement of water vapor δD and $\delta^{18}O$ using TDLAS: field deployment and application, Journal of Hydrology, 349, 489–500, https://doi.org/10.1016/j.jhydrol.2007.11.021, 2008.

Weng, Y., Hörhold, M., and Ritter, A.: Memory effects in isotopic water vapor sampling tubes: material comparison and equilibration times, Atmospheric Measurement Techniques, 17, 6193–6205, https://doi.org/10.5194/amt-17-6193-2024, 2024.

Zhang, B., Xu, Q., Gao, D., Wang, T., Xu, W., Huang, J., and Zuo, H.: Ecohydrological separation between tree xylem water and groundwater: insights from two types of forests in subtropical China, Plant and Soil, 480, 625–635, https://doi.org/10.1007/s11104-022-05607-x, 2022.