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Identification and correction of spectral contamination in $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ measured in leaf, stem, and soil water

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Plant water extracts typically contain organic materials that may cause spectral interference when using isotope ratio infrared spectroscopy (IRIS), resulting in errors in the measured isotope ratios. Manufacturers of IRIS instruments have developed post-processing software to identify the degree of contamination in water samples, and potentially correct the isotope ratios of water with known contaminants. Here, the correction method proposed by an IRIS manufacturer, Los Gatos Research, Inc., was employed and the results were compared with those obtained from isotope ratio mass spectrometry (IRMS). Deionized water was spiked with methanol and ethanol to create correction curves for $\delta^{18}\text{O}$ and $\delta^2\text{H}$. The contamination effects of different sample types (leaf, stem, soil) and different species from agricultural fields, grasslands, and forests were compared. The average corrections in leaf samples ranged from 0.35 to 15.73‰ for $\delta^2\text{H}$ and 0.28 to 9.27‰ for $\delta^{18}\text{O}$. The average corrections in stem samples ranged from 1.17 to 13.70‰ for $\delta^2\text{H}$ and 0.47 to 7.97‰ for $\delta^{18}\text{O}$. There was no contamination observed in soil water. Cleaning plant samples with activated charcoal had minimal effects on the degree of spectral contamination, reducing the corrections, by on average, 0.44‰ for $\delta^2\text{H}$ and 0.25‰ for $\delta^{18}\text{O}$. The correction method eliminated the discrepancies between IRMS and IRIS for $\delta^{18}\text{O}$, and greatly reduced the discrepancies for $\delta^2\text{H}$. The mean differences in isotope ratios between IRMS and the corrected IRIS method were 0.18‰ for $\delta^{18}\text{O}$, and -3.39% for $\delta^2\text{H}$. The inability to create an ethanol correction curve for $\delta^2\text{H}$ probably caused the larger discrepancies. We conclude that ethanol and methanol are the primary compounds causing interference in IRIS analyzers, and that each individual analyzer will probably require customized correction curves. Copyright © 2011 John Wiley & Sons, Ltd.

Stable isotope analyses of plant and soil water are routinely performed, and have been used to better understand water use patterns in terrestrial ecosystems,^[1,2] to partition the components of evapotranspiration,^[3–5] and to understand the controls on surface H_2O and CO_2 fluxes.^[2,6–8] In the past, the analysis of $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ in liquid water has been exclusively conducted using isotope ratio mass spectrometry (IRMS). Recently, the development of isotope ratio infrared spectroscopy (IRIS) has simplified the isotope analysis of water, allowing the simultaneous measurement of $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ in liquid water. Isotope ratio infrared spectroscopy analyzers do not require the chemical conversion of compounds into their elemental constituents prior to analysis unlike IRMS. Additional benefits of IRIS analyzers include cost, speed of analysis, and portability.^[9]

The analytical precision and accuracy of IRIS analyzers are similar to those of IRMS when analyzing pure water;^[9,10] however, it has recently been shown that there are discrepancies between the isotope ratios of plant and soil water measured with IRIS and IRMS.^[10,11] The conventional method

of cryogenic vacuum distillation (e.g. West *et al.*^[12]) for the extraction of water from plant and soil samples can co-distill organic materials (e.g. methanol and ethanol) that may interfere with the spectral signal for the IRIS methods, resulting in erroneous isotope values. Research by West *et al.*^[11] found deviations as large as 46.5‰ and 15.4‰ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively, from water extracted from a range of 12 plant and soil samples collected in or near Berkeley, CA, USA. Cleaning the samples with activated charcoal reduced, but did not eliminate, the deviations, since activated charcoal is inefficient in removing alcohols, glycols, strong acids, and bases.^[13]

In response to the observed spectral interference by organic materials in plant and soil samples, IRIS manufacturers have developed software to identify and quantify the presence of contaminants in water samples. Here, we use the Liquid Water Isotope Analyzer (LWIA) (DLT-100) from Los Gatos Research Inc., Mountain View, CA, USA with the Spectral Contamination Identifier (LWIA-SCI) post-processing software to identify and quantify spectral contamination, and correct the isotope values of contaminated water samples. The instrument manufacturer has proposed a method to correct the isotope ratios of contaminated samples if the contaminants are known. The correction method consists of spiking clean water with known contaminants and measuring the degree of contamination based on the output from the LWIA-SCI software. In this study, we test how well this correction method

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works. To our knowledge, this is the first attempt to correct the isotope ratios in plant and soil samples with known contamination using an IRIS analyzer.

The objectives of this study are to (1) quantify the measurement errors of $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ associated with spectral interference caused by organic contaminants; (2) compare the contamination effects of different sample types (leaf, stem, soil) and species from agricultural fields, grasslands, and forests; (3) correct the isotope ratios of contaminated water samples; and (4) test the accuracy of the corrections by comparing the results with those from the traditional IRMS technique.

EXPERIMENTAL

Sample collection and isotope analysis

Leaf, stem, and soil samples were collected from (1) the Rosemount Research and Outreach Center (RROC) in Rosemount, MN, USA, approximately 25 km southeast from Minneapolis;^[14] (2) the Marcell Experimental Forest (MEF) in northern Minnesota, USA;^[15] and (3) the Borden Forest Research Station (BFRS) in southern Ontario, Canada.^[16,17] Table 1 shows the plant species included in this study. No other study has examined the contamination effects of these species.

Following the sampling protocol given by the Moisture Isotopes in the Biosphere and Atmosphere (MIBA) program,^[18] we collected leaf, stem, and soil samples near midday (12:00 local standard time (LST)). Dry, healthy sunlit leaves were chosen for analysis. If a distinct major vein was visible, it was removed and discarded. Non-green stem samples were collected at the base of the plants. Soil samples were collected from approximately 10 cm below the soil surface. All samples were sealed in glass vials with parafilm, and frozen until water extraction on a custom-made vacuum glass line.

Cryogenic vacuum distillation^[12] was used to extract water from the plant and soil samples. During the process of vacuum distillation, water is evaporated from the plant or soil sample and frozen in a collection tube. During this process, organic compounds within the plant cells may co-distill with the water, causing spectral contamination when using

IRIS. Complete water extraction was ensured to avoid isotope fractionation. Plant and soil samples were weighed post-extraction, oven-dried, and weighed again. The oven-drying process was assumed to completely dry the sample. If there was a discrepancy between the post-extraction mass and the oven-dried mass of a sample, the sample was discarded. Each sample was pipetted into a small vial, sealed with parafilm, and refrigerated until isotope analysis. Individual plant samples with sufficient water for duplicate analyses were divided into two sub-samples to test the effect of activated charcoal on the spectral contamination. Following West *et al.*,^[11] activated charcoal was added in excess of 10% of the total mass of the sample and mixed well. After a minimum of 24 h, the samples with activated charcoal were filtered into new vials using a 0.45 μm filter.

The isotope analysis of all liquid water extracted from plant and soil samples was performed on a DLT-100 liquid water isotope analyzer (LWIA; Los Gatos Research Inc.) coupled to a HT-300A autosampler (HTA s.r.l., Brescia, Italy) at the Biometeorology Lab at the University of Minnesota. The manufacturer's specifications give a precision of $\pm 1.0\text{‰}$ for $\delta^2\text{H}$ and $\pm 0.25\text{‰}$ for $\delta^{18}\text{O}$. The LWIA calculates the spectral absorbance of $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ at infrared wavelengths using off-axis integrated cavity output spectroscopy (OA-ICOS). Because the analyzer measures the concentrations of the individual isotopologues and reports them in absolute ratios, it is necessary to include pre-calibrated internal laboratory standards within and throughout the autoruns to calibrate the unknown samples to Vienna Standard Mean Ocean Water (VSMOW). Standards for each autorun are selected based on the expected isotopic composition of the unknown samples and should bracket the range of unknown samples. Linear calibration equations are calculated using each set of standards throughout the run and used to correct unknown samples. All measured unknown water samples are calibrated to the known internal water standards and reported in delta (δ) notation relative to VSMOW [$\delta = (R_{\text{sample}}/R_{\text{VSMOW}} - 1) \times 1000$], where R_{sample} is the isotope ratio (e.g. $^{18}\text{O}/^{16}\text{O}$) of the sample and R_{VSMOW} is the isotope ratio of the standard, VSMOW. The standard deviation of the water standards throughout a typical autorun is better than 0.8‰ for $\delta^2\text{H}$ and 0.3‰ for $\delta^{18}\text{O}$, and is typically around 0.4‰ for $\delta^2\text{H}$ and 0.15‰ for $\delta^{18}\text{O}$.

Table 1. The different plant species examined in this study. Corn, soybean, big bluestem, purple clover, and creeping spearwort samples were collected from the RROC, cotton grass and leather leaf samples were collected from the MEF, and white ash, large-tooth aspen, and red maple samples were collected from the BFRS

Common name	Species	Sample type
Corn	<i>Zea mays</i>	leaf, stem, soil
Soybean	<i>Glycine max</i>	leaf, stem, soil
Big bluestem	<i>Andropogon gerardii</i>	leaf, stem, soil
Purple clover	<i>Trifolium pretense</i>	leaf, stem
Creeping spearwort	<i>Ranunculus flammula</i>	leaf, stem
Snap peas	<i>Pisum sativum</i>	leaf, soil
Cotton grass	<i>Eriophorum chamissonis</i>	leaf, stem
Leather leaf	<i>Chamaedaphne calyculata</i>	leaf, stem
White ash	<i>Fraxinus americana</i>	leaf
Large-tooth aspen	<i>Populus grandidentata</i>	leaf
Red maple	<i>Acer rubrum</i>	leaf

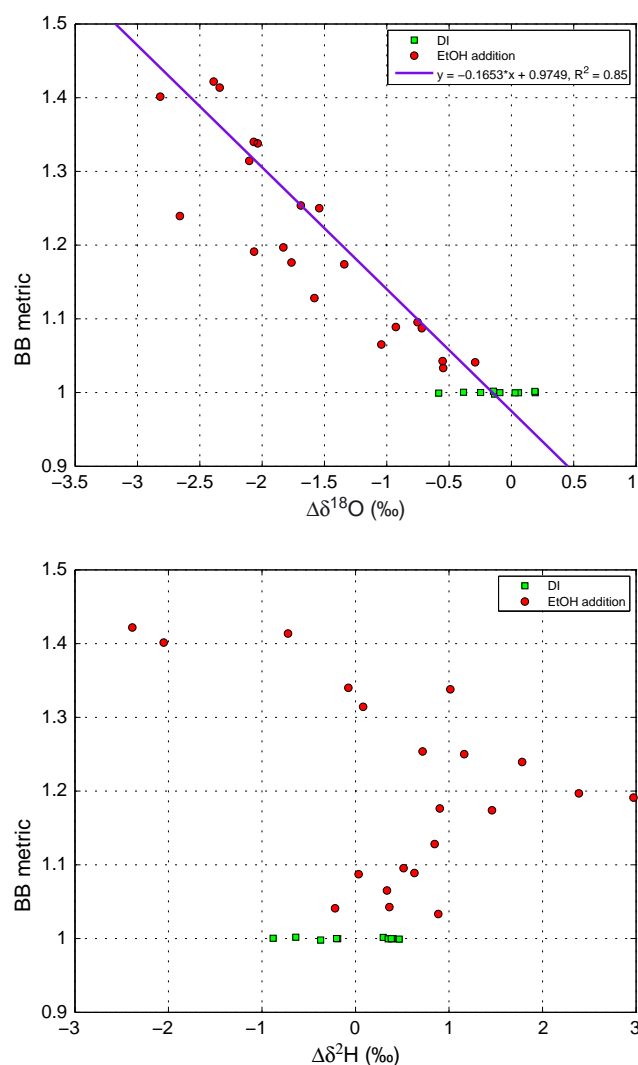


Figure 1. Ethanol correction curves for $\delta^{18}\text{O}$ (top) and $\delta^2\text{H}$ (bottom). Ethanol was added to deionized water, resulting in a linear relationship between the broad-band (BB) contamination metric from the LWIA-SCI software and the offset in $\delta^{18}\text{O}$ ($\Delta\delta^{18}\text{O}$) ($\text{BB} = -0.1653 \times \Delta\delta^{18}\text{O} + 0.9749$). There was no clear relationship between the BB contamination metric and the offset in $\delta^2\text{H}$ ($\Delta\delta^2\text{H}$).

Contamination identification and correction

If other compounds are present in the water samples that absorb at the same wavelengths as those used to detect the isotopes of hydrogen and oxygen, errors in the measured ratios of $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ may occur. It has been shown that organic molecules present in water extracted from plants interfere with the spectral signal using IRIS approaches, and can produce significant errors in the measured isotope ratios.^[10,11] It is likely that only molecules with similar absorption features in the same spectral region that also have an O-H bond will interfere with the measured $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ ratios.^[10] Therefore, the compounds most likely to cause contamination are methanol (MeOH) and ethanol (EtOH).

The LWIA Spectral Contamination Identifier (LWIA-SCI) software was developed to identify features in the LWIA spectra that are consistent with water contamination. Briefly,

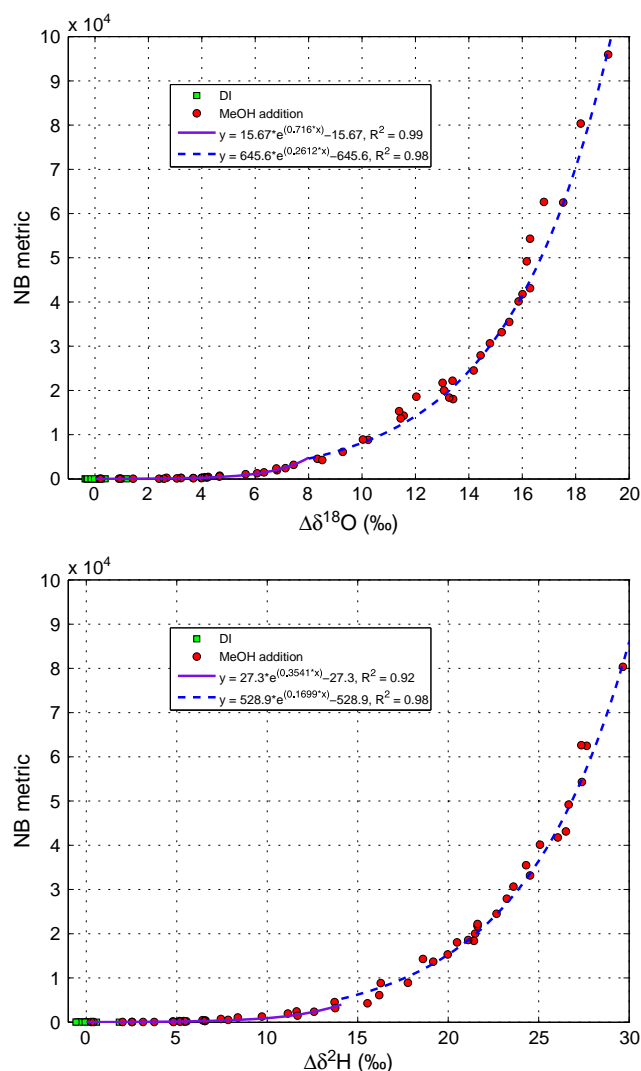


Figure 2. Methanol correction curves for $\delta^{18}\text{O}$ (top) and $\delta^2\text{H}$ (bottom). Methanol was added to deionized water to create relationships between the narrow-band (NB) contamination and the offsets in $\delta^{18}\text{O}$ and $\delta^2\text{H}$. To best describe the offset in $\delta^2\text{H}$ and $\delta^{18}\text{O}$ over the full range of contamination, two separate equations were used for $\delta^{18}\text{O}$ and δD . For $\delta^{18}\text{O}$, $\text{NB}_{\leq 4000} = 15.67e^{(0.716 \times \Delta\delta^{18}\text{O})} - 15.67$, and $\text{NB}_{>4000} = 645.6e^{(0.2612 \times \Delta\delta^{18}\text{O})} - 645.6$. For δD , $\text{NB}_{\leq 4000} = 27.3e^{(0.3541 \times \Delta\delta^2\text{H})} - 27.3$, and $\text{NB}_{>4000} = 528.9e^{(0.1699 \times \Delta\delta^2\text{H})} - 528.9$.

the recorded spectra from unknown samples are analyzed and compared with those from known clean samples (such as standards) to produce a metric of contamination from either narrow-band (e.g. MeOH) or broad-band (e.g. EtOH) absorbers. The metric of contamination indicates the likelihood or degree of spectral interference. If the contaminant(s) are known, it should be possible to correct the isotope ratios of contaminated samples based on the magnitude of the contamination.

In order to correct the isotope ratios of contaminated samples, deionized (DI) water was spiked with varying amounts of EtOH and MeOH to measure the isotope errors associated with the magnitude of the contamination metric. The approximate range of EtOH and MeOH concentrations used to create these correction curves was 0.5% to 5.0%, and

Table 2. Contamination effects by species/sample type. The second column (n) refers to the total number of samples. The third column (# cont.) refers to the number of contaminated samples. All values are reported in per mil (‰). The p-values shown in bold are significant at the 95% confidence level

Sample Name	n	# cont.	Before correction			$\Delta\delta^2\text{H}$			$\Delta\delta^{18}\text{O}$			After correction		p-values	
			mean $\delta^2\text{H}$	mean $\delta^{18}\text{O}$	sdev	mean	max	sdev	mean	max	sdev	mean $\delta^2\text{H}$	mean $\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$
corn leaf	26	22	-28.41	5.87	3.61	3.68	11.97	3.61	1.98	5.79	1.80	-32.09	3.88	0.3025	0.0568
soybean leaf	25	23	-20.48	7.19	8.61	6.03	34.76	8.61	3.45	20.94	5.01	-26.50	3.73	0.2096	0.0285
big bluestem leaf	7	4	-43.95	-1.94	0.32	0.35	0.83	0.32	0.28	0.65	0.25	-44.24	-2.22	0.9811	0.9076
clover leaf	7	7	-11.14	10.61	11.87	11.76	27.27	11.87	6.59	15.65	6.71	-22.89	4.02	0.2422	0.0389
spearwort leaf	5	5	-11.54	11.18	11.51	15.73	24.47	11.51	9.27	18.08	6.93	-27.26	1.91	0.2608	0.0166
snap pea leaf	3	3	-37.02	7.47	11.26	13.84	25.54	11.26	7.85	14.32	6.14	-50.86	-0.38	0.3073	0.0515
cotton grass leaf	14	5	-51.57	-1.35	3.18	2.48	6.41	3.18	1.13	2.67	1.35	-54.05	-2.49	0.8861	0.7014
leather leaf leaf	14	3	-49.77	1.61	4.33	2.65	7.65	4.33	1.43	4.04	2.26	-52.42	0.18	0.9360	0.7864
white ash leaf	26	26	-23.07	13.71	4.62	14.81	23.46	4.62	8.84	14.18	2.42	-37.88	4.90	0.0036	< 0.001
lg. tooth aspen leaf	26	26	-35.90	5.37	0.94	0.90	3.47	0.94	0.56	1.91	0.54	-36.80	4.81	0.8464	0.6809
red maple leaf	25	25	-32.79	8.99	0.44	0.44	4.66	0.43	0.26	0.94	0.25	-33.23	8.73	0.9198	0.8614
corn stem	7	7	-51.04	-6.27	1.77	1.77	4.94	1.54	0.47	3.11	1.69	-52.81	-6.73	0.5577	0.1836
soybean stem	10	9	-47.90	-2.27	8.70	7.07	28.11	8.70	4.27	17.24	5.29	-54.97	-6.54	0.0850	0.0081
big bluestem stem	8	3	-56.46	-4.49	5.52	6.62	10.17	5.52	3.81	5.79	3.12	-63.08	-8.30	0.8724	0.3485
clover stem	9	9	-40.27	1.33	12.87	13.70	34.63	12.87	7.97	20.83	7.54	-53.97	-6.64	0.0134	0.0014
spearwort stem	7	7	-48.16	-1.72	7.74	7.82	20.91	7.74	4.22	11.93	4.26	-55.44	-5.94	0.6343	0.2419
cotton grass stem	15	14	-64.97	-5.70	6.40	6.36	18.84	6.40	3.45	10.66	3.52	-71.34	-9.15	0.0680	0.0020
leather leaf stem	14	2	-60.23	-6.20	1.37	1.17	2.13	1.37	0.55	1.10	0.77	-61.40	-6.75	0.9691	0.9270
soil	45	0	-51.50	-5.70	x	x	x	x	x	x	x	-51.50	-5.70	x	x

45 ppmv to 0.2%, respectively. Based on these relationships, correction curves were derived to adjust the measured isotope ratio of water samples with varying degrees of contamination. Figure 1 presents the change in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ with the addition of EtOH. There was a clear linear relationship between the broad-band (BB) metric and the offset in $\delta^{18}\text{O}$ given by Eqn. (1):

$$\text{BB} = -0.1653 \times \Delta\delta^{18}\text{O} - 0.9749 \quad (1)$$

Unfortunately, there was no clear relationship between $\Delta\delta^2\text{H}$ and the BB metric; therefore, it was not possible to correct $\delta^2\text{H}$ in samples with BB contamination.

Figure 2 presents the change in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ with the addition of MeOH. Because the size of a MeOH molecule is more similar than EtOH to a water vapor molecule, MeOH has a stronger effect on the measured isotope ratios.^[10] To best describe the offset in $\delta^2\text{H}$ and $\delta^{18}\text{O}$ over the full range of contamination, two separate equations were used. Equations (2a) and (2b) describe the relationship between the narrow-band (NB) metric and $\Delta\delta^2\text{H}$ at the NB metric values of ≤ 4000 and >4000 , respectively:

$$\text{NB}_{\leq 4000} = 27.3e^{(0.3541 \times \Delta\delta^2\text{H})} - 27.3 \quad (2a)$$

$$\text{NB}_{>4000} = 528.9e^{(0.1699 \times \Delta\delta^2\text{H})} - 528.9 \quad (2b)$$

Equations (3a) and (3b) describe the relationship between the NB metric and $\Delta\delta^{18}\text{O}$ at metric values of ≤ 4000 and >4000 , respectively:

$$\text{NB}_{\leq 4000} = 15.67e^{(0.716 \times \Delta\delta^{18}\text{O})} - 15.67 \quad (3a)$$

$$\text{NB}_{>4000} = 645.6e^{(0.2612 \times \Delta\delta^{18}\text{O})} - 645.6 \quad (3b)$$

The LWIA-SCI software outputs the NB or BB metric for each individual sample. Based on those metrics, Eqns. (1)–(3) were used to solve for $\Delta\delta^{18}\text{O}$ and $\Delta\delta^2\text{H}$. The MeOH and EtOH equations corrected the isotope ratios in different directions. The addition of MeOH to DI water resulted in more *positive* isotope values, and the corrections needed to be *subtracted* from the original isotope values, while the addition of EtOH to DI water resulted in more *negative* isotope values, and the corrections needed to be *added* to the original isotope values. In samples with both NB and BB contamination, both correction curves ($\delta^{18}\text{O}$ only) were used to adjust the isotope ratios of the samples. In these instances, each correction curve was applied to the original value of $\delta^{18}\text{O}$, the NB and BB corrections were summed, and the final correction was used to adjust $\delta^{18}\text{O}$.

IRMS analysis and comparison

The IRIS stable isotope values of the 78 leaf samples from the Borden Experimental Forest were compared with the IRMS values in a blind comparison to test the accuracy of the correction method. In the blind comparison, the IRMS values were not known prior to applying the corrections to the IRIS analyzed isotope values. The CO_2 equilibration method was used to determine the $\delta^{18}\text{O}$ values of the leaf water samples, using a DeltaPlus XP mass spectrometer with a Gas Bench interface (both from ThermoFinnigan, Bremen, Germany).

Table 3. The effects of activated charcoal on the degree of contamination observed – all samples treated with activated charcoal (AC) are shown in parentheses. All values are reported in per mil (‰)

Sample type (AC)	Before correction		After correction		p-values	
	mean $\delta^2\text{H}$	mean $\delta^{18}\text{O}$	mean $\delta^2\text{H}$	mean $\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$
corn leaf	-29.19 (-28.09)	6.35 (6.44)	-33.15 (32.67)	3.76 (4.00)	0.9104	0.8874
corn stem	-51.43 (-51.04)	-6.07 (-6.14)	-53.62 (-53.00)	-7.07 (-7.05)	0.9260	0.9856
big blue stem	-51.02 (-60.26)	-2.75 (-7.66)	-60.45 (-60.26)	-8.17 (-7.66)	x	x
clover stem	-36.67 (-36.88)	2.91 (2.62)	-53.08 (-52.76)	-6.47 (-6.51)	0.9384	0.9868
soybean leaf	-6.80 (-6.81)	14.28 (13.11)	-22.59 (-22.06)	4.86 (3.98)	0.9712	0.7012
soybean stem	-47.90 (-48.25)	-0.98 (-2.09)	-57.13 (-56.99)	-6.64 (-7.49)	0.9833	0.4070

The $\delta^2\text{H}$ values were determined by a chromium reaction using a ThermoFinnigan MAT 253 mass spectrometer with an H-device at Yale University.^[16] The precision was 0.2‰ for $\delta^{18}\text{O}$ and 1.0‰ for $\delta^2\text{H}$.

Statistical analysis

To assess the significance of the difference between the uncorrected and corrected IRIS measurements, the uncorrected and corrected IRIS measurement results were compared using one-way analysis of variance (ANOVA) for both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ at the 95% significance level ($\alpha = 0.05$ level) for each species and sample type. The p-values are reported in Table 2. To determine whether the addition of activated charcoal to plant water samples prior to analysis had significant effects on the final corrected isotope ratios, the final isotope ratios of the samples with and without activated charcoal added were compared using one-way ANOVA at the 95% significance level. The p-values are reported in Table 3.

RESULTS

Contamination and correction

Figure 3 presents the average corrections by species and sample type. The error bars represent the 95% confidence interval. All the plant species exhibited some degree of spectral contamination. No contamination was observed in the soil samples (Table 2). The average corrections for stem samples among the species analyzed ranged from 1.17‰ to 13.70‰ for $\delta^2\text{H}$

and from 0.47‰ to 7.97‰ for $\delta^{18}\text{O}$. The lowest average corrections were observed in corn and leather leaf for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively, with the highest average corrections observed in clover. The maximum correction in a stem sample was 34.63‰ for $\delta^2\text{H}$ and 20.83‰ for $\delta^{18}\text{O}$ (clover). The average corrections for leaf samples ranged from 0.35 to 15.73‰ for $\delta^2\text{H}$ and 0.28 to 9.27‰ for $\delta^{18}\text{O}$. The lowest average corrections were observed in big bluestem and the highest average corrections were observed in greater creeping spearwort. The maximum correction in a leaf sample was 34.76‰ for $\delta^2\text{H}$ and 20.94‰ for $\delta^{18}\text{O}$ (soybean). Statistically significant differences between the uncorrected and corrected IRIS mean $\delta^{18}\text{O}$ values were observed in soybean leaves, clover leaves, spearwort leaves, white ash leaves, soybean stems, clover stems, and cotton grass stems. In the mean $\delta^2\text{H}$ values, statistically significant differences were observed in white ash leaves and clover stems. It is important to note that because of the large variations in contaminant levels within species, each sample needed to be corrected individually, not by using a blanket correction factor for each species.

According to West *et al.*,^[12] leaf water typically contains a higher fraction of organic contaminants than water extracted from stems. This was not necessarily the case with our samples. For corn, spearwort, and leather leaf, there was more contamination observed in the leaves than in the stems. On the other hand, for soybean, big bluestem, clover, and cottongrass, there was more contamination observed in the stems than in the leaves. For the two grasses sampled (big bluestem and cottongrass), the errors in the results for the stems were notably higher than those in the leaves.

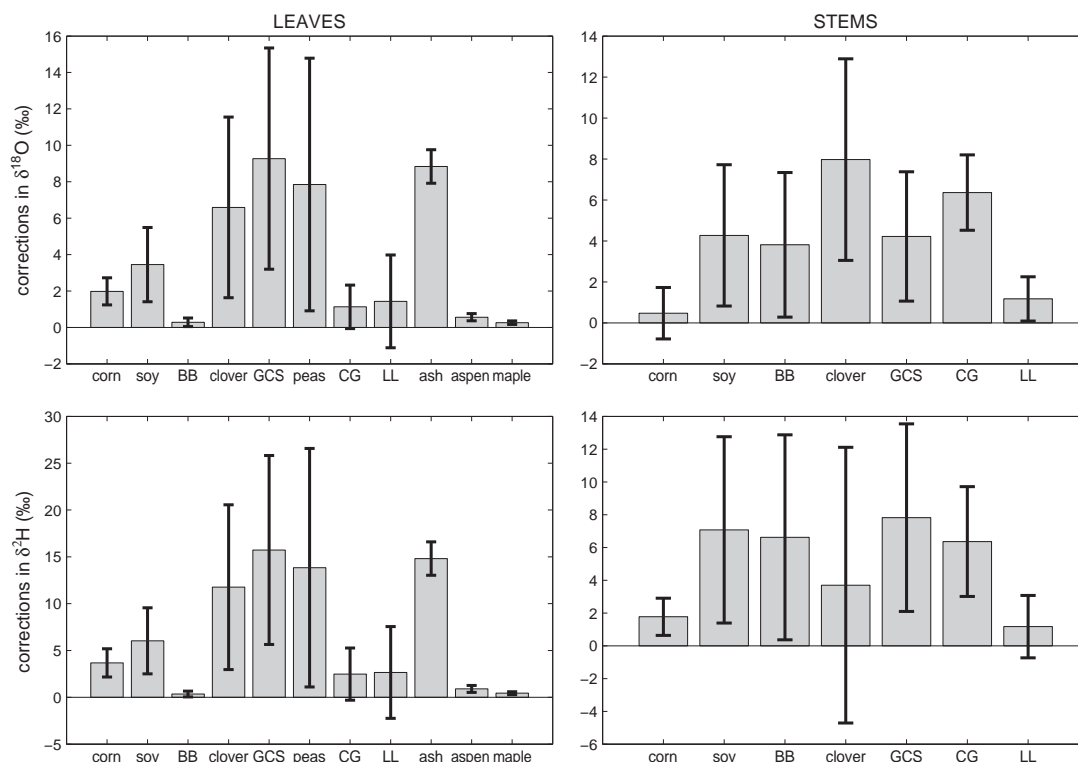


Figure 3. Average corrections for the leaf and stem samples analyzed. The error bars represent the 95% confidence interval. The average corrections in leaf samples ranged from 0.35 to 15.73‰ for $\delta^2\text{H}$ and 0.28 to 9.27‰ for $\delta^{18}\text{O}$. The average corrections in stem samples ranged from 1.17 to 13.70‰ for $\delta^2\text{H}$ and 0.47 to 7.97‰ for $\delta^{18}\text{O}$. There was no contamination observed in soil water.

Effect of activated charcoal

Cleaning plant samples with activated charcoal had minimal effects on the degree of spectral contamination observed in leaf and stem samples. Table 3 presents a summary of the isotope ratios and correction values for samples treated with and without activated charcoal. With the exception of one sample (big bluestem stem), activated charcoal reduced the average corrections from 9.72‰ to 9.28‰ for $\delta^2\text{H}$, and from 5.61‰ to 5.36‰ for $\delta^{18}\text{O}$, resulting in average correction reductions of 0.44‰ and 0.25‰ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively. Inexplicably, the activated charcoal removed all contamination from a big bluestem stem sample. On comparing the cleaned and uncleaned samples, the final isotope values of this stem sample agree well, with a small difference of 0.19‰ in $\delta^2\text{H}$ and 0.51‰ in $\delta^{18}\text{O}$. Overall, there were small differences between the final values of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in cleaned and uncleaned samples, with an average difference of 0.33‰ for $\delta^2\text{H}$ and 0.42‰ for $\delta^{18}\text{O}$. None of these differences were statistically significant. These results suggest that the main contaminants in plant samples are in fact methanol and ethanol, although other contaminants were removed with activated charcoal (big bluestem stem). We conclude that the process of cleaning plant samples with activated charcoal has minimal effects on this correction procedure. The use of activated charcoal produces a cleaner sample, however, and it is good laboratory practice to use activated charcoal because of the potential negative effects of injecting impure water samples into the analyzer (i.e. memory effects, clogged filters, sample cell integrity).^[13]

Comparison with IRMS

The accuracy of the correction method was tested by comparing the corrected IRIS isotope ratios of leaf samples with those of the same samples analyzed using IRMS in a blind comparison. The 78 leaf samples – from white ash, large tooth aspen, red maple – from the Borden Experimental Forest were used in this comparison. We assume that the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values measured via IRMS represent the 'true' isotope values of the leaf samples.^[12] Figure 4 presents the comparison of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in leaves measured with IRMS and with the corrected IRIS methods. Overall, the isotope corrections eliminated the discrepancies between $\delta^{18}\text{O}$ measured using IRIS and IRMS and greatly reduced the discrepancies in $\delta^2\text{H}$. The mean differences in isotope ratios between the IRMS and the (corrected) IRIS methods ($\delta_{\text{IRMS}} - \delta_{\text{IRIS}}$) were 0.18‰ for $\delta^{18}\text{O}$ and -3.39% for $\delta^2\text{H}$. Without the IRIS correction, the mean differences between IRMS and IRIS were -3.06% for $\delta^{18}\text{O}$ and -8.98% for $\delta^2\text{H}$, and as large as -12.84% and -28.24% for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively.

We further investigated the offset in $\delta^2\text{H}$ by analyzing a pure water sample using the same IRIS and IRMS methods to determine if the offset resulted from a bias caused by different water standards used in the Yale University and University of Minnesota labs. The isotope values of this water sample, based on 10 replicated samples using the IRIS method, were $-122.56 \pm 0.67\%$ for $\delta^2\text{H}$ and $-16.34 \pm 0.24\%$ for $\delta^{18}\text{O}$. The isotope values of this sample, based on 12 replications using the IRMS methods, were $-123.5 \pm 0.4\%$ for $\delta^2\text{H}$ and $-16.39 \pm 0.07\%$ for $\delta^{18}\text{O}$. There was excellent agreement between the two methods on the pure water sample (differences of 0.94‰ for $\delta^2\text{H}$ and 0.05‰ for $\delta^{18}\text{O}$), eliminating instrument bias as a cause of the $\delta^2\text{H}$ offset.

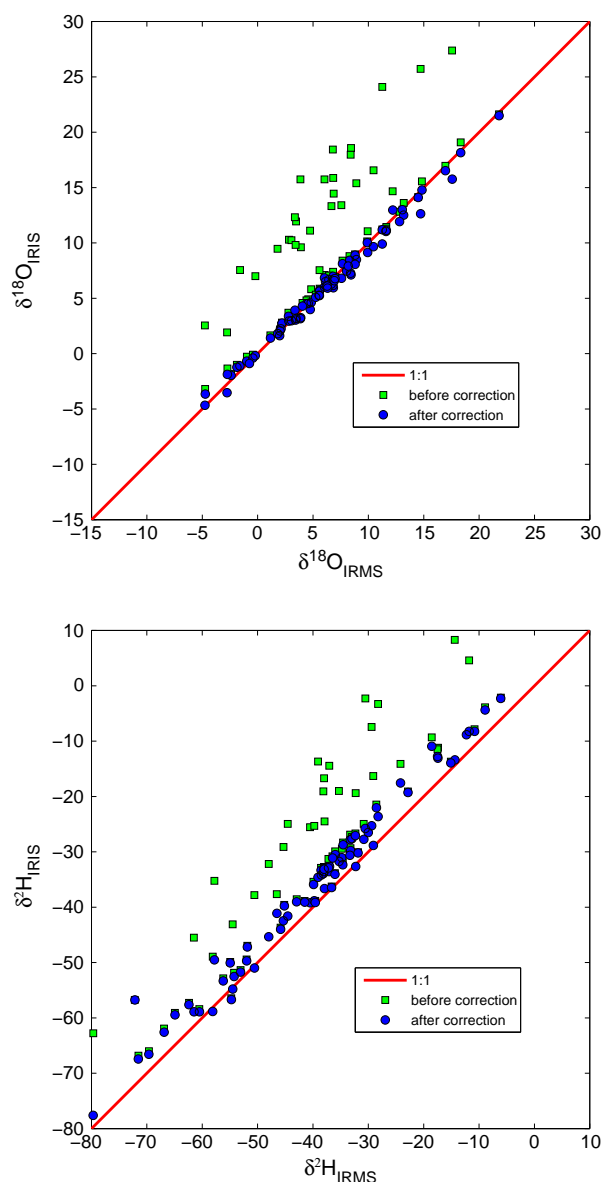


Figure 4. The corrected IRIS values of 78 leaf samples were compared with those for the same samples analyzed using IRMS in a double-blind comparison. Overall, the corrections eliminated the discrepancies between IRMS and IRIS for $\delta^{18}\text{O}$, and greatly reduced the discrepancies for $\delta^2\text{H}$. The mean differences in the isotope ratios between IRMS and corrected IRIS methods were 0.19‰ for $\delta^{18}\text{O}$ and -3.54% for $\delta^2\text{H}$. The inability to create an ethanol correction curve for $\delta^2\text{H}$ probably caused the larger differences.

DISCUSSION AND CONCLUSIONS

Using the proposed correction method, we were able to eliminate the errors in $\delta^{18}\text{O}$ and greatly reduce the errors in $\delta^2\text{H}$ caused by spectral contamination. We suspect that the incomplete corrections in $\delta^2\text{H}$ resulted from the inability to create a correction curve for ethanol contamination. There was no clear relationship between $\Delta\delta^2\text{H}$ and the broad-band

contamination metric (see Fig. 1). The offset could also be due to the interference of other contaminants. The accuracy of the corrected $\delta^{18}\text{O}$ values confirms the fact that ethanol and methanol are the primary contaminants causing spectral interference; however, additional contaminants may have been removed with activated charcoal.

In theory, these correction curves should be applicable to water samples analyzed on other water isotope instruments from Los Gatos Research (LGR). However, when comparing our correction curves with the example curves created by LGR in their SCI-LWIA manual, large differences are evident in both the magnitude and the direction of the corrections. Thus, it is likely that each individual analyzer will require custom-made correction curves. Further research is required to evaluate if these correction curves are stable over time.

Based on the comparison of the Borden Experimental Forest leaves analyzed by IRIS and IRMS, this correction method has an accuracy of -3.39‰ for $\delta^2\text{H}$ and 0.18‰ for $\delta^{18}\text{O}$. The standard deviation of the differences (IRMS – IRIS corrected) is 1.98‰ and 0.58‰ , respectively, which includes measurement noise in both instruments. We can conclude that the overall precision of the IRIS method with corrections is better than 1.98‰ for $\delta^2\text{H}$ and 0.58‰ for $\delta^{18}\text{O}$. It is important to note that not all species analyzed in this study by IRIS methods were compared with measurement by IRMS. It is therefore too soon to conclude that these correction curves will have the same accuracy and precision across all species. We recommend that the corrected values of each individual species are validated against traditional IRMS methods for complete confidence in these methods, and that the correction curve data (e.g. equations, R^2 values, range of contaminant levels) are reported for each individual analyzer.

Recently, West *et al.*^[19] recommended that the use of spectral contamination identification software become incorporated into IRIS standard data post-processing protocols to ensure data quality. We agree with this recommendation, but disagree with their conclusion that all contaminated IRIS data should be discarded. Instead, we recommend following our protocol of creating correction curves for known contaminants, and validating the corrected isotope values of new species or sample types against IRMS methods.

It should be noted that the correction curves in this experiment were created based on the degree of contamination observed in the plant water samples. In our plant samples, the maximum corrections were 34.76‰ for $\delta^2\text{H}$ and 20.94‰ for $\delta^{18}\text{O}$. Recently, Zhao *et al.*^[20] reported errors in IRIS measurements as large as 224‰ for $\delta^2\text{H}$ for some species. We have not investigated the accuracy of these particular correction curves at higher contamination levels.

There have been no instances of contamination when using optical techniques to conduct *in situ* measurements of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in water vapor.^[21,22] This is probably a non-issue because the concentrations of potential contaminants are very low in the atmosphere (e.g. MeOH concentration is 2 parts per billion (ppb) in the winter, and 7 ppb in the summer at the RROC Trace Gas Observatory).^[23] The atmospheric MeOH concentrations are an order of magnitude less than the minimum amounts observed to cause spectral contamination on the liquid water analyzer.

In conclusion, we have shown that it is possible to correct $\delta^{18}\text{O}$ values in plant water extracts that contain organic contaminants that cause spectral interference using IRIS. We used a liquid water isotope analyzer with spectral contamination identifier post-processing software to identify and quantify contamination in water samples. Correction curves for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ were created by spiking DI water with known contaminants, methanol and ethanol. It was possible to correct $\delta^{18}\text{O}$ for methanol and ethanol contamination, but it was only possible to correct $\delta^2\text{H}$ for methanol contamination. We analyzed water extracted from leaf, stem, and soil samples and found spectral contamination in all plant species, and no contamination in soil water. We compared our corrected IRIS measurements of leaf samples with IRMS measurements of the same samples and found exceptional agreement in $\delta^{18}\text{O}$ of 0.18‰ , within the margin of error of the instrument. There was a difference of -3.39‰ between the IRMS and IRIS methods, probably due to the inability to correct for ethanol contamination for $\delta^2\text{H}$. There is significant potential to use IRIS methods to analyze water extracted from leaves and stems; however, IRMS methods are still needed for IRIS quality validation, and it is likely that the correction curves may vary among instruments. Until an analytical solution is developed to remove all sample contaminants prior to injection into IRIS analyzers, we believe that this correction method presents a viable alternative to traditional IRMS methods for determining the isotope ratios of plant and soil waters.

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