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A simple greenhouse experiment to explore the effect of cryogenic water extraction for tracing plant source water

N. Orlowski[1,2] | A. Winkler[1] | J.J. McDonnell[2] | L. Breuer[1,3]

[1] Institute for Landscape Ecology and Resources Management (ILR), Research Centre for BioSystems, Land Use and Nutrition (iFZ), Justus Liebig University Giessen, Giessen, Germany

[2] Global Institute for Water Security, University of Saskatchewan, Saskatoon, Canada

[3] Centre for International Development and Environmental Research (ZEU), Justus Liebig University Giessen, Senckenbergstraße 3, 35392 Giessen, Germany

Correspondence
N. Orlowski, Institute for Landscape Ecology and Resources Management (ILR), Research Centre for BioSystems, Land Use and Nutrition (iFZ), Justus Liebig University Giessen, Giessen, Germany.
Email: natalie.orlowski@hydrology.uni-freiburg.de

Present Address
N. Orlowski, Chair of Hydrology, Albert-Ludwigs-University of Freiburg, Friedrichstraße 39, 79098 Freiburg, Germany.

Abstract

Stable isotopes of water (^{2}H and ^{18}O) are useful tracers for determining root water uptake depths. In such studies, plant and soil water are extracted most commonly by cryogenic vacuum distillation. However, recent studies have suggested that cryogenic extraction conditions (extraction time, temperature, and vacuum) and soil physico-chemical properties affect the isotopic composition of extracted soil water. Here, we perform a simple greenhouse trial with 2 plant species (*Taraxacum officinale* and *Pelargonium* spp.) in 2 soil types (clayey loam and sand) to test our ability to match plant water to its putative soil water source(s) by using different extraction conditions (30–240 min, 80–200 °C, 0.1 Pa). We irrigated plants with water of known isotopic composition, sampled root crowns and soils at 2 depths, and varied the cryogenic water extraction conditions.

Our isotope results from the sandy soils were unaffected by cryogenic extraction conditions. In contrast, extraction parameters affected the isotope composition of waters recovered from clayey soil. This influenced the estimates of plant water sourcing, where $\delta^{2}H$ and $\delta^{18}O$ returned different results from each other. With higher extraction temperatures and longer extraction times, we gradually extracted more enriched soil water, which reflected the source water of both plant species. Our results imply that longer extraction times and temperatures for clayey soils are needed to reduce fractionation effects during the extraction procedure. Future studies should explore how these effects apply to natural clay-rich soils as well as plant tissue isotope composition.

KEYWORDS

cryogenic extraction conditions, cryogenic water extraction, isotope fractionation effects, plant water uptake, soil water extraction

1 | INTRODUCTION

The relationship between soil water distribution and plant water availability is an area of active study (e.g., Couvreur, Vanderborght, Draye, & Javaux, 2014; Ellsworth & Sternberg, 2015; Liu et al., 2011). Much progress has been made on plant water uptake location and magnitude (e.g., Asbjornsen, Mora, & Helmers, 2007; Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002; Liu, Liu, Li, Duan, & Li, 2010; Simonin et al., 2014). Such information is used for irrigation scheduling in water-limited ecosystems (e.g., Shen, Zhang, Gao, & Peng, 2014; Zegada-Lizarazu & Iijima, 2004; Zhang, Shen, Sun, & Gates, 2011) or documenting soil water competition by different plants (e.g., Meißner, Köhler, Schwendenmann, & Hölscher, 2012; Williams & Ehleringer, 2000). Much of this progress has come from the use of stable isotopes of water (^{2}H and ^{18}O) for quantifying plant water uptake (e.g., Barbeta et al., 2015; Bertrand et al., 2014; Butt, Ali, Fazil, & Latif, 2010). Although there exist some notable exceptions (e.g., Ellsworth & Williams, 2007; Lin & Sternberg, 1993; Zhao et al., 2016), plant water uptake is

generally a nonfractionating process (Dawson & Ehleringer, 1991). The origin of a plant's water has been determined largely by matching its isotopic composition with a single potential water source or a mixture of subsurface water sources (groundwater, deep and shallow soil water, or surface water; e.g., Evaristo, McDonnell, & Clemens, 2017).

Cryogenic vacuum extraction is currently the most widely used technique to extract soil and/or plant water for isotope analysis (Orlowski, Breuer, & McDonnell, 2016) and for quantifying the relative contributions of water sources to plant water uptake (Rothfuss & Javaux, 2017).

But recent work has shown that cryogenic extraction for certain soil types can be problematic (e.g., Gaj, Kaufhold, Koeniger, et al., 2017; Meißner, Köhler, Schwendenmann, Hölscher, & Dyckmans, 2014; Oerter et al., 2014; Orlowski, Breuer, et al., 2016; Orlowski, Frede, Brüggemann, & Breuer, 2013). Cryogenic extraction conditions (such as time, temperature, and vacuum) along with physicochemical soil properties (e.g., clay and carbonate content, cation exchange capacity, or soil organic carbon) can significantly impact the extracted δ^2H and $\delta^{18}O$ soil water values (Gaj, Kaufhold, Koeniger, et al., 2017; Meißner et al., 2014; Oerter et al., 2014; Orlowski et al., 2013; Orlowski, Breuer, et al., 2016). We still do not know all the factors affecting water extractions from different substrates. These issues with cryogenic extraction in soils now call into question whether cryogenically extracted soil and plant water isotope composition can indeed be used for an unbiased assessment of plant's source water. Controlled experiments of plant water source(s) in the context of extraction effects and influences are much needed to mechanistically assess such impacts.

Here, we perform a simple, but fully controlled, greenhouse study to identify the ideal cryogenic extraction parameters (valid for our cryogenic extraction set-up) for precisely matching soil water signatures with plant water isotopic signatures. As such, our goal is to improve the extraction method for such ecohydrological applications involving plant water source apportionment. We compare the isotopic compositions of irrigation water with plant root crowns of common dandelion (*Taraxacum officinale*) and geranium (*Pelargonium* spp.) and the water recovered via cryogenic extraction from two contrasting soils (clayey loam and sand) used as potting substrate. We irrigate these plants with water of known isotopic composition. We then extract the soil water under varying cryogenic extraction conditions (80, 120, and 200 °C for 30, 60, 180, and 240 min each) using a system set-up by Orlowski et al. (2013). We then address the following specific research questions: (a) Can we reliably match root crown water $\delta^{18}O$ and/or δ^2H values from *T. officinale* and *Pelargonium* spp. with extracted soil water and/or irrigation water under varying extraction parameters? (b) Are the plant–soil water matching results the same for δ^2H and $\delta^{18}O$? (c) How do soil types affect source water matching?

2 | MATERIAL AND METHODS

2.1 | Experimental design

Two soil types with differing physicochemical properties were chosen as potting material for the greenhouse experiment: a local fine sand from Homberg-Ohm (Hesse, DE) and a clayey loam from the German State Research Institute for Agriculture (LUFA Speyer, 2015; Table 1).

Prior to potting, soils were oven dried (200 °C, 48 hr) to avoid possible memory effects with residual soil water. Such memory effects can be responsible for deviations between spike water and cryogenically extracted water as observed by Koeniger, Marshall, Link, and Mulch (2011) and Newberry, Prechsl, Pace, and Kahmen (2017). We recognize that our harsh drying conditions do not occur in nature. However, tightly bound soil water can indeed influence the isotopic composition of mobile soil water (Newberry et al., 2017), which we aimed to avoid by oven drying the soils at 200 °C. We followed the approach of Barnard, de Bello, Gilgen, and Buchmann (2006) who found the most suitable plant tissue to investigate for stable isotopic

TABLE 1 Soil characteristics of LUFA 2.4 (clayey loam) and sandy soil (means ± SDs)

Parameter	Clayey loam	Sand
pH value	7.1 ± 0.1	6.2 ± 0.1
Max. water holding capacity (%)	39.9 ± 0.5	23.7 ± 1
Bulk density (g cm^{-3})	1.2	1.5
Cation exchange capacity ($cmol_c$ kg^{-1})	25.9 ± 2.5	3–5
Carbonate content (mass-%)	—	<0.5
Particle size (mm) distribution according to German DIN (%)		
<0.002 (clay)	41.9	2.5
0.002–0.063 (silt)	36.6	4.8
0.063–2 (sand)	21.6	92.7
XRD analysis (%)		
Kaolinite	18.8	92.7
Illite	18.0	3.7
Chlorite	1.2	0.0
Vermiculite	43.4	1.1
Smectite	0.5	0.1
Mixed layered clays (Illite/Smectite/Vermiculite)	18.1	2.4
XRF analysis (%)		
SiO_2	65.1	98.2
TiO_2	0.4	0.1
Al_2O_3	8.8	0.9
Fe_2O_3	3.1	0.1
MnO	0.1	<0.001
MgO	1.5	0.0
CaO	5.3	0.0
Na_2O	0.9	<0.01
K_2O	1.7	0.0
P_2O_5	0.2	0.0
SO_3	0.1	<0.01
Cl	<0.002	<0.002
F	<0.05	<0.05

Note. The mineral composition of soil samples was determined via X-ray powder diffraction (XRD) using a Philips X'Pert PW 1830 equipped with a PW2273/20 tube and a theta/theta-goniometer (PANalytical, EA Almelo, NL) following Poppe, Paskevich, Hathaway, and Blackwood (2016). Values were not corrected for reference intensity ratios. X-ray fluorescence (XRF) characterization of the chemical composition (in % weight) was performed using an Axios spectrometer (PANalytical, EA Almelo, NL).

signatures is the root crown. We chose two taproot-establishing plant species: common dandelion (*T. officinale*) and geranium (*Pelargonium* spp.). Taproot-establishing plants were chosen in order to ensure a sufficient amount of plant sample material for water extraction and to minimize boundary effects caused by lateral root growth. To avoid intraspecific and interspecific source water competition, plants were grown as monocultures. The daily photoperiod for the entire experiment was 14 hr; temperature was on average 25 °C, and relative humidity was maintained at 60%. Each plant was grown in a single free-draining pot (three replicates per species, soil type, and cryogenic extraction condition; 0.25 m height, 0.21 m top diameter, 0.17 m bottom diameter, volume: 5.85 L). We sowed on April 10, 2014. In order to ensure that a vertical taproot establishment and that we generated a sufficient amount of plant sampling material, we buried PVC tubes (0.12 m length, 0.05 m ID) into the soil (Figure 1). Plants were irrigated with a single water source of known isotopic composition (δ^2H: −59.75 ± 1.31; $\delta^{18}O$: −8.66 ± 0.22; N = 42) through additional, perforated, flexible PVC-tubes (Figure 1; 0.07 m OD), buried 0.04 m into the soil to minimize boundary effects or evaporation of irrigation water. We minimized the evaporative effect at the soil surface by covering the pots with gravel (Figure 1). Gravel mulch has long been used to reduce soil surface evaporation (Yuan, Lei, Mao, Liu, & Wu, 2009). For example, Walker and Richardson (1991) covered the soil surface with a 0.1-m mulch layer. Zarebanadkouki, Kim, and Carminati (2013) mulched the soil with a 1-cm layer of quartz gravel with a grain size of 3 mm to minimize evaporation. In a study by Bariac, Gonzalez-Dunia, Tardieu, Tessier, and Mariotti (1994), covering plant pots reduced the difference between soil water $\delta^{18}O$ and root crown water $\delta^{18}O$ by on average 0.1%. We monitored the volumetric water contents of the soil via frequency domain sensor probes (ML3 ThetaProbe, Delta-T Devices Ltd, Cambridge, UK) installed in two soil depths (0.06 and 0.17 m below soil surface). Accuracy of the probes was ±0.01 m^3 m^{-3} (1%).

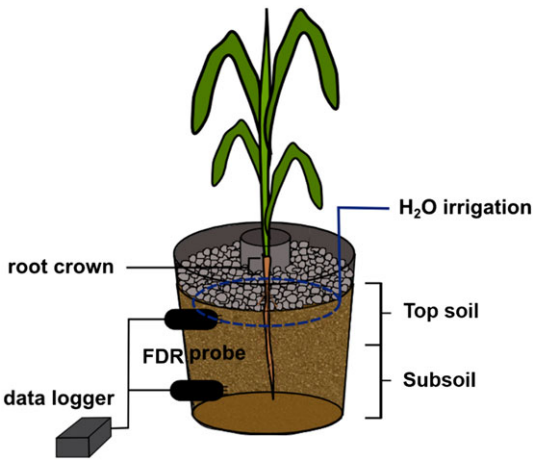

FIGURE 1 Experimental set-up of plant pots (0.25 m height, 0.21 m top diameter, 0.17 m bottom diameter, volume: 5.85 L) with PVC tubes (0.12 m length, 0.05 m ID) buried into the soil to support vertical root growth. Gravel covering the soil to prevent evaporation and perforated flexible PVC tubes for irrigation (0.07 m OD). Frequency domain sensor (FDR) probes for volumetric water content measurements were installed at two depths: 0.06 and 0.17 m below the soil surface. Root crowns, top and subsoil (0.06–0.16 m and 0.17–0.25 m, respectively), were sampled for stable water isotope analysis

Figure 2 shows the volumetric water content of soil and irrigation amounts applied to both soil types. To support plant growth, we applied liquid fertilizer together with the irrigation water which included a full complement of macronutrient and micronutrient (N:P:K ratio of 8:8:6; 0.05–0.1% by vol.). Fertilizer was applied once per week starting at the end of April 2014.

We sampled the root crowns and soils at two depths (0.06–0.16 m and 0.17–0.25 m) when roots matured enough to provide an adequate amount of sample material for water extraction (July 23 and 24, 2014). Plant and soil samples were transferred to amber glass tubes, capped, sealed with Parafilm®, and immediately frozen until cryogenic water extraction to avoid evaporative water losses.

2.2 | Cryogenic extraction conditions

The cryogenic extraction system of Orlowski et al. (2013) was utilized for water extractions. To identify plant source water, soil samples were extracted via cryogenic extraction at the following conditions: 80, 120, and 200 °C for 30, 60, 180, and 240 min at a static vacuum of 0.1 Pa. Plant samples were extracted at 98 °C for the duration of 180 min and a static vacuum of 0.1 Pa, respectively (Table 2). Gravimetric soil water analyses before and after oven drying of the extracted soils (105 °C, 24 hr) revealed complete water extraction in terms of weight.

2.3 | Isotopic analysis

δ^2H and $\delta^{18}O$ compositions were measured at the Institute for Landscape Ecology and Resources Management (Justus Liebig University Giessen, DE) according to the International Atomic Energy Agency standard procedure (Newman, Tanweer, & Kurttas, 2009) utilizing a Los Gatos Research DLT-100-Liquid Water Isotope Analyser (Los Gatos Research Inc., Mountain View, CA, USA). We followed the International Atomic Energy Agency standard procedure, which allows for drift and memory corrections. Isotopic ratios are reported in per mil (‰) relative to the Vienna Standard Mean Ocean Water (Craig, 1961). Precision of analyses was ±0.6‰ for δ^2H and ±0.2‰ for $\delta^{18}O$ (LGR, 2013). Since water extracts especially from woody plants can contain a high fraction of organic contaminants (Martín-Gómez et al., 2015; West, Goldsmith, Brooks, & Dawson, 2010), which might lead to spectral interferences when using isotope ratio infrared spectroscopy (Leen, Berman, Liebson, & Gupta, 2012; Schultz, Griffis, Lee, & Baker, 2011), all isotopic samples were checked for spectral interferences. Since Martín-Gómez et al. (2015) showed that postprocessing correction can significantly improve isotope ratio infrared spectroscopy accuracy with differences between mass spectrometry and isotope ratio infrared spectroscopy-corrected values falling within reasonable limits in most field-collected samples, we applied the Spectral Contamination Identifier postprocessing software (LWIA-SCI, Los Gatos Research Inc.). Martín-Gómez et al. (2015) further recommended the postprocessing correction as the first choice for analysis of samples of unknown contamination, allowing detailed ecohydrological studies at a reasonable cost. In our study, no plant water samples were found to be affected by organic contamination.

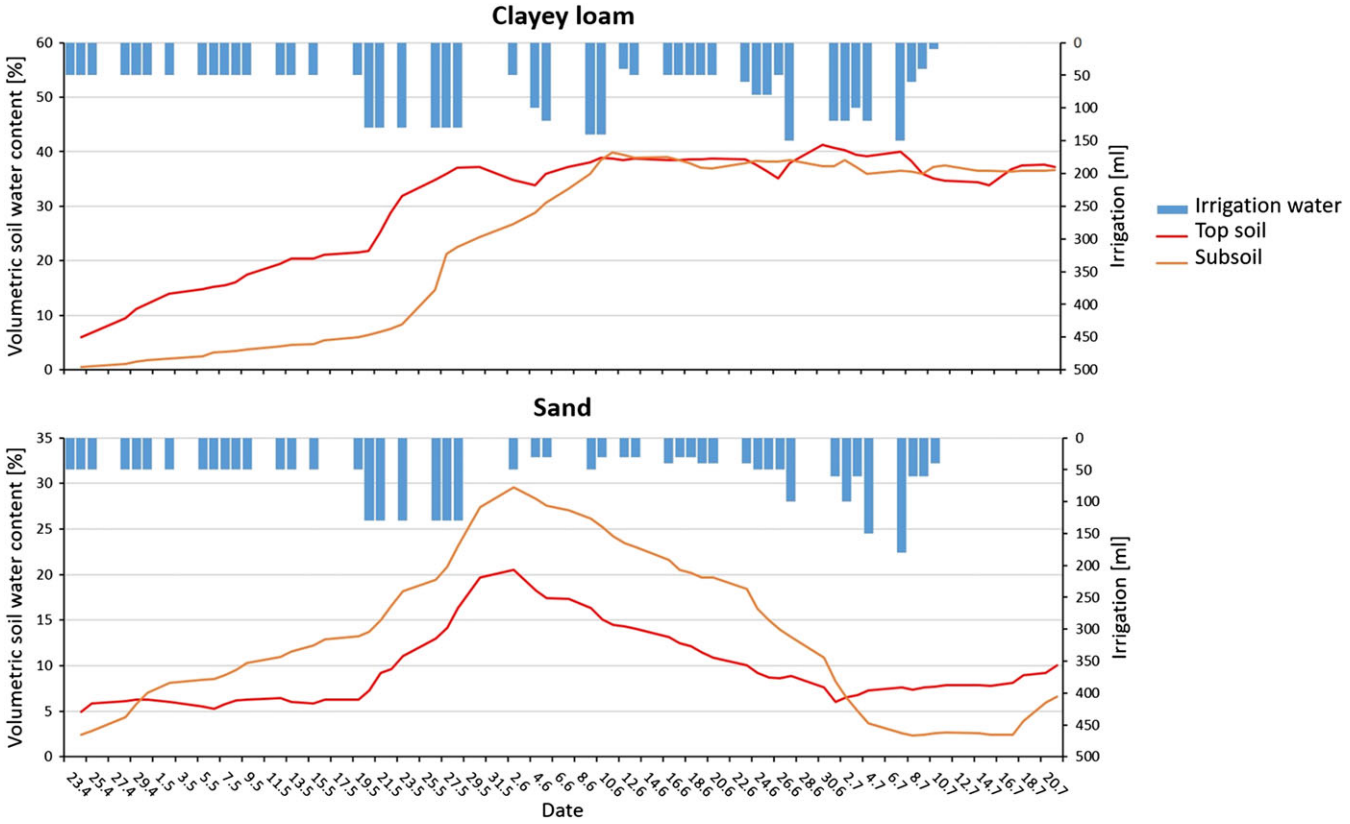

FIGURE 2 Two period moving average of volumetric soil water contents (orange and red lines) and irrigation amounts (blue bars) applied to clayey loam and sand pots and measured at two depths in the top and subsoil via frequency domain sensor probes. Sampling was conducted on July 23 and 24, 2014

2.4 | Statistical analysis

Since we irrigated the plants only with a single water source, we applied simple statistical analyses (utilizing RStudio Version 3.2.0., RStudio, Inc.) instead of multisource linear mixing models (e.g., as per Phillips & Gregg, 2003; Rothfuss & Javaux, 2017). All data were tested for normalcy (Shapiro–Wilk test). To investigate interactions of soil types and to determine the effects of extraction temperature and duration, ANOVA (analysis of variance) was performed. Tukey-HSD tests were run to specify significant differences between factors, separately for each soil type (with a significance determined by $p \leq .05$). To determine significant equivalences between root crowns and corresponding soil water isotopic signatures, a two one-sided test (TOST) was applied (equivalence margin for δ^2H and $\delta^{18}O = 2$). Since equivalences were only found for $\delta^{18}O$ values, multiple pairwise *t* tests were applied to compare δ^2H with the respective soil water ($p \leq .05$).

3 | RESULTS

3.1 | Isotopic variations

When comparing stable isotope values in dual isotope space, irrigation water plotted on the Global Meteoric Water Line, whereas all soil and plant water isotopic values plotted below and to the right of the Global Meteoric Water Line on a shallower slope (Figure 3). Generally, clayey loam soil evaporation water lines showed greater slopes (3.5–4.5) than sandy soil evaporation water lines (2.9–4.2). Isotopic signatures of root crown waters and extracted soil waters were therefore more enriched than those of irrigation water (Figure 3). This was particularly true for the $\delta^{18}O$ values of both soils. Isotopic fractionation due to evaporation leads to a stronger kinetic effect for ^{18}O compared with ^{2}H, resulting in evaporative enrichment of the water along an evaporation water line (e.g., soil evaporation water line) with a lower slope relative to the original water (Gonfiantini, 1986)—in our case, the irrigation water.

δ^2H values of both soils plotted closer to the irrigation water's δ^2H signature. On average, $\delta^{18}O$ values of plant water extracts deviated from the irrigation water by ±3.84‰, whereas δ^2H values showed a mean difference of ±4.59‰ to the irrigation water, which shows that δ^2H is generally less sensitive to kinetic fractionation effects (Garvelmann, Kuells, & Weiler, 2012). We observed the greatest isotopic differences between the irrigation water and the water extracted from geranium plants grown in clayey loam (−8.86‰ for δ^2H and −4.67 for $\delta^{18}O$; Figure 3).

Comparing the two soil types, shallow soil waters (0.06–0.16 m) for the sand were isotopically more enriched than waters from similar depths in the clayey loam. For the lower soil depth, there was no isotopic difference between the two soil types and less isotopic variation.

The isotopic range of water extracted from sand was broader than for clayey loam: standard deviations of $\delta^{18}O$ were almost double as high as for clayey loam (±0.78‰ vs. ±1.53‰) over all extraction settings and depths.

Comparing the soil and plant water isotopic signatures showed that dandelions grown in sand had the greatest isotopic differences to the soil water, and these plant waters were isotopically more

TABLE 2 Suitable extraction parameter settings (temperature and time) to match dandelions and geraniums water source grown in clayey loam and sand (for two soil depths: 0.06–0.16 m and 0.17–0.25 m, respectively) are highlighted in grey

			$\delta^{18}O$										$\delta^{2}H$									
		(°C)	80				120				200		80				120				200	
		(min)	30	60	180	240	30	60	180	240	30	60	30	60	180	240	30	60	180	240	30	60
Clayey loam	Dandelion	Top soil																				
		Subsoil																				
	Geranium	Top soil																				
		Subsoil																				
Sand	Dandelion	Top soil																				
		Subsoil																				
	Geranium	Top soil																				
		Subsoil																				

Note. No statistically significant matches were found for the remaining parameter settings (blank white boxes).

depleted (Figure 3). The opposite was the case when dandelions were grown in clayey loam. Geranium's root crown isotopic values generally plotted closer to potential soil water sources signatures.

3.2 | Extraction condition effects

Figure 3 shows how certain cryogenic extraction conditions (varying times and temperatures) affected the δ^2H and $\delta^{18}O$ values of clayey loam and sand water extracts. Temperature and time showed significant effects on both δ^2H and $\delta^{18}O$ for the clay loam (p = .00). The $\delta^{18}O$ values of clayey loam were also significantly different between the two depths (p = .00). The $\delta^{18}O$ values for the 200 °C extraction were significantly more enriched compared with lower extraction temperatures (Figure 3; p = .00). This was especially true for the shallow soil samples. Trends for δ^2H values were similar to $\delta^{18}O$ for the top soil: significant enrichment when soil water from clayey loam was extracted at 200 °C compared with lower extraction temperatures. In contrast, δ^2H values of clayey loam were statistically significantly depleted when extracted for 30 min when compared with longer extraction times (Figure 3). For the sandy soils, no significant extraction temperature effect or time effect was observed. However, shallow soil δ^2H values for the sandy soil were statistically significantly enriched when compared with the deeper soil samples (p < .01; Figure 3).

For extraction efficiency, we found no statistically significant differences between the weights of extracted samples (before and after oven drying) for any of the applied extraction conditions (p > .05). The water recovery rate of all samples was 99.5% to 99.9%.

3.3 | Plant source water

We observed greater root lengths for plants grown in sand than for those in clayey soils (Figure 4). Average root lengths in clayey loam soils were 0.10 m for geranium and 0.16 m for dandelion. In sandy soils, measured average root lengths were 0.17 m for geranium and 0.21 m for dandelion (Figure 4).

Dandelion's root crown isotopic signature matched the shallow clayey loam soil isotopic value for extraction parameter settings of 200 °C and 240 min for $\delta^{18}O$ and 200 °C and 180 min for δ^2H (Table 2).

We could find no statistically significant match between the clayey loam subsoil and dandelion's root crown isotope values. For dandelion in sand, three extraction condition settings for $\delta^{18}O$ worked to match top soil and plant water isotope values (high temperatures and longer extraction times). This was not the case for δ^2H where all extraction settings performed well (except 30 min at 200 °C) for matching dandelion's δ^2H values with its associated soil water (Table 2) in the sand pots. Since $\delta^{18}O$ and δ^2H returned different results, it was difficult to narrow down the ideal extraction parameter settings for dandelion's water source in sand.

Our results suggest that geranium grown in clay soil used water from the top soil. δ^2H values of geranium's root crowns reflected top soil signatures at extraction conditions of 200 °C and >180 min (Table 2, Figure 3). Several other parameter settings returned matches between geranium's $\delta^{18}O$ values and clay's top soil (Table 2). Both geranium and dandelion plants grown in the clayey loam seemed to

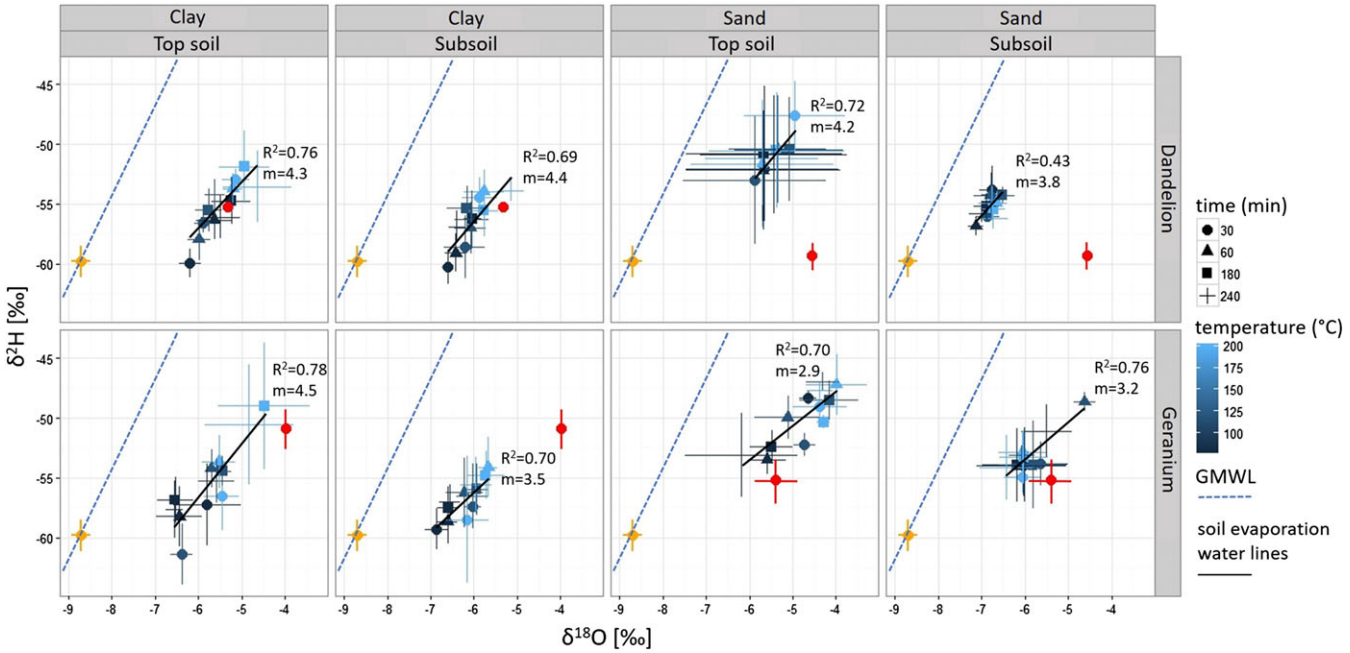

FIGURE 3 Dual isotope plots for both plant species (dandelion and geranium), soils (clayey loam and sand), and soil depths (top and subsoil), including irrigation water (orange dots) and root crown (red dots) isotopic values. X- and Y-error bars represent the isotopic variation of the replicates. The colour code represents applied extraction temperatures, and the symbol type characterizes extraction times. The Global Meteoric Water Line (GMWL) as per Rozanski, Araguás-Araguás, and Gonfiantini, (1993; blue dotted line) is provided as a reference as well as soil water evaporation lines (with R^2 and slope m; black lines)

take up water only from the top soil, because no statistical equivalences were found between the subsoil and root crown's isotopic signatures. This was underscored by plants' rooting length (Figure 4). Both plant species developed a shorter rooting system when grown in clayey loam. In comparison with the clayey loam (with few exceptions), sandy soil resulted in a wide range of different extraction parameter settings that were able to match geranium root crown isotopic composition for both soil depths and isotopes. However, this made it difficult to clearly identify a specific water source for geranium in sand based on both isotopes. Consequently, it was difficult to determine an ideal water extraction parameter set. The same was true for dandelion grown in sand.

4 | DISCUSSION

4.1 | Plant source water

Both plant species seemed to take up water from the shallow soil layer when grown in clayey loam, although only few extraction parameter settings worked for matching plants and soil isotopic composition. When grown in sand, several different extraction parameter settings were identified to recover the isotopic signature of both plants (also depending on which isotope was considered). However, this made it difficult to identify and optimize a set of extraction parameters based on time, temperature, and vacuum conditions for both soil types. Therefore, we were not able to reliably match root crown water $\delta^{18}O$ and/or $\delta^{2}H$ values from dandelion and geranium with the extracted soil water source, even though our testing of the cryogenic system guaranteed stable extraction conditions (Orlowski et al., 2013).

Some isotope studies have found a correlation between maximum root density (in the top soil) and water uptake depth for grasses and shrubs (Liu et al., 2011; Roux, Bariac, & Mariotti, 1995). Other studies have shown that xylem water covers similar regions in a dual isotope plot as the top soil (e.g., Bertrand et al., 2014; Goldsmith et al., 2012) and that root water uptake is governed by root distribution and the hydraulic conductivity of the roots during wet periods. Still others have identified soil water availability as the main driver during dry periods (Asbjornsen, Shepherd, Helmers, & Mora, 2008; Ellsworth & Sternberg, 2015; Hallett, Gordon, & Bengough, 2003; Song, Zhu, Li, & Yu, 2014; Zarebanadkouki, Ahmed, & Carminati, 2016). Also, Zarebanadkouki et al. (2016) showed that root and soil conductivities can vary over time and in space and are influenced by complex processes and soil–root interactions.

Despite this paper work, our results from a fully controlled greenhouse study were complex. The sand in our study dried out towards the end of our experiment despite our efforts in reducing evaporation by gravel cover. Soil water was therefore affected by evaporative isotope effects. Plants developed different rooting systems and lengths in the sand compared with plants grown in the clayey loam, which further affected plants' water uptake patterns (in addition to the drier soils; Figure 4). Thus, the drier sandy soils together with different root distributions in the pots (Figure 4) could be responsible for the difficulties in determining exact extraction parameter sets for plant water uptake depths. When comparing isotopic signatures from plant and soil waters with the applied irrigation water, it was apparent that plants used water which was altered by soil properties (clayey soil) and evaporation processes (sandy soil). Other studies showed similar deviations of extracted plant water isotopic composition from potential soil water sources (e.g., Bowling, Schulze, & Hall, 2017; Brooks, Barnard,

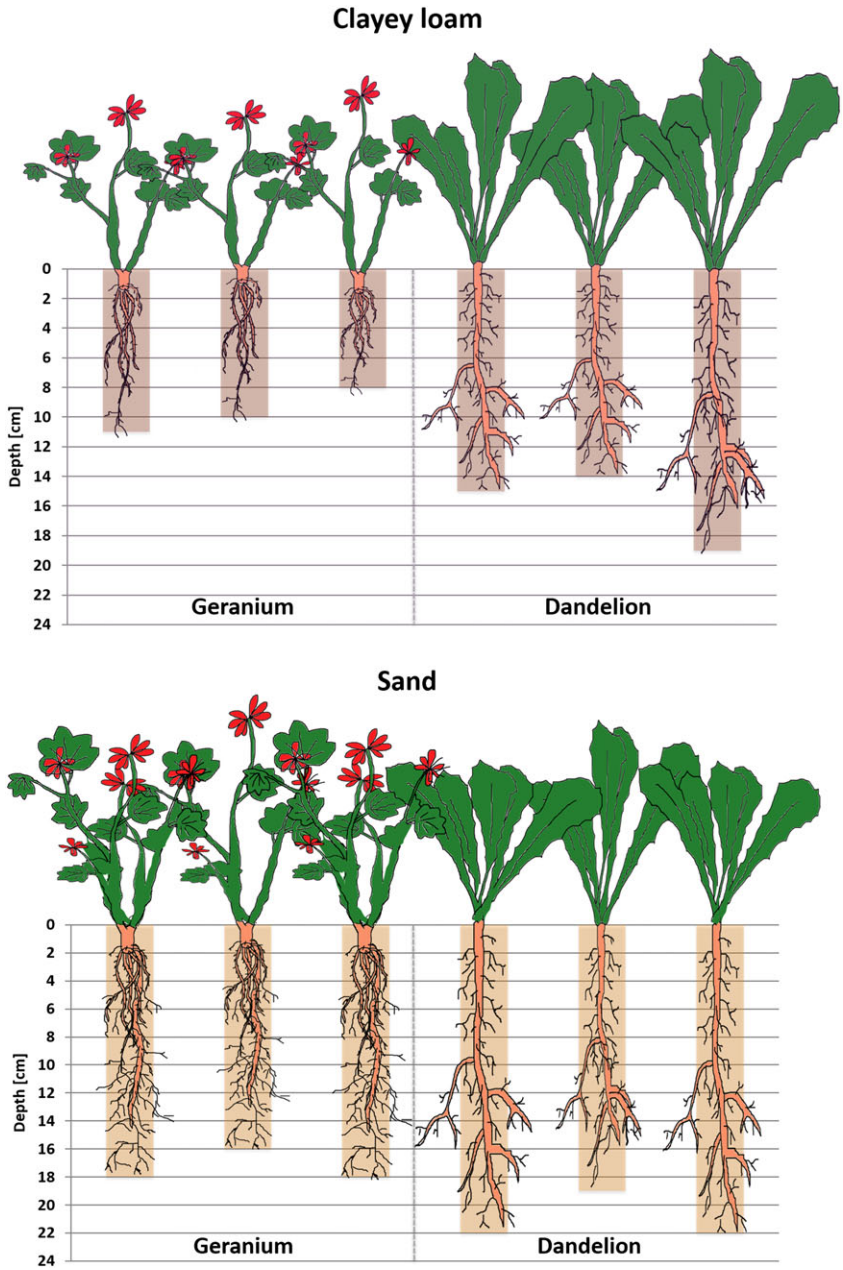

FIGURE 4 Root distribution and length (cm) as well as plant size of geranium and dandelion grown in clayey loam and sand

Coulombe, & McDonnell, 2010; Hervé-Fernández et al., 2016) using different cryogenic extraction systems and parameters. However, little is known about rhizosphere water dynamics (Carminati, 2014) which are technically challenging to capture in situ (Rudolph-Mohr, Vontobel, & Oswald, 2014). Zarebanadkouki et al. (2013) tested a new method to measure the local fluxes of water into and along the root system of transpiring lupine (*Lupinus albus*) roots grown in sand. They monitored the transport of deuterated water (D_2O) into lupine roots by means of time-series neutron radiography and found out that water uptake was not even uniform along roots. Water uptake was higher in the upper soil layers than in the lower ones. Rudolph-Mohr et al. (2014) proposed that new multi-imaging approaches combining fluorescence and neutron radiography could be beneficial in more precisely unravelling the dynamic processes occurring at the soil–root interface. In our study, the plant–soil water matching results for $\delta^{18}O$ and $\delta^{2}H$ were not the same. Others have stated the importance of measuring and reporting both isotopes in plant water uptake studies to reduce uncertainties and to investigate potential ecohydrological separation (e.g., Evaristo, McDonnell, Scholl, Bruijnzeel, & Chun, 2016; McDonnell, 2014; Meißner et al., 2014; Orlowski et al., 2013). Isotope fractionation effects were observed recently for halophytes and woody plants, for example, on the tree level (e.g., Martín-Gómez, Serrano, & Ferrio, 2016; Zhao et al., 2016). Further, stem transpiration can isotopically enrich the xylem water due to limited leaf transpiration in early growing stages along with decreased hydraulic gradients (Martín-Gómez et al., 2016; Sperry, Alder, & Eastlack, 1993). During our study, we only sampled root crown tissues. Therefore, we could not determine within-plant fractionation effects.

We observed $\delta^{18}O$ to be more sensitive to fractionation effects and different results in terms of plant's water sources were obtained from both isotopes (Table 2). Such differences have previously been observed by Meißner et al. (2014) and Orlowski et al. (2013). However, there is a lack of data on isotopic fractionation during plant water uptake under unsaturated soil water conditions, as noted recently by

Vargas, Schaffer, Yuhong, and Sternberg (2017). There is currently a pressing need to better quantify coupled ecosystem and hydrologic functioning during periods of water limitation (Jenerette, Barron-Gafford, Guswa, McDonnell, & Villegas, 2012). However, deuterium fractionation on the plant level as observed by Zhao et al. (2016) is likely a common phenomenon, at least in dryland ecosystems, in addition to their studied coastal regions. Thus, plant–soil water matching from isotope data gained under unsaturated conditions might still be problematic. We argue that such observed isotopic fractionation effects potentially lead to errors in water source calculation. This miscalculation in plant's water source could be quite large and could lead to misinterpretations of the role different plant species play in hydrologic processes at the ecosystem or larger scales (Zhao et al., 2016). For past studies, it remains difficult to find out whether results are affected by isotope fractionation effects on the plant or soil level.

Further, we know that cryogenically extracted water from soil samples with low water content shows significant isotopic deviation from labelled input water during spiking experiments (Meißner et al., 2014; Orlowski, Pratt, & McDonnell, 2016). This isotopic effect is assumed to be larger at low water contents (Ingraham & Shadel, 1992; Oshun, Dietrich, Dawson, & Fung, 2016). Vargas et al. (2017) recently found that plants in their controlled irrigation experiment took up preferentially ^{1}H and ^{16}O, leaving the remaining pool of water in the soil enriched in ^{2}H and ^{18}O. They suggested that such discrimination effects might increase at lower water contents (under unsaturated conditions). Since the final water content in our clayey soil pots was around 30% (close to maximum water holding capacity; see Table 1), we could exclude such water content driven isotope effects. This might have played more of a role for the sandy soil, as water contents at the end of the experiment were generally lower. The dandelion sandy soil pots showed 7.2 ± 1.8% final water content and the geranium sandy soil pots 9.0 ± 1.3% final water content. This could have caused the isotopic shift of extracted total water towards more positive δ values. We further observed a difference in water content between the top and subsoil for the sand pots. This was not the case for the clayey soils (Figure 2).

Covering the pots with gravel obviously did not completely prevent evaporation from the soils. Future studies should consider using a different cover material, for example, a plastic mulch or reflective foil that could avoid undesired heat build-up through solar radiation. Plastic mulch further helps to conserve water. Thus, the evaporation driven kinetic isotope fractionation might be a reason why the isotopic values showed a wider spread in the top soil of the sand and why they were more enriched in comparison with the clayey loamy soils. Water can evaporate from higher permeable sand far more easily than from lower permeable clay soils where water is hold more tightly because of smaller pores (Barnes & Allison, 1988; Or, Lehmann, Shahraeeni, & Shokri, 2013). Although transpiration may be the dominant component of evapotranspiration (Jasechko et al., 2013; Lawrence, Thornton, Oleson, & Bonan, 2007), in terms of isotopic behaviour, soil water uptake by plants is (with exceptions) a nonfractionating process which does not affect the remaining soil water. During evaporation, however, kinetic fractionation comes into play, which enriches the remaining soil water pool in heavy isotopes. We hypothesize that kinetic fractionation is responsible for the isotopic enrichment in the top soil of the sand. Beyond the soil water pressure or soil water content, the instantaneous evaporation process from a drying soil is affected by additional factors such as wind, thermal gradients, vapour pressure gradients, and vapour transfer, as well as storage in the soil (Brutsaert, 2014).Thus, water content and evaporation affected our isotope results in the sandy and clayey soils in different ways due to their different moisture release curves and how those functions controlled their interaction with the additional factors.

4.2 | Extraction condition and soil type effects

Currently, neither a standardized cryogenic extraction system set-up nor a standard operating procedure for using the cryogenic extraction technique exists (Orlowski, Breuer, et al., 2016). Thus, different cryogenic extraction conditions have been applied over the past decades to answer ecohydrological research questions. As a general trend, we found that with longer extraction times and higher temperatures, isotopically more enriched water was gradually obtained. This was in agreement with findings by Orlowski, Breuer, et al., 2016. This trend was more pronounced for clayey loam. Since the early work of Araguás-Araguás, Rozanski, Gonfiantini, and Louvat (1995) and Walker, Woods, and Allison (1994) we know that extraction temperatures have a major influence on the extracted isotopes and that applied extraction temperatures are likely to mobilize both hygroscopic (Koeniger et al., 2011) and biologically relevant water (Sprenger, Herbstritt, & Weiler, 2015). Since cryogenic extraction is something of a brute force approach (Orlowski, Breuer, et al., 2016), it does not discriminate between mobile and more tightly bound water.

As outlined by Orlowski, Breuer, et al. (2016) and others (e.g., Goebel & Lascano, 2012; Koeniger et al., 2011; Orlowski et al., 2013; West, Patrickson, & Ehleringer, 2006), cryogenic extraction time can influence the isotopic composition of water that is collected and isotope fractionation occurs if the extraction process is not conducted until completion (Raleigh distillation; Barnes & Turner, 1998). For the sandy soil, we found neither a temperature nor a time effect but nevertheless, these parameters affected the isotope results of the clayey loam. As recently shown by Gaj, Kaufhold, Koeniger, et al. (2017), Gaj, Kaufhold, and McDonnell (2017), higher temperatures should be applied to clay-rich soils to improve isotope extraction results. This recommendation is supported by our findings. For the clayey soil, matches with plants isotope signatures were mainly possible when higher temperatures and longer extraction times were applied.

Physicochemical soil properties have been shown to be responsible for various isotopic fractionation effects in soil water extracts (Araguás-Araguás et al., 1995; Gaj, Kaufhold, Koeniger, et al., 2017; Meißner et al., 2014; Oerter et al., 2014; Orlowski, Breuer, et al. 2016; Oshun et al., 2016). When comparing cryogenically recovered water from clayey and sandy soils, past and more recent findings suggest that isotopic fractionation effects are more pronounced for soils with a large fraction of small pores (<0.002 mm; Barnes & Turner, 1998), that is, clayey soils (e.g., Koeniger et al., 2011; Orlowski et al., 2013). New data from Gaj, Kaufhold, and McDonnell (2017) suggested that temperatures between 200 °C and 300 °C should be used for water extractions of clay-rich soils. They attributed this to the

dehydration of monovalent cations at temperatures of about 200 °C and dehydration of bivalent cations between 200 and 300 °C.

In our study, δ values of clayey loam water were significantly enriched when extracted at 200 °C compared with δ values extracted at lower temperatures. We recognize that water released under such harsh extraction conditions (e.g., crystalline or interlayer water from clay minerals) might not be an easy accessible water pool for plants. Nevertheless, Palacio, Azorín, Montserrat-Martí, and Ferrio (2014) showed that crystallization water of gypsum rocks can be a relevant water source for plants.

We also found out that ^{2}H and ^{18}O were affected differently. Oxygen is a highly reactive element that interacts and exchanges with other oxygen atoms in the surroundings, whether solid (e.g., clays), liquid (e.g., water), or gaseous (e.g., CO_2; Hervé-Fernández et al., 2016). The $\delta^{18}O$ values of water adsorbed to the clay mineral surface depends on the constitution of cations (Mg^{2+}, Ca^{2+}, and K^{+}) and on the soil water content (Oerter et al., 2014). Oerter et al. (2014) tested this by creating mineral–water mixtures with deionized water of known isotopic composition at 5–32% gravimetric water content. The clayey loam is rich in Vermiculite (2:1 clay) with a medium shrink-swell capacity but a high cation exchange capacity (CEC; Table 1), which has been identified to cause isotope fraction effects (Meißner et al., 2014; Oerter et al., 2014). With the sandy soil having a negligible clay fraction (2.5%) and low CEC (Table 1), we did not expect to see any isotope fractionation effects linked to CEC. As for mineral–water interactions, H-bonding, charge-dipole attraction, ligand–ligand repulsion, and van der Waals interactions are the main drivers (Schoonheydt & Johnston, 2013). The interlayers of 2:1 minerals consist of (hydrated) cations, organic material, hydroxide octahedra, and/or hydroxide octahedral sheets, and their abundance is characterized by the interlayer distance and charge (Meunier, 2005). We therefore attribute the observed isotope effects in the clayey loam extracts in our study also to mineral–water interactions characterized by the negative charge of the clay mineral surface due to isomorphous substitution and the abundance of exchangeable cations. Gaj, Kaufhold, Koeniger, et al. (2017) recently presented postcorrection possibilities for isotope data based on physicochemical soil properties. However, their correction procedure was only valuable for certain clay-rich soils. Nevertheless, the precision of cryogenically recovered isotope results was better when higher extraction temperatures (205 °C) were applied to soils with elevated clay contents (34.5% and 48.0%; Gaj, Kaufhold, Koeniger, et al., 2017).

A critical community exploration of issues during water extractions of different soils is still embryonic. Here, we argue that future studies should look deeper into the question of how the presence of certain clay minerals affects the cryogenically recovered isotope results and continue the work of Gaj, Kaufhold, Koeniger, et al. (2017). It would also be desirable that future studies try to explicitly unravel processes and soil properties that cause isotope fractionation during cryogenic extraction and include various different soils across the soil classification diagram following Orlowski, Breuer, et al. (2016).

For future studies that use cryogenic extraction in order to determine plant water sources in different soil types, it is perhaps most important to define extraction parameter settings that mirror plant water uptake depth. However, extraction parameters should then be adjusted individually for the respective extraction system used in such studies. To date, it has been difficult to narrow down the observed fractionation effects to a single main factor influencing isotope results or to differentiate between the various effects (i.e., the soil properties and extraction conditions). When plant isotopic signatures from a controlled greenhouse experiment are already difficult to interpret in terms of potential water sources, we argue that field data will have even more uncertainty. It is therefore crucial to consider soil properties, as well as cryogenic extraction parameters when it comes to matching plant water sources with soil isotopic composition, especially for clay-rich soils.

5 | CONCLUSIONS

Cryogenic water extraction is the most widely used method in ecohydrological plant water uptake studies. We used a simple greenhouse experiment with *T. officinale* and *Pelargonium* spp. to test the effects of varying cryogenic extraction conditions and physicochemical soil properties on estimating plant water source depth. Extraction conditions (temperature and time) had a significant effect on isotope results of the clayey loam extracts but little to no effects were observed for the sandy soil. For water extracts of both soils, longer extraction times and higher temperatures resulted in enriched isotopic signatures, which might relate to the extraction of more tightly bound water under those conditions. Our results suggest that choosing correct cryogenic extraction parameter settings is crucial for matching soil with plant water isotopic signatures. However, such choice is not straightforward yet. Observed isotopic fractionation issues occurred for the clayey soil and discrepancies were observed when either ^{2}H or ^{18}O was used to determine plant's source water depth. Our simple greenhouse experiment was not able to unravel mechanisms responsible for these isotope fractionation effects. Field studies are now needed to shed light on clay mineral composition and their effects on water mixing processes in the subsurface. Such toggling back and forth between field experiments and then lab experiments to further test hypotheses could be a profitable way to proceed. Overall, our results suggest extending extraction conditions to longer times and higher temperatures for problematic clay-rich soils in order to match soil isotope results with plant's source water. In order to understand from which soil water source plants take up their water, we need to have a sound understanding of the interactions between water (mobile and higher tension water) and the overall soil compartment. Current lab-based water extraction techniques remain one of the biggest challenges in achieving this goal. New in situ measurement methods of soil and plant water isotopic composition that provide direct and continuous data might overcome isotope fractionation issues with lab-based extraction methods. However, when cryogenic water extraction is applied, it is key to consider the physicochemical properties of the soil and to adapt the extraction parameters to the respective soil material individually for each extraction system. Future studies are needed to explore the effects of varying cryogenic extraction parameters on plant tissue isotope composition. Greenhouse hydroponic systems may be useful to bypass issues we see with soil water extractions. Such studies could also help unravel the dynamic processes occurring at the soil–root interface.

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ORCID

N. Orlowski http://orcid.org/0000-0003-4368-4580

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