Received: 21 February 2019 | Revised: 12 April 2019 | Accepted: 17 April 2019

DOI: 10.1002/rcm.8470

RESEARCH ARTICLE

WILEY Rapid Communications in Mass Spectrometry

^{17}O-excess as a detector for co-extracted organics in vapor analyses of plant isotope signatures

Magali F. Nehemy[1] | Cody Millar[1] | Kim Janzen[1] | Marcel Gaj[1] | Dyan L. Pratt[1] | Colin P. Laroque[1,2] | Jeffrey J. McDonnell[1]

[1] Global Institute for Water Security, School of Environment and Sustainability, University of Saskatchewan, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada

[2] Department of Soil Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada

Correspondence
M. F. Nehemy, Global Institute for Water Security, School of Environment and Sustainability, University of Saskatchewan; Saskatoon, Canada.
Email: magali.nehemy@usask.ca

Funding information
NSERC Discovery Grant; NSERC CREATE for Water Security Program

Rationale: The stable isotope compositions of hydrogen and oxygen in water (δ^2H and δ^{18}O values) have been widely used to investigate plant water sources, but traditional isotopic measurements of plant waters are expensive and labor intensive. Recent work with direct vapor equilibration (DVE) on laser spectroscopy has shown potential to side step limitations imposed by traditional methods. Here, we evaluate DVE analysis of plants with a focus on spectral contamination introduced by organic compounds. We present ^{17}O-excess as a way of quantifying organic compound interference in DVE.

Methods: We performed isotopic analysis using the δ^2H, δ^{18}O and δ^{17}O values of water on an Off-Axis Integrated Cavity Output Spectroscopy (IWA-45EP OA-ICOS) instrument in vapor mode. We used a set of methanol (MeOH) and ethanol (EtOH) solutions to assess errors in isotope measurements. We evaluated how organic compounds affect the ^{17}O-excess. DVE was used to measure the isotopic signatures in natural plant material from *Pinus banksiana*, *Picea mariana*, and *Larix laricina*, and soil from boreal forest for comparison with solutions.

Results: The ^{17}O-excess was sensitive to the presence of organic compounds in water. ^{17}O-excess changed proportionally to the concentration of MeOH per volume of water, resulting in positive values, while EtOH solutions resulted in smaller changes in the ^{17}O-excess. Soil samples did not show any spectral contamination. Plant samples were spectrally contaminated on the narrow-band and were enriched in ^{1}H and ^{16}O compared with source water. *L. laricina* was the only species that did not show any evidence of spectral contamination. Xylem samples that were spectrally contaminated had positive ^{17}O-excess values.

Conclusions: ^{17}O-excess can be a useful tool to identify spectral contamination and improve DVE plant and soil analysis in the laboratory and *in situ*. The ^{17}O-excess flagged the presence of MeOH and EtOH. Adding measurement of δ^{17}O values to traditional measurement of δ^2H and δ^{18}O values may shed new light on plant water analysis for source mixing dynamics using DVE.

1 | INTRODUCTION

The use of the stable isotope ratios of hydrogen and oxygen (δ^2H and $\delta^{18}O$ values) as a tracer of plant water uptake is increasing rapidly.[1-4] Investigations of ecohydrological processes using such techniques have improved our understanding of soil water dynamics[5-8] and patterns of plant water use.[9-12] The isotopic composition of xylem water provides an integrated measure of the plant water source, and hinges on mixing model results in dual isotope space to determine the contributions from different sources (e.g. SIAR,[13] MixSIR,[14] IsoSource,[15] RAPID[16]). These mixing models, in turn, rely on extracted liquid samples obtained from plant sampling and subsequent extractions of xylem water.

However, traditional analyses of xylem water constrain our understanding of ecohydrological processes. Cryogenic vacuum distillation[17] (CVD) used to extract water from xylem is costly, labor-intensive, and time-consuming, which limits investigations to "snapshot" observations of plant water use.[18] Limited temporal observations may not reflect actual water source, because of long residence times of water in the stem of tree species.[19,20] Dynamic water uptake processes of plants can only be observed through high-frequency measurements of isotope composition.[21] Thus, current heterogeneity and dynamics of ecohydrological and physiological processes are masked, and much uncertainty exists in isotopic measurements of plant water sources.[22] Yet another issue with CVD is that it extracts all the water contained in the sampled plant material, including intracellular water[21,22] and pools that may not be contributing to the transpiration stream.

Recent work has shown that direct vapor equilibration (DVE) measurements of plant water sources can be made using laser spectroscopy,[21,23] which facilitates the sidestepping of methodological limitations imposed by traditional liquid extraction methods. These include lowering cost and improving sampling resolution. Measurements of the hydrogen and oxygen isotopic compositions of plant water can be conducted in vapor mode using isotope ratio infrared spectroscopy (IRIS) combined with the DVE method.[24,25] This is carried out through isotopic measurements of an equilibrated head space above sampled plant material,[23] or via *in situ* measurements.[21] Vapor measurement may allow for a more direct assessment of the transpiration stream, instead of the bulk plant water isotopic measurement seen with CVD.[21-23] *In situ* vapor measurements provide the possibility to carry out simultaneous assessment of plant water and soil water isotopic compositions. These high-frequency coupled measurements can reveal the dynamics and mechanisms of plant response to changes in water availability.[21]

Whilst high-frequency measurements of isotopes in plants bring opportunities to improve our understanding of ecohydrological processes,[22] plants produce a wide range of organic compounds that are often co-extracted with water using CVE, including methanol (MeOH) and ethanol (EtOH). These compounds introduce errors when measured using quick inexpensive laser spectroscopy and thereby impose a methodological challenge to advancements of plant water isotopic analysis.[26-28] MeOH and EtOH have different interference effects on laser spectroscopy analysis: MeOH causes narrowband spectral interference[29] while EtOH causes broadband interference.[29] MeOH and EtOH have already been used across different methodologies in liquid laser spectroscopic isotopic analyses to identify spectral interference, and ranges of errors in relation to distilled water.[26-28] The presence of organic compounds in xylem will vary depending on plant species, development stage and phenological phase.[30-33] Some organic compounds share similar laser absorption features with water and will result in spectral interference.[27,34] If organic contamination is not detected during laser spectrometric analysis, the δ-values will be systematically biased and inaccurate.[27] Spectral contamination flagging software exists to identify interference by organic compounds (Spectral Contamination Identifier (LWIA-SCI) – Los Gatos Research, Inc., Mountain View, CA, USA; and ChemCorrectTM – Picarro Inc., Santa Clara, CA, USA) in liquid mode, and it is being applied to plant water studies.[28,35] However, no systematic narrowband or broadband spectral contamination metrics are being generated for organic compound identification in vapor mode to date.

Here we explore the use of $\delta^{17}O$ values in the context of plant water analysis and organic contaminants during DVE-OA-ICOS analysis. We show for the first time how ^{17}O-excess values can be used to identify samples containing organic compounds that would otherwise obscure plant water source analysis. Similar to the deuterium excess (d-excess),[36] deviations from the known relationship between $\delta^{17}O/\delta^{16}O$ and $\delta^{18}O/\delta^{16}O$ ratios are defined as[37]:

$$^{17}O - \text{excess} = \delta'^{17}O - 0.528\ \delta'^{18}O \quad (1)$$

where $\delta'^{17}O = \ln(\delta^{17}O + 1)$, $\delta'^{18}O = \ln(\delta^{18}O + 1)$, and 0.528 is the slope of the Global Meteoric Water Line.

^{17}O-excess is known to be relatively insensitive to temperature compared with the d-excess and can provide new information on kinetic fractionation processes.[38-40] Thus, we hypothesize that the ^{17}O-excess may help to identify organic compounds in water vapor. This approach may help the scientific community better understand fractionation processes occurring within plants.

We conducted spectral contamination experiments using MeOH and EtOH as these are constituents of most organic compounds found in plants,[33] and have been used in previous studies for the same purpose.[27,28,41] We used solutions of MeOH and EtOH, and natural samples to test our system for flagging and to address the question, "Can ^{17}O-excess identify samples that are influenced by the presence of organic compounds?".

2 | EXPERIMENTAL

We used spiked water with known concentrations of MeOH and EtOH to determine the influence of organic compounds on ^{17}O-excess and measured isotope ratios via DVE. We collected xylem samples from three tree species to verify whether spectral contamination in natural solutions could be also identified by ^{17}O-excess.

2.1 | Ethanol and methanol solutions

We prepared a set of solutions with different concentrations of MeOH (Fisher Chemical, Fisher Scientific, Hampton, NH, USA, 99.9% by volume) and EtOH (Commercial Alcohols, Greenfield Global Inc., Toronto, ON, Canada, 100% by volume) diluted in distilled water with δ^2H −126.83‰, $\delta^{18}O$ −15.65‰, and $\delta^{17}O$ −7.50‰.

Ten MeOH and EtOH contaminant mixtures (% v/v) with five replicates each ($n = 5$) were prepared using the same reference distilled water. We kept the five replicates of the distilled water under the same conditions as the prepared solutions. The MeOH concentrations were 0.001%, 0.002%, 0.004%, 0.008%, 0.016%, 0.032%, 0.064%, 0.128%, and 0.256%, and the EtOH concentrations were 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.25%, 0.5%, 1%, 5%, and 10%. We used different contaminant ranges for MeOH and EtOH based on our pilot experiments which showed that spectral analysis is more sensitive to the presence of MeOH, even in small volumes.

2.2 | Xylem and soil samples

Xylem samples were collected from three tree species to verify whether spectral contamination in natural samples could also be identified by ^{17}O-excess. Our study sites (Boreal Ecosystem Monitoring Research Sites – BEMRS) are located at the southern limit of the Canadian boreal forest ecozone in Saskatchewan. The Old Jack Pine site (OJP, 53.92 N, 104.69 W) has a forest stand of jack pine (*Pinus banksiana* Lamb) trees that are approximately 114 years old, while the Old Black Spruce site (OBS, 53.98 N, 105.12 W) forest stand is inhabited by black spruce (*Picea mariana* (Mill.) BSP) and eastern larch (*Larix laricina* (Du Roi) K. Koch) trees that are approximately 140 years old. The climate in the region is continental with a long and dry cold season from October to March, and short growing season from April to September.

We used three permanent sampling plots within a 20 m radius at the OJP and OBS sites to collect xylem and soil samples. We collected xylem and soil samples on two sampling dates in April and May 2018. Both xylem and soil samples were analyzed via DVE. We sampled xylem from all three plots, from five different individual trees per month ($n = 15$). Because eastern larch trees were sparse in the plots, we were only able to collect two trees in April and one in May. In each plot, we collected suberized branches with stem diameters between 1.5 and 3 cm from healthy looking trees. An extendable tree pruner was used to collect the selected branches. We immediately removed the outer and inner bark of the branch in the field, cut them into smaller segments, and stored only the xylem components in sealed glass vials. We collected bulk soil water by sampling soil at ten different depths per plot using a soil auger. We collected soil samples every 20 cm at the OJP site, up to 200 cm, and every 10 cm at the OBS site up to 100 cm ($n = 30$). The difference in depth was chosen according to water table depths at each site. The ten sampling depths were selected to observe soil water isotopic composition and variation with depth. The soil samples were stored in 25-mL Nalgene™ HDPE bottles until vapor analysis. The bottles were filled completely to minimize headspace and mixing of atmospheric vapor with sampled soil over the storage period. All samples were sealed in the field with Parafilm®, transported in coolers, and subsequently stored in the laboratory at 4°C.

2.3 | Isotope analyses

The isotopic compositions (δ^2H, $\delta^{18}O$ and $\delta^{17}O$ values) of the MeOH and EtOH solutions, and the soil and xylem samples were measured using a TIWA-45EP OA-ICOS instrument (Los Gatos Research)[42] in vapor mode, following protocols developed by Wassenaar et al.[24] Samples were transferred to leak-proof plastic bags (17.78 cm × 20.38 cm, Model No. S-5855, Uline, USA), and immediately inflated with dry air. For plants, we used all branch material collected in one vial, approximately 10–12 g, and for soil approximately 200 g. For MeOH and EtOH solutions we used 10 mL of solution per bag. After inflation, bags were left for 24–48 h at room temperature (~22°C) to allow sample water to equilibrate with dry air in the headspace of the inflated bag.

After equilibration, we sampled the bag headspace for ~3 min intervals, or until the water content stabilized at ~28,000 to 30,0000 ppm V H_2O, at which point the δ^2H, $\delta^{18}O$, and $\delta^{17}O$ raw values were recorded.[42] The isotope signatures are reported in δ-notation, in per mil (‰). The δ-value denotes the measured isotope ratio of sample (R_{sample}) for δ^2H ($^2H/^1H$) and $\delta^{18}O$ ($^{18}O/^{16}O$) in reference to Vienna Standard Mean Ocean Water (VSMOW) ($R_{reference}$):

$$\delta^2H \text{ or } \delta^{18}O \text{ or } \delta^{17}O = \left(\frac{R_{sample}}{R_{reference}} - 1\right) \quad (2)$$

where R_{sample} is the $^2H/^1H$ or $^{18}O/^{16}O$ or $^{17}O/^{16}O$ ratio of the measured sample, and $R_{reference}$ is the $^2H/^1H$ or $^{18}O/^{16}O$ or $^{17}O/^{16}O$ ratio of the VSMOW reference.[43,44]

The laboratory reference waters had δ-values of −200.26‰, −26.00‰, and −14.08‰ for the depleted reference, and 9.70‰, 0.57‰, and −0.10‰ for the enriched reference water, for ^2H, ^{18}O, and ^{17}O, respectively. A third control standard with known δ-values (δ^2H −137.0‰, $\delta^{18}O$ −17.4‰, and $\delta^{17}O$ 9.2‰) was measured every eight samples for quality control and quality assurance of the measurements. The TIWA-45EP analyzer in vapor mode, using a 30 s reading period, has a precision of: ±1.8 for δ^2H values and ±0.3 for $\delta^{18}O$ and $\delta^{17}O$ values.

2.4 | Spectral contamination and statistical analysis

Statistical analyses were performed using R 3.5.1 software.[45] We calculated the mean and standard deviations of the three isotopologues, for each solution of MeOH and EtOH, and the

natural samples. The δ-values of the solutions were compared against the distilled reference water. The difference in δ-values between distilled water and the solutions was treated as a measure of trueness. We used the Kruskal-Wallis[46] and post hoc Dunn's test[47] to determine which of the solutions resulted in significantly different isotopic measurements from the control ($p \leq 0.05$). The *p*-values were adjusted with the Benjamini-Hochberg method.[48] We also assessed the trueness of measured isotopic composition per concentration of MeOH, EtOH, and per tree species by calculating the produced error – the difference between the measured δ-value of the solution and that of the reference water. For the xylem samples, the error was calculated as the difference between the mean δ-values per species of samples identified as spectrally contaminated, and those of xylem samples found not to be contaminated.

Finally, we compared the ^{17}O-excess with the qualitative Spectral Fit Residual plot on the TIWA-45EP OA-ICOS instrument (Los Gatos Research; see supporting information). The Spectral Fit Residual plot, which has not been previously reported in the literature, was enabled for this study. This plot produces a graphical image that allows the user to visually identify the presence of spectral contamination in the sample. The user categorizes samples as 'contaminated' or 'not contaminated' by examining changes in slope, and residual noise of the spectra. The Spectral Fit Residual plot also enables the user to distinguish between narrow- and broadband spectral contamination based on responses to contaminants (MeOH and EtOH). We used the Spectral Fit Residual plots produced with distilled water and MeOH/EtOH in water as a qualitative parameter for the identification of spectral contamination in soil and xylem samples.

3 | RESULTS

3.1 | ^{17}O-excess as a flagging tool for narrow- (MeOH) and broadband (EtOH) spectral contamination

The ^{17}O-excess of MeOH solutions increased linearly with increasing concentrations of MeOH per volume of water, resulting in positive ^{17}O-excess values (Figure 1A). EtOH solutions resulted in smaller changes in ^{17}O-excess than MeOH and conversely the values were negative (Figure 1B). Solutions of MeOH produced larger errors on the measured isotopic composition (Table 1), and in relation to the meteoric water line (Figure 2). Deviations in the δ^2H and δ^{18}O values caused by MeOH skewed the results; they plotted below the meteoric water line in dual isotope ratio space (Figure 2A). For EtOH, the isotope ratios were skewed to the left, plotting above the meteoric water line (Figure 2B). The isotopic composition of the MeOH water mixtures differed substantially from that of the original distilled water (Table 1). MeOH introduced positive bias to the δ^2H, δ^{18}O, and δ^{17}O values of distilled water. The MeOH interference on δ-values was statistically significant, starting at 0.008% for δ^{17}O ($p < 0.02$), for δ^{18}O ($p < 0.02$), and for δ^2H ($p < 0.02$) values. EtOH introduced positive and negative bias depending on concentration, and the EtOH influences were statistically significant at 0.25% ($p < 0.001$) for δ^{17}O value, but not at 0.5% ($p > 0.05$). The presence of EtOH did not affect the δ^2H and δ^{18}O values in the observed range of 0.001 to 0.25%. For statistical analyses, we did not include MeOH concentrations above 0.016%, and EtOH above 5%. These solutions produced unreliable measurements as seen on the Spectroscopic Absorbance plot during analysis, with values below zero during vapor analysis (see Figure S3, supporting information).

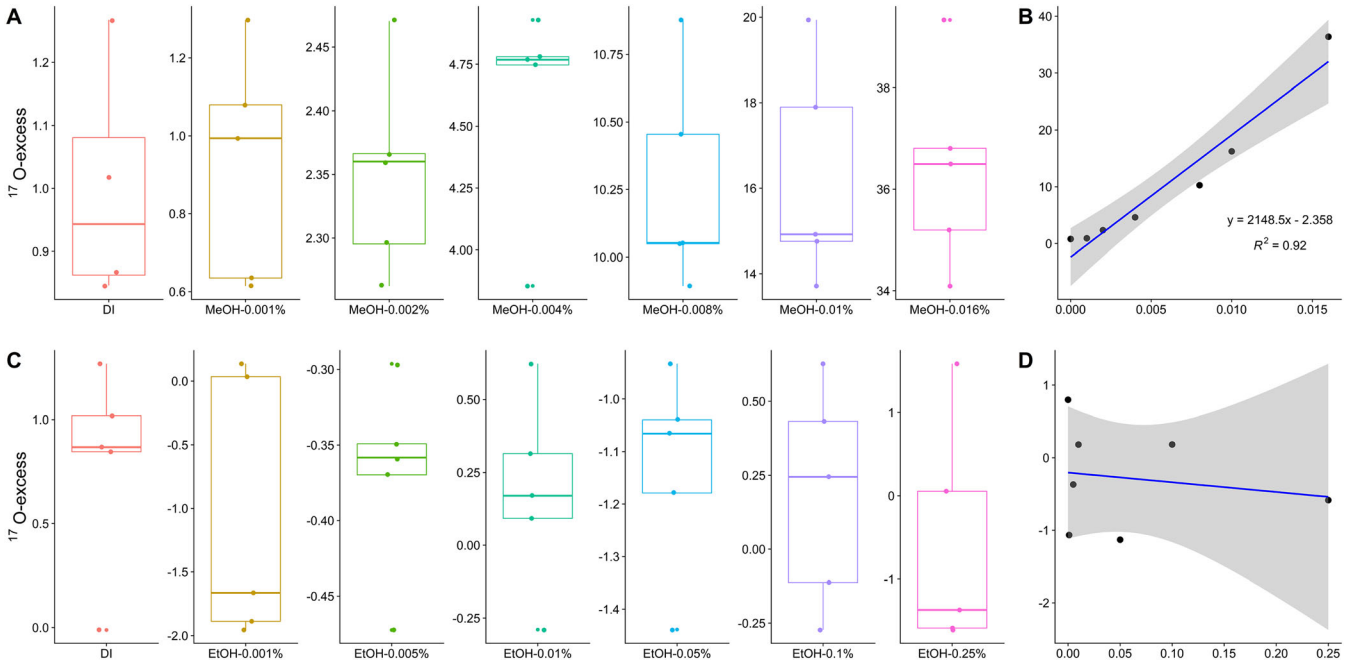

FIGURE 1 Spectral contamination results on measured isotopic composition of distilled water with different concentrations of MeOH and EtOH. A and C show changes in ^{17}O-excess (per meg) per concentration of MeOH and EtOH, respectively (note changes in y axis). B and D show linear relationships between ^{17}O-excess and MeOH and EtOH, respectively [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Methanol (MeOH) and ethanol (EtOH) with mean isotopic composition (δ^2H, $\delta^{18}O$, and $\delta^{17}O$ values) per concentration (% v/v), standard deviation (sd). Calculated error (‰) and ^{17}O-excess

	No. total samples	% v/v	δ^2H	sd	Error (‰)	$\delta^{18}O$	sd	Error (‰)	$\delta^{17}O$	sd	Error (‰)	^{17}O-excess	sd	Error (‰)
Pure water	5	0	−130.127	1.40		−16.05	0.24		−7.51	0.09		1.0	0.19	
MeOH	5	0.001	−123.83	±1.75	−6.30	−14.07	±0.64	−1.98	−6.53	±0.29	−0.98	0.92	±0.29	0.08
	5	0.002	−125.28	±1.10	−4.85	−13.67	±0.17	−2.37	−4.91	±0.06	−2.61	2.35	±0.08	−1.35
	5	0.004	−122.63	±2.74	−7.50	−11.57	±0.27	−4.47	−1.53	±0.43	−5.98	4.62	±0.43	−3.62
	5	0.008	−113.44	±1.62	−16.68	−6.45	±0.33	−9.60	6.87	±0.29	−14.39	10.27	±0.40	−9.27
	5	0.01	−94.84	±8.24	−35.29	1.36	±0.68	−17.40	17.11	±2.48	−24.62	16.24	±2.59	−15.24
	5	0.016	−93.54	±1.44	−36.58	5.58	±0.33	−21.63	40.11	±1.96	−47.62	36.39	±1.97	−35.39
EtOH	5	0.001	−124.073	±3.26	−6.05	−15.37	±0.64	−0.67	−9.20	±1.14	1.69	−1.07	±1.06	2.1
	5	0.005	−128.407	±1.30	−1.72	−16.12	±0.16	0.07	−8.91	±0.12	1.40	−0.37	±0.06	1.4
	5	0.01	−123.023	±6.71	−7.10	−14.65	±0.94	−1.39	−7.58	±0.40	0.07	0.18	±0.33	0.8
	5	0.05	−130.806	±2.20	0.68	−16.33	±0.29	0.28	−9.78	±0.20	2.26	−1.13	±0.19	2.1
	5	0.1	−112.63	±8.34	−17.50	−15.80	±1.10	−0.24	−8.19	±0.23	0.68	0.18	±0.37	0.8
	5	0.25	−130.63	±3.32	0.50	−18.19	±0.35	2.15	−10.23	±1.22	2.71	−0.59	±1.39	1.6

The use of the Spectral Fit Residual plot alone was insufficient to determine the presence of contaminants. We found it difficult to identify spectral contamination of organic compounds using the Spectral Fit Residual plot for low concentrations of MeOH (0.004%) and EtOH (0.1%), while the $\delta^{17}O$ value was sensitive to organics at the lowest concentration (MeOH 0.001%).

3.2 | ^{17}O-excess and natural samples

Soil water samples from both sites did not show any spectral contamination on the Spectral Fit Residual plot, nor were they flagged by the ^{17}O-excess (Figure 3). The average soil water ^{17}O-excess value was 0.56, and −0.28 for the OJP and OBS sites. The highest ^{17}O-excess was 0.95 at 200 cm depth, at OJP (Figure 3A). The lowest ^{17}O-excess value was −0.77 at 10 cm depth, at OBS (Figure 3B).

We identified spectral contamination in xylem samples using the Spectral Fit Residual plot (Figure 4). Spectrally contaminated xylem water showed increases in ^{17}O-excess values (Figure 4), and narrowband spectral contamination. The average ^{17}O-excess value for contaminated xylem was 7.27, and 20.51 for *P. mariana* and *P. bankisiana*, respectively (Figure 4). The average ^{17}O-excess value of xylem free of spectral contamination was 0.85 and 0.47 for *P. mariana* and *P. bankisiana*. Of the tree species sampled, most of the spectral contamination was shown in *P. bankisiana* at 66.7% of samples followed by *P. mariana* at 46.7%. *L. laricina* did not show any spectral contamination in vapor mode by the applied method, with a ^{17}O-excess of 1.55.

Table 2 shows a comparison of the isotopic compositions of samples identified with and without spectral contamination. Spectrally contaminated xylem showed large errors in isotopic composition, compared with samples free of contamination (Table 2). The δ-values of xylem samples identified as free of organic contamination were more depleted in ^2H, ^{18}O, and ^{17}O than the spectrally contaminated xylem samples. Xylem samples free of spectral contamination had isotope ratios close to those of soil water, and plotted close to the meteoric water line (Figure 5).

4 | DISCUSSION

4.1 | ^{17}O-excess and spectral contamination

The experiments with MeOH and EtOH revealed the sensitivity of ^{17}O-excess to the presence of organic contaminants when conducting spectral analysis via DVE. The ^{17}O-excess flagged the presence of both organic compounds, with more sensitivity to

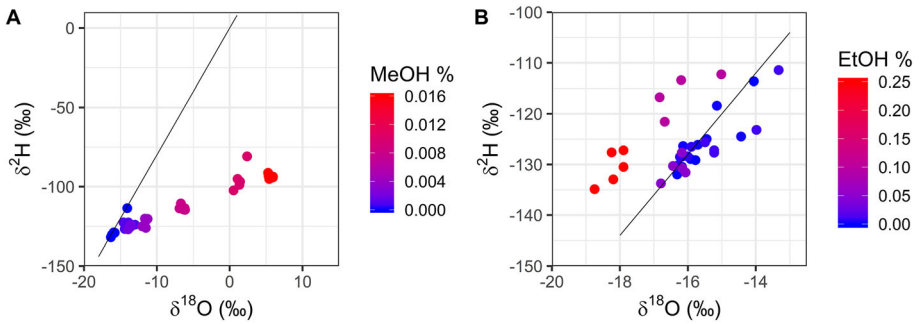

FIGURE 2 Dual isotope ratio space results for MeOH (A) and EtOH (B). Black line represents the global meteoric water line (GMWL) [Color figure can be viewed at wileyonlinelibrary.com]

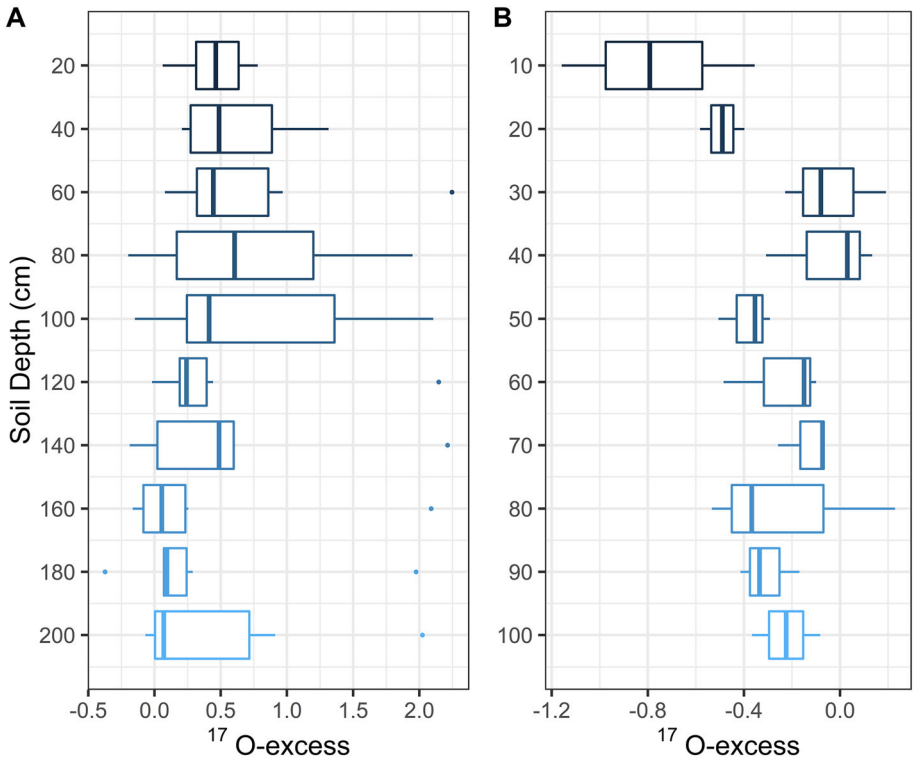

FIGURE 3 Soil ^{17}O-excess (per meg) from OJP (A) and OBS (B) [Color figure can be viewed at wileyonlinelibrary.com]

MeOH than EtOH. Although there are no previous reports on the effects of organic compounds on the ^{17}O-excess our results are consistent with previous spectral contamination investigations in the literature with respect to laser spectroscopy. Brand et al,[27] West et al,[26] and Martín-Gómez et al[28] have reported pronounced effects from MeOH and EtOH contamination on δ^2H and δ^{18}O values of reference water when measuring isotopes in liquid mode for both isotope ratio infrared spectroscopy (IRIS) and isotope ratio mass spectrometry (IRMS) analysis; MeOH is known to have a more similar structure to water than EtOH does, resulting in stronger spectral contamination and larger errors on isotope composition.[25]

While MeOH produced positive ^{17}O-excess values, EtOH produced more negative ^{17}O-excess values. These findings are also in agreement with results found by Schultz et al.[41] While these

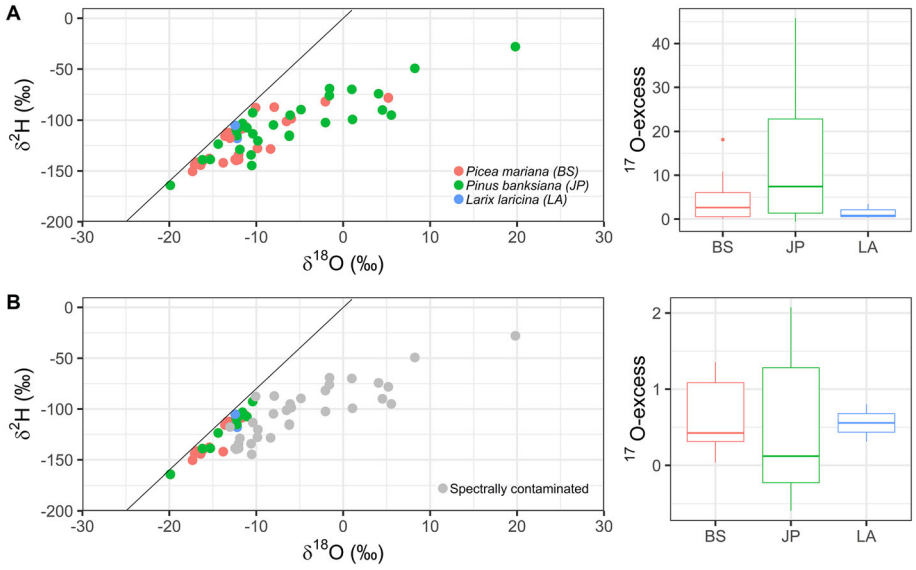

FIGURE 4 Xylem samples in dual isotope ratio space and respective ^{17}O-excess (per meg): A results for all species; B the same samples, but distinguishing between spectrally contaminated (grey) identified visually on the 'spectral fit residual plot', and not contaminated. Note changes in ^{17}O-excess axis [Color figure can be viewed at wileyonlinelibrary.com]

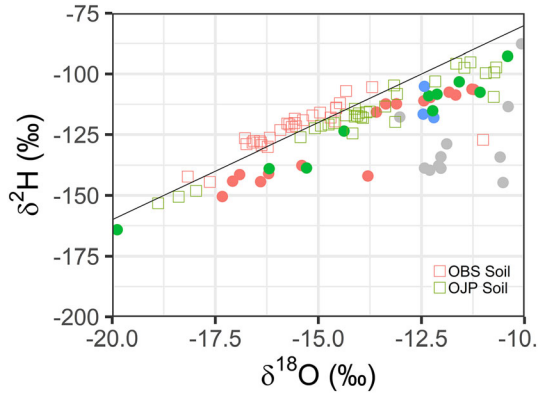

FIGURE 5 Xylem and soil samples plotted together in dual isotope ratio space with focus on the isotope range of soil water. Xylem water are represented by filled circles, *P. banksiana* (green), *P. mariana* (red), and *L. laricina* (blue). Xylem samples in grey were visually identified as spectrally contaminated by the 'spectral fit residual plot'. Soil samples from both sites (OBS and OJP) are represented by squares. Global meteoric water line (GMWL) in black [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Xylem samples from black spruce (*P. mariana*), jack pine (*P. banksiana*), and eastern larch (*L. laricina*) with mean δ-values and standard deviation (sd) from samples identified as free of spectral contamination and samples identified with spectral contamination by the current method. Errors were calculated per species

Free of spectral contamination								Spectral contamination								
Species	No. total samples	δ^2H	sd	$\delta^{18}O$	sd	$\delta^{17}O$	sd	Species	No. total samples	δ^2H	sd	Error δ^2H	$\delta^{18}O$	sd	Error $\delta^{18}O$	$\delta^{17}O$	sd	Error $\delta^{17}O$
P. mariana	16	-124.43	± 17.25	-14.00	± 2.22	-6.57	± 1.33	*P. mariana*	14	-114.10	± 23.84	**10.33**	-8.53	± 5.05	**5.46**	2.77	± 6.15	**9.33**
P. banksiana	10	-120.15	± 21.43	-13.55	± 2.90	-6.71	± 1.48	*P. banksiana*	20	-95.77	± 28.87	**24.37**	-2.28	± 7.97	**11.27**	19.63	± 18.47	**26.34**
L. laricina	3	-113.24	± 7.07	-12.36	± 0.14	-5.03	± 1.66											

authors measured neither $\delta^{17}O$ nor ^{17}O-excess values, they used EtOH and MeOH to develop correction curves and assess off-set in isotopic measurement introduced by organics in liquid water samples. They also showed that samples identified by narrowband spectral contamination (MeOH) resulted in more positive $\delta^{18}O$ and δ^2H values, meaning that a correction factor had to be subtracted from the δ-values. Broadband spectral contamination (caused by EtOH) produced results that were more positive mainly to $\delta^{18}O$ values, and a correction factor had to be added to the measured δ-values. Our results show that a small concentration of MeOH was enough to introduce statistically significant errors to isotopic measurements during DVE analysis. In previous studies on liquid mode analysis in laser spectrometry, higher concentrations of MeOH (0.05% v/v) and EtOH (8% v/v) tested were shown to induce statistically significant errors to isotopic measurements.[27,28,49] The interference of organic compounds could be more pronounced on DVE than on liquid mode, but smaller concentrations need to be systematically tested on liquid mode.

4.2 | ^{17}O-excess and the TIWA-45EP OA-ICOS analyzer spectral fit residual plot

TIWA-45EP OA-ICOS offers a quick and reliable method to measure $\delta^{17}O$ values compared with IRMS.[42] The TIWA-45EP OA-ICOS analyzer Spectral Fit Residual plot produced satisfactory qualitative indicators of spectral contamination. This was done via visual qualitative inspection of the Spectral Fit Residual plot during the analysis of contaminated and non-contaminated water solutions. We validated our observations of contamination using the Spectral Fit Residual plot against calculated errors on isotope ratio measurements compared with the non-contaminated reference

water value. We found it challenging to determine spectral contamination with any precision using the visual inspection of the Spectral Fit Residual plot when the level of organic compounds in water was very low (below 0.004% for MeOH). ^{17}O was the most sensitive isotope to the presence of organics. The ^{17}O-excess as an organic contamination detection tool introduces a systematic step to quantitatively inform errors produced during isotopic analysis. ^{17}O-excess values enabled the identification of spectral contamination at the low concentrations tested in this study (0.002% for MeOH). Thus, the use of a third isotope helps avoid unknown error from organic contamination and is independent of human judgment of trying to observe variations on the Spectral Fit Residual plot.

The next step should be to develop metrics for the correction of samples with indicated contamination during isotope analysis via DVE. A mix of organics should be also investigated to verify whether there is interference of one over the other. Measurements of ^{17}O may offer an opportunity for the development of correction curves. Post-processing correction for liquid mode analysis is already used by the scientific community. The correction can be done by available software, or developed through experimental analysis with MeOH and EtOH, based on measured metrics from the absorbed spectrum,[28,29,41] and this has been applied to isotope measurements of plant water in liquid mode (e.g. Barbeta et al[35]). The scientific community would benefit from similar software for vapor mode analysis.

4.3 | Proof of concept with xylem and soil water samples

We used xylem and soil samples collected from the boreal forest to test ^{17}O-excess as a contamination flagging tool. Our soil water isotopic analyses via DVE, and using the Spectral Fit Residual plots as a visual cue, did not identify any spectral contamination. The ^{17}O-excess values from soil were similar to values produced by distilled water samples. ^{17}O-excess did not indicate spectral contamination for soil water analyzed via vapor mode and was in agreement with observations made by Spectral Fit Residual plots. Soil water isotopic composition (δ^2H and $\delta^{18}O$ values) did not show deviations from the meteoric water line.

Previous plant water investigations in liquid mode via laser spectrometry have shown that organic compounds can be flagged by available software.[28,41,50] However, there are no spectral files generated, nor is software available for post processing or screening vapor generated isotope data. Without these tools, researchers cannot quantitatively identify isotope measurements that have been compromised by spectral interference from organic compounds. In our xylem plant water analyses, we show that it is possible to identify contaminated samples using ^{17}O-excess. Xylem samples visually identified as contaminated with the Spectral Fit Residual plots showed positive ^{17}O-excess values. The ^{17}O-excess values of contaminated samples were above those identified for distilled water, and soil. Spectrally contaminated samples showed more positive $\delta^{18}O$ and δ^2H values than uncontaminated xylem. Previous work found similar results during laser spectrometric analysis of plant water in liquid mode.[41,45] Plant water samples contaminated by organic compounds analyzed by OA-ICOS resulted in results that were enriched in ^{18}O and ^2H in relation to corrected plant water samples,[44] or IRIS analysis.[50]

All contaminated xylem samples showed narrowband spectral contamination. The ^{17}O-excess value from spectrally contaminated xylem samples correlated to the ^{17}O-excess value of 0.01% for MeOH. The strong linear relationship between the concentration of MeOH (narrowband spectra) and the ^{17}O-excess values suggests that it may be possible to quantify the concentration of organics in samples utilizing ^{17}O-excess. This should be further investigated with post-processing correction curves. Taken together, the δ^2H, $\delta^{18}O$, and $\delta^{17}O$ values offer a potential tool to assess spectral interference of organics in vapor mode analysis through deviations of ^{17}O-excess. The $\delta^{17}O$ value can be measured simultaneously in vapor mode, without adding extra costs or additional analyses. To move forward with the DVE approach and analysis of plant water, we need to implement a systematic way to verify and quantify the presence of organic compounds.

5 | CONCLUSIONS

Use of the $\delta^{17}O$ value together with δ^2H and $\delta^{18}O$ values can provide additional information about spectral contamination during plant water analysis in vapor mode via OA-ICOS. Organic compounds are naturally present in the xylem of tree species and are an important part of phenological and physiological processes. However, their possible influence over observed plant-water isotopologues in vapor mode has not been fully investigated. The use of $\delta^{17}O$ values has the potential to improve analysis in vapor mode by improving the reliability of observed isotopic results. With this approach, we avoid introducing contaminated xylem samples to mixing model analysis, and further misinterpreting plant-water sources. Additional studies are nonetheless required to identify which organics interfere in the $^{17}O/^{16}O$ isotopic ratio measurements, as well as whether other different organic compounds affect the observed $\delta^{17}O$ values.

ACKNOWLEDGEMENTS

The authors thank Taylor Kosokowsky, Becket Stark, and Bruce Johnson for their assistance during field work and laboratory analyses. They also thank Geoff Kohler for his input and earlier discussions. This research was supported by an NSERC Discovery grant to JJM, and by the NSERC CREATE program.

ORCID

Magali F. Nehemy https://orcid.org/0000-0002-2212-3592
Cody Millar https://orcid.org/0000-0002-2171-581X
Marcel Gaj https://orcid.org/0000-0003-0304-8328

REFERENCES

1. Sprenger M, Tetzlaff D, Buttle J, et al. Storage, mixing, and fluxes of water in the critical zone across northern environments inferred by stable isotopes of soil water. *Hydrol Processes*. 2018;32(12):1720-1737. https://doi.org/10.1002/hyp.13135
2. Martin J, Looker N, Hoylman Z, Jencso K, Hu J. Differential use of winter precipitation by upper and lower elevation Douglas fir in the northern Rockies. *Glob Chang Biol*. 2018;24(12):5607-5612. https://doi.org/10.1111/gcb.14435
3. Brinkmann N, Seeger S, Weiler M, Buchmann N, Eugster W, Kahmen A. Employing stable isotopes to determine the residence times of soil water and the temporal origin of water taken up by *Fagus sylvatica* and *Picea abies* in a temperate forest. *New Phytol*. 2018;219(4):1300-1313. https://doi.org/10.1111/nph.15255
4. Barbeta A, Peñuelas J. Relative contribution of groundwater to plant transpiration estimated with stable isotopes. *Sci Rep*. 2017;7(1):10580. https://doi.org/10.1038/s41598-017-09643-x
5. Sprenger M, Seeger S, Blume T, Weiler M. Travel times in the vadose zone: Variability in space and time. *Water Resour Res*. 2016;52(8):5727-5754. https://doi.org/10.1029/2004WR003899
6. Sprenger M, Tetzlaff D, Buttle J, et al. Measuring and modeling stable isotopes of mobile and bulk soil water. *Vadose Zo J*. 2018;17(1):170149. https://doi.org/10.2136/vzj2017.08.0149
7. Oerter EJ, Bowen G. In situ monitoring of H and O stable isotopes in soil water reveals ecohydrologic dynamics in managed soil systems. *Ecohydrology*. 2017;10(4):e1841. https://doi.org/10.1002/eco.1841
8. Rempe DM, Dietrich WE. Direct observations of rock moisture, a hidden component of the hydrologic cycle. *Proc Natl Acad Sci*. 2018;115(11):2664-2669. https://doi.org/10.1073/pnas.1800141115
9. Evaristo J, Jasechko S, McDonnell JJ. Global separation of plant transpiration from groundwater and streamflow. *Nature*. 2015;525(7567):91-94. https://doi.org/10.1038/nature14983
10. Evaristo J, McDonnell JJ. Prevalence and magnitude of groundwater use by vegetation: A global stable isotope meta-analysis. *Sci Rep*. 2017;7(1):44110. https://doi.org/10.1038/srep44110
11. Hervé-Fernández P, Oyarzún C, Brumbt C, et al. Assessing the 'two water worlds' hypothesis and water sources for native and exotic evergreen species in south-central Chile. *Hydrol Process*. 2016;30(23):4227-4241. https://doi.org/10.1002/hyp.10984
12. McCutcheon RJ, McNamara JP, Kohn MJ, Evans SL. An evaluation of the ecohydrological separation hypothesis in a semiarid catchment. *Hydrol Process*. 2016;31(4):783-799. https://doi.org/10.1002/hyp.11052
13. Parnell AC, Inger R, Bearhop S, Jackson AL. Source partitioning using stable isotopes: Coping with too much variation. *PLoS One*. 2010;5(3):e9672. https://doi.org/10.1371/journal.pone.0009672
14. Moore JW, Semmens BX. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecol Lett*. 2008;11(5):470-480. https://doi.org/10.1111/j.1461-0248.2008.01163.x
15. Phillips DL, Gregg JW. Source partitioning using stable isotopes: Coping with too many sources. *Oecologia*. 2003;136(2):261-269. https://doi.org/10.1007/s00442-003-1218-3
16. Ogle K, Tucker C, Cable JM. Beyond simple linear mixing models: Process-based isotope partitioning of ecological processes. *Ecol Appl*. 2014;24(1):181-195.
17. Koeniger P, Marshall JD, Link T, Mulch A. An inexpensive, fast, and reliable method for vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry. *Rapid Commun Mass Spectrom*. 2011;25(20):3041-3048. https://doi.org/10.1002/rcm.5198
18. Berry ZC, Evaristo J, Moore G, et al. The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward. *Ecohydrology*. 2017;11(3):e1843. https://doi.org/10.1002/eco.1843
19. Gaines KP, Meinzer FC, Duffy CJ, Thomas EM, Eissenstat DM. Rapid tree water transport and residence times in a Pennsylvania catchment. *Ecohydrology*. 2016;9(8):1554-1565. https://doi.org/10.1002/eco.1747
20. Meinzer FC, Brooks JR, Domec J-C, et al. Dynamics of water transport and storage in conifers studied with deuterium and heat tracing techniques. *Plant Cell Environ*. 2006;29(1):105-114. https://doi.org/10.1111/j.1365-3040.2005.01404.x
21. Volkmann THM, Haberer K, Gessler A, Weiler M. High-resolution isotope measurements resolve rapid ecohydrological dynamics at the soil-plant interface. *New Phytol*. 2016;210(3):839-849. https://doi.org/10.1111/nph.13868
22. Penna D, Hopp L, Scandellari F, et al. Tracing ecosystem water fluxes using hydrogen and oxygen stable isotopes: Challenges and opportunities from an interdisciplinary perspective. *Biogeosci Discuss*. 2018;15(21):6399-6415. https://doi.org/10.5194/bg-2018-286
23. Millar C, Pratt D, Schneider DJ, McDonnell JJ. A comparison of extraction systems for plant water stable isotope analysis. *Rapid Commun Mass Spectrom*. 2018;32(13):1031-1044. https://doi.org/10.1002/rcm.8136
24. Wassenaar LI, Hendry MJ, Chostner VL, Lis GP, About M, Article T. High resolution pore water δ2H and δ18O measurements by H_2O(liquid)-H_2O(vapor) equilibration laser spectroscopy. *Environ Sci Technol*. 2008;42(24):9262-9267.
25. Hendry MJ, Schmeling E, Wassenaar LI, Barbour SL, Pratt D. Determining the stable isotope composition of pore water from saturated and unsaturated zone core: Improvements to the direct vapor equilibration laser spectroscopy method. *Hydrol Earth Syst Sci Discuss*. 2015;19(11):4427-4440. https://doi.org/10.5194/hessd-12-6241-2015
26. West AG, Goldsmith GR, Brooks PD, Dawson TE. Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. *Rapid Commun Mass Spectrom*. 2010;24(14):1948-1954. https://doi.org/10.1002/rcm
27. Brand WA, Geilmann H, Crosson ER, Rella CW. Cavity ring-down spectroscopy versus high-temperature conversion isotope ratio mass spectrometry; a case study on δ^2H and $\delta^{18}O$ of pure water samples and alcohol/water mixtures. *Rapid Commun Mass Spectrom*. 2009;23(12):1879-1884. https://doi.org/10.1002/rcm
28. Martín-Gómez P, Barbeta A, Voltas J, et al. Isotope-ratio infrared spectroscopy: A reliable tool for the investigation of plant-water sources? *New Phytol*. 2015;207(3):914-927. https://doi.org/10.1111/nph.13376
29. Leen BJ, Berman ESF, Liebson L, Gupta M. Spectral contaminant identifier for off-axis integrated cavity output spectroscopy measurements of liquid water isotopes. *Rev Sci Instrum*. 2012;83(4):044305. https://doi.org/10.1063/1.4704843
30. Vanhatalo A, Chan T, Aalto J, et al. Tree water relations can trigger monoterpene emissions from Scots pine stems during spring recovery. *Biogeosciences*. 2015;12(18):5353-5363. https://doi.org/10.5194/bg-12-5353-2015
31. Mozaffar A, Schoon N, Bachy A, et al. Biogenic volatile organic compound emissions from senescent maize leaves and a comparison

with other leaf developmental stages. *Atmos Environ*. 2018;176:71-81. https://doi.org/10.1016/j.atmosenv.2017.12.020

32. Laothawornkitkul J, Taylor JE, Paul ND, Hewitt NC. Biogenic volatile organic compounds in the atmosphere. *Biog Volatile Org Compd Earth Syst*. 2009;183:27-51.
33. Salomón RL, Rodríguez-Calcerrada J, Staudt M. Carbon losses from respiration and emission of volatile organic compounds – The overlooked side of tree carbon budgets. In: *Oaks Physiological Ecology. Exploring the Functional Diversity of Genus* Quercus *L.* Vol.7 New York, NY: Springer; 2017:327-359.
34. Hendry MJ, Richman B, Wassenaar LI. Correcting for methane interferences on δ^2H and $\delta^{18}O$ measurements in pore water using H_2O (liquid)-H_2O (vapor) equilibration laser spectroscopy. *Anal Chem*. 2011;83(14):5789-5796. https://doi.org/10.1021/ac201341p
35. Barbeta A, Jones SP, Clavé L, et al. Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrol Earth Syst Sci Discuss*. 2019;23:2129-2146. https://doi.org/10.5194/hess-2018-631
36. Dansgaard W. Stable isotopes in precipitation. *Tellus*. 1964;16(4):436-468. https://doi.org/10.3402/tellusa.v16i4.8993
37. Barkan E, Luz B. Diffusivity fractionations of $H_2{}^{16}O/H_2{}^{17}O$ and $H_2{}^{16}O/H_2{}^{18}O$ in air and their implications for isotope hydrology. *Rapid Commun Mass Spectrom*. 2007;21(18):2999-3005. https://doi.org/10.1002/rcm
38. Landais A, Barkan E, Yakir D, Luz B. The triple isotopic composition of oxygen in leaf water. *Geochim Cosmochim Acta*. 2006;70(16):4105-4115. https://doi.org/10.1016/j.gca.2006.06.1545
39. Landais A, Barkan E, Luz B. Record of $\delta^{18}O$ and ^{17}O-excess in ice from Vostok Antarctica during the last 150,000 years. *Geophys Res Lett*. 2008;35(2):1-5. https://doi.org/10.1029/2007GL032096
40. Angert A. Kinetic ^{17}O effects in the hydrologic cycle: Indirect evidence and implications. *Geochim Cosmochim Acta*. 2004;68(17):3487-3495. https://doi.org/10.1016/j.gca.2004.02.010
41. Schultz NM, Griffis TJ, Lee X, Baker JM. Identification and correction of spectral contamination in $^2H/^1H$ and $^{18}O/^{16}O$ measured in leaf, stem, and soil water. *Rapid Commun Mass Spectrom*. 2011;25(21):3360-3368. https://doi.org/10.1002/rcm.5236
42. Berman ESF, Levin NE, Landais A, Li S, Owano T. Measurement of $\delta^{18}O$, $\delta^{17}O$, and ^{17}O-excess in water by off-axis integrated cavity output spectroscopy and isotope ratio mass spectrometry. *Anal Chem*. 2013;85(21):10392-10398. https://doi.org/10.1021/ac402366t
43. Craig H. Standard for reporting concentration of deuterium and oxygen-18 in natural waters. *Science*. 1961;133(3467):1833-1834.
44. Coplen TB. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Commun Mass Spectrom*. 2011;25(17):2538-2560. https://doi.org/10.1002/rcm.5129
45. R Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing; 2018.
46. Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis stable. *J Am Stat Assoc*. 1952;47(260):583-621.
47. Dunn OJ. Multiple comparisons using rank sums. *Dent Tech*. 1964;6(3):241-252.
48. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc*. 1995;57(1):289-300.
49. Wassenaar LI, Terzer-Wassmuth S, Douence C, Araguas-Araguas L, Aggarwal PK, Coplen TB. Seeking excellence: An evaluation of 235 international laboratories conducting water isotope analyses by isotope-ratio and laser-absorption spectrometry. *Rapid Commun Mass Spectrom*. 2017;32(2):393-406. https://doi.org/10.1002/rcm.8052
50. West AG, Goldsmith GR, Matimati I, Dawson TE. Spectral analysis software improves confidence in plant and soil water stable isotope analyses performed by isotope ratio infrared spectroscopy (IRIS). *Rapid Commun Mass Spectrom*. 2011;25(16):2268-2274. https://doi.org/10.1002/rcm.5126

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Nehemy MF, Millar C, Janzen K, et al. ^{17}O-excess as a detector for co-extracted organics in vapor analyses of plant isotope signatures. *Rapid Commun Mass Spectrom*. 2019;33:1301–1310. https://doi.org/10.1002/rcm.8470