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Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters

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The use of isotope ratio infrared spectroscopy (IRIS) for the stable hydrogen and oxygen isotope analysis of water is increasing. While IRIS has many advantages over traditional isotope ratio mass spectrometry (IRMS), it may also be prone to errors that do not impact upon IRMS analyses. Of particular concern is the potential for contaminants in the water sample to interfere with the spectroscopy, thus leading to erroneous stable isotope data. Water extracted from plant and soil samples may often contain organic contaminants. The extent to which contaminants may interfere with IRIS and thus impact upon data quality is presently unknown. We tested the performance of IRIS relative to IRMS for water extracted from 11 plant species and one organic soil horizon. IRIS deviated considerably from IRMS for over half of the samples tested, with deviations as large as 46‰ (δ^2H) and 15.4‰ (δ^{18}O) being measured. This effect was reduced somewhat by using activated charcoal to remove organics from the water; however, deviations as large as 35‰ (δ^2H) and 11.8‰ (δ^{18}O) were still measured for these cleaned samples. Interestingly, the use of activated charcoal to clean water samples had less effect than previously thought for IRMS analyses. Our data show that extreme caution is required when using IRIS to analyse water samples that may contain organic contaminants. We suggest that the development of new cleaning techniques for removing organic contaminants together with instrument-based software to flag potentially problematic samples are necessary to ensure accurate plant and soil water analyses using IRIS. Copyright © 2010 John Wiley & Sons, Ltd.

The recent development of new analytical techniques for the stable isotope analysis of water has resulted in the availability of several methods based on fundamentally different technologies. These can be broadly separated into two classes: (1) traditional isotope ratio mass spectrometry (IRMS) methods, during which water is converted into H_2, CO, or equilibrated with CO_2 and thereafter analyzed in gaseous form by IRMS[1–4] and (2) more recent isotope ratio infrared spectroscopy (IRIS), where photo absorption by H_2O molecules is measured and the isotopologues of H_2O are calculated via spectroscopy. Two commercially available IRIS analyzers – based on off-axis integrated cavity output spectroscopy (OA-ICOS, Los Gatos Research) and wavelength-scanned cavity ring-down spectroscopy (WS-CRDS, Picarro Inc.) – are already in use.[5,6]

IRIS offers many advantages over IRMS in terms of ease of use, cost and the potential of field portability.[7,8] IRIS methods have been shown to produce similar results to IRMS when analyzing pure water;[5,6,9] however, there is little information about how robust these methodologies are for waters that may contain organic contaminants. Trace amounts of contaminants are unlikely to have a large effect on the isotopic value of a water sample measured by IRMS due to the relatively small mass contribution that they make to the total amount of ^{1}H, ^{2}H, ^{16}O and ^{18}O isotopes in the sample. However, the same cannot be said for IRIS, as this is not a mass-based method of analysis. Instead, it is possible that spectral interference by contaminants may have a large effect on the calculated isotopic composition of the sample. Such an effect was shown for water/alcohol mixtures,[9] but there have been no other published studies examining other contaminants. With the likely increase in studies using IRIS to analyze water samples from a variety of sources, it is important that IRIS techniques be tested for robustness.

In the environmental sciences, stable isotope analyses are routinely performed on water samples extracted from organic or inorganic matrices. Common applications include the analysis of water extracted from plants and soils.[10] The process of water extraction from plants or soils can either introduce organic contaminants to the sample[11] or co-distil them with the desired water.[12] The majority of these organic contaminants can be removed from the extracted sample though adsorption onto activated charcoal fragments (polar carbon with a high surface area). However, trace amounts of

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organic contaminants may remain in the water sample. To date, there have been no studies that have examined the relative performance of IRIS and IRMS methods in analyzing extracted plant water samples.

Our study aimed to test whether the data obtained by IRIS were comparable with those from IRMS for identical plant water samples, and whether this was influenced by the use of activated charcoal to remove organic contaminants. Our approach was to use leaf-water extracts from a range of plant species likely to have a diversity of plant secondary compounds. Identical samples were analyzed for ^{2}H/H and ^{18}O/^{16}O ratios using two different IRMS methods and two different IRIS methods.

We hypothesized that IRIS data would differ from IRMS values for water extracts untreated with activated charcoal, but that this difference would disappear when samples were cleaned with activated charcoal. Significant differences between IRIS and IRMS for cleaned samples would indicate that IRIS analyses were highly sensitive to the presence of organic contaminants and that our current method of cleaning extracted water samples – using activated charcoal – is insufficient to solve this problem.

EXPERIMENTAL

Sample collection and water extraction

We selected a range of plant species likely to contain a diversity of plant secondary compounds for our tests (Table 1). We sampled leaves rather than woody tissues. Leaf water extracts typically contain a higher fraction of organic contaminants relative to xylem water and thus provided the most stringent test of these methods. Leaf samples were obtained from plants growing on and around the University of California, Berkeley campus. All samples were collected on the same day, placed in plastic bags, and transferred to the laboratory for immediate water extraction. Soil samples were collected from below the litter layer in the first 10 cm of the organic horizon, placed in glass vials and similarly transferred for water extraction.

Water was extracted from leaves and soil by cryogenic vacuum distillation.[12] The process of cryogenic vacuum distillation extracts all the water from a sample and freezes it into a collection tube. During this process, all compounds that are volatile under the sample-tube conditions (vacuum and high temperature) will be co-distilled with the water. In the case of plant samples, this is likely to include cell

contents, as the cells are lysed by freezing the sample in liquid nitrogen prior to extraction. Thus, extracted plant water may contain high levels of organic contaminants, as was observed for many species in the study, where the extracted water sample had a strong odour and a milky appearance, indicating the presence of organic contaminants. Several extractions per species were necessary in order to collect sufficient water for duplicate analyses. These extracts were subsequently pooled, resulting in one large water sample per species. From this pooled, untreated water sample, 0.5 mL aliquots were pipetted into smaller vials. Activated charcoal was added to half of these vials to adsorb organics in the water. We added activated charcoal in excess of 10% of the total mass of the sample (e.g. at least 1 mg per 10 mL). The vials with activated charcoal (hereafter referred to as 'cleaned') were agitated periodically over 24 h, following which they were pipetted, through a 0.2 µm filter, into new vials. If a cleaned sample was not clear and odourless, the activated charcoal treatment was repeated until the sample was both clear and odourless.

Sufficient numbers of vials were created for three sample sets. Each set consisted of replicates of both untreated and cleaned samples per species, together with two calibration standards and a quality control standard. A standard to check the effect of adding activated charcoal was also included. This consisted of pure deionized water and it was treated in the same manner as the plant and soil water samples. N = 5 for all replicates.

Where sample sets were analyzed on more than one instrument, pierced septa were replaced immediately after analysis to prevent any evaporation.

Isotopic analyses

Identical water samples were analyzed by five different isotopic analysis methods in this study. These methods are described briefly below:

IRMS methods

Method 1: Chromium combustion using an H/Device (labelled in results as 'HDEV')

Microliter quantities of water were injected into an H/Device (HDEV, ThermoFinnigan, Bremen, Germany) coupled to a Delta Plus mass spectrometer (ThermoFinnigan). Injected H_2O was reduced to H_2 gas in a hot chromium reactor and the ^{2}H/H ratio of this gas was then measured by mass spectrometry.[2,3] Any contaminants injected with the water

Table 1. Plant species used in this study

Common name	Species	Family
Alder	*Alnus rhombifolia*	Betulaceae
Baccharis	*Baccharis pilularis*	Asteraceae
Bay	*Umbellularia californica* (Hook. & Arn.) Nutt.	Lauraceae
Brugmansia	*Brugmansia* sp. (cultivated hybrid, formerly *Datura*)	Solanaceae
Citrus	*Citrus* sp. (cultivated hybrid, likely *C. reticulata*)	Rutaceae
Eucalyptus	*Eucalyptus globulus* Labill.	Myrtaceae
Ginkgo	*Ginkgo biloba*	Ginkgoaceae
Grass	*Poa* sp. (*cultivated hybrid*)	Poaceae
Maple	*Acer macrophyllum* Pursh	Aceraceae
Pine	*Pinus radiata* D. Don	Pinaceae
Redwood	*Sequoia sempervirens* (D. Don) Endl.	Cupressaceae

sample might have produced H_2 gas in the reactor through predictable chemistry. Contaminants will provide a detectable impact on the $^2H/H$ ratio of the sample only if: (1) they contribute a large fraction of the total H in the sample, and (2) the $^2H/H$ ratio of the contaminant-derived H is different from that of the H_2O. The H/Device analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley, CA, USA.

Method 2: High-temperature pyrolysis (labelled in results as 'TCEA')

Microliter quantities of water were directly injected into a temperature conversion/elemental analyzer (TCEA, ThermoFinnigan) coupled to a Delta Plus XL mass spectrometer (ThermoFinnigan). Injected H_2O was pyrolyzed to H_2 and CO gas in the presence of excess C and the $^2H/H$ and $^{18}O/^{16}O$ ratios of these gases were measured by IRMS.[1] Any contaminants injected with the water sample might have produced H_2 and CO gas in the reactor. These contaminants could affect the measured isotopic ratio in a similar manner to that described for the HDEV above. The TCEA analyses were conducted at SIRFER, University of Utah, Salt Lake City, UT, USA.

Method 3: CO_2 headspace equilibration using a GasBench II (labelled in results as 'GB')

The $\delta^{18}O$ values of water samples were measured by CO_2 headspace equilibration.[4] Water samples were left to equilibrate with a 0.2% CO_2 headspace for 48 h at 21–23°C. Following equilibration, the vials were inserted into a GasBench II (GB, ThermoFinnigan) connected to a Delta Plus XL mass spectrometer. The GasBench II was modified with a 10-port injection valve allowing a 0.2% CO_2 reference injection to follow each CO_2 sample injection. This reference CO_2 peak eluted 30 s after the sample CO_2 peak and was used to correct for any volatile contaminants in the sample injection. This correction, together with the fact that the measurement was carried out by mass spectrometry focused on the ions of m/z 44 and 46, should minimize the effect of volatile contaminants in the sample. GB analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley.

IRIS methods

Method 4: Off-axis integrated cavity output spectroscopy (labelled in results as 'LGR')

The analyses were performed on a liquid water isotope analyzer (DLT-100) from Los Gatos Research (Mountain View, CA, USA). Microliter quantities of water were injected into a vaporization chamber and then passed into an infrared absorbance cavity. The isotope ratios (δ^2H and $\delta^{18}O$) are calculated from the spectral absorbance at specific wavelengths using off-axis integrated cavity output spectroscopy (OA-ICOS).[6] Contaminants included in the injected sample may interfere with the calculated isotope ratio if they are (1) volatile enough to enter the absorbance cavity and (2) absorb in the wavelengths used to identify isotopologues of water. This is potentially a mass-independent effect – small amounts of contaminant may have a large effect on the calculated isotope ratio. The OA-ICOS analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley, USA.

Method 5: Wavelength-scanned cavity ring-down spectroscopy (labelled in results as 'PIC')

The analyses were performed on a water isotope analyzer (L1102-i) from Picarro Inc. (Sunnyvale, CA, USA). Microliter quantities of water were injected into a vaporization chamber and then passed into an infrared absorbance cavity. The isotope ratios (δ^2H and $\delta^{18}O$) are calculated from the spectral absorbance at specific wavelengths using wavelength-scanned cavity ring-down spectroscopy (WS-CRDS).[5] Contaminants included in the injected sample may interfere with the calculated isotope ratio in a manner similar to that described for the OA-ICOS method above. The WS-CRDS analyses were conducted at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley, USA.

For all methods, the isotope ratios are expressed in ‰ as:

$$\delta^N E = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000 \qquad (1)$$

where N is the heavy isotope of element E and R is the ratio of the heavy to light isotope ($^2H/H$ or $^{18}O/O$). On all the instruments, two calibration standards were used to adjust the delta values relative to V-SMOW.

For the purposes of our analysis, we assume that the long-term precision is no better than ±1.5‰ for δ^2H and ±0.15‰ for $\delta^{18}O$. Any differences below these thresholds are regarded as insignificant.

Data standardization and analysis

Isotope data produced on different instruments were standardized using two quality control (QC) standards that were not used for correcting the data relative to V-SMOW. These standards were pure waters that had been untreated by activated charcoal. Analysis of variance (ANOVA) of these QC standards showed that the data from the instruments run in Berkeley (HDEV, GB, LGR, PIC) either did not significantly differ or demonstrated a very small effect size (Table 2). However, there was a significant difference for data from the TCEA, run in Salt Lake City, with an effect size greater than typical instrument error (2.5‰ δ^2H and 0.7‰ $\delta^{18}O$). For purposes of comparison, a mean offset was applied to the data from each instrument relative to the mean of the Berkeley instruments (Table 2).

All subsequent analyses were performed using the mean (n = 5) of all replicates. Where the difference of two means was calculated, the error was propagated as:

$$\varepsilon_{(A-B)} = \sqrt{(\varepsilon_A{}^2) + (\varepsilon_B{}^2)} \qquad (2)$$

where A and B are the two means being subtracted and ε is the error associated with those means.

Assumption of 'true' value for leaf water

Verifying the true isotope ratio for leaf water is challenging, as the isotopic composition of leaf water is influenced by both biological and physical factors.[13–17] Attempting to directly verify the true isotope ratio for a variety of species would have required a logistically challenging glasshouse experiment. Instead, we applied the assumption that the true isotope ratio of extracted plant and soil water was represented by samples cleaned using activated charcoal and measured by IRMS. This assumption has formed the

Table 2. Isotope data from quality control (QC) standards used to standardize between different instruments. For each instrument, the means ± 1SE are shown for N = 10 (QC1) and N = 5 (QC2). See Experimental section for description of the instruments. *HDEV is for δ^2H, GB for δ^{18}O. The letter following the mean represents statistically different means (post-hoc Tukey HSD test). ANOVA results are listed below the means. Offset from the mean refers to the difference between the instrument value and the standardized value (see Experimental section for details). Offset applied is average correction applied to all data from that instrument and is the average of the offsets from the two QC standards

Instrument type	δ^2H (‰) QC1	QC2	δ^{18}O (‰) QC1	QC2
HDEV & GB*	-37.8 ± 0.23^a	-91.9 ± 0.40^a	-5.00 ± 0.04^a	-12.83 ± 0.01^a
LGR	-36.2 ± 0.17^b	-93.7 ± 0.13^{ab}	-5.03 ± 0.03^a	-13.07 ± 0.04^a
PIC	-36.7 ± 0.13^b	-92.5 ± 0.13^b	-5.14 ± 0.03^a	-12.91 ± 0.08^a
TCEA	-34.0 ± 0.28^c	-90.6 ± 0.23^c	-4.24 ± 0.06^b	-12.30 ± 0.09^b
F-stat	56.7	18.5	103	27.5
DF	37	19	38	19
p	<0.0001	<0.0001	<0.0001	<0.0001
Offset from mean				
HDEV & GB*	−0.2	0.8	0.06	0.11
LGR	0.4	−1.0	0.03	−0.14
PIC	−0.1	0.2	−0.09	0.03
TCEA	2.6	2.1	0.82	0.64
Offset applied	δ^2H (‰)	δ^{18}O (‰)		
HDEV & GB*	−0.07	0.08		
LGR	−0.14	−0.05		
PIC	0.21	−0.03		
TCEA	2.49	0.73		

basis of many environmental studies over several decades.[18,19] To provide a test of this assumption, we measured cleaned leaf and soil water by two different IRMS methods for both δ^2H (HDEV and TCEA) and δ^{18}O (GB and TCEA). We reasoned that if these two methods returned identical results for cleaned samples, our assumption was justified.

Once adjusted by the QC standards (described above), the isotope ratios for cleaned samples were very consistent across different IRMS methods, falling within ±1.5‰ for δ^2H and ±0.15‰ for δ^{18}O (Figs. 1 and 2). This is comparable with the long-term precision on these instruments. Thus, there appeared to be no systematic difference between the different IRMS methods, supporting our assumption that they represent the true value of the extracted water.

RESULTS

Deviation of IRIS values from mean IRMS values

The IRIS values for untreated samples (i.e. extracted water not treated with activated charcoal) deviated from the mean IRMS values for 9 of the 12 plant and soil samples, with deviations as large as 46.5‰ (δ^2H) and 15.4‰ (δ^{18}O) being obtained (Table 3, Figs. 1 and 2).

These deviations largely remained – albeit slightly reduced in magnitude – for samples cleaned with activated charcoal. For cleaned samples, 7 of the 12 plant and soil samples showed significant deviations from the IRMS mean with deviations as large as 35.5‰ (δ^2H) and 11.8‰ (δ^{18}O) being obtained (Table 3, Figs. 1 and 2).

The species that showed the largest deviations on the IRIS methods were alder, *Baccharis*, bay, *Brugmansia*, *Citrus*, grass and maple. There appeared to be no large deviations between
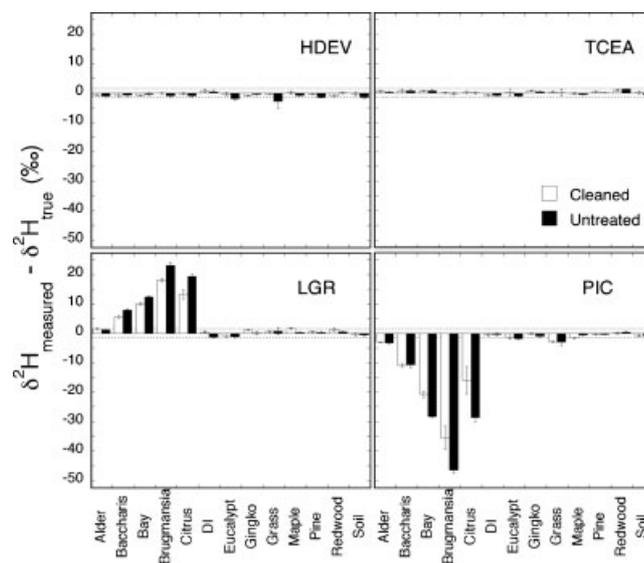

Figure 1. Difference in measured δ^2H and the assumed true value for all samples. The true value was assumed to be the mean of samples cleaned with activated charcoal analyzed by IRMS. Text in each panel refers to the measurement method (see Methods for description). Horizontal dotted lines represent minimum instrument error on either side of the mean (1.5‰ for δ^2H). Note that all cleaned samples measured by IRMS (HDEV and TCEA) fall within these limits, indicating very low variance between these methods. The large differences seen for both untreated and cleaned samples measured by IRIS (LGR, PIC) indicate the possibility of residual organic contaminants in the water samples causing spectral interference.
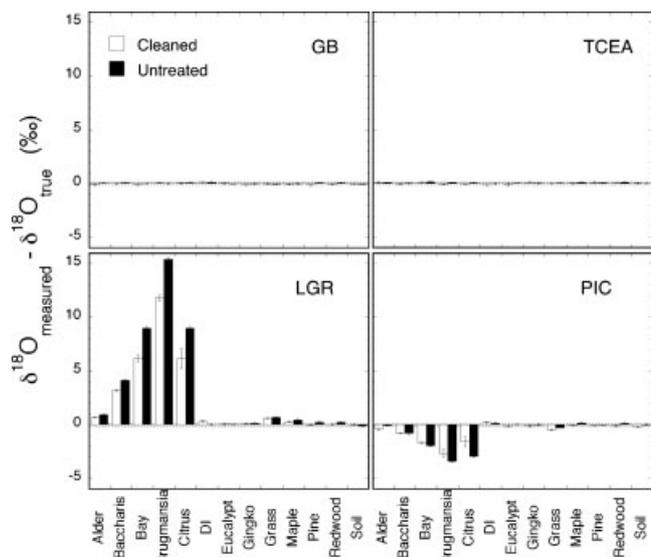
Figure 2. Difference in measured $\delta^{18}O$ and the assumed true value for all samples. The true value was assumed to be the mean of samples cleaned with activated charcoal analyzed by IRMS. Text in each panel refers to the measurement method (see Methods for description). Horizontal dotted lines represent minimum instrument error on either side of the mean (0.15‰ for $\delta^{18}O$). Note that all cleaned samples measured by IRMS (HDEV and TCEA) fall within these limits, indicating very low variance between these methods. The large differences seen for both untreated and cleaned samples measured by IRIS (LGR, PIC) indicate the possibility of residual organic contaminants in the water samples causing spectral interference.

the IRIS and IRMS values for *Eucalyptus*, *Gingko*, pine and redwood despite the presence of high levels of organic contaminants in the untreated samples.

Interestingly, when the IRIS value for a species deviated from the IRMS mean, it was seen for both IRIS methods, with the same relative magnitude but the opposite sign (Figs. 1 and 2). For example, the samples with the largest deviation on the LGR instrument also had the largest deviation on the PIC instrument, but in opposite directions. The LGR tended to report more positive values and the PIC more negative, a trend consistent for both δ^2H and $\delta^{18}O$.

Effect of cleaning with activated charcoal

There was a small effect of cleaning with activated charcoal for samples analyzed by IRMS (Figs. 3 and 4). For δ^2H, the largest cleaning effects were seen for grass (−2.4‰ HDEV) and *Eucalyptus* (−1.7‰ HDEV) leaves. For $\delta^{18}O$, the largest effects were seen for pine (0.2‰ GB) and *Brugmansia* (0.2‰ TCEA). However, for the majority of species, there was no effect of cleaning with activated charcoal, with cleaned and untreated samples lying close to the IRMS mean (Figs. 1 and 2).

A much larger cleaning effect was seen for the IRIS data (Figs. 3 and 4). For δ^2H, the largest cleaning effects were seen for bay (2.4‰ LGR and −7.6‰ PIC), *Citrus* (6.1‰ LGR and −12.6‰ PIC) and *Brugmansia* (4.9‰ LGR and −11‰ PIC) leaves. The same species showed the largest effects for $\delta^{18}O$, namely bay (2.8‰ LGR and −0.3‰ PIC), *Citrus* (2.8‰ LGR and −1.4‰ PIC) and *Brugmansia* (3.6‰ LGR and −0.8‰ PIC). In the majority of cases, the effect of cleaning with

Table 3. Deviations from mean IRMS cleaned value for all the analytical techniques tested in this study. See Experimental section for description of the techniques. *HDEV is for δ^2H and GB for $\delta^{18}O$. Values in bold represent deviations larger than 1.5‰ (δ^2H) and 0.15‰ ($\delta^{18}O$), which we consider to be a significant effect size based on long-term IRMS precision

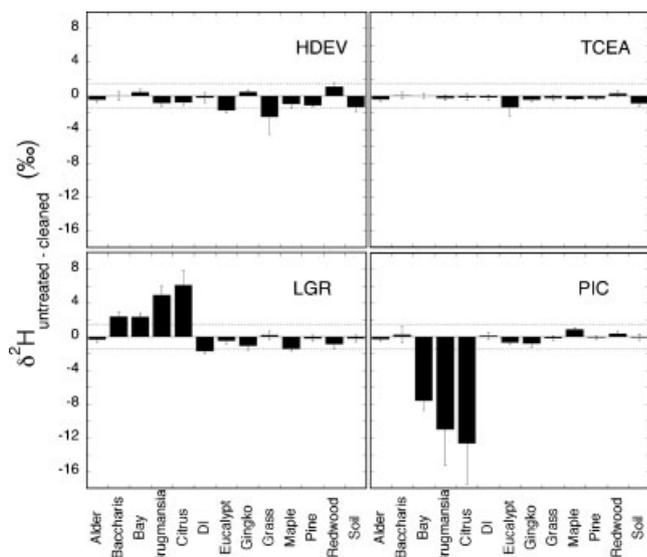|		UNTREATED				CLEANED			
	Species	HDEV & GB*	TCEA	LGR	PIC	HDEV & GB*	TCEA	LGR	PIC
δ^2H	Alder	−1.1	0.3	1.2	**−3.3**	−0.7	0.7	1.5	**−3.0**
	Baccharis	−0.7	0.8	**8.0**	**−10.7**	−0.7	0.7	**5.5**	**−10.9**
	Bay	−0.3	0.8	**12.5**	**−28.3**	−0.7	0.7	**10.1**	**−20.7**
	Brugmansia	−0.9	−0.2	**23.1**	**−46.5**	−0.1	0.1	**18.1**	**−35.5**
	Citrus	−1.0	0.1	**19.4**	**−28.6**	−0.2	0.2	**13.3**	**−16.0**
	Eucalyptus	**−2.0**	−1.0	−1.1	**−1.9**	−0.3	0.3	−0.7	−1.2
	Gingko	−0.4	0.4	0.1	−0.9	−0.8	0.8	1.1	−0.2
	Grass	**−2.8**	0.1	0.8	**−3.0**	−0.4	0.4	0.6	**−2.9**
	Maple	−0.8	−0.5	0.3	−0.6	0.1	−0.1	**1.7**	−1.5
	Pine	−1.5	0.1	0.4	−0.4	−0.4	0.4	0.5	−0.3
	Redwood	0.1	1.3	0.5	0.5	−1.0	1.0	1.4	0.1
	Soil	−1.5	−0.6	−0.5	−0.8	−0.2	0.2	−0.4	−0.7
$\delta^{18}O$	Alder	0.04	0.12	**0.94**	−0.08	−0.12	0.12	**0.67**	**−0.42**
	Baccharis	0.09	0.03	**4.13**	**−0.78**	0.01	−0.01	**3.19**	**−0.79**
	Bay	0.00	**0.17**	**8.96**	**−1.97**	−0.12	0.12	**6.13**	**−1.69**
	Brugmansia	0.03	0.09	**15.39**	**−3.42**	0.09	−0.09	**11.77**	**−2.65**
	Citrus	0.08	0.06	**8.97**	**−2.95**	0.03	−0.03	**6.16**	**−1.59**
	Eucalyptus	−0.02	0.06	0.03	0.02	0.05	−0.05	0.08	**−0.15**
	Gingko	−0.01	0.06	0.13	−0.01	−0.09	0.09	0.10	−0.12
	Grass	−0.05	0.03	**0.69**	**−0.28**	−0.02	0.02	**0.61**	**−0.47**
	Maple	0.00	0.11	**0.46**	0.15	−0.07	0.07	**0.26**	−0.09
	Pine	0.09	0.08	**0.24**	−0.03	−0.09	0.09	−0.01	−0.06
	Redwood	0.08	0.13	**0.24**	0.14	−0.08	0.08	0.00	−0.10
	Soil	−0.04	0.02	−0.14	−0.01	−0.03	0.03	−0.01	**−0.21**

Figure 3. Difference in δ^2H between samples without organics removed (untreated) and those treated with activated charcoal (cleaned). Text in each panel refers to the measurement method (see Methods for description). Horizontal dotted lines represent minimum instrument error on either side of the mean (1.5‰ for δ^2H).
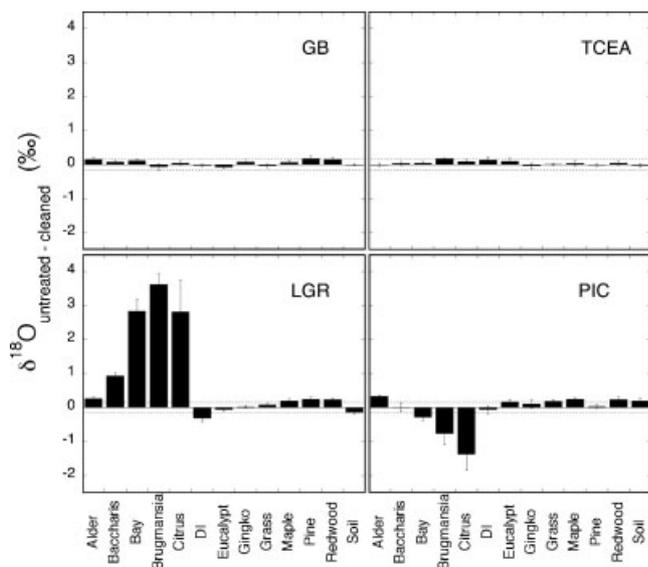

Figure 4. Difference in $\delta^{18}O$ between samples without organics removed (untreated) and those treated with activated charcoal (cleaned). Text in each panel refers to the measurement method (see Methods for description). Horizontal dotted lines represent minimum instrument error on either side of the mean (0.15‰ for $\delta^{18}O$).

activated charcoal was to reduce the deviation from the IRMS mean.

DISCUSSION

Our results offer striking evidence of the sensitivity of IRIS methods to the presence of organic contaminants in water

samples. We were forced to reject our initial hypothesis – that cleaned samples would be indistinguishable on IRIS and IRMS methods – as there was considerable discrepancy between cleaned samples analyzed on IRIS and IRMS for 7 of the 12 species. The similar relative effects seen for both IRIS instruments – albeit in opposite directions –suggests a generic susceptibility of IRIS to the presence of organic contaminants in water extracted from plants. The opposite direction of the effects measured on the IRIS instruments is alarming. Unfortunately, as the technical information on the spectral wavelengths employed by the CRDS instruments is proprietary information – and thus not known to the authors – we cannot provide sound working hypotheses for the large isotope effects we observed or why the two instruments gave opposite results. However, our results indicate the real possibility of including and propagating errors of a large magnitude if combining analyses using different technologies.

The discrepancy between IRIS and IRMS did not exist for all species. For some species with large quantities of organic contaminants in the extracted water (e.g. pine and *Eucalyptus*) there was no difference between the IRIS and mean IRMS value. This would suggest that only some organic contaminants interfere with IRIS and that with sufficient testing, it may be possible to analyze certain plant water extracts by IRIS. To this we add a note of caution. The concentration and composition of plant secondary compounds can vary considerably within a species depending on ontogenetic, biotic and environmental factors.[20–23] Without regular crosschecking on IRMS, the IRIS user is not yet able to detect this effect and, as illustrated in our data, the potential errors are large. Thus, it would appear that the analysis of plant waters using IRIS requires extreme caution and that IRIS may not be a suitable method of analysis for certain species at present.

While we have demonstrated the potential problems with plant extracts, similar problems may be present for other types of samples. To deal with this problem, we would urge manufacturers of IRIS instruments to provide a 'spectral diagnosis tool' that would flag samples for which spectral interference from contaminants may have occurred. This would allow researchers to screen their samples for possible interference and then follow up with IRMS crosschecks. Until these tools are available, we would urge caution in utilizing IRIS methods for the analysis of water samples that may contain trace contaminants.

Effectiveness of activated charcoal in cleaning water samples

Cleaning plant and soil water samples with activated charcoal has become standard practice, yet there has been no published test of the necessity or efficacy of this treatment. In this study we were able to examine the effect of using activated charcoal on IRMS and IRIS analyses.

Our data suggest that cleaning with activated charcoal is less important than previously thought for analysis by IRMS methods. The presence of organic contaminants did alter the isotopic ratio of some samples measured by IRMS; however, this was a surprisingly small effect that only occurred in a few species (Figs. 3 and 4). The maximum differences

between untreated and cleaned samples on IRMS methods were $-2.4‰$ (δ^2H) and $0.18‰$ ($\delta^{18}O$). These values are very close to the estimates of machine precision and might be regarded as insignificant.

The small differences between untreated and cleaned samples – as analyzed by IRMS – indicate a lack of sensitivity of IRMS to the presence of organic contaminants in water. Since the IRMS methods are mass-dependent, the effect of organic contaminants on the isotopic ratio of the sample depends both on the relative mass of H and O derived from organic contaminants in the sample and on the difference in isotopic ratio of the contaminant-derived H and O from that of the water. For most samples, it is likely that there was insufficient mass of H and O from the organic contaminants relative to that of water to significantly affect the isotopic ratio of the sample. However, it is also possible that the isotopic ratio of these organic contaminants was not markedly different from that of the water. Although biosynthetic pathways should result in organic compounds having an isotopic ratio markedly different from that of plant water,[24] it is possible that the H – and to a lesser extent the O[25,26] – of organic contaminants undergo exchange with water during the distillation process. This would act towards minimizing the apparent effect of cleaning with activated charcoal. A final possibility exists, that the activated charcoal was ineffective at removing organic contaminants from the water sample. Two lines of evidence suggest that this was not the case. First, adding activated charcoal to extracted samples had the effect of removing all odour and all discoloration of the water sample. Opaque and milky samples with strong odour became clear and odourless after the activated charcoal treatment. Secondly, the activated charcoal treatment had a large effect for the IRIS data (Figs. 3 and 4), acting to reduce the deviation from the IRMS mean. Thus, we are left to conclude that activated charcoal did remove most organic contaminants from the sample and that the lack of sensitivity is due to either lack of sufficient mass or exchange. In either case, the activated charcoal treatment appears not to be extremely important for IRMS analyses with the exception of studies where the highest precision is required.

Our study provides strong evidence that the IRIS methods were highly sensitive to the presence of even small amounts of organic contaminants in a sample. The addition of activated charcoal did reduce the deviation from the mean IRMS value; however, activated charcoal alone was insufficient to yield data comparable with the mean IRMS values. Alternative cleaning methods may prove effective in removing this effect, but these will need to be thoroughly investigated.

CONCLUSIONS

The use of IRIS for the stable isotope analysis of water offers great promise for researchers looking for low-cost, high-precision and portable analyzers. However, our data suggest that this technology is highly susceptible to interference from organic contaminants and that caution is needed in analyzing samples that may contain some form of organic contaminant. We would suggest that further testing on the influence of sample contaminants and techniques for their removal needs to be performed before this technology is adopted for widespread use. We also suggest that IRIS instruments should be equipped with a 'spectral diagnosis tool' that flags data with potential spectral interference. Without such a tool, users may routinely fail to exclude erroneous data. In the meantime, we recommend regular crosschecks with IRMS, especially in cases where novel samples are being analyzed or contamination is suspected.

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