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An inexpensive, fast, and reliable method for vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry

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The stable isotopes of water (hydrogen and oxygen isotopes) are of utmost interest in ecology and the geosciences. In many cases water has to be extracted directly from a matrix such as soil or plant tissue before isotopes can be analyzed by mass spectrometry. Currently, the most widely used technique for water is cryogenic vacuum extraction. We present a simple and inexpensive modification of this method and document tests conducted with soils of various grain size and tree core replicates taken on four occasions during 2010. The accuracies for sandy soils are between 0.4‰ and 3‰ over a range of 21‰ and 165‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. Spiking tests with water of known isotope composition were conducted with soil and tree core samples; they indicate reliable precision after an extraction time of 15 min for sandy soils. For clayey soils and tree cores, the deviations were up to 0.63‰ and 4.7‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. This indicates either that the extraction time should be extended or that mechanisms different from Rayleigh fractionation play a role. The modified protocol allows a fast and reliable extraction of large numbers of water samples from soil and plant material in preparation for stable isotope analyses. Copyright © 2011 John Wiley & Sons, Ltd.

Water isotope (e.g.: deuterium, ^{2}H; oxygen-18, ^{18}O) studies have become increasingly important in the fields of hydrology, soil science, (paleo)-ecology, and forensics. Commonly, there is a need to process large sample numbers with high resolution in time and space, e.g., to characterize rainfall patterns, runoff processes, soil water movement, or water isotope footprints in plant, animal and human tissue, or the fossilized remains thereof.[1] Various methods have been developed to enable water isotope analyses by mass spectrometry from substrates after extraction, e.g., centrifugation, squeezing, distillation and equilibration techniques.[2–14]

Vacuum extraction is the most commonly used technique for water extractions from unsaturated soil samples, xylem, plant water, and cell water (e.g. [5,15–18]). During vacuum extraction a cold trap (liquid nitrogen) acts as a water-vapor sink and water diffuses rapidly under low-pressure conditions from the substrate into a sample vessel. Most laboratories use a glass extraction device and flame seal the glass tube after extraction or use valved devices with a glass-metal connector (e.g.: Ultra-Torr®; Swagelok, Solon, OH, USA). Recent improvements in soil and plant water extraction techniques have optimized extraction times and enabled a high sample throughput[9,15–17] (up to 72 samples per day[16]). Intercomparison tests for extraction methods have been published in multiple studies including discussion of the strengths and limitations of the different methods.[5,6,10,16,18,19] Those studies highlight that the time required for water extractions constrains sample throughput, which makes such extractions laborious and expensive. Given the importance of water isotopes in enabling scientific advances in a wide range of disciplines, improved extraction methods are needed.

The purpose of this study is to describe a modification of the vacuum extraction method that is easy to adopt and provides significantly improved sample throughput. The method is inexpensive and allows a rapid extraction of large numbers of samples, and has been demonstrated to recover water of known isotopic composition from spiked soil and tree core samples.

EXPERIMENTAL

Modified vacuum extraction

The vacuum extraction method was modified by replacing the extraction apparatus with a pair of Valco Exetainer® vials (Labco Ltd., High Wycombe, UK) connected with a 1.56 mm stainless steel capillary (i.d. 0.95 mm) inserted through the septa. Valco Exetainer® vials (12 mL borosilicate, round- bottomed, product no. 938 W) are routinely used in gas chromatography and for bench applications. Exetainers have been extensively tested and validated for the storage and transport of gas samples by Glatzel and Well[20] and are relatively inexpensive.

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The samples to be extracted were inserted into Exetainer® vials with caps and septa and connected to a second empty vial using a capillary tube (Fig. 1, right-hand side). The sample was then frozen with liquid nitrogen (approx. –196 °C) to prevent loss of water vapor during evacuation. Care should be taken to avoid breaking the glass vials during freezing. The septum and cap should not be cooled because the septum will leak if frozen. Leaks, if present, can be recognized because it will be impossible to evacuate the vials sufficiently. The connected and frozen vials were subsequently evacuated using a syringe needle that was connected to an evacuation system (Fig. 1, left-hand side). For this procedure, a vacuum pump (Edwards® rotary vane vacuum pump type E2M2, Crawley, UK) connected with a valve and vacuum sensor (Thermovac® sensor TTR 91 and vacuum meter, OC Oerlikon Management AG, Pfäffikon, Switzerland) to a glass apparatus was used. The glass apparatus was connected with a simple syringe injection needle and vacuum-sealed with a PTFE/silicone septum. To hasten the extraction, we submerged the sample Exetainers into beakers that were filled with distilled water and heated close to the boiling point of water (~90 °C) over a hot plate. The beakers were surrounded by Dewar flasks filled with liquid nitrogen and placed within reach of the capillary tubes. The lower end of the empty vial just reached the surface of the liquid nitrogen (Fig. 1, right-hand side) and was maintained in that position throughout the procedure. With this setup, several vials could be extracted at the same time (up to 3 samples per beaker and 12 vials per hot plate). After the water in the sample had been quantitatively transferred to the cold trap, vial extractions were stopped manually by pulling the capillary from the Exetainers. Immediately following removal of the capillary, the pierced septa was replaced with caps while the water was still frozen, and the vials were wrapped with paraffin tape (Parafilm® M; Brand GmbH, Wertheim, Germany) to minimize water loss during storage. After evacuation, the water samples were stored in a refrigerator until measurement, but no longer than a few days.

Soil samples

Soil was collected (1) from sand dunes (Ss) at Borkum island in Germany; (2) from silt deposits at Borkum island (Su); and (3) from a core with a high clay content (Ut) collected during a drilling project at Heidelberg, Germany (150 m depth). The grain size distribution of the soil material was measured with a laser diffractometer (LS13320; Beckman Coulter, Pasadena, CA, USA), which resolves particle sizes from 0.04 to 2000 μm. The grain size distributions for the experimental soils are plotted in Fig. 2.

The soil extraction tests relied on a spiking procedure with water of known isotopic composition that was analyzed multiple times to increase the accuracy. The soils were first oven-dried at 110 °C for 24 h to remove most of the water. Water of known isotopic composition was added and the water content adjusted to represent natural field conditions. The respective $\delta^{18}O$ and $\delta^{2}H$ values of the added water to test extraction of waters with different isotope compositions were: spiked waters S1 –22.25‰ and −175.2‰, S2 –8.06‰ and −58.9‰, and S3 –1.15‰ and −7.6‰. The gravimetric water content of samples was adjusted using a balance (classic AB265-S; Mettler Toledo, Columbus, OH, USA). Gravimetric analyses (balance precision 0.01 mg) were conducted before and after extraction by weighing empty and filled vials and estimating the residual water loss after extraction (heating in an oven at 110 °C for 24 h).

Soil water extraction tests were conducted in three separate experiments. The first experiment (A) used water isotope compositions over a range of 21‰ and 165‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. The second experiment (B) compared extraction times of 2.5, 5, 7.5, 10, 15, 20, and 40 min whereas experiment (C) compared soils with water content of 4.8, 7.0, 8.9 and 12.6% (by weight). The fourth experiment (D) compared the three substrate types: sandy soil (Ss), silt sand (Su4) and silt clay (Ut2).

Plant samples

Xylem samples were collected with an increment corer from three tree species in a radius of 10 km around Hannover in Germany. These samples were used to test the paired-vial extraction method described earlier. Each time six replicate samples were taken at about 1.5 m above ground from all sides of the stem. Two cores per vial provided enough liquid (~0.5 mL) for the extraction tests (12 cores per tree were taken for six replicates). We collected samples from Norway spruce (*Picea abies*) on 13th June 2010 (day of year 2010 or day 164, Deister, 52.266°N, 9.4997°E, 280 meters above sea level

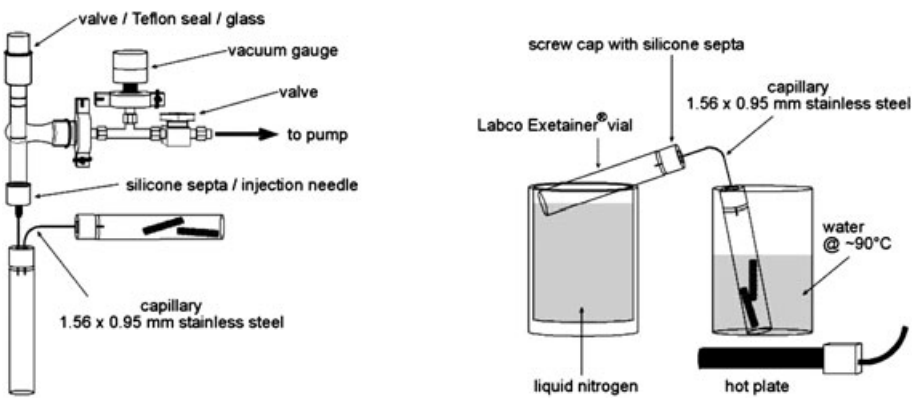

Figure 1. Schematic of the modified vacuum extraction method used for this study with an evacuation unit (left) and a cryogenic water trap (right). Exetainer vials with silicone septa and screw caps were connected with stainless steel tubing to extract water from soil and plant samples.

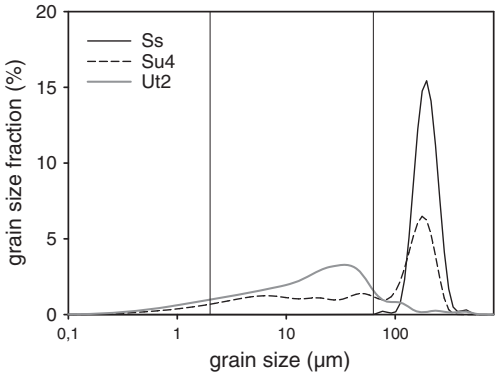

Figure 2. Characterization of soil types (sandy sand Ss, silt sand Su4, and clayey silt Ut2) used for the soil extraction experiment. The grain size distribution is described by plots of grain fraction (%) vs. grain size (µm) of the substrate.

(m a.s.l.)). The second sampling was conducted on 4th July 2010 (day 189, west of Hannover, 52.368°N, 9.6324°E, 61 m a.s.l.) from a Turkish hazel tree (*Corylus colurna*). On 18th July 2010 (day 199) and 15th November 2010 (day 312, east of Hannover, 52.406°N, 9.8326°E, 58 m a.s.l.) tree cores were collected from a Scots pine tree (*Pinus sylvestris*). Each sample was placed in an Exetainer vial, sealed with a screw cap and Paraffin tape in the field and stored frozen in the laboratory until extraction. After the extraction of natural water from the tree cores collected in November, we spiked the cores with water S3 but only increased the water content to about 2/3 of the original value. The samples were equilibrated after each spike step for 24 h at 30 °C in Exetainer® vials prior to extraction. The lower water content was adjusted so as not to over-saturate the samples. Each spike test was conducted twice.

Isotopic analyses

All stable isotope measurements were conducted at the LIAG Stable Isotope Ratio Mass Spectrometry (IRMS) laboratory in Hannover, Germany. A 0.5 mL liquid sample was used to measure the $^{18}O/^{16}O$ ratios and the $^{2}H/H$ ratios from the same sample. The $^{18}O/^{16}O$ ratios of the water samples were measured using an automated equilibration unit (Gasbench II; ThermoFinnigan, Bremen, Germany)[21] in continuous flow mode, connected to a ThermoFinnigan Delta XP isotope ratio mass spectrometer. For $^{2}H/H$ analyses, we used a fully automated chromium reduction system at 800 °C (H/Device, ThermoFinnigan),[22,23] directly coupled to the dual-inlet system of the ThermoFinnigan Delta XP isotope ratio mass spectrometer. Isotope values are given in the standard delta notation in per mil (‰) versus VSMOW according to Eqn. (1):

$$\delta = \left[\left(\frac{R_{SA}}{R_{ST}}\right) - 1\right] * 1000 \quad (1)$$

where R_{SA} (−) denotes the isotope ratio of $^{18}O/^{16}O$ or $^{2}H/H$ of a sample and R_{ST} (−) those of the standard. The data sets were corrected for machine drift during the run and normalized to the VSMOW/SLAP scale by assigningvalues of 0‰ and −55.5‰ ($\delta^{18}O$) and 0‰ and −428‰ ($\delta^{2}H$) to VSMOW and SLAP, respectively. For normalization two laboratory standards that were calibrated directly against VSMOW and SLAP were measured in each run. The external analytical reproducibility for the IRMS system, defined the standard deviations of a control standard during all runs, were better than 0.10‰ and 0.8‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively.

RESULTS

Extraction with water of known isotope composition

During tests (A) approximately 10% (by weight) of waters S1, S2, and S3 was added to oven-dried sandy substrate (Ss). For each of the test waters, five aliquots were prepared and extracted using the previously described protocol with extraction times of approximately 15 min. The standard deviations for all isotope compositions were less than 0.14‰ for $\delta^{18}O$ and 1.5‰ for $\delta^{2}H$, which are both slightly higher than the analytical reproducibility (Fig. 3(A)). The observed measurement accuracy for the extracted water was in good agreement with the actual values. For water (S1), the most depleted in heavy isotopes, the deviation from the actual values was highest (+0.23‰ and −1.6‰ for $\delta^{18}O$ and $\delta^{2}H$).

Extraction time

Extraction times of 2.5 to 40 min were used during tests (B) to optimize the duration of the extraction procedure. Five replicates were prepared with substrate Ss and adjusted to a soil water content of about 10% (by weight) with water S2. The isotope ratio measurements reflect depleted values of the heavy isotopes and thus large fractionation for extraction times of 2.5 to 5 min (Fig. 3(B)). In both cases incomplete recovery was observed (Table 1) which explains the observed fractionation. For extraction times between 7.5 and 15 min, the results indicate isotope values within the analytical reproducibility (Fig. 3(B)). When extractions took longer than 15 min, the $\delta^{18}O$ values indicate that the samples tend to be slightly more depleted in ^{18}O. This minor depletion may be due to leakage to the atmosphere. Based on these results, all subsequent extractions lasted 15 min.

Variable water content

Figure 3(C) shows results derived from extractions carried out with S2 test water at variable water content on sandy soil (Ss). The samples were extracted for 15 min. The observed standard deviations are between 0.07‰ and 0.19 ‰ for $\delta^{18}O$ values and between 0.8‰ and 1.7‰ for $\delta^{2}H$ values (Table 2). The accuracy was lower for samples with 7.0% water, but there was no general trend in either precision or accuracy across the range of water contents.

Varied substrates

Three sets of tests were conducted with varying natural soils containing different amounts of clay and silt. The measured isotope values and standard deviations increase with increasing silt content of the material, while the precision of the extraction declined. The results from substrate Su4 (silt sand from tidelands at Borkum) and substrate Ut2 (silt layer from a core at Heidelberg) (Fig. 2) indicate that the described extraction protocol cannot be used to obtain reliable isotope values from such substrates (Fig. 3(D)).

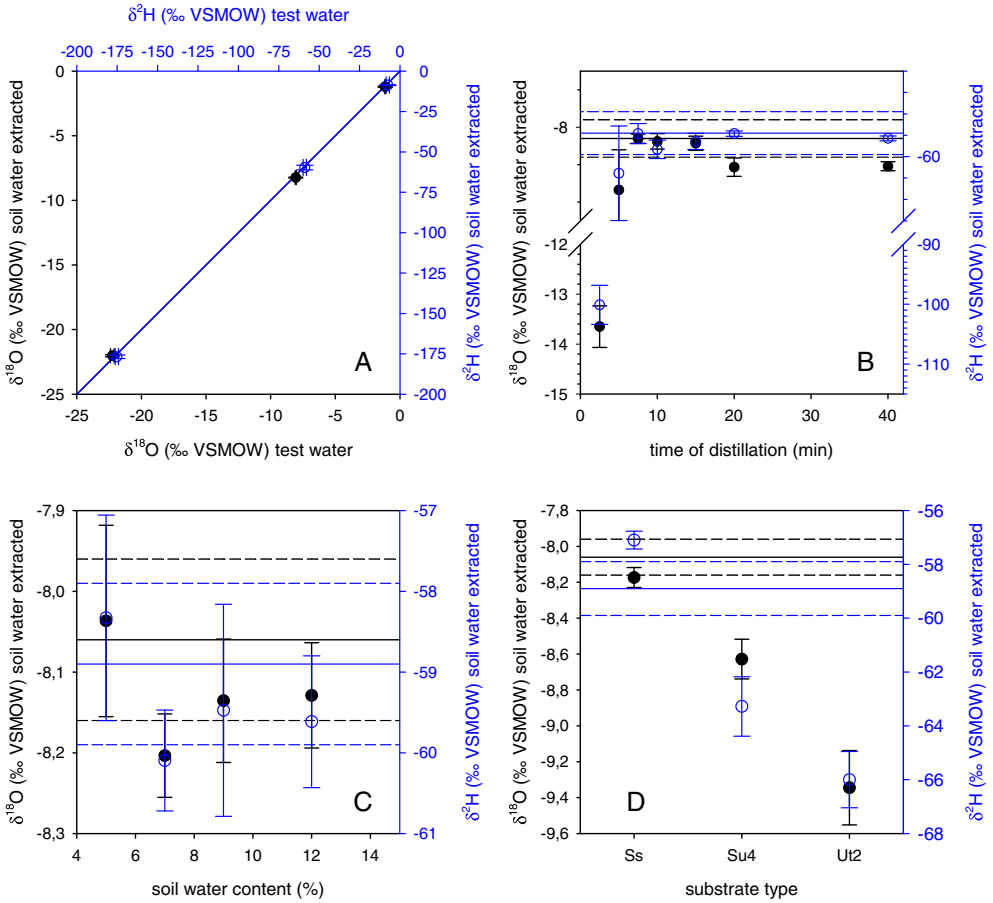

Figure 3. Results of the soil extraction tests for $\delta^{18}O$ (black color, closed symbols) and $\delta^{2}H$ (blue color, open symbols) with variable (A) isotope concentrations (solid is 1:1 line), (B) time of distillation, (C) soil water content, and (D) substrate type (sandy sand Ss, silt sand Su4, and clayey silt Ut2). Lines in (B), (C) and (D) represent mean (closed line) and standard deviations (open lines) of known water S2.

Test series with tree cores

Xylem water data are presented in Fig. 4 and Table 2.

The standard deviations of the measured values of the extracted water from the tree core samples are higher than those described for the prior tests with soil substrate. The $\delta^{18}O$ values range between −10.55‰ and −8.75‰ with SDs between 0.45‰ and 0.63‰. The $\delta^{2}H$ values range between −70.3‰ and −82.8‰ with SDs between 1.5‰ and 4.7‰. The summer xylem values are more depleted in heavy isotopes than those in November and thus indicate a phase shift in comparison with the precipitation that was measured in Hannover (weekly samples were weighted by weekly precipitation amount to yield monthly isotope compositions of −6.37‰ and −45.9‰, –4.05‰ and −27.4‰, –5.80‰ and −39.9‰, and −11.70‰ and −86.5‰ in June, July, August and November 2010 for $\delta^{18}O$ and $\delta^{2}H$, respectively). Hannover precipitation generally shows isotope values lower than −10‰ and −75‰ during November to February for $\delta^{18}O$ and $\delta^{2}H$, respectively, whereas summer values (May to October) tend to be higher. This phase shift probably results from root uptake of winter precipitation stored at depth in the soil profile.

The results for the spike water tests are shown in Fig. 4(B), where the filled symbols refer to $\delta^{18}O$ values (left-hand scale) and the open symbols refer to $\delta^{2}H$ values (right-hand scale). The horizontal lines indicate the concentrations of the applied spike water. The standard deviations of six replicate samples were smaller than those obtained for the extraction from natural samples. For $\delta^{18}O$, the spike water S3 extraction showed about 0.2‰ more positive waters for $\delta^{18}O$ (S3-1 and S3-2). The values of the extracted water were 0.7‰ more enriched in ^{18}O than the spike water S1 in the first extraction test (S1-1) and 0.6‰ more depleted in ^{18}O in the second test (S1-2). For $\delta^{2}H$, good accuracy was not reached for either spike water during the first extraction test. For spike water S3 a 3‰ more negative and for S1 a 6‰ more positive $\delta^{2}H$ value was obtained after the second extraction.

DISCUSSION

The precision of isotopic analyses of the water extracted from soil samples was expressed in standard deviations of 0.02 to 0.42‰ and 0.2 to 3.3‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. The spiking tests showed that the experimental protocol was problematic for soil samples that contain large proportions of clay and silt. Gravimetric analyses that were conducted prior to and after the soil extractions showed an almost complete recovery of spike water except for very short (e.g. <10 min) extraction

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Table 1. The effect of spike water isotope concentration, extraction time, water content, and substrate type on measured recovery (%), $\delta^{18}O$ and $\delta^{2}H$ values and standard deviations

		N (–)	Recovery (%)	$\delta^{18}O$ (‰ VSMOW)	$\delta^{2}H$ (‰ VSMOW)
(A) Concentration	S1	5	101.14 ± 0.23	−22.02 ± 0.14	−176.8 ± 1.1
	S2	5	100.55 ± 0.73	−8.24 ± 0.03	−59.7 ± 1.5
	S3	5	100.79 ± 0.15	−1.23 ± 0.05	−8.7 ± 0.2
(B) Extraction time (min)	2.5	5	96.62 ± 0.43	−13.65 ± 0.42	−100.1 ± 3.3
	5	5	99.92 ± 0.11	−8.43 ± 0.29	−61.5 ± 2.5
	7.5	5	100.02 ± 0.07	−8.06 ± 0.02	−58.9 ± 0.5
	10	5	100.06 ± 0.04	−8.12 ± 0.10	−59.3 ± 0.9
	15	5	100.07 ± 0.06	−8.09 ± 0.04	−59.3 ± 0.4
	20	5	100.07 ± 0.06	−8.21 ± 0.02	−58.8 ± 0.7
	40	5	100.01 ± 0.06	−8.21 ± 0.02	−58.8 ± 0.7
(C) Water content (%)	4.8	5	101.43 ± 0.99	−8.04 ± 0.12	−58.3 ± 1.3
	7.0	5	100.89 ± 0.80	−8.23 ± 0.07	−59.5 ± 1.5
	8.9	5	100.50 ± 0.60	−8.22 ± 0.19	−60.1 ± 1.7
	12.6	5	100.47 ± 0.40	−8.13 ± 0.07	−59.6 ± 0.8
(D) Substrates	Ss*	5	99.98 ± 0.01	−8.17 ± 0.06	−57.1 ± 0.3
	Su4[a]	5	99.66 ± 0.02	−8.63 ± 0.11	−63.3 ± 1.1
	Ut2[a]	5	99.61 ± 0.10	−9.34 ± 0.21	−66.0 ± 1.0

*sandy sand; **silt sand; ***clayey silt

Table 2. Mean values and standard deviations of water content and isotope values from natural tree cores and spike tests conducted with water S1 and S3 (-1 and -2 refer to sequential equilibration of the same sample)

		N (–)	Water content (mg)	$\delta^{18}O$ (‰ VSMOW)	$\delta^{2}H$ (‰ VSMOW)
spruce	doy 164	6	0.45 ± 0.14	−10.55 ± 0.63	−82.8 ± 4.7
birch	doy 185	6	0.42 ± 0.04	−10.36 ± 0.58	−81.6 ± 3.2
pine	doy 199	6	0.39 ± 0.02	−10,08 ± 0.50	−81.8 ± 2.5
pine	doy 312	6	0.40 ± 0.04	−8.75 ± 0.45	−70.3 ± 1.5
spike tests	S3-1	6	0.32 ± 0.03	−1.09 ± 0.26	−20.8 ± 2.6
	S3-2	6	0.26 ± 0.03	−0.91 ± 0.25	−10.3 ± 1.7
	S1-1	6	0.30 ± 0.01	−21.54 ± 0.33	−135.1 ± 1.2
	S1-2	6	0.41 ± 0.04	−22.85 ± 0.49	−168.9 ± 1.4

doy: day of year

times (Table 1). The gravimetric analyses that were conducted for the fine-textured soils (Table 1 – D) show that almost as much water was removed as was added prior to the extraction. This indicates that hygroscopic water that is stored in the interstices between surfaces of adjoining clay particles might be the reason for the observed memory effects. The observed deviation from the spike water is probably due to real memory and not associated with insufficient extraction. However, other studies have reported comparable standard deviations for extraction of soil and plant materials (summarized in Table 3). Our results indicate that the modified vacuum extraction method is comparable with other methods.

Other methods that produce comparable results have different advantages and disadvantages. Azeotropic distillation with kerosene or toluene[2–6] produces similar standard deviations to the vacuum extraction method. Because organic solvents can alter CO_2 equilibration during IRMS measurements of ^{18}O as well as bias analyses with laser-based spectroscopy instruments through interference with the absorption spectra of water,[7–9] azeotropic distillation can be problematic for such instruments. Direct equilibration methods developed for IRMS[10–13] have the advantages of simplicity and good reproducibility when applied to saturated substrates but some of these methods are restricted to ^{18}O analyses. For micro-distillation methods and offline preparation of water samples, an isotope ratio mass spectrometer equipped with a dual-inlet system is necessary, which is a technical restriction for some laboratories. An equilibration method of pore water and water vapor for direct measurements of water vapor with laser spectroscopy instruments was described recently by Waassenaar *et al.*,[14] where the drilling core material was directly equilibrated in Ziploc®

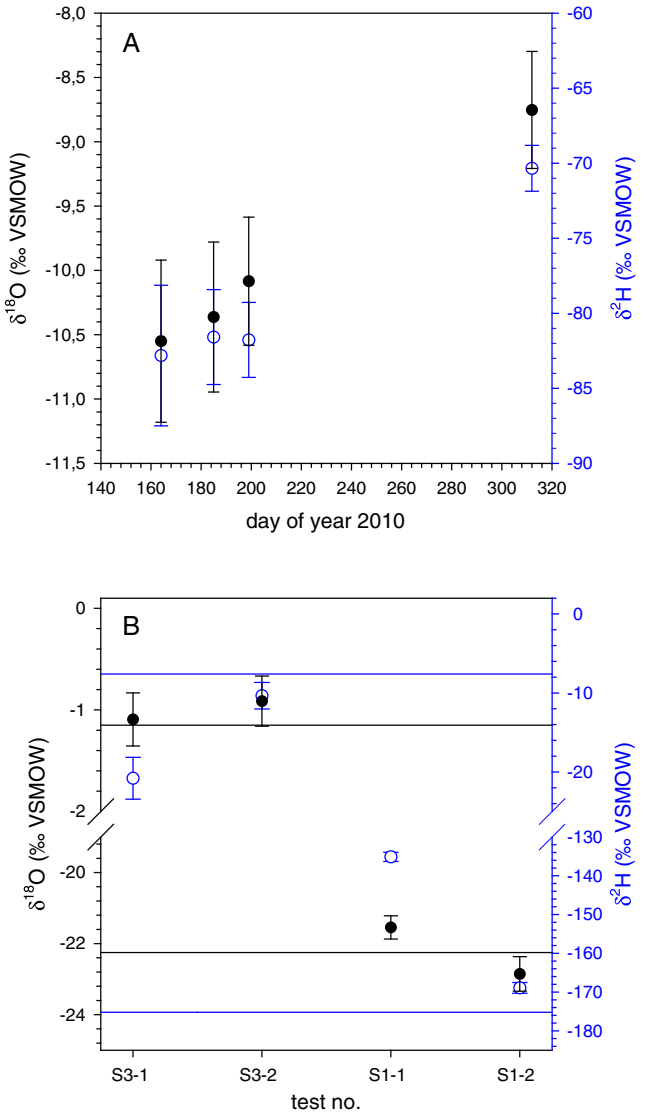

Figure 4. Xylem water extracted from tree cores (a) collected 13 June, 4 July, 18 July and 11 November 2010 from spruce, birch and pine trees close to Hannover, and (b) isotope values of water after equilibration twice with known waters (S1 and S3).

bags (S.C. Johnson & Son Inc., Racine, Canada). They showed good results for saturated cores with some restrictions for samples with low water contents.

Extraction of the tree cores using the described protocol found standard deviations of 0.4 to 0.6 and 1.5 to 4.7 for $\delta^{18}O$ and $\delta^{2}H$, respectively, for the natural samples. Some of this variation may be due to real differences in stable isotope composition within the stems of a tree; for example, standard deviations of 2.5‰ and 5‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively, for eight randomly sampled trees of one species have been observed.[25] Comparing the isotope values of water extracted from trees with those of precipitation suggests an approximately 3- to 6-month lag between precipitation deposition and transport and uptake to the point of sampling in the tree. This may be due to late season dependence on water that infiltrated deep into the soil at the time of snowmelt. Spiking tests conducted with the tree cores found smaller standard deviations of 0.25 to 0.49‰ and 1.2 to 2.6‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. Interestingly, the real values of the added waters S1 and S3 were reached for $\delta^{18}O$ whereas for $\delta^{2}H$ a lag was observed. Following the spike with water S1, the extracted water is considerably more enriched in the heavy isotopes than the original spike water. This could be due to other mechanisms such as hydrogen exchange with plant material (e.g., in cell walls) or equilibration with cell water. Alternatively, the spiking experiments may simply demonstrate that hydrogen exchange with other pools in the samples cause a memory effect that is real and would need to be accounted for when the isotopic concentration of water shifts substantially. This could also be tested with plant material and spike water in combination with isotope analyses of organic material.

The extraction time tests for sandy material show that an extraction time of 15 min is sufficient. Extraction times that are necessary for the vacuum extraction method and an optimization of the extraction parameters were presented by West *et al.*[15]. For their method they estimated extraction times of 30 min for sandy soils, 40 min for clay soils and 60 to 70 min for tree cores for a minimum of 98% recovery of water. Longer extraction times for tree core samples would probably lead to better precision with our protocol. Gravimetric analyses that were conducted prior to and after the soil extractions showed an almost complete recovery of spike water except for very short extraction times (Table 1). The gravimetric analyses that were conducted for the fine-textured soils (Table 1 – D) show that almost as much water was removed as added prior to the extraction. This indicates that hygroscopic water that is stored in the interstices between surfaces of adjoining clay particles probably causes the memory effects observed in this study. The observed deviation from the spike water is probably due to due to real memory and not associated with extraction problems.

Earlier studies reported a depletion of extracted water with increasing clay content[19] and intercomparison studies of laboratories with different extraction methods showed both an increasing variation as the water content of the soils decreased and that the variation of values were greater for clays than for sands.[6] West *et al.*[15] reported that vacuum distillation and toluene extractions were problematic for dry soils that have a high proportion of bound water. We did not observe a strong influence of water content, but extractions with the described method may fail for material with high proportions of small particle sizes.

An evacuation threshold level of 23 mTorr was used in this study. It would be possible to adjust to lower pressures with stronger pumps or with a longer evacuation time, but here we aimed to test extractions using a relatively simple protocol and set of equipment. The evacuation threshold was generally reached in less than 1 min. West *et al.*[15] used an evacuation pressure of approximately 60 mTorr, Vendramini and Sternberg[16] 10 mTorr and Peters and Yakir[17] 1 mTorr for vacuum extraction experiments on plant and soil samples, indicating that this method was within the ranges used by other protocols.

During vacuum extraction, especially of dry and humus-rich materials, temperature-resistant soil bacteria can survive the extraction procedure. Bacterial growth in extracted water samples might then cause elevated CO_2 concentrations. *In situ* production of CO_2 will overprint the added CO_2/He mixture that is necessary for measurements with the water equilibration process using, e.g., the ThermoFinnigan Gasbench method. The resultant high CO_2 concentrations cause out of

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Table 3. Summary of other extraction studies, with extraction method and materials used, and derived accuracies (standard deviation, SD) for $\delta^{18}O$ and $\delta^{2}H$

Studies performed	Extraction method	Material used	SD	
			$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)
Turner and Gailitis[24]	micro-distillation (Zn)	soils		0.2–1.7
Revesz and Woods[4]	azeotropic distillation	soils	0.2	2
Walker *et al*.[6]	azeotropic distillation	soils	0.3	1.2
Scrimgeour[10]	direct equilibration	soils and twigs	1.3	7
Araguas-Araguas *et al*.[19]	vacuum extraction	clayey soils	0.3	3
Hsieh *et al*.[11]	direct equilibration	soils	0.3 - 0.4	
McConville *et al*.[12]	direct equilibration	soils	0.1	
Koehler *et al*.[13]	direct equilibration	saturated geological media	0.3	1
West *et al*.[15]	vacuum extraction	clayey soils	0.15	0.69
This study	modified vacuum extraction	sandy soils	0.4	3
		tree cores	0.63	4.7

range IRMS signals and errors during isotope measurements. In other experiments we found such problems during the water extraction of dry soil samples with very high humus content (data not presented), but not with soil material used during this study. Therefore, it was not necessary to sterilize samples prior to the analyses. Options for chemically sterilizing (e.g. mercuric chloride $HgCl_2$, sodium azide NaN_3, copper sulfate $CuSO_4$) samples to prevent bacterial growth with chemicals have been discussed in the literature.[26] Other alternatives might be to place activated charcoal in the sample[9] or to use filters (e.g. 0.45-µm syringe filters).

This method of water extraction might be used to extracts samples for analysis using laser spectrometry, but there are issues related to organic interferents that would need to be considered if this method were applied to plant tissues. In this respect, this method shares the strengths and weaknesses of other cryogenic distillation methods.

CONCLUSIONS

We modified the standard vacuum extraction method of soils and plants so that large numbers of samples can be extracted at the same time. The method worked very well for sandy soils, but there appeared to be some residual water in the spiking trials with tree cores and with soils rich in silt or clay. Such materials might need longer extraction times to obtain a representative water sample, and hence this method should be used with caution for samples containing high proportions of silts and/or clays. The described method is easier to apply and less expensive than other water extraction methods. It has great potential for combined hydrological and ecological studies focusing, for example, on unsaturated zone processes,[27] groundwater recharge, and plant water uptake mechanisms.[25,28] It is possible to apply the modified vacuum extraction method to samples with low water content or when many replicate samples must be processed. For sandy soils we showed that a large amount of samples processed within a short time produced excellent results.

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