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Two common pitfalls in the analysis of water-stable isotopologues with cryogenic vacuum extraction and cavity ring-down spectroscopy

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Abstract

Water stable isotopologue analysis is widely used to disentangle ecohydrological processes. Yet, there are increasing reports of measurement uncertainties for established and emerging methods, such as cryogenic vacuum extraction (CVE) or cavity ring-down spectroscopy (CRDS). With this study, we investigate two pitfalls, that potentially contribute to uncertainties in water-stable isotopologue research. To investigate fractionation sources in CVE, we extracted pure water of known isotopic composition with cotton, glass wool or without cover and compared the isotopologue results with non-extracted reference samples. To characterise the dependency of δ^2H and δ^{18}O on the water mixing ratio in CRDS, which is of high importance for in-situ applications with large natural variations in mixing ratios, we chose samples with a large range of isotopic compositions and determined δ^2H and δ^{18}O for different water mixing ratios with two CRDS analysers (Picarro, Inc.). Cotton wool had a strong fractionation effect on δ^2H values, which increased with more ^{2}H-enriched samples. δ^2H and δ^{18}O values showed a strong dependency on the water mixing ratio analysed with CRDS with differences of up to 34.5‰ (δ^2H) and 3.9‰ (δ^{18}O) for the same sample at different mixing ratios. CVE and CRDS, now routinely applied in water stable isotopologue research, come with pitfalls, namely fractionation effects of cover materials and water mixing ratio dependencies of δ^2H and δ^{18}O, which can lead to erroneous isotopologue results and thus, invalid conclusions about (ecohydrological) processes. These practical issues identified here should be reported and addressed adequately in water-stable isotopologue research.

KEYWORDS
cotton, CRDS, Isotopic fractionation, δ^{18}O, δ^2H

1 | INTRODUCTION

Water-stable isotopologues are a powerful tool to trace a variety of ecohydrological processes, such as plant water uptake or soil water infiltration.[1–4] To extract water from plant and soil material, cryogenic

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vacuum extraction (CVE)[5,6] is still widely used,[7] although new methods have been developed.[2,8–12] In addition, recent studies have questioned the validity of CVE due to extraction biases in δ^2H values[13,14] and problematic comparability between laboratories.[15] Potential reasons for extraction biases include different extraction times,[7,16–18] extraction temperature,[19,20] sample preparation protocols or leaky vacuum systems.[15] It has also been demonstrated that soil type, composition and water content can have a significant impact on water stable isotopologue composition when CVE is applied.[19,21–24]

While the literature is abundant on these issues, a rarely reported problem in CVE is the material, which is used to cover the plant or soil sample to prevent particles from being drawn into the vacuum pump or the extracted water sample. This material is present during the entire extraction process, experiences the same (temperature) conditions and is passed by the extracted water vapour on the way to the cold trap. Therefore, inert glass wool is generally suggested and used.[16,22,25] However, many studies do not report the used covering materials in CVE. This raises the question of whether glass wool or other materials are used, which could have severe consequences for isotopologue analysis if they are of an organic nature. Indeed, some studies report the use of other (organic) cover materials, such as fleece[7] or cotton wool,[17] although, for example, cotton contains hydrogen and oxygen itself and is considered hygroscopic.[22,26,27] In addition, cellulose is the major constituent of cotton wool.[28,29] Wassenaar and Hobson[30] experimentally determined ∼23% of hydrogen in cotton to be exchangeable at 130°C, which is close to the temperature used in CVE.

Thus, it is likely that there are contamination or fractionation effects of such organic (cover) materials on isotopic composition during CVE. Similar fractionation/contamination effects have been described in other studies, which were often related to the presence of organic materials with exchangeable hydrogen, such as plant material itself.[13,30–32]

After CVE, extracted water samples are often analysed with laser spectrometers, such as cavity ring-down spectroscopy (CRDS) instruments. While these instruments offer a fast and inexpensive measurement of water-stable isotopologues, they come with other issues compared to isotope-ratio mass spectroscopy, such as organic contaminations[33–36] or water mixing ratio dependencies.[37,38] For example, Picarro Inc., one of the main manufacturers of CRDS analysers, states that one of the current models (L2130-*i*) has a recommended measurement range of 1000–50,000 ppm.[39] Yet, it has been demonstrated that the analysed water mixing ratio in CRDS has a strong impact on obtained δ^2H and δ^{18}O values,[37,38,40] which has high relevance for studies, where the isotopic composition is measured directly (and is difficult to control) in the water vapour, such as soil evaporation, soil water vapour, atmospheric water vapour, plant transpiration or xylem water in the gas phase.[12,41,42] With the development and application of new water stable isotopologue measurement techniques,[2,8,10,12,43,44] which are based on the direct measurement of water vapour of different mixing ratios, the water mixing ratio-dependency of stable water isotopologues with CRDS is gaining relevance. The direct equilibration method to sample, for instance, soil water vapour is based on thermodynamic equilibrium and

full water vapour saturation, which is controlled by the ambient temperature that typically varies considerably under natural conditions. This means that a change in soil temperature from 15 to 25°C results in a change of 9000 ppm. Thus, isotopologue values should be corrected to a target value of ∼ 18,000 ppm (default mixing ratio in Picarro software), which requires an instrument-specific calibration. However, few studies correct or at least report instrument-specific corrections of the isotopic compositions for different water mixing ratios,[12,43,44] which might lead to misinterpretations of ecohydrological processes due to an erroneous analysis.

Both, fractionation or contamination during CVE and water mixing ratio dependencies during water stable isotopologue analysis via CRDS might considerably contribute to uncertainties in water stable isotopologue research. Thus, the objectives of this study were to 1) investigate the effect of two different cover materials with and without organics for CVE on δ^2H and δ^{18}O values and 2) determine the water mixing ratio dependency of δ^2H and δ^{18}O values for water stable isotopologue analysis with CRDS.

2 | EXPERIMENTAL

2.1 | Sample material, preparation and CVE

To test the effects of cotton wool on δ^2H and δ^{18}O values, we chose water samples with a wide range of isotopic compositions (Table 1). These included a variety of in-house standards, tap water (Freiburg, Germany) and varying precipitation samples from a Mediterranean site (Portugal).[45,46] Every sample was divided into four subsamples with ∼ 0.75 mL each. The first subsample was immediately filtered with a glass fibre syringe filter (0.7 µm, Syringe Filter; Membrane Solutions) to remove potential particle contaminations, especially of precipitation samples, and set aside as reference (a). The three other subsamples were frozen (-20°C) immediately in 1.5 mL glass vials (LLG) and prepared for CVE as in Kübert et al.[2] Freezing sample material (e.g. soil, xylem) is a common practice to avoid the loss of water vapour during the evacuation procedure of the sampling line. To ensure the frozen status of the sample, the vials were additionally inserted in liquid N_2 (−196°C) right before extraction.[47] As cover material, we used (b) commercially available cotton wool (elkos FACE; EDEKA), (c) glass wool (extra fine, Karl Hecht GmH & Co KG) or (d) none at all. CVE was conducted on a custom-made extraction line designed by R. Siegwolf (similar to Ehleringer & Dawson[3]), which allowed the extraction of 20 samples simultaneously. Sample lines were evacuated to remove any residual water vapour in the extraction lines before the samples were lowered into the water bath. In the water bath, samples were heated to 95°C for 90 min and connected to U-tubes immersed in a cold trap filled with liquid N_2. During extraction, the whole system was connected to a pump with a vacuum of 0.1 mbar (XDS10 vacuum pump; Edwards).[2] After CVE, U-tubes containing the frozen water were sealed airtight until samples were completely defrosted. Then, the extracted water was filtered with a glass fibre syringe filter (0.7 µm, Syringe Filter; Membrane Solutions), transferred into 1.5 mL glass vials

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TABLE 1   Water samples were used to test the effect of cotton and glass wool on δ^2H and δ^{18}O values during cryogenic vacuum extraction. Samples were (A) only filtered (reference), (B) extracted with cotton wool, (C) extracted with glass wool and (D) extracted without cover material. δ^2H (+1 SD) and δ^{18}O (+1 SD) values are given for the sample, which was only filtered (A) and measured directly with a Picarro 2130i laser spectrometer.

Sample ID	Sample description	δ^{18}O (‰)	δ^2H (‰)
1	Standard—Light	−25.05 ± 0.01	−102.44 ± 0.05
2	Standard—Medium	−9.23 ± 0.02	−63.75 ± 0.04
3	Standard—Heavy	−5.14 ± 0.03	−9.66 ± 0.03
4	Standard—Superheavy	−0.37 ± 0.02	54.32 ± 0.08
5	Standard—Light 2	−15.15 ± 0.02	−111.22 ± 0.03
6	Standard—Heavy 2	2.89 ± 0.00	1.77 ± 0.05
7	Tap water Alps	−24.96 ± 0.02	−101.99 ± 0.08
8	Tap water Black Forest	−9.27 ± 0.03	−63.58 ± 0.08
9	Tap water University of Freiburg	−9.37 ± 0.01	−64.46 ± 0.13
10	Distilled water University of Freiburg	−9.34 ± 0.03	−63.94 ± 0.12
11	Precipitation Vila Viçosa 12 Jan 2018	−7.65 ± 0.05	−48.23 ± 0.07
12	Precipitation Vila Viçosa 12 Jun 2018	−2.00 ± 0.04	−6.12 ± 0.17
13	Precipitation Vila Viçosa 16 May 2018	0.07 ± 0.01	8.87 ± 0.08
14	Precipitation Vila Viçosa 25 Sep 2019	−2.02 ± 0.02	−4.97 ± 0.04
15	Precipitation Vila Viçosa 24 Oct 2018	−7.71 ± 0.03	−47.03 ± 0.10
16	Precipitation Vila Viçosa 5 Feb 2019	−4.72 ± 0.01	−24.77 ± 0.05
17	Precipitation Vila Viçosa 21 Oct 2019	−3.28 ± 0.03	−20.20 ± 0.05
18	Precipitation Vila Viçosa 3 Dec 2019	−5.88 ± 0.03	−33.76 ± 0.09
19	Precipitation Vila Viçosa 17 Dec 2019	−7.80 ± 0.02	−46.85 ± 0.06
20	Groundwater Vila Viçosa 23 Mar 2020	−5.35 ± 0.01	−32.08 ± 0.06

(LLG) and immediately measured with the reference samples (a) on an A0325 autosampler and a V1102-i vaporization module operated at 140°C connected to a Picarro 2130-*i* CRDS instrument (Picarro Inc.) with N_2 as carrier gas. In total, nine injections of each sample were used, of which the first six were discarded to avoid carry-over effects of the previous sample. All four subsamples (a–d) were measured consecutively in a random order. Care was taken that the water mixing ratio for measurements was very similar between samples at ∼18,000 ppm. For calibration, we used four in-house water standards (Sample ID 1–4 in Table 1), which are regularly calibrated against the international standards VSMOW-2, SLAP-2 and GRESP (IAEA). No organic contamination was detected by ChemCorrect (Picarro Inc.). Hydrogen and oxygen stable isotopic ratios are reported relative to the values of the Vienna Standard Mean Ocean Water and

expressed as isotopic composition (respectively, δ^2H and δ^{18}O) in per mill (‰).[48]

2.2 | Sample material for water mixing ratio test

To investigate the dependency of δ^2H and δ^{18}O on the analysed water mixing ratio in CRDS, we selected four in-house standards (Sample ID 1–4 in Table 1) that span a wide isotopic range (δ^2H: −102.90‰/δ^{18}O: −25.13‰, −64.01‰/−9.28‰, −10.27‰/−5.22‰ and 53.89‰/−0.40‰). We measured with different sample volumes on the Picarro 2120-*i* CRDS instrument coupled to an A0325 autosampler and a V1102-i vaporization module (Picarro Inc.) and dry air as carrier gas (see above). The sample volume can be adjusted in the

Picarro program Autosampler Control. The program allows us to run a sequence of measurements with different methods (i.e. in our study sample volumes) automatically. The program's default is 1.8 μL. The nine chosen amounts varied between 0.2 and 3.1 μL to cover a wide range of water concentrations. For each amount and sample, eight injections were conducted, of which the first three were discarded for analysis. This procedure was repeated with a second Picarro 2130-*i* CRDS instrument with eight different sample volumes between 0.25 and 3.1 μL to compare the performance of the CRDS models. The results of this analysis are reported and illustrated as uncalibrated raw values to demonstrate the variability of δ^2H and δ^{18}O before an instrument-specific calibration is applied.

2.3 | Statistical analysis

We compared δ^2H and δ^{18}O values of water samples extracted with cotton, glass wool or without cover material and reference samples with a linear mixed effect model (*lme*)[49] with treatment (reference—cotton wool—glass wool—without cover) as fixed and sample ID (see Table 1) as a random factor. In case of statistical differences, a post-hoc pairwise comparison of the means with the function *emmeans* with a Bonferroni adjustment in the package *emmeans*[50] in R was applied.[51] As the requirements for a parametric test were not always met, we repeated the analysis with the non-parametric Friedman Test (*friedman_test*) in the R package *rstatix*[52] and the Wilcox Test (*wilcox_test*) with a Bonferroni adjustment for a pairwise comparison of the different treatments.[51]

To visualize the offset of δ^2H and δ^{18}O values from the reference samples, we applied a linear mixed effect model (*lme*)[49] with measured δ^2H/δ^{18}O values (reference) and treatment (cotton wool—glass wool—without cover) as fixed and sample ID as a random effect.

In addition, we calculated the deviation of the isotopic composition of samples extracted with cotton wool, glass wool and without cover material from the isotopic composition of the reference sample:

$$\Delta\delta = \delta\,(measured) - \delta\,(reference)$$

and applied a linear model (*lm*) between $\Delta\delta^2$H/$\Delta\delta^{18}$O and δ^2H/δ^{18}O of the reference samples to test for increasing/decreasing deviations with more enriched/depleted samples for each type of cover material separately.

For the relationship of δ^2H and δ^{18}O values and water mixing ratios in CRDS, we applied non-linear third-order logarithmic regression in the form of:

$$\delta = a + b * \ln(WMR) + c * (\ln(WMR))^2 + d * (\ln(WMR))^3,$$

where δ is the δ^2H or δ^{18}O (in‰), WMR is the water mixing ratio (in ppm), and a, b, c and d regression parameters. For consistency, we used the same non-linear regression for both isotopes and for both CRDS models. We are aware that the application of this regression may result in an overfitting. However, the sole purpose of this regres-

sion was to better visualize the trends of δ^2H and δ^{18}O values with increasing water mixing ratio and not to provide a water mixing ratio correction matrix. All graphics and the non-linear third-order logarithmic regression were conducted with SigmaPlot (Version 14, Systat Software GmbH).

3 | RESULTS

3.1 | Effect of cover material on δ^2H values

CVE with cotton instead of glass wool or without cover had a clear and significant effect on the isotopic composition of the extracted water samples (Figure 1 and Table 2). δ^2H values were significantly more depleted ($p < 0.001$, Figure 1A) for cotton extracted compared to reference samples, with an average deviation of 7.2 ± 4.7‰ (mean ± standard deviation, SD). For example, at δ^2H values of +54.3‰ (Sample ID 4, Table 1), cotton-extracted water samples were depleted by 14.3‰ compared to reference samples (Figures 1A and 2A). For ^{2}H-depleted samples, such as the light in-house standard (δ^2H = −102.44‰, Sample ID 1), deviations were less pronounced (Figures 1A and 2A). The increasing fractionation effect with more enriched samples for cotton wool extracted samples was significant ($p < 0.001$, $R^2 = 0.71$, Figure 2A). δ^2H values of water extracted with glass wool (average deviation: δ^2H: 0.8 ± 0.8‰, δ^{18}O: 0.1 ± 0.1‰, Figure 1C) or without cover material (average deviation: δ^2H: 0.8 ± 0.8‰, δ^{18}O: 0.1 ± 0.1‰, Figure 1E) did not differ strongly from reference samples (both $p > 0.05$, Figures 1 and 2A and Table 2). Interestingly, δ^2H values of water extracted with glass wool also deviated stronger from the reference sample with increasing enrichment (Figure 2A). This trend was weak and only marginally significant ($p = 0.06$, $R^2 = 0.14$). For δ^2H values of water extracted without cover, this pattern was not evident ($p > 0.05$) (Figure 2A).

3.2 | Effect of cover material on δ^{18}O values

For δ^{18}O, the values after extraction with cotton wool, glass wool or without cover did not differ significantly from the reference values (Figure 1B,D,F and Table 2). However, similar trends as for δ^2H values were evident for the different cover materials. δ^{18}O values for samples extracted with cotton wool showed the strongest deviation from the reference values (0.3 ± 0.2‰). This deviation was lowest at δ^{18}O values of ∼ −9‰ and significantly increased with more enriched or more depleted samples ($p < 0.001$, $R^2 = 0.90$, Figure 2B). This trend was also evident in a minor deviation from the 1:1 line in Figure 1B. Samples extracted with glass wool had a low deviation from the reference samples in their isotopic composition (0.1 ± 0.1‰, Figure 1D). However, this deviation significantly increased with more enriched samples ($p < 0.05$, $R^2 = 0.21$). This trend was, as for δ^2H, only weak for δ^{18}O (Figure 2B). Samples extracted without cover material had similar deviations from the reference values in δ^{18}O (0.1 ± 0.1‰, Figure 3C) compared to samples extracted with glass wool, yet no significant trend
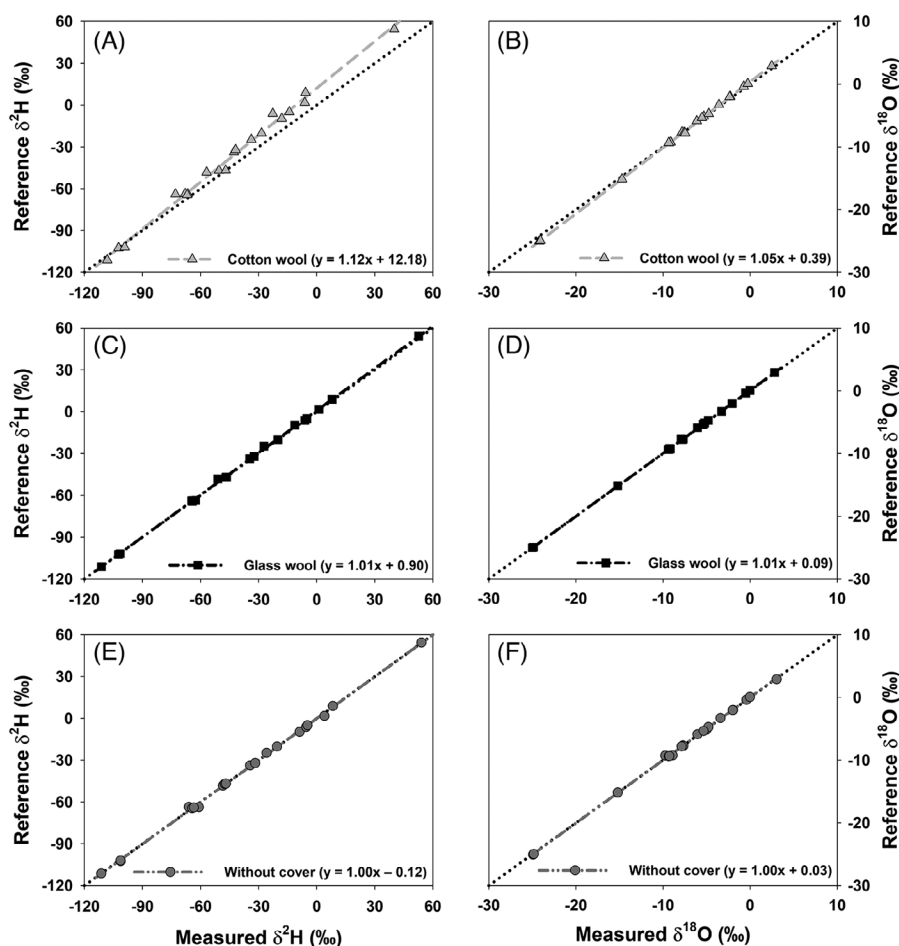
FIGURE 1 Relationship of reference and extracted δ^2H values, as well as the relationship of reference and extracted δ^{18}O values for cotton wool (A, B), glass wool (C, D) and without cover (E, F) for cryogenic vacuum extraction with 1:1 line (dotted) and linear mixed effect model. All regressions were highly significant $(p < 0.001)^{***}$.

TABLE 2 Average values with a standard deviation of δ^2H and δ^{18}O values for the four different treatments of the 20 water samples: Reference, extracted with cotton, extracted with glass wool and extracted without cover material. Different letters indicate statistical differences at a significance level of $p < 0.05$ (linear mixed effect model & Friedmann Test) for each isotope separately.

Treatment	Reference	Cotton	Glass wool	Without cover
δ^2H (‰)	-39.00 ± 41.23^a	-45.63 ± 36.64^b	-39.53 ± 40.81^a	-38.85 ± 41.16^a
δ^{18}O (‰)	-7.57 ± 7.25^a	-7.56 ± 6.89^a	-7.61 ± 7.21^a	-7.57 ± 7.22^a

was evident $(p > 0.05)$. In summary, the trends were similar for both isotopes, ^{2}H and ^{18}O, although they were more significant for ^{2}H. Samples extracted without cover material showed the best agreement with and did not differ significantly from the reference samples (Figures 1 and 2 and Table 2).

3.3 | Effect of water mixing ratio in CRDS on δ^2H and δ^{18}O values

For both isotopes, ^{2}H and ^{18}O, the measured water mixing ratio had a strong impact on both evaluated CRDS models (Figures 3 and 4). With increasing water mixing ratios, the CRDS instrument

measurements indicated more enriched isotopic compositions compared to the true value (except for δ^{18}O values of the light standard, Figure 4A). This effect levelled off for δ^2H values above 10000 ppm compared to the increase below 10000 ppm (Figure 3). For δ^{18}O values, the obtained isotopic composition increased almost linearly after 10000 ppm (Figure 4). Changes in measured isotopic compositions below 10,000 ppm were mostly non-linear for both isotopes and CRDS instruments. The comparison of the CRDS models clearly indicated that the measurements with the L2130-i were less variable over the measured water mixing ratio range for both isotopes (Figures 3 and 4). Please note that the values reported are uncalibrated raw values for both instruments.
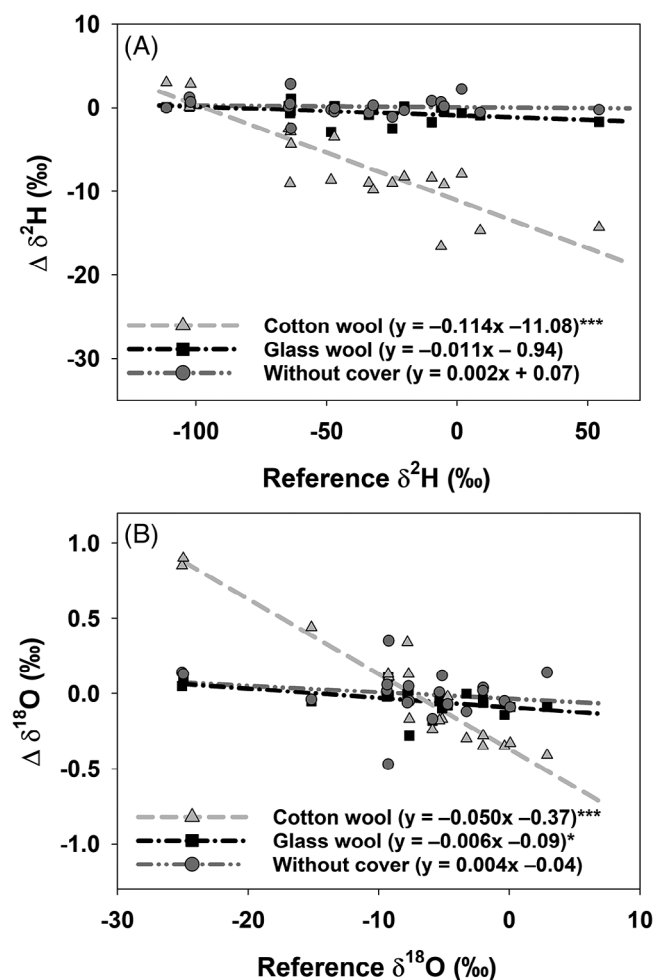
FIGURE 2   Relationship of reference δ^2H and offset ($\Delta\delta^2H$) of extracted and reference δ^2H value (extracted—reference) (A) and relationship of reference $\delta^{18}O$ and offset ($\Delta\delta^{18}O$) of extracted and reference $\delta^{18}O$ value (measured—reference) (B) with linear regression. Significance level (linear regression) given at $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

4   DISCUSSION

4.1   Cotton wool strongly fractionates during CVE

Cotton wool as cover material had a strong effect on the isotopic composition of the analysed samples. The depleted δ^2H values compared to the reference samples are in agreement with other studies testing fractionation effects for cotton[53] or other organic materials.[14,32] Here, different mechanisms played a role. As potentially 30% of the hydrogen atoms are exchangeable in cellulose,[54,55] such hydrogen exchange with the extracted water vapour could explain the strong fractionation effects for ^2H. At higher temperatures, the kinetic energy of molecules increases, which can facilitate isotope exchange.[14] However, isotope exchange with cellulose as the sole explanation for the fractionation effects cannot elucidate the patterns observed for $\delta^{18}O$ of samples extracted with cotton wool. A variety of studies concluded that fractionation effects caused by CVE do not affect $\delta^{18}O$ values of

samples,[13,14,53] which is in contrast to our results. Indeed, the effect (Figure 2B) for $\delta^{18}O$ was small but still significant. Thus, in addition to hydrogen exchange, there seems to be a mechanism affecting both stable isotopologues of water. Here, the hygroscopicity of cotton wool[22,26,27] might play a decisive role. As we did not dry the cotton wool prior to the extraction, adsorbed atmospheric water vapour was potentially replaced with water vapour originating from the water sample during CVE. Chen et al. found the isotopic composition of water, which was adsorbed by organic surfaces, including cotton, was clearly depleted in ^2H compared to unconfined water.[32] They attributed this to "surface effects", where water adsorbed by large surfaces is isotopically different from bulk water molecules due to structural and energetic differences.[32] Such adsorption and surface effects would also be highly relevant for $\delta^{18}O$ and could, thus, explain our results.

As we observed similar patterns for both isotopes of glass wool, although of much lower magnitudes (Figure 2), surface effects might also explain these patterns. Richard et al. demonstrated that surface effects were also present for porous silica tubes as adsorbent,[56] which are chemically similar to glass wool. Nevertheless, deviations of δ^2H and $\delta^{18}O$ values of water samples extracted with glass wool from reference water samples were very small.

In conclusion, these results illustrate the strong fractionation effect of cotton wool on water isotopic composition. They also demonstrate the urgent need to report if and what kind of cover materials are used in CVE. If possible, any cover material should be avoided. In cases, where a cover material is necessary (i.e. for soil samples), glass wool is an appropriate choice. In addition, test runs of CVE systems with pure water of different isotopic compositions are strongly recommended.

4.2   CVE works well—for pure water samples

While extraction with both, cotton- and glass wool, showed (small) fractionation effects, isotopic compositions of samples extracted without cover did not demonstrate any difference from reference samples. This illustrates that CVE is by no means an unsuitable technique as suggested in recent years.[15] It did not produce any significant measurement uncertainties with our protocol. This statement is only valid for the extraction of pure water samples and cannot inform on effects occurring with plant[13,14,31] or soil[23,24] materials (which was not the aim of this study). However, even if fractionation effects do occur, they do not necessarily invalidate study results obtained by using CVE.[22,57] On one hand, some experiments reporting fractionation effects were conducted under artificial conditions, such as spiking experiments testing the effect of soil properties on the isotopic composition during CVE.[22] Here, soil samples are artificially oven-dried at 105°C, and rewetted with water of known isotopic composition, which is then extracted cryogenically.[15,23] Such high temperature and fast drying do not represent natural conditions and processes and thus, might lead to different isotopic results. On the other hand, (measurement) uncertainties can rarely be excluded from studies and should be accounted for.[57] Apart from measurement uncertainties, practical considerations should also be reviewed. For example, in-situ methods are rather ideal
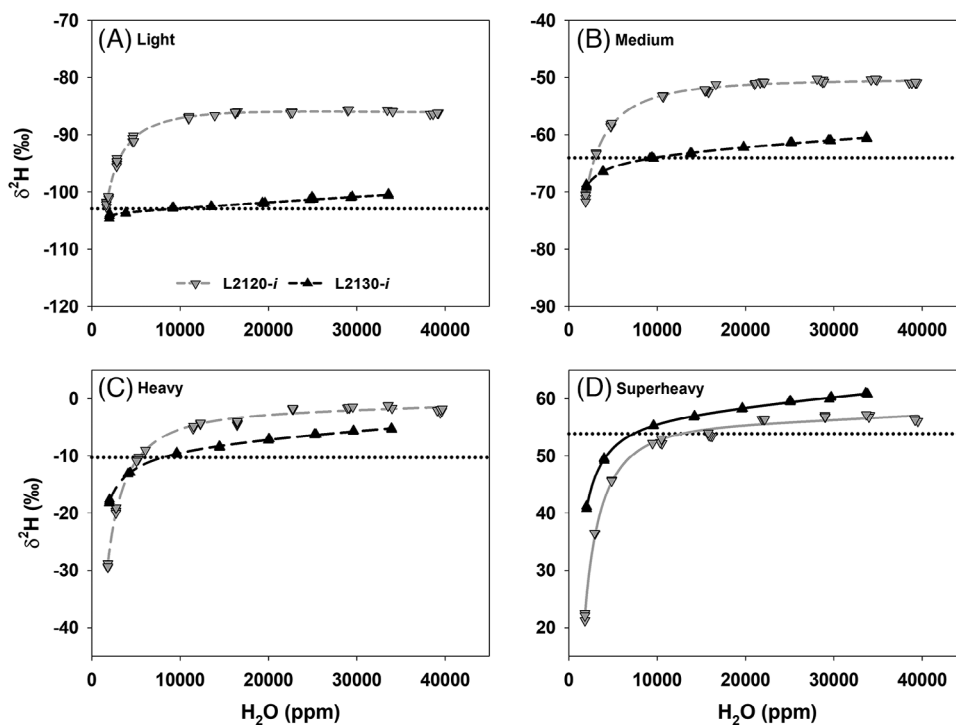
FIGURE 3 Relationship of uncalibrated δ^2H and water mixing ratios measured with two Picarro cavity ring-down spectroscopy (CRDS) instruments (L2120-*i* and L2130-*i*) for four water samples with different isotopic compositions (Table 1, sample ID 1–4): (A) light, (B) medium, (C) heavy and (D) superheavy. The dotted line represents the true value as calibrated with IAEA standards.
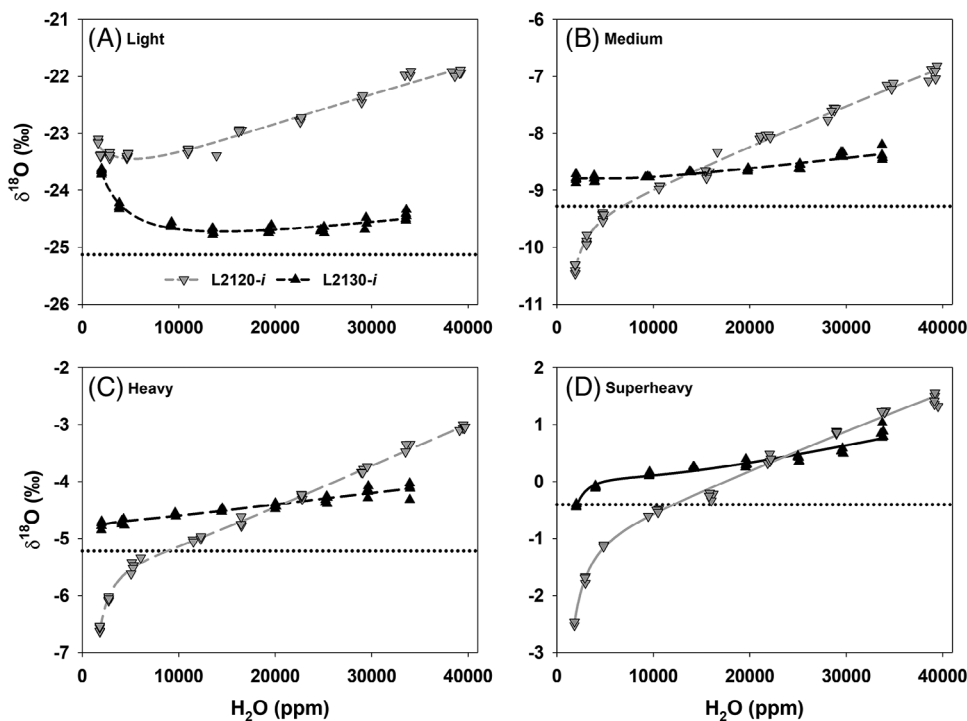

FIGURE 4 Relationship of uncalibrated $\delta^{18}O$ and water mixing ratios measured with two Picarro cavity ring-down spectroscopy (CRDS) instruments (L2120-*i* and L2130-*i*) for four water samples with different isotopic compositions (Table 1, sample ID 1–4): (A) light, (B) medium, (C) heavy and (D) superheavy. The dotted line represents the true value as calibrated with IAEA standards.

for small-scale, mechanistic studies,[2,12] which cannot (yet) cover the spatial heterogeneity of water stable isotopologue composition naturally occurring in ecosystems. Here, destructive sampling and CVE can be, for instance, combined with in-situ methods to address both the spatial and temporal resolution.[12]

δ^2H and δ^{18}O values are strongly dependent on the analysed water mixing ratio in CRDS

Our results demonstrate that newly developed methods[8,11] to measure the isotopic compositions directly in the water vapour need to correct for water mixing ratio effects and isotope dependencies.[37] Such methods have little control over the water mixing ratio and measure over a large range of natural variations in mixing ratios, so measurements must be made at the given water mixing ratio and cannot easily be adjusted in-situ to, for example, ~18,000 ppm, as recommended by Picarro. This is a known problem[37,38] and corrections are commonly applied.[11,12,58] However, there are several issues we would like to point out with this study. From our data, it is evident that water mixing ratios below 10,000/5000 ppm (δ^2H/δ^{18}O) yield variable and non-linear changes in isotopic compositions for both, δ^2H and δ^{18}O values in CRDS, which makes corrections more difficult. Thus, low water mixing ratios, although given as an appropriate measurement range in CRDS,[39] should be treated with caution, as they might yield unreliable results. As an alternative, if such low water mixing ratios are unavoidable, such as in cooler environments, the CRDS instrument should be characterised thoroughly for this range. As demonstrated, these effects are clearly instrument-specific[37] and might even change over time. Therefore, the water mixing ratio dependency of each instrument should be individually determined. We recommend determining this dependency based on at least three water isotope standards, spanning a wide isotopic range that covers the expected measurement range, and in steps of, for example, 0.4 μL.[12] We also recommend making use of the Picarro Autosampler Control and autosampler to run an automated sequence. Alternatively, head space calibration in combination with a dilution of dry air or N₂ can be used. We further suggest first correcting the water mixing ratio dependency, for example, to correct all isotopic compositions to, for instance, the target value of ~ 18,000 ppm (default mixing ratio in Picarro software). The water mixing ratio correction should be based on a water isotope standard in a similar isotopic range to the measured value to be corrected. Then, the newly derived isotopic composition can be calibrated against a calibration curve, determined for the target mixing ratio range (e.g. ~ 18,000 ppm) and ideally from at least three water standards, spanning a wide isotopic range of the expected measurement values.[12,37] If these steps are followed, new in-situ methods provide an enormous advantage and can provide insights into ecohydrological dynamics and processes on a (sub) daily basis.[42,43,59]

5 | CONCLUSION

The usage of the inappropriate cover material for CVE and negligence of different water mixing ratios for CRDS measurements can strongly modify the obtained results for δ^2H and δ^{18}O. If not corrected ade-quately, these results might lead to erroneous or invalid conclusions about (ecohydrological) processes. Thus, we strongly recommend following and reporting strict measurement protocols in water-stable isotopologue research with CVE and CRDS.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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