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

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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 bags, and 500 mL Aluminum 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}). 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 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 $\delta^{2}H$). In the detailed testing of the most suitable container type - the infusion glass bottles – 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 have been used as tools to understand hydrological processes in ecological 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 are essential to 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, which requires the extraction of material from forests and the combination of different techniques such as cryogenic vacuum extraction, centrifugation or others (Orlowski et al., 2018; Kübert et al., 2020). This technique has been questioned due to its impact on effects 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, it continues to be widely used in studies to monitor hydrological processes in ecosystems (Barbeta et al., 2022; Koeniger et al., 2011).

To obtain continuous measurements, 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 limitations that constrain its deployment in challenging environments (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 extractions (Magh et al., 2022; Gralher et al., 2021; Kübert et al., 2020; Galewsky et al., 2016). Water vapor sampling refers to the collection of isotopically equilibrated water vapor 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 instead of being sent to the laser spectrometer. Water vapor sampling is more flexible (not limited to a small number of trees compared to true *in situ*), more cost-effective (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 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 validated it with results showing 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 condition 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 select the optimal sampling method for analyzing isotopes in water vapor, several different experiments were carried out in the laboratory, as shown in Fig. 1. The study was conducted in two consecutive phases. In phase 1 we tested different storage containers in two different experiments (subsequently referred to as experiments 1 and 2). We evaluated three different sample storage containers: 250 mL glass bottles, 500 mL Aluminum-zip bags, and 1 L FlexFoil bags. In experiment 1 we simply filled these with dry synthetic air at three different storage temperatures (4, 20–25, and 40 °C) and at three storage durations before measurement (6 hours, 1 day, and 3 days) in order to evaluate hermeticity (i.e., the container's airtightness). In experiment 2 we used isotopic references (standards) and filled the sampling containers with equilibrated water vapor. We then repeated the procedure as in experiment 1 in order to assess isotopic stability. For the final decision on the most suitable sampling container for water vapor analysis, we defined six decision criteria (Fig. 1).

Using the most suitable container, we carried out two more experiments (experiments 3 and 4) in Phase 2. Here, we expanded the parameters to include 7-day storage, tested two syringe sizes and 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 assurance of isotopic equilibrium. The technical details are provided in the following sections.

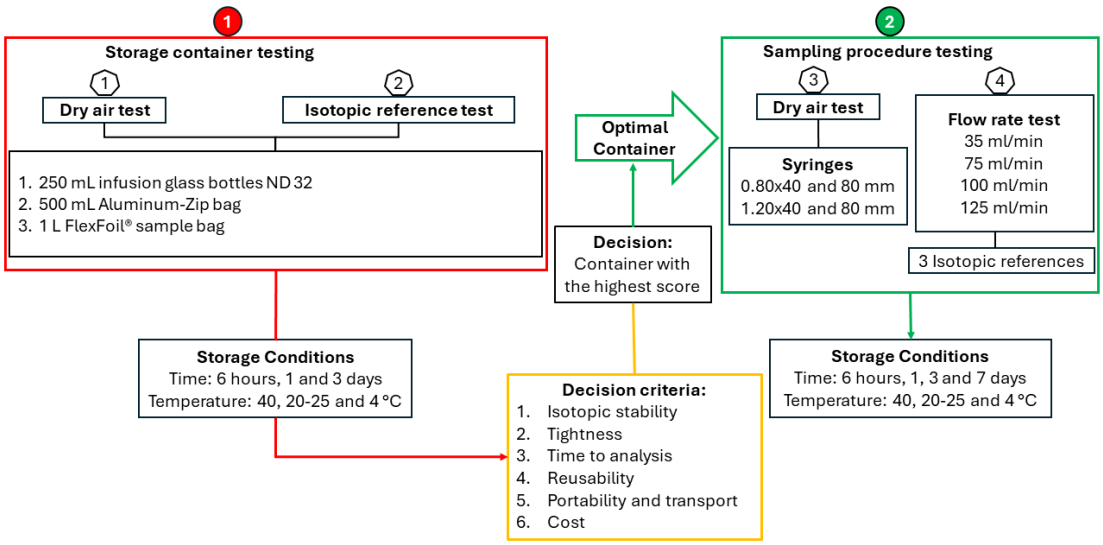


Figure 1. Illustration of the experimental design for evaluating water vapor storage and sampling. Phase 1 (red box) was dedicated to testing storage containers, and Phase 2 (green box), focused on optimizing the sampling procedure. Gray hexagons indicate the four experiments carried out: [1] dry air 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 (orange box).

The methodological framework outlined in Figure. 1 was implemented using specific setups designed for water vapor sampling and analysis with a CRDS isotope analyzer, as detailed in Fig. 2.

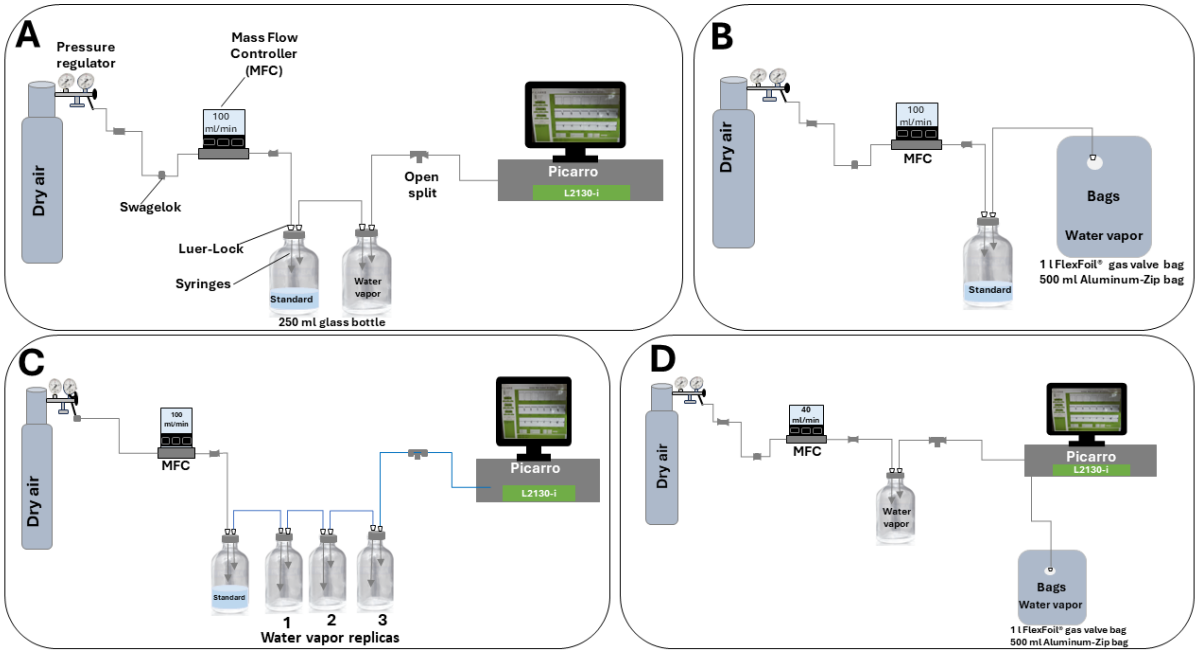


Figure 2. Technical implementation of the experiments. The schemes show the technical components used throughout the experimental stages: (A) setup for continuous generation of reference water vapor from a liquid isotopic standard and direct analysis via laser spectroscopy (Picarro L2130-i); (B) configuration for filling 1 L FlexFoil gas valve bags and 500 mL Aluminum-zip storage bags with reference vapor; (C) system for simultaneous filling of three 250 mL 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 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 their ability to maintain the isotopic stability of water vapor (Fig. 1). To this end, two tests were carried out: one with dry air (experiment 1) and the other with a reference with known isotope values (experiment 2).

The dry air and water vapor samples were subjected to three temperature conditions. Two of these were controlled and maintained constant temperatures: a Fridge at 4°C and an Oven at 40°C. The third condition – room temperature –, where the temperature fluctuated between 20 and 25 °C, was uncontrolled and corresponded to the normal ambient temperature of our laboratory. Three storage times were tested for each temperature condition: 6 hours, 1 and 3 days.

2.2.1 Experiment 1 - Tightness of the containers

Before the experiment several preparatory measures were taken. The 250 mL 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 rinsed 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 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 volatile organic compounds. 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 amass flow controller. The air was passed through PTFE tubing (outer diameter: 1/8") connected to a special syringe via 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, the 250 mL 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 (was prepared in five replicates).

The 500 mL Aluminum-zip bags were filled in six minutes under the same flow conditions by inserting the syringe directly into the silicone septum of the bag. For the 1 L FlexFoil 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 (was prepared in five replicates).

2.2.2 Experiment 2 -Isotopic reference test

The same sampling configuration and container types were used as described above. In this experiment, the key difference was the use of saturated water vapor from a known isotopic reference to rinse the containers. Each container type (250 mL glass bottle, 1 L FlexFoil 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 was chosen at 100 mL min^{-1} for all containers. The sampling time was 12 minutes for glass bottles and 6 minutes for bags. 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. Decision criteria (Fig. 1) to ensure objectively, in total six evaluation criteria were defined. These were divided into critical (criteria 1-3) and non-critical (criteria 4-6) categories. The 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) time until analysis; 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%), time to analysis (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 container identified as optima in 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 isotopic 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 stored simultaneously but measured only at their respective storage times. This approach also confirmed the importance of avoiding excessively long delays between sampling and measurement.

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) and four storage times: 6 hours, 1 day, 3 and 7 days. In experiment 4, the experimental conditions included four sampling flow rates (35, 75, 100 and 125 mL min^{-1}) and three isotope references (Table 1) for water vapor sampling. The series started at 35 mL min^{-1}, which corresponds roughly to the operating flow rate of our CDRS isotope analyzer. Water vapor samples were collected from three isotopic references using four different flow rates. The vapor samples were stored for 6 hours, 1 day, 3 days, and 7 days at different temperatures: Oven, Room, and Fridge (Fig. 1).

The purpose of this phase was to determine the most reliable combination of syringe size and sampling flow rate that would ensure isotopic precision under the experimental conditions of temperature and storage time.

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 - 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 for the glass bottles. Two syringe sizes (0.80 x 40 mm, 80 mm, and 1.20 x 40 mm, 80 mm) were used to assess whether the opening created in the septum affected container tightness and sampling flow rate. Tests were conducted using 250 mL glass bottles previously filled with dry air. After sampling with both syringes, the bottles containing air samples were stored under different temperature and storage time conditions as part of the sampling procedure tests. 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 using a test with known isotope concentrations. Three reference standards were used: ALBI1 (enriched in deuterium oxide), BS (intermediate isotopic composition), and KEI (light isotopic composition).

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 conditions in the laboratory (Fig. 1). Sampling was performed at four different flow rates: 35, 75, 100, and 125 mL min^{-1}. Each combination of standard and flow rate was repeated three times to ensure the stability of the results. The reliability of this test allows the uncertainty of the sampling method to be validated and the flow rate(s) to be identified that may have a significant impact on the accuracy of the isotope concentrations measured with water vapor. Before sampling, the 250 mL glass bottles were cleaned by placing them in an oven at 60-80 °C for 24 hours. They were then stored in a dry place (aluminum box with insulation) with a butyl stopper to prevent mixing with atmospheric moisture. They were also rinsed with a strong, dry stream of air for 1 to 2 minutes before use to ensure that the inside of the bottle was completely clean and dry. They were then sealed by placing an aluminum cap on the butyl stopper and pressing down with sealing pliers to ensure a secure and tight seal. During water vapor sampling, the temperature was monitored to ensure that it remained stable and recorded with a sensor (Sensirion AG, Sht4x Smart Gadget) that allowed a uniform temperature to be maintained throughout the laboratory. Approximately 100 ml of the reference standard was placed in a 250 mL glass bottle to ensure headspace sampling. During the sampling time, we achieved a dynamic equilibrium and the isotope exchange reached a fractionation factor defined by the temperature.

The sampling system implemented makes it possible to inject the air stream passing through the bottle containing the reference standard to equalize and exchange the isotopes in the water vapor flushing the sampling bottles. A synthetic dry air cylinder was used, which had a pressure regulator and was connected to an electronic mass flow controller. This was used to regulate or inject the air flow used for sampling. The connections were made with 1/8" PTFE tubing. The end connected to the mass flow controller had Swagelok connectors and the other had a Luer lock adapter with a large syringe inserted through the septum into the glass bottle. Then another shorter syringe was inserted to deliver the water vapor into the bottles. The three replicates were withdrawn simultaneously for 35 minutes, i.e., the water vapor flushed the three bottles at the same time, ensuring that all three bottles received the same amount of water vapor passed through tubing and syringes. The bottle with the standard injected the water vapor into the first replica, then into the second bottle, and finally into the third bottle, which was connected directly to the CRDS to measure the sample and obtain an initial reference. Before connecting to the Picarro, we performed an open split to avoid pressure on the devices. The configuration of the sampling system is shown in the diagram below (Fig. 2C).

For each of the reference standards (3), samples were taken and subjected to the procedures described in the section on experimental conditions. For each combination of time, flow rate and standards, 36 samples were taken (4 combinations). A total of 144 samples were taken per temperature reservoir, giving a total number of 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 measured during sampling. This was done to achieve a homogeneous temperature inside the bottles and to prevent condensation.

The measurements were performed 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 1 L FlexFoil 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 Picarro (see Fig. 2D).

The 250 mL 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 or 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 two minutes of each measurement were discarded due to possible memory effects in the system, as were the last two minutes, since the water vapor concentration (ppm) is decreasing over time when using stored samples which could influence the isotope values and introduces water vapor concentration effects.

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 expressed 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 standard.

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 (Oven, Room and Fridge) and container type on the stability of isotope values. Differences between groups were visualized with boxplots and statistically evaluated using non-parametric tests such as the Kruskal-Wallis test, as the data did not follow a normal distribution.

To validate the precision of the isotope results, 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 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 bags showed the least variability, with values of approximately ~100 and ~600 ppm across all storage conditions and times, with no significant increase.

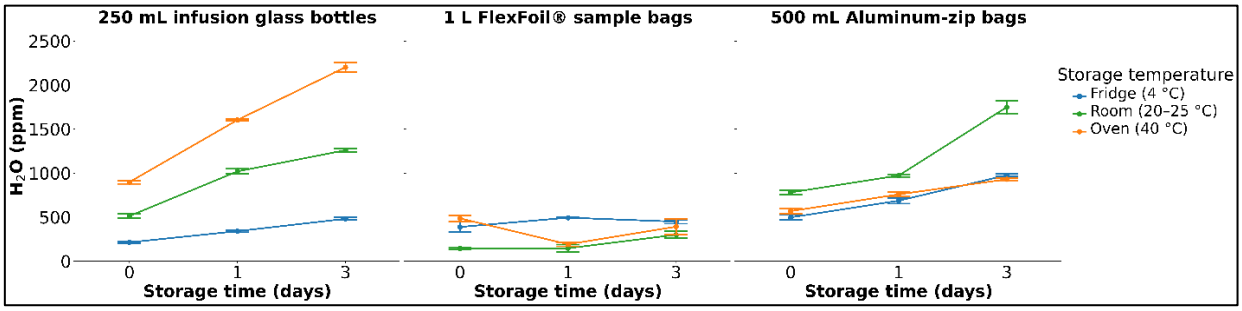


Figure 3. The graph shows the changes in water vapor concentration (H_2O, in ppm) in dry air samples after different storage times (0, 1, and 3 days) in three types of containers (250 mL infusion glass bottles, 1 L FlexFoil sample bags and 500 ml Aluminum-zip bags) and under three temperature conditions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

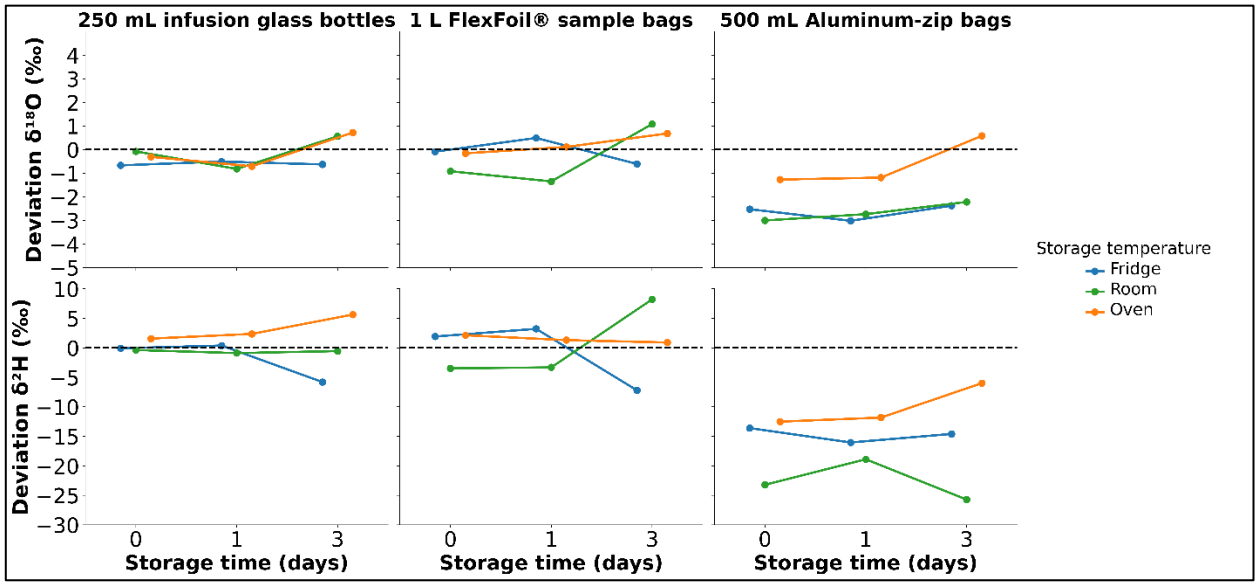
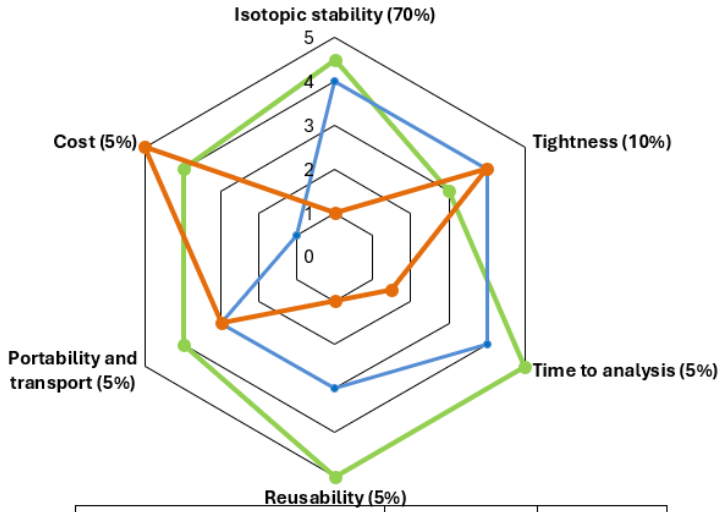


Figure 4. Isotopic deviations ($\delta^{18}O$ and $\delta^{2}H$) in water vapor samples stored in different containers (250 ml infusion glass bottles, 1 L FlexFoil sample bags, and 500 ml Aluminum-Zip bags) at three storage temperatures (Fridge, Room, Oven) for 0, 1 and 3 days. The dashed line represents 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 we carried out in order to find the optimal container.



Containers	Score	Symbology
250 ml infusion bottle with ND 32	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 time to analysis (5%). Non-critical criteria: reusability (5%), portability and transport (5%) and cost (5%).

The 250 ml glass bottles ND 32 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 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 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 for water vapor samples and the measurement of $\delta^{18}O$ and $\delta^{2}H$ isotopes. 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 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 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.

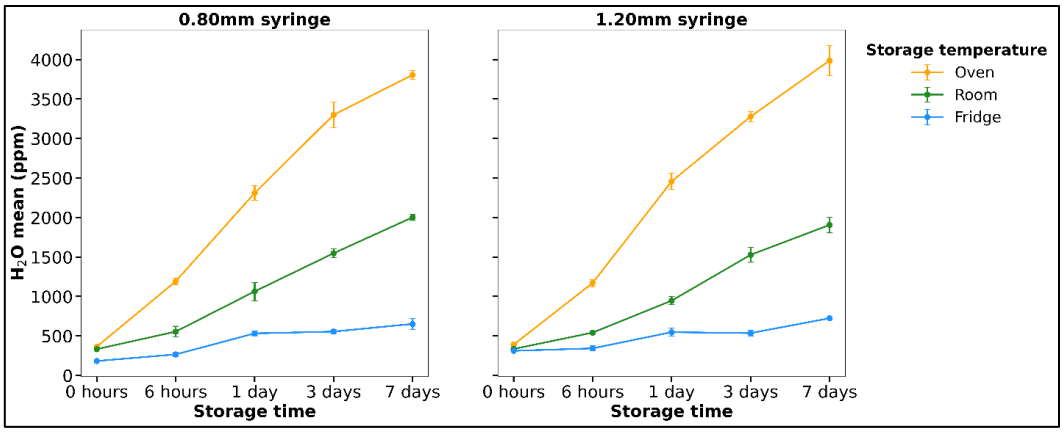


Figure 6. Absolute H_2O concentration (ppm) in synthetic dry air samples stored in 250 ml glass bottles and analyzed with small and large syringes at different temperatures and times. The initial H_2O at 0 hours was approximately 200–300 ppm; the reported values represent the total measured concentration, 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 reached a plateau at approximately 25 minutes for the 100 and 125 mL min^{-1} runs; at 75 mL min^{-1}, a plateau was observed around 31 minutes, while at 35 mL min^{-1}, the concentration (ppm) did not fully stabilize within 35 minutes. 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 standard 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 25o 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).

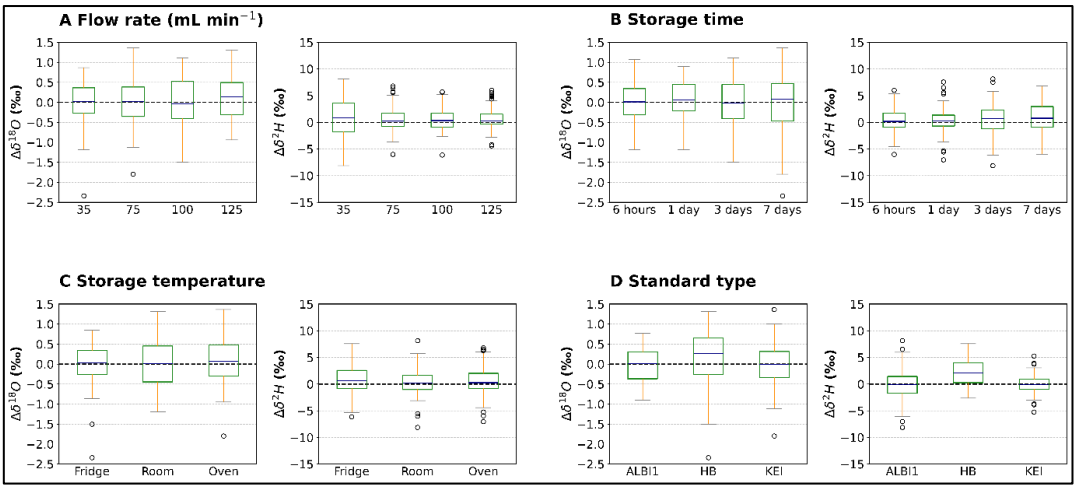


Figure 7. Isotopic deviations ($\Delta\delta^{18}O$ and $\Delta\delta^2H$) of water vapor samples under different factors: A) Flow rates (mL min^{-1}**), B) Storage time, C) Storage temperature, and D) Standard type. Boxplots represents the median (blueline), the interquartile range (green box)**

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 glass bottles (ND 32).

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.

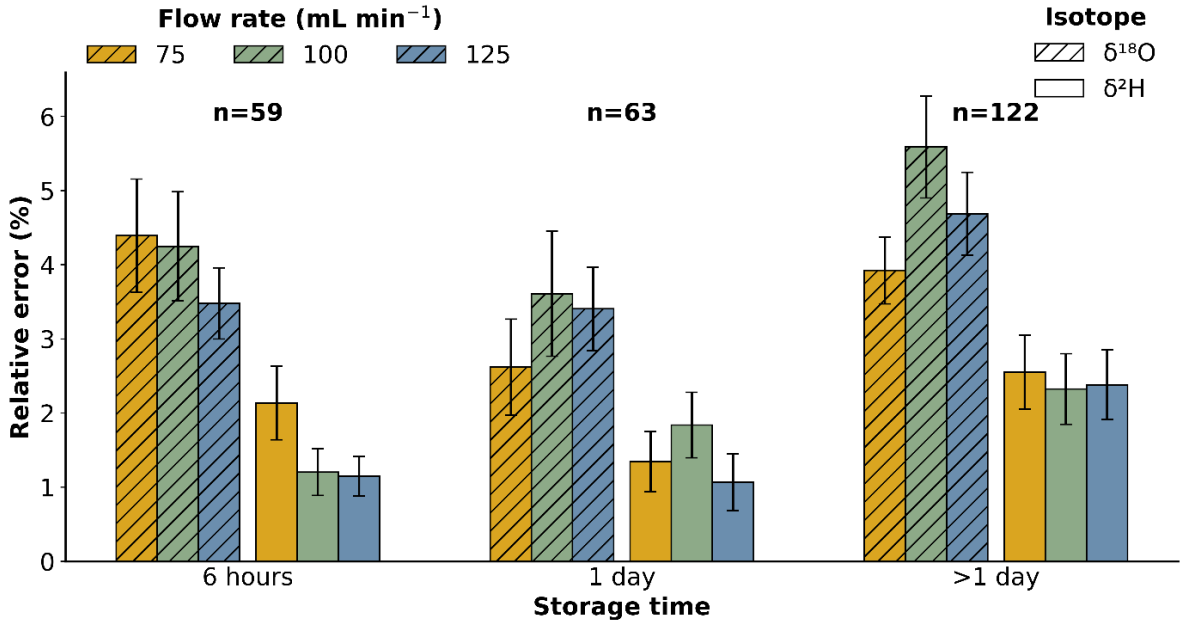


Figure 8. Relative error (%) for $\delta^{18}O$ (hatched bars) and $\delta^{2}H$ (solid bars) as a function of storage time (6 hours, 1 day, and >1 day) and sampling flow rates (75, 100, and 125 mL min^{-1}). The number of observations (*n*) for each storage category is shown above the bars. Error bars 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 glass bottles showed the largest in H_2O. 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. However, this aspect did not seem to effect water vapor isotope values substantially because in the stability test the 250 mL glass bottles performed best here. 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 bags remained the most stable system, with low variability and no significant increase across storage treatments.

To evaluate whether this small diffusive exchange affects the isotope values, we tested how well an isotopic reference could be reproduced. The 250 mL glass bottles provided the most stable values, particularly for $\delta^{18}O$; noticeable $\delta^{2}H$ deviations occurred only after 3 days of storage. While FlexFoil bags effectively limited exchange, deviations from the reference water (especially in $\delta^{2}H$) were detectable after 1 day, this indicates 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 $\delta^{2}H$ is more sensitive than $\delta^{18}O$ to storage-related effects, consistent with previous storage assessments (e.g., Magh et al., 2022). Temperature changes that promoting condensation, evaporation, and incomplete internal equilibration, along with potential isotope exchange with the bag material (Herbstritt et al., 2023), likely contributed to the greater variability in bag systems.

The combined evaluation of container tightness, vapor storage performance, and practical criteria such as analysis time, reusability, cost, portability, and transport, enabled the selection of the most suitable container for water vapor sampling. Although secondary criteria were less decisive (see Fig. 5), they supported the final ranking. The 250 mL glass bottles preserved the isotope values best when they are analyzed rather fast after sampling, could be reused after drying at 60–80 °C, and were practical for field campaigns because large numbers could be transported in insulated cases. If longer storage times are required - for instance when sampling at a remote site - we recommend to seal the needle pinches after sampling. Although this was not tested here, it is something that will much likely improve the performance of the glass bottles further.

Preservation of the original isotopic signature was the most important criterion. Accordingly, the 250 ml glass bottles achieved the highest overall score (85%) and the greatest short-term isotopic stability. Despite higher increases in internal H_2O vapor concentration during storage, isotope values remained stable, indicating that early concentration changes did not substantially affect the vapor signal. Significant $\delta^{2}H$ shifts became evident only after 3 days, mainly under cold and warm storage conditions, whereas ambient storage remained comparatively stable. Because the stored vapor originated from a fully saturated source (>20000 ppm H_2O), limited early diffusive exchange likely had little influence on isotopic composition, consistent with Magh et al. (2022). In contrast, both types of bags showed greater isotopic variability under the filling and storage conditions applied in this study.

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 bags. Therefore, the production of the aluminum bags might have an influence if they are not pre-treated.

In our study, 1 L FlexFoil bags were the second-best option, with a score of 75% (Fig. 5). They performed well in leak-tightness tests but showed greater isotopic deviations than glass bottles, especially at ambient temperature. Additionally, their higher cost and larger volume hinder transport logistics and durability when many samples are needed. Another important aspect is reuse, which 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 new. They also identified a storage time–dependent memory effect, indicating that reuse may be problematic when broad isotopic ranges or consecutive samplings are involved. This is particularly relevant tracer experiments, where cross-contamination between samples may compromise data quality.

Finally, 500 mL Aluminum-zip bags showed the largest isotopic deviations in our study and only moderate 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 here, 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 glass bottles remained the most robust option due to reusability, economic proficiency, practical transport capacity, and comparatively stable isotope fluctuation.

4.2 Protocol and optimal container for water vapor sampling and storage

Methods for measuring the stable isotopes of water are constantly being improved. Water vapor sampling is a new way to overcome reported limitations and potential biases of extraction-based methods (Kübert et al., 2020; Mennekes et al., 2021). However, it requires proper implementation and rigorous, efficient sampling procedures. To reduce uncertainties in water vapor sampling and analysis, we propose a refined method and protocol that control the factors most affecting the isotopic signature and ensure data quality. According to our results, it is essential to consider container type, flow rate, storage time, and temperature conditions; this priority has also been highlighted by other authors (Van Duren, 2004; Magh et al., 2022; Herbstritt et al., 2023; Bagheri et al., 2021; Benetti et al., 2017). 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 the importance of maintaining controlled temperature conditions to minimize measurement errors, particularly for δ^2H, due to its greater sensitivity to kinetic and diffusive processes (Horita and Wesolowski, 1994; Lamb et al., 2017; Wei et al., 2022; Weng et al., 2024; Wen et al., 2008).

The results confirmed a progressive increase in H_2O vapor concentration; 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 initiated 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, indicating that short storage time are important to minimize isotopic drift (see Table 3).

Our results regarding the 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 $\delta^{18}O$); Magh et al., (2022) indicate that measurements can be made up to 3 days later, emphasizing stability over short periods and noting an increasing bias at 7 days, consistent with our observations. In contrast, Havranek et al., (2023) implemented an automated soil water isotope storage system based on 650 ml flasks, that maintained reliable isotope values (±0.9 ‰ for $\delta^{18}O$ and ±3.7 ‰ for δ^2H) for substantially longer periods under laboratory and field conditions. This extended stability may be related to the larger bottle volume, although such systems may be less practical for handling and rapid field sampling. Regardless, analysis within the first day should be considered the most robust and conservative time window, although storage up to 3 days may still provide reliable results under controlled conditions. More prolonged storage, according to our findings, may lead to isotopic deviations, particularly under temperatures variations (4 to 40 °C), suggesting progressive vapor exchange and fractionation over time. Therefore, the system developed by Havranek et al., (2023) was 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 result further shows that $\delta^{18}O$ was consistently more stable than δ^2H and can therefore be used more reliably for ecohydrological applications. Accordingly, precise measurements require control of sampling flow rate, storage time, and temperature, with the best performance obtained under moderate to high flow rate, 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). Minimize 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 demonstrated that 250 mL glass were the most reliable system for collecting and storing vapor samples for isotope analysis, outperforming 1 L FlexFoil bags and 500 ml Aluminum-zip bags. Our findings show that the isotopic stability of water vapor is influenced by container type, 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 can still provide 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 thermal variation. This indicates that faster sample processing, preferably within 1 day, in the most robust strategy to minimize isotopic deviations. Overall, our systematic testing and developed protocol provides a framework for sampling water vapor for stable isotope analysis, which has great potential for ecohydrological research. In particular, it could serve as semi- *in situ* alternative for measuring soil and plant water isotopes when true *in situ* measurements are not feasible. It could be further used for sampling of 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 and thereby contribute to isotopic fractionation. Increasing replicate number and modestly extending 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 glass bottles by minimizing potential 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.

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