Technical note: A fast and objective autosampler for direct vapor equilibration isotope measurements

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Abstract.

To investigate water movement in environmental systems, stable water isotopes (^{2}H and ^{18}O) are commonly used tracers. Analyzing the isotopic ~~composition~~ ratios of water bonded to substances like soil or plant tissue necessitates ~~its extraction~~ extraction or equilibration of water prior to analysis. One such method, direct vapor equilibration, is popular due to its cost-effectiveness and straightforward sample processing. However, sample analysis requires ~~a~~ significant manual labor, thereby limiting the number of samples that can be analyzed. This limitation is compounded by the fact that stored samples undergo isotopic ~~composition~~ changes over time, and in addition manual measurements may lack objectivity. The operator currently has to decide subjectively if the measurements are stable, and then record the analyzer readings. To address these challenges, we have developed a ~~device~~ system that automates the analysis process. Our vapor autosampler for ~~vapour~~ vapor samples, named VapAuSa, features a modular design ~~with~~ allowing up to 350 ports for direct vapor equilibration samples. These ports sequentially connect the prepared samples to the analyzer, enabling continuous automatic measurements. Within the accompanying software, measurement criteria can be specified, facilitating objective analysis. The developed system was tested by co-measuring 90 soil samples and 21 liquid water samples of known δ-values. VapAuSa measurements have a ~~similar uncertainty~~ negligible measurement bias (δ^2H and δ^{18}O both < 0.1e-12 ‰) and similar measurement repeatability compared to manual analysis of identical samples (VapAuSa δ^2H= ~~+/-~~ ± 4.5 ‰ δ^{18}O = ~~+/-~~ ± 0.58 ‰ vs. manual δ^2H= ~~+/-~~ ± 5.7 ‰ δ^{18}O = ~~+/-~~ ± 0.37 ‰). However, the increased sample throughput minimizes storage-induced isotopic changes. Moreover, VapAuSa triples sample throughput per week while reducing direct labor time to just 10 % of manual processing.

1 Introduction

Stable water isotopes (δ^{18}O and δ^2H) have found widespread application as tracers in earth and environmental system sciences. They are applied ~~in many studies~~ to elucidate storage and redistribution processes of water in various hydrological compartments. In soils, stable water isotope analysis revealed different flow-process along hillslopes like lateral- and preferential-flow as well as mixing (Garvelmann et al., 2012; Thomas et al., 2013; Peralta-Tapia et al., 2015). They were also used to ~~obtain~~ estimate soil properties by inversely modeling soil-water isotope profiles, better representing the soil properties than traditional pedotransfer functions (Sprenger et al., 2016). In addition, the tracking of vertical infiltration using soil isotope profiles has allowed for the quantification of groundwater recharge rates (Filippini et al., 2015; Chesnaux and Stumpp, 2018; Boumaiza et al., 2020). Analyzing the ~~distribution~~ ratios of stable water isotopes in groundwater has revealed hydrogeological differences and solute transport mechanisms (Hendry and Wassenaar, 2009; Hendry et al., 2011a; Hendry and Wassenaar, 2011; Stumpp and Hendry, 2012). Moreover, the use of stable water isotopes in plants has shed light on plant water uptake, water transit times, and ~~partitioning effects~~water partitioning, providing valuable insights into which sources of water plants utilize for various purposes (Bertrand et al., 2014; Smith et al., 2020; Kuhlemann et al., 2020).

~~While most isotope analyzers are build to measure the isotopic composition of liquid water , extracting water~~ To measure the stable water isotope ratios of water bound to substances or tissues, such as soil, sediment or plant material, ~~is still not directly possible. The most popular extraction methods are~~ an extraction or equilibration to vapor is needed. One such method is direct vapor equilibration laser spectroscopy (DVE-LS) (Wassenaar et al., 2008)~~and cryogenic vacuum distillation (CVD). For CVD the sample is heated inside a vial so all water evaporates. Next, another vial, which is cooled and contains a vacuum, is connected to the first. This causes the vapor to transfer to the cooled vial and condense (Ingraham and Shadel, 1992). The condensate can then be analysed.~~

~~By contrast, in~~ . In DVE-LS, the water ~~vapour of a samples~~ vapor of a sample is measured and recalculated to its liquid ~~composition~~ isotope ratios using calibration standards. The process works as follows: (1) The sample (soil, plant, etc.) is placed in an inflatable, sealable and ~~diffusion tight bag~~ gas diffusion-tight bag (Pratt et al., 2016). Many different bag-materials ~~are~~ have been used, however most are insufficient due to diffusive losses. These losses can be minimized with aluminum laminated bags (Gralher et al., 2021).~~(2)~~ Regarding identical treatment of calibration standards (Werner and Brand, 2001), liquid water of known isotopic composition is also filled into identical bags. (~~3~~2) The sample and calibration standard bags are inflated with dry air and ~~heat~~ sealed. Then, a silicone blot is added to each bag as a septum, needed for airtight measurements later. (~~4~~3) Samples are then stored under constant climatic conditions (~~climatized~~ ideally in a air-conditioned room where they are ~~also~~ later analyzed) so liquid and gas phase within the bags can ~~isotopically equilibrate~~reach isotopic equilibrium (Wassenaar et al., 2008). Equilibration time varies between studies but for aluminum laminated bags, 48 hours are optimal for soil samples (Gralher et al., 2021). (~~5~~4) After equilibration, the vapor in the bag's headspace is analyzed using off-axis integrated cavity output spectroscopy (OA-ICOS) or cavity ring-down spectroscopy (CRDS). To facilitate this analysis, a cannula connected to the analyzer's inlet port is inserted into the ~~septum. (6~~silicone septum of the bag. (5) After analysis, the measurements are referenced to the VSMOW-SLAP scale using the co-measured calibration standards ~~(Craig, 1961)~~(Craig, 1961; Pratt et al., 2016).

Advantages of DVE-LS compared to other extraction methods include low technical effort and minimal material requirements, making it a cost-effective option (Millar et al., 2018). Additionally, only a small sample ~~volumesimilar to that of CVD~~ volume is necessary, allowing for high spatial and temporal resolution sampling (Wassenaar et al., 2008; Garvelmann et al., 2012). Furthermore, minimal handling is required, reducing the risk of sample damage (Wassenaar et al., 2008). Due to these favorable attributes, DVE-LS finds application in various contexts. For instance, all ~~the~~ findings presented in the first paragraph were obtained through the application of DVE-LS. The ~~popularity~~ wide application of DVE-LS led to numerous methodological improvements, most focused around container material, equilibration time (Gralher et al., 2021)~~and~~ , the correction of

~~changes in~~ variations of the carrier gases (Gralher et al., 2016, 2018) or co-extracted substances which interfere with the laser spectrometer analysis ~~(Hendry et al., 2011b; Nehemy et al., 2019)~~(Hendry et al., 2011b).

However, the method still lacks automation in the analysis process. Manual measurement of each sample is time-consuming, imposing limitations on ~~large-scale~~ high number sampling, as sample storage can alter the isotopic signature (Gralher et al., 2021). Moreover, the current DVE-LS analysis routine lacks objectivity. There is no standardization for analysis time and measurement stability criteria, leading to potential variations in results among different labs and operators analyzing the same sample (?Ceperley et al., 2024). To address these challenges and enhance both the speed and objectivity of the analysis, we introduce our fully autonomous vapor sampler system, named "VapAuSa". This innovative system enables high-throughput sample processing with significantly reduced manual labor compared to the prevailing procedure.

2 Design

This section provides a summary of the sampler design. More in-depth information such as the list of materials, technical drawings, circuit diagram, code, and manuals, necessary for building the VapAuSa, can be found in the Supplement and on https://gitlab.rz.uni-freiburg.de/hydrology/vapausa~~(last access: 11 July 2023)~~.

2.1 Hardware

The VapAuSa features a modular design comprising standalone boxes ~~that can also~~ holding the sample bags that can be combined to larger sample ~~units~~setups. Each box is equipped with 24 ports, of which 23 are designated for sample bags, and ~~1~~ one is reserved for flushing the system with ambient air to prevent moisture ~~buildup~~build-up and thus memory effects. The ports consist of valves (Model E3O10A-1W024; Clippard) which regulate vapor flow to the cavity of the ring-down isotope analyzer (in our case a L2130-*i*, Picarro Inc.). The valves are attached to CNC-cut aluminum valve-blocks organized in groups of eight and interconnected by 1/8 inch PTFE tubing (Figure 1). ~~For~~ The 23 sample bags are connected with cannulas (2.1 mm diameter * 80 mm, B.Braun STERICAN) to the valves through 1/8 inch PTFE tubing. The tubes are secured by super glue (LOCTITE 406 and 770, Henkel Adhesives) into the cannula and linked to the valve block via flangeless fittings (XP-301X; IDEX) (see Figure 1). We experimented with using 1/16 inch tubes and a gas-drying cartridge at the flushing valve, but this did not yield improved measurements.

2.2 Circuit board, electronic components and Firmware

The first box of each VapAuSa is controlled by the primary module, while all subsequent boxes are controlled by extension modules. We have designed a circuit board that ~~, depending on the equipped electronic components,~~ can be used to build either of these two configurations, depending on the electronic components installed. The primary and extension modules share most parts but are equipped with different types of micro-controllers and connectors. This distinction arises because only the primary module ~~is the only one directly~~ can directly be connected to the power supply and to the Picarro. Each extension module is equipped with a DIP-switch that can be used to assign a unique address between 1 and 16 (the primary module is assigned

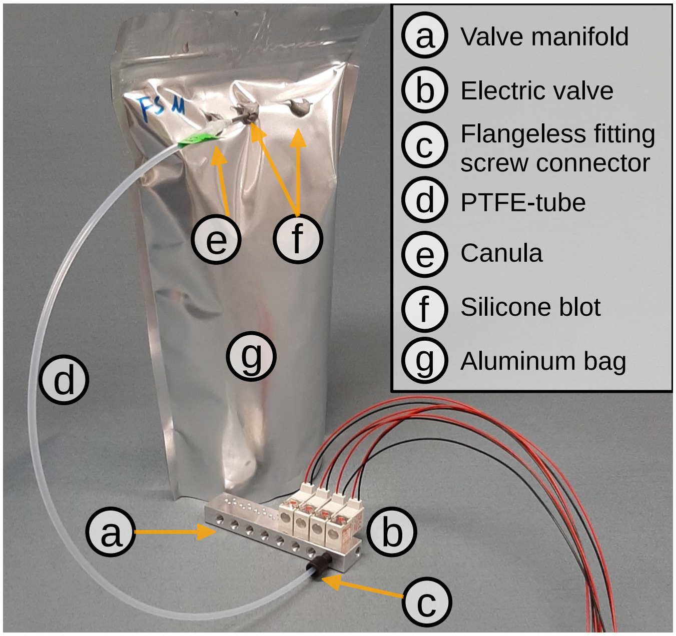

Figure 1. Basic setup of the VapAuSa: A cannula ⓔ is superglued onto a PTFE-tube ⓓ, which is connected to a CNC-cut valve manifold ⓐ via a flangeless fitting connector (XP-301X; IDEX) ⓒ. In this example image, only four of the eight positions on the valve manifold are equipped with electric valves (E3O10A-1W024; Clippard) ⓑ. The cannula is inserted to the laminated aluminum bag ⓖ via a silicone blot ⓕ, which acts as a seal after the bag was been pierced by the cannula.

address 0). In total, this allows for up to 17 boxes in one VapAuSa, capable of accommodating 391 samples simultaneously. The primary module and all extension modules can be daisy-chained one after another, with communication ~~between~~ among them facilitated through a RS485-bus system. Communication and power supply for all extensions are achieved using off-the-shelf Ethernet cables.

A detailed part list for the required electronic components to equip the circuit boards can be found in the appendix of this paper. Circuit boards schematics (created with Fritzing) and the firmware for the micro-controllers of primary board and extensions can be found under https://gitlab.rz.uni-freiburg.de/hydrology/vapausa.

2.3 Software

The software used for the VapAuSa is based on software that was developed for in-situ stable water isotope sampling (Seeger & Weiler, 2021). It was designed to operate directly on a Piccaro stable water isotope analyser (model ~~L2120-i, L2130-i or L2140-i~~L2120-*i*, L2130-*i* or L2140-*i*) and consists of a collection of modular Python3 (Python-Software-Foundation, 2022) scripts whose purpose is to fulfil two main tasks:

a) Monitoring the measurements: By frequently reading out and parsing the instrument's log-files, the software is aware of the currently measured values of sample gas volumetric vapor content (H_2O), δ^{18}O and δ^{2}H (see ④ in ~~Fig.~~ Figure 4). Through a

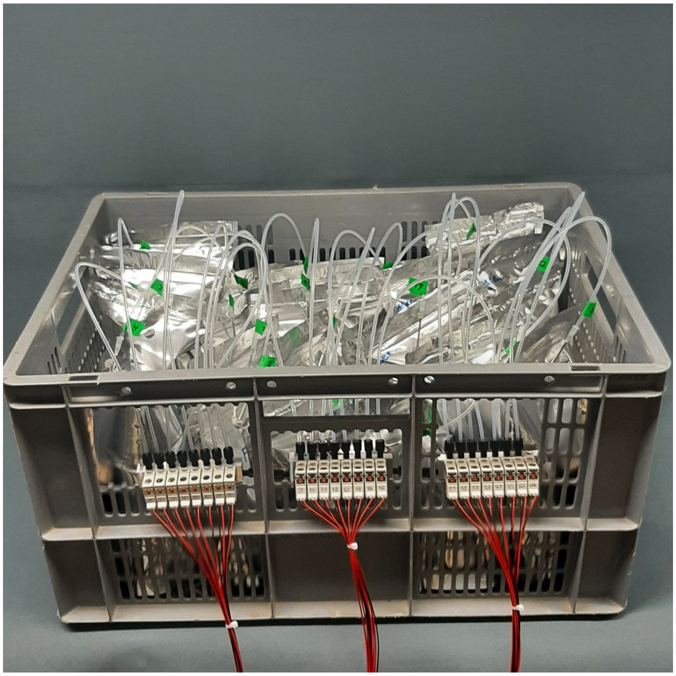

Figure 2. VapAuSa standalone box holding the 23 sample bags with the 24 valves~~and 23 bag ports~~

configuration file, the user can define stability criteria, specifying trends and standard deviations of the mentioned measurement parameters over a user-defined time span (⑤ in ~~Fig.~~ Figure 4). This establishes an objective metric for the automated detection of stable plateaus during measurements. This module can operate independently and is potentially suitable for manual DVE-LS measurements, since it provides instant one-click summary statistics for ~~freely-selectable~~ user-selectable time spans. In contrast, the standard Picarro analyzer GUI often requires tedious zooming in and out to achieve the same purpose.

b) Controlling the valves and sampling: Using another configuration file, the user can assign custom names (sample IDs) to specific valve slots. Each valve slot is uniquely ~~adressed~~ addressed by a combination of boxID and slotID (e.g., `2#5` addresses the fifth valve slot of the second extension box). The user has the flexibility to set a maximum sampling time for each valve and determine whether sampling may conclude before that time if all stability criteria are met. Additionally, the user can establish a custom sequence for all defined valve slots, enabling repeated measurements from specific slots (e.g., standards) within a single sequence. ~~Within~~ During an active sequence, each measurement phase is preceded by a ~~flush~~ flushing phase during which the valve block of the currently active slot is flushed with ambient air until a ~~customizable~~ defined H_2O ~~value~~ concentration (ppm in the analysis chamber) is undershot or a maximum flush time has been reached. Upon running the main script, a graphical user interface (GUI) ~~is generated, visualizing~~ visualizes the recent measurements (④ in ~~Fig.~~ Figure 4). This GUI also allows the user to manually open certain valves by clicking on the corresponding buttons (① in ~~Fig.~~ Figure 4). The GUI automatically starts the predefined sequence (③ in ~~Fig.~~ Figure 4) and generates a log file that documents each valve switch and the reason why it occurred (timeout, fulfillment of all stability criteria, or manual click). By combining the analyzer log file with the valve

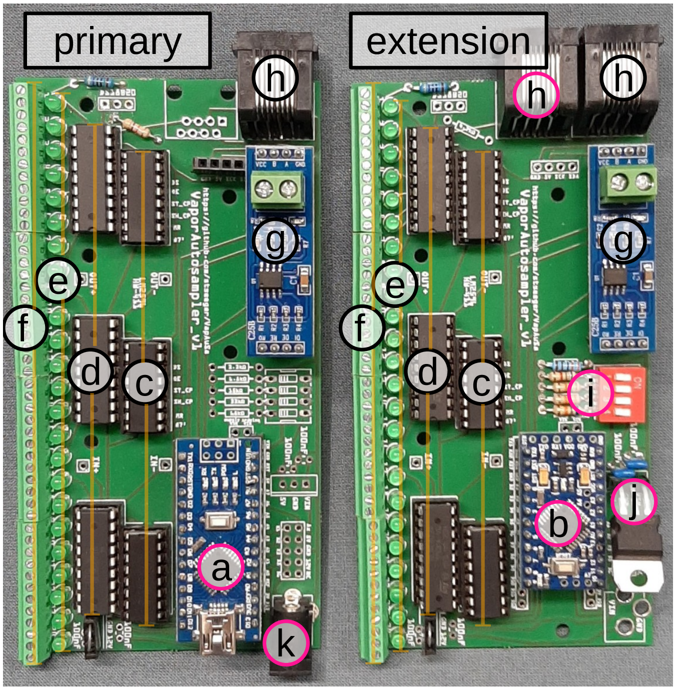

Figure 3. VapAuSa primary module (left) and an extension module (right). Based on identical circuit boards, differences in equipped components (pink circles) lead to the two kinds of modules used in the VapAuSa sytsem. ⓐ Arduino Nano, ⓑ Arduino ProMini, ⓒ Shift-Register, ⓓ ULN2803 TransisorArray, ⓔ Status LEDs, ⓕ Screw terminals, ⓖ RS485-Max communication module, ⓗ Ethernet port, ⓘ Address selection DIP-Switch, ⓙ 5V voltage regulator, ⓚ 12VDC power jack.

log file, it is possible to automatically aggregate and process the relevant parts of the analyzer's measurements. The software identifies a connected VapAuSa primary module by scanning all available Serial-COM ports and then sends the appropriate `valve boxID#slotID` commands to the primary module (firmware described in Sec. 2.2) in response to user inputs or progression through the predefined sequence.

An example call of the `valve` command might be `valve 2#5`, which means that the fifth valve of the second extension box should be opened. The receiving primary module would relay this command to all connected extensions. Subsequently, all boxes that are not the second extension would close all of their valves and the second extension would open its fifth valve.

3 Proof of Concept

3.1 Soil Water Test~~SetupWe analyzed the accuracy of the VapAuSa by measuring different~~

To evaluate the effectiveness of the VapAuSa for soil samples, we conducted a comparative study involving both manual and autosampler measurements of identical soil sampling bags. We drilled 11 soil cores to refusal depth using an electrical auger (Makita HM1810), resulting in a total of 90 soil samples of different depths. These samples were then placed into

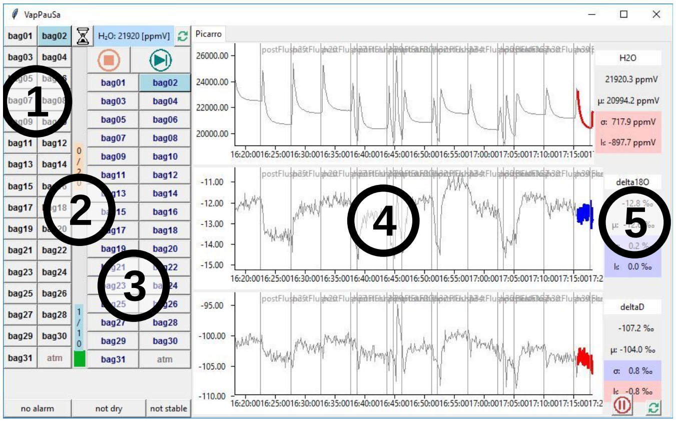

Figure 4. Screenshot of the VapAuSa GUI. ① Buttons for all valves defined in the configuration file, ② Flush and measurement progress bars for currently active valve, ③ Buttons for valve sequence, ④ Recent measurement values for H_2O, δ^{18}O and δ^2H, ⑤ ~~Fulfilment~~ Fulfillment of stability criteria (red=not fulfilled, blue=fulfilled) for H_2O, δ^{18}O and δ^2H

Table 1. Stability criteria for the VapAuSa measurements. Only if standard deviation (sd) and trend are all below the given thresholds within evaluation time (eval. time), VapAuSa switches to the next sample.

	sd	trend [unit/eval. time]	eval. time [s]
H_2O [ppmv]	100.0	150.00	120
δ^{18}O [‰]	0.2	0.08	120
δ^2H [‰]	0.7	0.30	120

aluminum-laminated plastic bags (WEBER Packing GmbH; CB400-420BRZ; 500 ml) and initially sealed with a ziplock. Afterwards, the bags were inflated with dry air, heat-sealed, and equipped with two silicone blots to ensure each measurement started with a "fresh" septum. The prepared bags were then stored in a climate-controlled room maintained at 20°C ± 1°C for 48 hours. All measurements were performed on cavity ring-down spectrometers (L2120-*i* and L2130-*i*, Picarro Inc.). The bags were punctured with a cannula and connected to the analyzers. Standards of known isotopic ratios were co-measured to calibrate the results. To compare the measurement methods, 12 bags were measured manually first and then by the autosampler, while the remaining were measured by the autosampler first and then manually. The VapAuSa was programmed to activate valves for 10 minutes each, with 5 minutes of system flushing between samples. Stability criteria were defined as shown in table 1, which represent the threshold after which the VapAuSa switches to the next sample. To assess the measurements, we calculated the difference for each bag by subtracting the calibrated δ values measured by VapAuSa from the calibrated *delta* values measured manually.

3.2 Liquid Water Test

Since the real isotopic ratios of soil water is currently impossible to determine (Koeniger et al., 2011; Orlowski et al., 2013, 2016; Gaj et al. , we assessed the measurement repeatability and bias of the VapAuSa by measuring different liquid DVE-LS samples. The test samples were created according to the suggested protocol by Wassenaar et al. (2008) and Gralher et al. (2021). Seven samples (10 ml each) of three isotopically distinct water sources were filled into 1 L aluminum laminated plastic bags. Subsequently, we inflated the bags with dry air, heat-sealed and equipped them with silicone septa. Then the bags were placed in a climate controlled room (20°C ~~+/-~~ ± 1°C) for 48 hours. Following this period, we randomly distributed the samples in the VapAuSa to minimize possible memory effects. To connect the bags to the isotope analyzer (cavity ring-down; ~~L2130i~~L2130-*i*, Picarro Inc.), we inserted the cannulas into the septa, puncturing the bags and ~~ensuring airtight vapor flow~~ enabling vapor flow to the analyzer. The VapAuSa was programmed ~~to activate valves for 10 minutes each, with 5 minutes of system flushing between samples. Stability criteria were defined as~~ identical to the soil water tests with the stability criteria shown in table 1.

~~Stability criteria for the VapAuSa measurements. Only if standard deviation (sd) an trend are all below the given thresholds within evaluation time (eval. time), VapAuSa switches to the next sample.~~ ~~H_2O 100.0 150.00 120 δ^{18}O 0.2 0.08 120 δ^{2}H 0.7 0.30 120~~

To test a wide isotopic range we selected water samples ~~of 3 different signatures: heavy~~ with three distinct signatures: relatively high δ values (sea-water, δ^{18}O = 0.7 ± 0.035 ‰ δ^{2}H = 0.3 ± 0.256 ‰), medium δ values(tap-water of Freiburg, δ^{18}O = -9.3 ± 0.035 ‰ δ^{2}H = -64.3 ± 0.256 ‰) and ~~light~~ low δ values (snow-melt water, δ^{18}O = -16.6 ± 0.035 ‰ δ^{2}H = -125.8 ± 0.256 ‰). These ~~compositions were gained~~ reference isotope ratios were derived by measuring sub-samples on a liquid isotope analyser (~~L2130-i~~ L2130-*i* with attached vaporizer unit A0211, Picarro Inc.) and are considered the samples "true" value throughout the analysis. After ~~VapAuSa analysis~~the samples were analysed on the VapAuSa, we calibrated the results to these liquid measurements.

~~We determined the measurement uncertainty as one standard deviation (1 σ).~~ To quantify the ~~measurement accuracy associated with the VapAuSa~~ deviations of the VapAuSa measurements, the results (~~$\delta^{18}O_a$~~δ-values VapAuSa) were subtracted by the respective liquid analyzer results (~~$\delta^{18}O_l$):~~

$$Accuracy\ \delta^{18}O = \delta^{18}O_a - \delta^{18}O_l$$

~~We~~ δ-values liquid). Measurement bias was then calculated as the mean deviation of all samples. We determined the measurement repeatability as one standard deviation (1 σ) for each of the three water sample groups. We also assessed the VapAuSa performance by comparing the measurement variability ~~with~~ of 230 manual measurements from identical water sources. ~~To calculate the accuracy, we again subtracted~~ There we again calculated the deviations as the manual measurements (δ-values hand) subtracted by the respective liquid ~~measurements from the manual data~~analyzer results (δ-values liquid).

3.3 Results

3.3.1 Soil Samples

The ~~VapAuSa measurements are subject to some uncertainty, however, 60% of the measurements stabilized before the switch time threshold. While we programmed the VapAuSa to analyze each sample for 10 minutes, most measurements met the stability criteria (Table 1)within 8 minutes. This reduction in overall runtime from 5 to 4 hours was observed across 21 sample measurements. In comparison to~~ distribution of the soil water measurements showed overall good agreement, with a similar mean and standard deviation for the manual and automatic measurements, indicating consistent measurements and no bias attributable to the VapAuSa, thus ruling out systematic measurement errors (Figure 5). Although the standard deviation was relatively high, it is similar across the measurements and suggests that the variance is likely due to the bags being measured twice, a theme that will later be discussed. Specifically, bags sampled first by the autosampler and then manually had a lower standard deviation (0.961 in δ18O and 6.25 in δ2H) compared to those measured manually first (1.44 in δ18O and 9.14 in δ2H). Additionally, the ~~results from the liquid analyzer, the autosampler exhibits a 1 σ uncertainty of +/-~~ coefficient of determination varied depending on the initial measuring device: when measured by hand first and then by the autosampler, the r^2 was a low 0.31, whereas if the autosampler measured first, the r^2 was 0.71 (Figure 6).

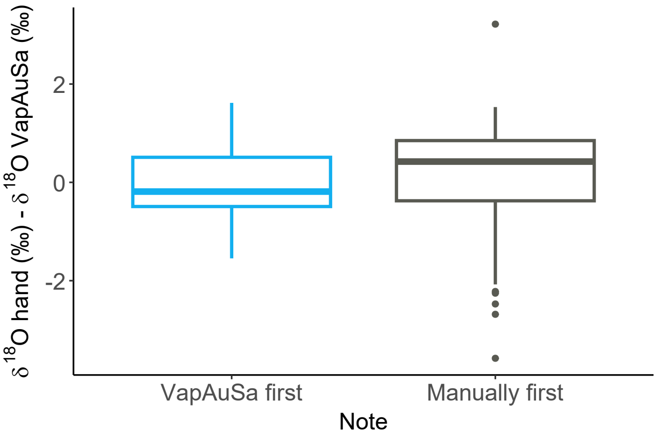

Figure 5. Differences of samples measured manually and with VapAuSa, depending which method measured first.

There is a clear divergence of the r^2 from soil bags measured first manually versus first with the autosampler. The soil samples which were measured first by the autosampler were often measured directly afterwards manually, while the first manually measured samples were placed in the autosampler and measured up to six hours later due to their positioning in the system. There is always a risk that once a bag is pinched and the cannula is pulled out, the seal might not be tight, potentially leading to higher uncertainty with the hand-first bags due to the extended time allowed for leakage through the pinched septum. Since the uncertainties among bags measured first manually and those measured by the autosampler are similar, this suggests that the largest uncertainty is induced by measuring the bags twice. If the VapAuSa would decrease measurement precision, this would manifest as a consistent bias, either always increasing or decreasing the δ values.

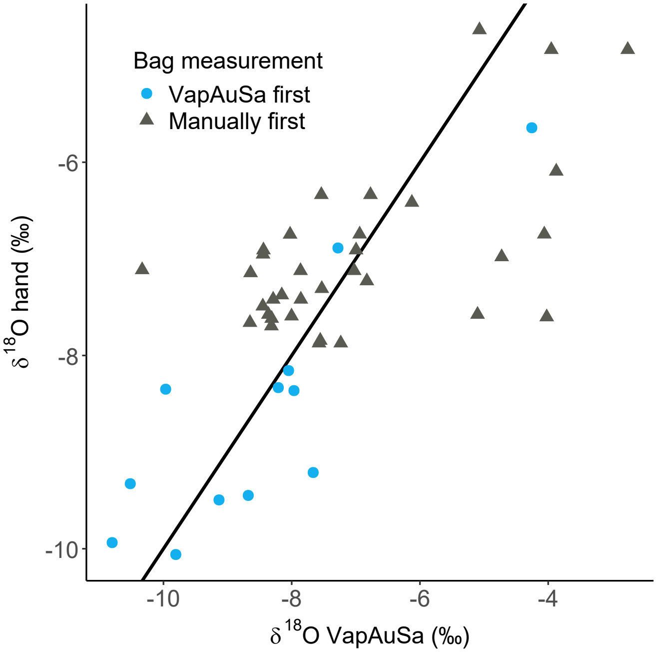

Figure 6. Relation of VapAuSa soil water measurements to manual measurements. 12 samples were measured by autosampler first, the rest manually first.

3.3.2 Water Samples

The measurement bias (mean difference of VapAuSa measurement to liquid value) was extremely low; -3.9e-15 ‰ for δ^{18}O and 7.1e-14 ‰ for δ^{2}H. Average repeatability across all samples was ± 0.58 ‰ for δ^{18}O and ~~+/-~~ ± 4.5 ‰ for δ^{2}H. ~~Across~~ However across the different water-sources, VapAuSa showed ~~the highest uncertainties for light water with +/-~~ differing repeatabilities. The largest repeatability range was measured for the low δ value water with ± 0.82 ‰ for δ^{18}O and ~~+/-~~ ± 7.0 ‰ for δ^{2}H. It ~~best performed in~~ performed best for the isotopic range of the medium and high ~~signatures~~ δ values with similar deviations of around ~~+/-~~ ± 0.47 ‰ for δ^{18}O and ~~+/-~~ ± 3.2 ‰ for δ^{2}H (Figure ~~??~~7).

~~Measurements from 3 water samples of different isotopic composition, analyzed using the VapAuSa and the liquid mode of a Picarro L2130i. Liquid analyzer accuracy: +/- 0.035‰ for δ^{18}O and +/- 0.256 ‰ for δ^{2}H~~

~~Manual analysis had a measurement uncertainty~~ Also 60% of the measurements stabilized before the evaluation time threshold. While we programmed the VapAuSa to analyze each sample for 10 minutes, most measurements met the stability criteria (Table 1) within 8 minutes. This reduction in overall runtime from 5 to 4 hours was observed across 21 sample measurements. The manual analysis showed a measurement repeatability (1 σ) of ~~+/-~~ ± 0.37 ‰ for δ^{18}O and ~~+/-~~ ± 5.7

‰ for δ^2H, being in a similar range as those of the VapAuSa (Results are shown in Figure 7). The ~~high~~ increased uncertainty of the VapAuSa in the isotopically ~~light~~ low range is also visible in manual measurements. While the manual ~~uncertainty~~ repeatability range is 50 % lower in δ^{18}O than that of the VapAuSa, it is 130 % higher for δ^2H. ~~Therefore this error probably does not stem from the VapAuSa but rather from the DVE-LS extraction method in general.~~

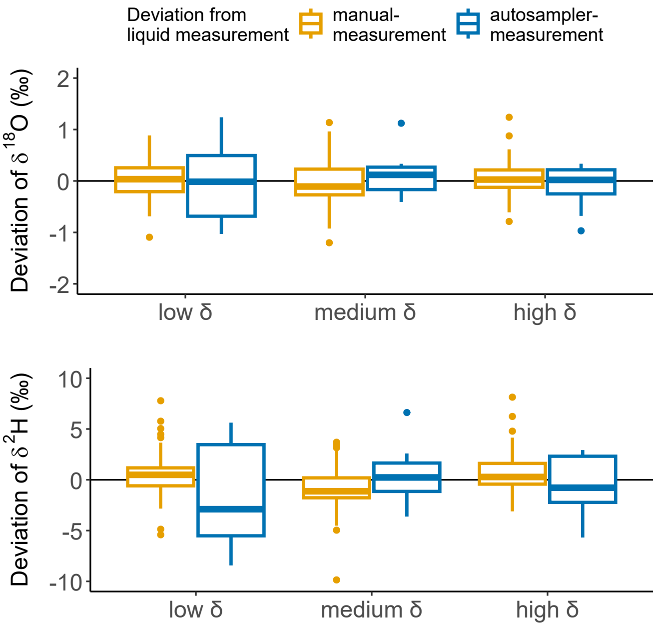

Figure 7. Deviation of VapAuSa and manual measurements from liquid measurements of 3 water samples of different ~~isotopic composition~~isotope ratios

4 ~~Discussion~~

As observed with the two times measured soil samples, pinched bags can alter their isotopic ratios. Therefore, it is crucial to evaluate the effect of the duration a sample remains in the autosampler, from the time it is placed and pinched by the cannula to the time of measurement. In the liquid water test, the time between pinching and measurement extended up to 4 hours. When analyzing the drift over time, the slopes for each bag measurement varied. The slopes of the water source with lower and higher δ values indicated an enrichment in heavier isotopes at rates of 0.31 ‰ per hour and 0.20 ‰ per hour, respectively, while the medium δ value water source showed depletion at a rate of -0.04 ‰ per hour. The slope for the high δ value is the only one that is statistically significant at $p<0.05$, while the slope of the medium and low δ water sources are not statistically significant. Therefore, no overall trend could be identified, suggesting that the drift is of minor importance to the measurement accuracy. However, this effect can always be analyzed and corrected for with the co-measured calibration standards.

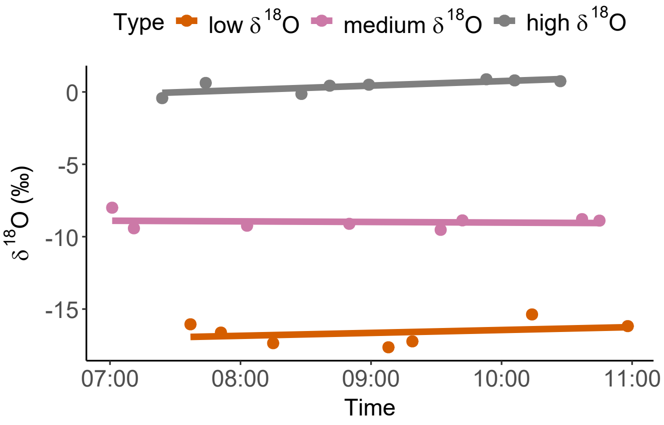

Figure 8. VapAuSa measurements of three water sources, each dot representing an individual bag. Isotopic drifts are indicated by the regression lines with slopes of 0.31 ‰ per hour for low *δ* values, -0.04 ‰ per hour for medium *δ* values and 0.20 ‰ per hour for high *δ* values.

4 Discussion

4.1 Accuracy

~~Sprenger et al. (2015) pointed out in their review that accuracies for different extraction methods vary strongly. Even when comparing the same extraction method among different studies, vastly different accuracy values are named. This comes for two reasons: the chosen extraction medium and the selected accuracymetric.~~

Oven dried soils spiked with water of known isotopic signatures are often used to examine extraction and equilibration performance (West et al., 2006; Wassenaar et al., 2008; Volkmann and Weiler, 2014). Accuracies ~~for this method~~ reported for DVE-LS vary between 0.7-1.03 ‰ for δ^2H and 0.15-0.3 ‰ for δ^{18}O ~~for DVE-LS (Wassenaar et al., 2008; Volkmann and Weiler, 2014) and 0.69-3 ‰ for δ^2H and 0.15-0.4 ‰ for δ^{18}O for CVD (West et al., 2006; Koeniger et al., 2011)~~(Wassenaar et al., 2008; Volkmann and W . However, the soil texture can alter the extracted signatures. While ~~isotopic signatures~~ the *δ* values of extracted water from sandy soils is often close to the known ~~composition~~isotope ratios, a high clay content changes the signatures (Koeniger et al., 2011; Orlowski et al., 2013, 2016; Gaj et al., 2016). This clay-induced error ~~(10 +/- 2 ‰ for δ^2H and 2 +/-1 ‰ for δ^{18}O applying CVD) is also extraction-method specific. While DVE-LS depletes signatures , CVD samples are enriched (Orlowski et al., 2016).~~ depletes signatures in DVE-LS (Orlowski et al., 2016). Since a true accuracy assessment using spiked soil samples is currently impossible, we chose to evaluate the usability of VapAuSa for soil samples by comparing hand and autosampler measurements of natural soil samples.

~~Another method to examine a systems performance is the water-to-water extraction, which we also applied in this study. The main benefit is the exclusion of soil~~ In order to exclude soils as an uncertainty variable ~~, which gives a more precise estimate~~

~~on the systems accuracy. For CVD,~~ in testing the VapAuSa system measurement accuracies (both measurement repeatability and bias), we chose a water-to-water ~~extraction has a precision of 0.2 ‰ for δ^{18}O and 0.8 ‰ for δ^{2}H (Orlowski et al., 2016).~~

~~Another comparability issue between extraction methods is the applied accuracy metric. While some studies give the accuracy (as in how good a known sample signature can be reproduced after extraction) (Koeniger et al., 2011; Orlowski et al., 2013, 2016), most only give the measurement repeatability (as in how measurements of multiple samples of same signatures deviate from each other)(West et al., 2006; Wassenaar et al., 2008; Mueller et al., 2014; Volkmann and Weiler, 2014). We think repeatability is inferior to accuracy, as it hides the overall measurement inaccuracies. For that reason we chose to present our results as accuracys.~~

~~Comparing our results to the~~ equilibration of known liquid samples. This approach has not been previously applied to DVE-LS, making its classification challenging. However, water-to-water ~~CVD extraction accuracys stated by (Orlowski et al., 2016) (~~extraction has been assessed in the context of cryogenic vacuum distillation, which can serve as a benchmark. In such assessments, water-to-water extraction exhibited a measurement repeatability of 0.2 ~~‰~~ ‰ for δ^{18}O and 0.8 ~~‰~~ ‰ for δ^{2}H ~~), our~~ (Orlowski et al., 2016). In comparison, the DVE-LS ~~extractions perform worse. However, this lower measurement accuracycomes with a~~ autosampler as well as the manual measurements demonstrated lower accuracy. But the VapAuSa offers advantages in higher sample throughput and ~~less manual labor~~ reduced manual labor compared to the cryogenic method.

4.2 ~~Manual labor in CVD and DVE-LS~~

~~Assessing sample preparation of CVD and DVE-LS regarding consumables, technical equipment and manual labor, DVE-LS is the least expensive and least time consuming method (Orlowski et al., 2016). However once the samples are prepared, the liquid samples gained through CVD can be autonomously processed by the isotope analyzers liquid autosampler. With the VapAuSa, the formerly manual analysis step is now also fully automated. Therefore,DVE-LS now comes at the same analysis autonomy like liquid CVD samples but at lower sample preparation costs (approx. 3 minutes manual labor per sample).~~ Because of the larger repeatability range, the applicability of the VapAuSa depends on the δ-value ranges of the water source ratio differences of interest. This issue has been previously discussed in the context of the uncertainty introduced by cryogenic extraction (Allen and Kirchner, 2022). The conclusion was that two sources can be differentiated in a mixing model only if the δ-value span between them is substantially larger than the uncertainty added by cryogenic extraction. This principle also applies to the VapAuSa. When the δ-value range of the end members is small,another extraction method with lower uncertainties should be chosen. However, if the δ-value range is substantially larger than the repeatability uncertainty, VapAuSa is a good choice as it increases sample throughput.

4.2 Storage Time

~~While in some cases the VapAuSa measurement uncertainty is higher compared to manual analysis, this uncertaintymight cancel out due to lower sample storage time. The higher weekly sample throughputleads to quicker analysis, thus causing less isotopic driftsduring storage. Over time, these changes appear~~ Sample storage leads to isotopic drifts, which occur over time with every DVE-LS ~~sample container materials~~container material. Even aluminum laminated bags sealed by their ziplock (as they would be during storage) have an isotopic drift of 0.042 ‰ per day for δ^{18}O at room-temperature (Gralher et al., 2021). ~~One can assume that even when storing the samples in a fridge the samples isotopic composition also shifts.~~ To minimize these shifts one ~~needs to~~ should analyse the samples as quick as possible. Manual DVE-LS sample analysis is limited to about 180 samples per week. However VapAuSa can analyze up to 120 samples per day thus enabling up to 500 sample measurements per week. Relating the throughput to storage ~~unceartainty~~drifts, analyzing 400 DVE-LS samples (2 weeks of manual analysis vs. 3.5 days VapAuSa analysis) adds about 0.6 ‰ storage induced uncertainty to manual δ^{18}O analysis , however only 0.17 ‰ storage induced uncertainty to VapAuSa δ^{18}O measurements. Therefore, ~~when measuring lots of DVE-LS samples is needed, the basic~~ with larger sample volumes, the measurement uncertainty of ~~the VapAuSa quickly cancels out due to minimal storage induced shifts~~VapAuSa may be lower than manual analysis due to shorter storage times.

4.3 Objectivity

By defining (and documenting!) stability criteria, measurements are less dependent on person specific skills and preferences regarding the detection of a "stable" ~~measurements~~measurement. The subjective influence of the operator diminishes even further, as the system treats all samples equally, without ~~mental~~ fatigue after several hours of measurements. ~~Additionally, the automated measurement eliminates the effect of the operators body heat, which even in an air conditioned lab setting has the potential to warm samples above the temperature at which the sample should have equilibrated for 48 hours.~~ Also, the ~~measurement~~ monitoring part of the VapPauSa-GUI can be used ~~on its own~~ separately and does not require any other hardware than the isotopic analyzer itself. We think it might even ~~be suited to facilitate and objectify~~ help to improve manual DVE-LS measurements, as it provides instant one click summary statistics for freely ~~selectable~~ defined time spans. Meanwhile the standard Picarro-analyzer GUI requires ~~some~~ tedious zooming in and out for that purpose. On top of that, the possibility to define objective stability criteria should also help to reduce the a subjective component of manual DVE-LS measurements.

4.4 Don’t buy cheap

To gain the mentioned VapAuSa benefits, we want to stress the importance of using appropriate, ~~vapor capable~~ vapor-resistant and vapor-impermeable materials. Even a tiny amount of ambient air continuously leaking into the system can cause major errors with water vapor analysis. ~~The~~ Our first prototype was build using valves designed for liquids with the connection between valve and cannula only plugged into each other and secured with Parafilm (PARAFILM® M). While this system ~~cost~~ costs a fraction of our current setup, it eventually developed a leak. This lead to ambient air mixing with the sample vapor, causing wrong measurements~~which needed correction~~. Therefore, it is important to use adequate materials like gas-rated valves and secure all connections either by screw-fittings or superglue. Only this can ensure reliable measurements.

5 Conclusions

VapAuSa ~~may be a helpful tool~~ is a helpful system for all disciplines applying DVE-LS. ~~The automatic analysis could also be beneficial for researchers who apply cheaper, more diffusive polyethylene bags for their DVE-LS samples. There, the shorter~~

~~storage time could be compensated by the increased sample throughputof the VapAuSa~~While the VapAuSa comes with certain measurement uncertainties, those measurements are less prone to personal measurement errors, less labor intensive and have an increased throughput. In cases where the source δ-value range is larger than VapAuSa uncertainty, the larger sample throughput can be a great benefit. So far we tested the system with liquid and soil samples only, however investigating plant samples (which have an even shorter maximum storage time) would be an interesting next step. Generally we think the VapAuSa is a valuable addition to the tool-sets of isotope geoscientists, enabling ~~large scale, high volume sampling~~ high number sampling and measurement, needed for advancing our understanding of environmental systems.

Code availability. All of the scripts that compose the VapAuSa-GUI can be found under https://gitlab.rz.uni-freiburg.de/hydrology/vapausa.

Author contributions. JP, SS and MW designed the experiment. SS developed the circuit boards and firmware of the auto sampler as well as the VapAuSa software. JP conducted the experiments and data analysis and wrote the first draft. BH analyzed the standard samples. SS, BH and MW contributed to writing the final manuscript.

Competing interests. At least one of the (co-)authors is a member of the editorial board of Hydrology and Earth System Sciences.

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