Correcting for Biogenic Gas Matrix Effects on Laser-Based Pore Water-Vapor Stable Isotope Measurements

Benjamin Gralher,* Barbara Herbstritt, Markus Weiler, Leonard I. Wassenaar, and Christine Stumpp

The isotopic composition ($\delta^2$H, $\delta^{18}$O) of pore water is an invaluable tracer for the minimally invasive study of subsurface water flow and transport processes. Here, we evaluated a method for pore water isotope analysis that combines laser-based isotope analyzers and water-vapor isotope equilibration using evaporation-proof metalized sample bags. We tested inflation atmospheres (dry air vs. pure N₂) and the impact of biogenic gas (CO₂, CH₄) accumulation for storage times of up to 4 wk. Samples were analyzed with a water isotope analyzer (Picarro L2120-i) and a gas chromatograph. Air-inflated water vapor samples showed a greater range of gas matrix effects ($\delta^{18}$O: 9.63‰; $\delta^2$H: 21.7‰) than N₂-inflated samples ($\delta^{18}$O: 7.49‰; $\delta^2$H: 10.6‰) induced by nonuniform buildup of biogenic CO₂ starting immediately after sample preparation. However, only air-inflated samples could be reliably corrected using instrument-specific sensitivity factors that were empirically determined by interpretation of periodically repeated isotope measurements. Corrected water isotope data were confirmed by similarity with local precipitation and suction cup isotope data. Residual uncertainties were well below the natural variations of soil water isotope values and independent of storage time, thus allowing for consistently reliable interpretations of soil water isotope profiles. We conclude that, especially for pore water sampling that requires small sample volumes and/or long storage times, metalized sample bags should be used to prevent evaporation notwithstanding the enhanced buildup of biogenic gases. Further, if gas matrix effects cannot be excluded, air inflation is preferred over pure N₂ as only in that case can reliable postcorrections be performed by using internal data only.

Abbreviations: CRDS, cavity ring-down spectrometry; DVE-LS, direct vapor equilibration laser spectrometry; GC, gas chromatography; GMWL, global meteoric water line; IC-excess, line-conditioned excess; LMWL, local meteoric water line; LWV, analyzer-recorded line width variable; OA-ICOS, off-axis integrated cavity output spectrometer.

Environmental tracers like the isotopic composition of water ($\delta^2$H, $\delta^{18}$O) have proven to be valuable tools in hydrology covering all parts of the water cycle. In subsurface hydrology, stable isotopes of water have been successfully applied to determine soil evaporation (Allison, 1982; Braud et al., 2009), groundwater recharge rates and sources (Adomako et al., 2010; Blasch and Bryson, 2007; Koeniger et al., 2016), flow paths (Garvelmann et al., 2012; Stumpp and Hendry, 2012; Uchida et al., 2006), mixing processes (Thomas et al., 2013), transit times (Stumpp et al., 2009; Timbe et al., 2014), root water uptake patterns (Rothfuss and Javaux, 2017), and hydraulic lift (Meunier et al., 2018). Kendall and McDonnell (1998) and Vitvar et al. (2005) presented extensive summaries of stable isotope applications in catchment hydrology. Sprenger et al. (2016) placed special emphasis on pore water isotope applications.

In traditional pore water isotope profiling studies, substantial resources were required for permanent and inflexible field installations, such as lysimeters or suction cups, or extensive laboratory work performing pore water extraction from core samples. Typical restrictions imminent to such extraction methods include their failure at low gravimetric...
water contents, their lack of suitability for certain soil types, or the comparatively large soil sample volumes necessary to obtain sufficient extracted water for conventional isotope-ratio mass spectrometry (Orlowski et al., 2016). As a consequence, pore water isotope studies are restricted by the tradeoff between analytical effort and achievable profile resolution. For the meaningful interpretation of pore water profiles, however, appropriate spatial depth resolutions that record naturally occurring variations of isotope signatures are needed. For many soil profiles, these requirements are centimeter-scale resolution, for instance, in the case of slow subsurface water flow velocities induced by low precipitation input or low hydraulic conductivity. Complex geology, multidirectional subsurface flow, or heterogeneous infiltration patterns can require further increase of spatial sampling resolution and thus create experimental limitations. Therefore, effective isotope studies can only be performed with methods that allow for high throughput of small-sized samples.

With the advent of laser-based techniques of isotope analysis like cavity ring-down spectrometry (CRDS) (Gupta et al., 2009) or off-axis integrated cavity output spectrometry (OA-ICOS) (Baer et al., 2002), direct and fast measurement of water in the vapor phase became feasible. This led to new methods of pore water isotope analysis, for example, via direct vapor equilibration laser spectrometry (DVE-LS) (Hendry et al., 2015; Wassenaar et al., 2008). Direct vapor equilibration laser spectrometry bypasses many of the previous laborious preparation steps while increasing the number of soil or rock samples that can be processed per day. However, the method’s simplicity can be complicated by sample storage, whereby biogenic, spectrally interfering gases may build up inside the sample storage containers. Under oxic conditions, this refers mainly to CO$_2$, which in natural soils can range between atmospheric levels (400 × 10$^{-6}$ mol mol$^{-1}$) and extremes of 20% (Johnson et al., 2013). Although there seems to be little influence of CO$_2$ on OA-ICOS laser systems (Sprenger et al., 2017), it has been demonstrated that CRDS laser systems are highly sensitive to background gas compositional changes (Gralher et al., 2016; Johnson and Rella, 2017). However, this effect could be corrected by using analyzer-recorded readings combined with specialty gases (N$_2$, CO$_2$, and/or synthetic air) for the determination of analyzer sensitivity (Gralher et al., 2016). Furthermore, the presence of CH$_4$ (Hendry et al., 2011), alcohols (Brand et al., 2009), or H$_2$S (Malowany et al., 2015), which may appear in anoxic or contaminated sites, has been reported to affect CRDS isotope readings.

Originally, the DVE-LS method was tested using an OA-ICOS instrument and food-grade plastic freezer bags (e.g., Ziploc). In the meantime it has become evident that these sample bags are not diffusion tight (Hendry et al., 2015; Herbstritt et al., 2014). On one hand, this has the advantage that elevated concentrations of biogenic headspace CO$_2$ are quickly leveled out, headspace conditions inside the container never become anoxic, and thus more troublesome biogenic gases like CH$_4$ are not produced at all. On the other hand, this will inevitably lead to diffusional water loss that eventually becomes substantial, depending on sample size and water content, ultimately leading to significant evaporative enrichment of heavy isotopes (Hendry et al., 2015). Comparatively small soil or rock sample volumes, however, are highly susceptible to evaporation-induced effects that can be avoided by the application of metalized, evaporation-safe sample bags. The downside of these diffusion-tight bags is the potential buildup of spectrally interfering biogenic gases whose effects and “correctability” are not yet fully investigated.

Consequently, the aim of this study was to investigate the impact of longer-term sample storage on the interpretation of DVE-LS data from natural soil samples using a background-gas-sensitive isotope analyzer. More specifically, we tested the feasibility of a proposed postprocessing data correction scheme that accounts for key biogenic background gases using diffusion-tight sample containers and different inflation atmospheres. We utilized the temporal changes in analyzer readings of periodically repeated isotope measurements for the determination of background gas sensitivities and compared calculated biases with headspace gas mixing ratios obtained by gas chromatography (GC). Subsequently, we compared our corrected DVE-LS data with independently obtained isotope data from the area under investigation. Given the results, we propose strategies for mitigation of potential storage-induced effects resulting from biogenic gases.

**Materials and Methods**

Natural soil (Cambisol) was sampled in August 2013 in two campaigns 1 wk apart at various sites near Miesbach, Upper Bavaria, Germany. For this area, biennial, monthly integrated water-stable isotope time series were available from precipitation and soil water sampled via a network of tensiometer-controlled suction cups installed at four different stations, each at two or three different depths (0.3, 0.6, and 0.9 m). Suction cup data covered the frost-free time period of April through November, whereas precipitation data covered the entire timeframe. Detailed descriptions of site characteristics and installations are found in Stoewer et al. (2015). Eleven depth profiles of soil samples were taken during the first (n = 4) and second (n = 7) sampling campaigns at a vertical resolution of 10 cm down to maximum depths of 40 to 100 cm using a hand auger (7-cm diam., Eijkelkamp). Aliquots (∼150 mL, n = 80) with volumetric water contents ranging from 0.2 to 0.4 m$^3$ m$^{-3}$ were transferred into metalized sampling bags of tri-ply, adhesive laminated sheets including one 12-μm layer of aluminum foil (1-L volume, Item no. CB400-420 BRZ, Weber Packaging, http://www.weber-packaging.de) for storage and subsequent DVE-LS analysis. Special care was taken to avoid evaporation during sampling and storage. That meant quick sample handling (<1 min), avoidance of open sunlight, and storage in a cooler while in the field. At the end of each sampling day and immediately upon arrival in the laboratory, sampling bags
from the first campaign were inflated with pure N₂ gas, whereas bags from the second campaign were inflated with pressurized air of atmospheric composition. All bags were inflated to full size at ambient pressure and heat sealed immediately thereafter. A small blot of silicone was placed on the outside to later serve as the septum. These metalized bags have a licensed food safety that guarantees inert material and indicates that no interaction with the interior is taking place. They had been found to be failure-safe and evaporation safe when correctly heat sealed, with total water losses below the detection limit (0.1 g) during the course of 5 wk, as compared with 0.5 g wk⁻¹ for the Ziploc bags originally employed for DVE-LS analysis (Herbstritt et al., 2014). Therefore, post-equilibration changes of the true isotopic composition of soil water and headspace vapor were excluded during the course of this experiment.

Schematics of the sample preparation and analysis setup are presented in Fig. 1. Isotope analysis (δ²H, δ¹⁸O) of the headspace water vapor of all samples was conducted with a hollow needle inserted through the silicone blot and directly connected by a 0.32-cm (1/8-inch) perfluoroalkoxy (PFA) tube to the sample inlet port of a Picarro L2120-i water-stable isotope analyzer. Using the analyzer-controlled flow rate of ∼35 mL min⁻¹, individual sample analyses lasted until stable readings of vapor concentration and isotopic data were observed. Stable readings were defined as δ-values and H₂O slopes close to zero and standard deviations of <150 ppm by volume for vapor concentration, <0.25‰ for δ¹⁸O, and <1.5‰ for δ²H, related to 1-min-averages using the analyzer’s statistics tool in the graphical user interface. Sample to sample time was ∼5 min. Measurements were conducted 3 d after inflation (t₀) and repeated after 2 (t₂) and 4 wk (t₄), ensuring persistent isothermal storage. Three different in-house standard waters with distinct isotopic compositions (−1.57, −10.73, and −16.75‰ for δ¹⁸O; −11.6, −76.5, and −113.5‰ for δ²H) served as calibration standards, and 20 mL of each were added into three metalized bags, amounting to a total of nine individual standards. Simultaneously to the sample bags, these standards were also inflated using either pure N₂ or pressurized air and stored in the same temperature-controlled environment as the soil samples to ensure identical conditions during isothermal equilibration between the isotopically known liquid phase and the repeatedly analyzed gaseous phase. Bulk water contents of samples (27–80 mL) and standards (20 mL) were sufficient to exclude measurable enrichment of heavy isotopes in the liquid phase after evaporation of a small fraction of water (∼17 μL) needed for bulk vapor saturation and isotopic equilibration of headspace atmospheres. During analysis, each subset of five soil samples was followed by one of the standards that cycled through the different isotopic compositions.

Readings during stable isotope analyses also included the analyzer-recorded line width variable h₂o_y_eff_a, which will be referred to as LWV hereafter. It changed systematically with carrier gas composition only and thus facilitated a postprocessing correction of carrier gas effects, as detailed in Gralher et al. (2016). Uncalibrated readings of isotope values (δ) were corrected using the expression

\[
\delta_{\text{corrected}} = \delta_{\text{observed}} - m_i \times (\text{LWV} - \text{LWV reference})
\]

Reference values of LWV were calculated by averaging the respective readings taken during standard analyses of the same campaign (i.e., inflation atmosphere). Subscript i is used here as a control variable referring to the different isotope ratios (1 = δ¹⁸O, 2 = δ²H). The sensitivity factors \(m_{i,1} = \Delta\delta^{18}O/\Delta\text{LWV}\) and \(m_{1,2} = \Delta\delta^{2}H/\Delta\text{LWV}\) were also calculated for each inflation atmosphere separately. They are identical to the slopes of the linear regression models that are obtained by plotting differences (Δ) as observed over time (t₂ − t₀, t₄ − t₀) in uncalibrated isotope readings (δ¹⁸O and δ²H, vertical axis) against the respective...
changes of LWV (horizontal axis) of all samples subjected to the same inflation atmosphere. Calibrated isotope data were then obtained following published methods (Wassenaar et al., 2008) and reported in delta notation (‰) referenced to the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation (VSMOW-SLAP) scale (Craig, 1961a). The general distribution of uncorrected and corrected DVE-LS data in a dual-isotope cross-plot was compared with that of local suction cup data to validate the results. Furthermore, precipitation data were used for the construction of a local meteoric water line (LMWL), which is the local equivalent of the global meteoric water line (GMWL) (Craig, 1961b). The LMWL was taken as reference for the calculation of line-conditioned excess (lc-excess) values (Landwehr and Coplen, 2004).

Repeated isotope analyses ($t_2$ and $t_4$) were accompanied by GC analyses. For this purpose, 25 mL of each sample bag’s headspace was sampled with a gas-tight syringe and transferred into a SRI 8610C gas chromatograph (SRI Instruments) capable of analyzing CO$_2$, N$_2$, and CH$_4$ concentrations. Mixing ratios of the gaseous constituents were calculated by comparing the areas under the respective chromatograms. Calibration was facilitated by assuming that all gaseous constituents of individual samples added up to 100% or that the N$_2$ mixing ratio remained constant compared with atmospheric levels (78.08%) in the cases of N$_2$ and air inflation, respectively.

Results

Dinitrogen Inflation

The correlations between the changes in uncalibrated isotope readings ($\Delta^18$O and $\Delta^2$H) and the LWV during the course of 2 and 4 wk after pure N$_2$ inflation of DVE-LS samples can be seen in Fig. 2. There were clear linear relationships in the case of $\Delta^18$O, but the slopes were inconsistent over time (97.6 vs. 112.5‰ ppm$^{-1}$), with the data clusters centering near the origin. Given the higher $R^2$ in the case of $t_4 - t_0$, this slope ($m_{1.1}$) was considered appropriate to be used for correction (Eq. [1]) of all $\Delta^18$O data prior to calibration. However, $\delta^2$H data did not show such a clear linear relationship after 2 or 4 wk. Therefore, no correction was applied to uncalibrated $\delta^2$H data. After 2 and 4 wk, changes in $\Delta^18$O readings spanned ranges of 4.92 and 7.49‰, respectively, whereas changes in $\delta^2$H readings spanned ranges of 7.7 and 10.6‰. The lack of corrected $\delta^2$H data vitiated a qualitative comparison of isotope data from N$_2$-inflated DVE-LS samples with available precipitation and suction cup data in the dual-isotope space. No correlation was found between changes in isotope readings and CO$_2$ mixing ratios (data not shown). In one topsoil sample, small concentrations of 0.03 and 0.21% of CH$_4$ were detected via GC analysis after 2 and 4 wk of sample storage, respectively. The mean delta values of this sample’s isotope readings were biased by up to $\sim$0.0 and $\sim$300‰ in the case of $\Delta^18$O and $\delta^2$H, respectively, along with $\sim$7- and 40-fold increases in measurement uncertainty. This sample was therefore excluded from further consideration.

Air Inflation

Figure 3 shows the relations between changes in uncalibrated isotope readings ($\Delta^18$O and $\Delta^2$H) and the line width variable LWV during the course of 2 and 4 wk in the case of air-inflated DVE-LS samples. There were consistently strong coefficients of determination and matching slopes ($m_{1.1}$) in the case of $\Delta^18$O. Slopes of $\delta^2$H data ($m_{1.2}$) observed over time were variable (−113.7 vs. −171.6‰ ppm$^{-1}$). All data clusters predominantly led toward positive LWV values. Given the higher $R^2$ values, $t_4 - t_0$ data were taken for correction (Eq. [1]) of uncalibrated isotope data subjected to air inflation. Changes in uncorrected $\Delta^18$O readings spanned ranges of 5.97 and 9.63‰ during the course of 2 and 4 wk, respectively. The respective changes of $\delta^2$H readings spanned ranges of 16.2 and 21.7‰.

The effect of the applied corrections accounting for the bias due to gas matrix changes after air inflation can be seen in Fig. 4. Virtually all of the uncorrected data plotted well below the LMWL ($\delta^2$H = 7.53 × $\Delta^18$O + 3.45‰) and extended from there toward higher $\Delta^18$O and lower $\delta^2$H values. Clearly, this effect increased with storage time. For topsoil samples, the average vertical deviations of the LMWL at $t_0$, $t_2$, and $t_4$ were $\sim$27.9 ± 9.1, $\sim$65.4 ± 21.0, and $\sim$85.3 ± 39.0‰, respectively. Below the 10-cm soil depth, the respective deviations were $\sim$9.0 ± 5.5, $\sim$15.8 ± 10.1, and $\sim$17.7 ± 12.5‰. Maximum numbers were consistently found in topsoil samples, followed by an exponential decrease with depth, consistent with lc-excess values (Fig. 4, right). Corrections clearly resulted in isotope data plotting closer to the LMWL and the data from suction cups. Average lc-excess values after correction covering all depths were $\sim$2.6 ± 2.7, $\sim$2.5 ± 3.1, and $\sim$2.5 ± 2.0‰ for $t_0$, $t_2$, and $t_4$, respectively. This was in agreement with lc-excess values of the available local precipitation (0.0 ± 3.5‰) and suction cup data (1.2 ± 1.5‰).

Figure 5 reveals the correlation between our applied corrections and background gas CO$_2$ mixing ratios in air-inflated samples. Linear relationships were observed that were positive in the case of $\Delta^18$O and negative in the case of $\delta^2$H. Only minor differences in slopes were found, regardless of soil depth and storage time. This applied to both $\Delta^18$O and $\delta^2$H. All regression line offsets were close to the origin. Maximum mixing ratios of 14.1 and 19.4% after 2 and 4 wk, respectively, were found in topsoil samples. No CH$_4$ was found in any of the air-inflated DVE-LS sample bags during the course of 4 wk.

In Fig. 6, corrected isotope data obtained after different storage times of air-inflated samples were compared with one another. All data plotted close to their 1:1 lines. Absolute deviations from the 1:1 line for $t_2$:$t_0$ were 0.16‰ for $\Delta^18$O and 2.9‰ for $\delta^2$H averaged across all soil depths. The respective numbers for $t_4$:$t_0$ were
Fig. 2. Correlations between changes in uncalibrated isotope readings (left: $\delta^{18}$O, right: $\delta^{2}$H) and analyzer-recorded line width variable (LWV) during the course of 2 (top) and 4 wk (bottom) observed after pure nitrogen inflation. $t_0$, 3 d after sample inflation (initial measurement); $t_2$, 2 wk after initial measurement; $t_4$, 4 wk after initial measurement.

Fig. 3. Correlations between changes in uncalibrated isotope readings (left: $\delta^{18}$O, right: $\delta^{2}$H) and analyzer-recorded line width variable (LWV) during the course of 2 (top) and 4 wk (bottom) observed after air inflation. $t_0$, 3 d after sample inflation (initial measurement); $t_2$, 2 wk after initial measurement; $t_4$, 4 wk after initial measurement.
The observed natural range of corrected soil water isotope values was 5.55‰ for δ18O and 42.7‰ for δ2H. The respective signal (i.e., the natural range) to noise (i.e., the average residual) ratio varied from 23 to 35 and from 14 to 28 for δ18O and δ2H, respectively.

**Discussion**

Our experiment simulated the conditions that DVE-LS samples may be subjected to after extensive and/or remote sampling campaigns whereby storage times of weeks or longer can elapse prior to isotope analysis. To prevent evaporative water loss and subsequent corruption of isotope data due to enrichment of heavy isotopes in the liquid phase, it is critical to store samples in diffusion-tight sample bags for longer times. The concomitant is potential, nonuniform accumulation of biogenic gases in natural soil or rock samples during this storage time and the potential spectral effects on laser-based water isotope analyses. On the timescale of our study, these storage-induced effects of biogenic gases were corrected by interpretation of repeated isotope measurements without significant loss of data quality. However, the choice of headspace inflation gas restricted the “correctability” of isotope data from DVE-LS samples undergoing background gas compositional changes.

**Dinitrogen Inflation**

Only the changes of δ18O revealed a clear correlation with changes in the LWV that justified the calculation of an analyzer-specific sensitivity factor for subsequent postcorrection of N2–inflated DVE-LS samples. This allowed for interpretations based on δ18O alone, such as depth profiles for the calculation of vertical...
subsurface flow and transport parameters. However, it restricted the possibility to confirm corrected data with isotope data differing in represented space and time. In addition, any interpretations performed exclusively in the dual-isotope space, such as multiple endmember mixing analysis or evaporation estimates (Allison, 1982; Rothfuss and Javaux, 2017; Stumpp and Hendry, 2012), were impossible in this case. This constituted a tremendous loss of information that could be derived from soil water isotope data. The sensitivity factor for $\delta^{18}$O here ($m = 112.5 \text{ ppm}^{-1}$) was significantly different from that found for an identical instrument.
Therefore, the time of flushing during bag inflation (5–10 s) may (i.e., anoxic conditions) is used as an inflation atmosphere. (here, 4 wk) rather than snapshots. 

Although it was expected that individual analyzers would display unique sensitivity factors, the magnitude of difference was remarkable. Additionally, the fact that the data cluster extended equally toward positive and negative ΔLWV values in this case made corrections unreliable. For this reason, and due to the fact that the calculated sensitivity factor differed over time, it would have been essential to confirm corrected isotope data and thus the applied sensitivity factors by independent means. In our case, however, a direct quantitative comparison of corrected δ18O data was impossible, as DVE-LS data represented a snapshot of soil water isopes subject to, for example, depth-dependent time lags and mixing or dispersion as compared with precipitation data. A 1:1 comparison with suction cup data from the same depths was impossible, as these represent time periods (here, 4 wk) rather than snapshots.

During sample preparation, close attention was paid to quick sample handling to avoid evaporation, for obvious reasons. Therefore, the time of flushing during bag inflation (5–10 s) may have been too short to remove all the air that inevitably enters the sampling bags on opening. Presumably, this resulted in non-uniform air–N2 mixtures as inflation atmospheres. However, the biogenic evolution of such mixtures was not considered in the proposed correction scheme. Such an evolution between more than two endmembers would have required a multistep correction and consequently the identification of other analyzer-recorded correction variables beyond the LWV or additional GC analyses. This would have constituted a significant impairment of the current user friendliness of the DVE-LS method. Additionally, CH4 was encountered in one of these samples after only 2 wk of storage. This indicated further restrictions that are inherent when pure N2 (i.e., anoxic conditions) is used as an inflation atmosphere.

### Air Inflation

In contrast with the first part of this study, clear correlations were found between temporal changes of both uncalibrated isotope readings (δ18O and δ2H) and LWV in the case of air-inflated DVE-LS samples. This allowed for a straightforward correction of the entire dataset without necessitating further laboratory installations and specialty gases beyond air as the inflation atmosphere. The calculated slopes used for correction better matched the ones found in the previous study (Gralher et al., 2016), as compared with the N2 part of this study, but they were still not fully matching (δ18O: 83.5 vs. 85.7‰ ppm⁻¹, δ2H: 171.6 vs. −213.0‰ ppm⁻¹). Again, it was anticipated that individual CRDS analyzers may have differing sensitivities. In the case of δ2H, the respective slope changed over time. However, the corrected isotope data and thus the selected analyzer-specific sensitivity factors could be confirmed by taking the general distribution in the dual-isotope space and le-excess values of independent isotope datasets as the critical measures. Furthermore, this supported the assumption that extended storage did not change the true isotopic composition of soil water and headspace vapor. Rather, an ostensible isotope effect induced by measurement artifacts was encountered. In some samples, such gas matrix effects became evident after only 3 of storage (t0), which is the time commonly proposed for equilibration of DVE-LS samples (Wassenaar et al., 2008). The fact that the biases continuously increased over time did not have an impact on the overall quality of the corrected data. Average absolute deviations among corrected data after different storage times were, in most cases, well below the DVE-LS method’s reported uncertainties of 0.40‰ for δ18O and 2.0‰ for δ2H (Wassenaar et al., 2008) and consistently more than one order of magnitude below the natural range of observed soil water isotope values. This is despite biases of air-inflated samples spanning greater range than those of N2-inflated samples. Potential evaporative enrichment of heavy isopes inside the sample bags after vapor loss through leaking silicone blots would have shifted data above the respective 1:1 lines in Fig. 6, which was apparently not the case.

Regardless of soil depth and precorrection biases, average deviations from the LMWL after corrections matched the uncertainties of deuterium excess or le-excess values based on generally accepted individual isotope ratio measurement uncertainties (0.2‰ for δ18O, 1‰ for δ2H). Furthermore, they coincided with deviations of the isotope datasets obtained by other means. They were much smaller than the observed natural variability, thus allowing for consistently meaningful interpretations of the desired pore water isotope depth profiles. This held likewise for both isotope ratios investigated. In general, restrictions may apply for deeper soil layers where the seasonal amplitudes are dampened due to mixing and dispersion, thus approaching analytical uncertainties. However, that is also the case without background gas effects.

Incomplete flushing during inflation—resulting in an unknown mixture of air and inflation atmosphere, as is suspected problematic in the case of N2—did not seem to have an additional effect when air was used. The resulting air–air mixture still followed the same evolution toward 20% CO2, which was assumed anyway. In this context, however, it is suggested that in situ assays of water isotope observations based on diffusive or advective sampling of ambient atmospheres (Rothfuss et al., 2013; Volkmann and Weiler, 2014) should preferably use dry (zero) air as carrier and for dilution purposes. These assays were developed to investigate soil gas environments that may, for instance, vary between the endmembers air and 20% CO2 (Johnson et al., 2013). Additional endmembers would unnecessarily introduce the same problems encountered in the N2 inflation part of this study. Therefore, these assays must use air to remain between the naturally default gas mixing endmembers and thus allow for reliable postcorrection of background gas effects without the need of further analyses.

Although no CH4 was encountered in air-inflated samples during the course of this experiment, it cannot be excluded that this could eventually happen, as some topsoil samples were already approaching 20% CO2. This constitutes the maximum mixing ratio that is expected to be encountered in natural soils (Johnson et al., 2013) and can therefore be considered the transition toward anoxic...
conditions after air inflation. However, the presence of such a wide range of CO₂ mixing ratios should be perceived as an advantage. Given the strong and consistent correlation between CO₂ mixing ratios and the applied corrections with offsets close to the origin, it can be deduced that CO₂ was the key culprit encountered in our experiment. Thus, the observed biases were almost exclusively the result of microbial respiration in the natural soil samples. Unsurprisingly, the negative exponential decrease with depth of the observed bias profiles looked similar to typical depth profiles of microbial biomass and organic C distribution (Anderson and Domsch, 1989; Fierer et al., 2003; Jobbágy and Jackson, 2000). Consequently, a bias profile may be suitable as a low-resource proxy for depth profiles of microbial activity and organic C (turnover). Of course, this only holds true when punctual calibration and the principle of identical treatment of all samples regarding storage history and sample size, for example, are applied. Since microbial activity is conventionally evidenced by respiratory CO₂ output (Anderson and Domsch, 1978), this would be another advantage of diffusion-tight storage containers, as only they ensure reliable CO₂–correlated data. These data are then collected with minimal additional effort (e.g., calibration) by means of a background-gas-sensitive (here, water-stable isotope) analyzer.

Correction of the headspace gas matrix effects as performed in the air inflation part of this study prevented a variety of misinterpretations from being made. Obviously, sensitivity factors regarding the different isotope values had opposing signs, causing δ¹⁸O values to appear enriched and δ²H values to appear depleted in their heavy isotopes. Consequently, biased isotope data were shifted with a negative slope orthogonally below the commonly used references GMWL or LMWL in dual-isotope space. Therefore, uncorrected isotope data would have led to the misinterpretation of subsurface evaporative enrichment. Furthermore, back-calculating of pre-evaporation source water using the humidity-dependent, persistently positive slope of a so-called evaporation line would have pointed in the direction of isotopically lighter water (i.e., the intersection of the evaporation line and LMWL; e.g., Gibson et al., 1993, and Fig. 3.14 in Leibundgut et al., 2009) potentially precipitated during colder seasons. Due to nonuniform biases, however, this presumed source water would have appeared to remain in the topsoil, whereas at slightly lower depths, apparently "younger" water was found that was paradoxically less affected by evaporation, although it must have been introduced during a warmer season. This apparent inversion of water age in depth profiles would erroneously be explained by upward flow or predominant, only seasonally active preferential flow. Of course, further estimates of water budgets based on the miscalculated mean isotopic composition and travel times and directions based on apparent age distributions, as well as resulting groundwater management strategies or vulnerability assessments, would have been equally invalid. Therefore, corrections of ostensible isotope effects due to background gas compositional changes are critical when applying the DVE-LS method in vadose zone isotope studies.

**Conclusion**

We provided evidence that water-vapor isotope data from air-inflated DVE-LS samples were spectrally compromised in as little as 3 d. The changes in the headspace gas composition of the sampling bags were caused by microbial activity and generation of CO₂. This CO₂ strongly interfered with water isotope spectra of the CRDS analyzer. Without proper data corrections, this would have led to misinterpretations of soil water isotope data. The analyzer-observed variable LWV allowed for consistent corrections without the need for exact knowledge of the biogenic gas concentrations. This is notwithstanding that the influence of CO₂ evolution began immediately after sample preparation and increased with storage time. Corrected isotope data were verified by their similarity with local precipitation and suction cup data. The sensitivity factors used for corrections are probably unique for different CRDS analyzers. Consequently, they need to be determined individually for other CRDS analyzers, which can easily be facilitated and repeated for reasons of potential drift detection using exclusively analyzer-recorded readings of measurements repeated for sufficiently long timespans. Nitrogen-inflated samples, however, could not be corrected using the same approach, and so N₂ should be avoided. We suggest that dry air (i.e., oxic conditions) is preferable over N₂ as an inflation atmosphere when background gas effects cannot be excluded. Further, the use of pure N₂ causes anoxic conditions, which could lead to the risk of CH₄ production after short equilibration times. This would impair both precision and accuracy of isotope data beyond acceptable limits. The effect of other gaseous constituents produced under anoxic conditions, such as N₂O from nitrate or H₂S from sulfate, remains unknown.

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