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

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ABSTRACT

RATIONALE: The stable isotope compositions of hydrogen and oxygen in water (δ^2 H 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 sidestep limitations imposed by traditional methods. Here, we evaluate DVE analysis of plants with a focus on spectral contamination introduced by organic compounds. We present ¹⁷O-excess as a way of quantifying organic compound interference in DVE.

METHODS: We performed isotopic analysis using the δ^2 H, δ^{18} O and δ^{17} O values of water on a 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 ¹⁷O-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 ¹⁷O-excess was sensitive to the presence of organic compounds in water. ¹⁷O-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 ¹⁷Oexcess. Soil samples did not show any spectral contamination. Plant samples were spectrally contaminated on the narrow-band and were enriched in ¹H and ¹⁶O 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 ¹⁷O-excess values.

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

Key words: plant water; laser spectroscopy; direct vapor equilibration; spectral contamination; ¹⁷O-excess

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1. Introduction

The use of the stable isotope ratios of hydrogen and oxygen (δ²H and δ¹⁸O values) as a tracer of plant water uptake is increasing rapidly¹⁻⁴. Investigations of ecohydrological processes using such techniques have improved our understanding of soil water dynamics⁵⁻⁸ and patterns of plant water use⁹⁻¹². 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 of different sources (e.g. SIAR¹³, MixSIR¹⁴, IsoSource¹⁵, RAPID¹⁶). These mixing models, in turn rely on extracted liquid samples

However, traditional analyses of xylem water constrain our understanding of ecohydrological processes. Cryogenic vacuum distillation¹⁷ (CVD) used to extract water from xylem is costly, labor intensive, and time-consuming, which limits investigations to "snapshot" observations of plant water use¹⁸. 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 composition²¹. Thus, current heterogeneity and dynamics of ecohydrological and physiological processes are masked, and much uncertainty exists in isotopic measurements of plant water sources²². 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 material²³, or via *in situ* measurements²¹. 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 availability²¹.

Whilst high frequency measurements of isotopes in plants brings opportunities to improve our understanding of ecohydrological processes²², 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 analysis²⁶⁻²⁸. MeOH and EtOH have different interference effects on laser spectroscopy analysis: MeOH causes narrowband spectral interference²⁹ while EtOH causes broadband interference²⁹. 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 water²⁶⁻²⁸. The presence of organic compounds in xylem will vary depending on plant species, development stage and phenological phase³⁰⁻³³. 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 inaccurate²⁷. 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 narrow-band or broad-band spectral contamination metrics are being generated for organic compound identification in vapor mode to date.

Here we explore the use of δ^{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 ¹⁷Oexcess 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 δ^{17} O / δ^{16} O and δ^{18} O / δ^{16} O ratios are defined as:

$${}^{17}\text{O-excess} = \delta^{17}\text{O} - 0.528 \,\delta^{18}\text{O}^{-37}$$
[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.

¹⁷O-excess is known to be relatively insensitive to temperature compared with the dexcess and can provide new information on kinetic fractionation processes³⁸⁻⁴⁰. Thus, we hypothesize that the ¹⁷O-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 plants³³, and have been used in previous papers for the same purpose^{27,28,41}. We used solutions of MeOH and EtOH, and natural samples to test our system for flagging and address the question, "Can ¹⁷O-excess identify samples that are influenced by the presence of organic compounds?".

2. Materials and Methods

We used spiked water with known concentrations of MeOH and EtOH to determine the influence of organic compounds on ¹⁷O-excess and measured isotope ratios via DVE. We collected xylem samples from three trees species to verify whether spectral contamination in natural solutions could be also identified by ¹⁷O-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^2 H -126.83 ‰, δ^{18} O -15.65 ‰, and δ^{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 in 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%, 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 trees species to verify whether spectral contamination in natural samples could also be identified by ¹⁷O-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) tree species 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 10 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 25mL NalgeneTM 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 (δ²H, δ¹⁸O and δ¹⁷O 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 al²⁴. Samples were transferred to leak proof plastic bags (17.78 cm x 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 h at room temperature (~ 22⁰C) to allow sample water to equilibrate with dry air in the inflated bag's headspace.

After equilibration, we sampled the bag headspace for ~3 min intervals, or until water content stabilized at ~28,000 to 30,0000 ppm V H₂0, at which point the δ^2 H, δ^{18} O, and δ^{17} O raw values were recorded⁴². The isotope signatures are reported in δ -notation, in per mil (‰). The δ -value denote the measured isotope ratio of sample (R_{sample}) for δ^2 H (²H/¹H) and δ^{18} O (¹⁸O/¹⁶O) in reference to Vienna Standard Mean Ocean Water (VSMOW) (R_{reference}):

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

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

The laboratory reference waters had δ -values of -200.26 ‰, -26. ‰, and -14.08 ‰ for the depleted reference, and 9.70 ‰, 0.57‰ and -0.10 for the enriched reference water, for ²H, ¹⁸O, and ¹⁷O, respectively. A third control standard with known δ -values (δ ²H -137.0 ‰, δ ¹⁸O -17.4 ‰, and δ ¹⁷O 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: \pm 1.8 for δ ²H values, and \pm 0.3 for δ ¹⁸O and δ ¹⁷O values.

2.4 Spectral Contamination and Statistical Analysis

Statistical analyses were performed using R 3.5.1 software⁴⁵. 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-Wallis⁴⁶ and post hoc Dunn's test⁴⁷ 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 method⁴⁸. 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 ¹⁷O-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 broad-band spectral contamination based on responses to contaminants (MeOH and EtOH). We used the Spectral Fit Residual plots produced with distilled water and MeOH/EtOH water as a qualitative parameter for the identification of spectral contamination in soil and xylem samples.

3. Results

$3.1\ ^{17}\text{O}\text{-excess}$ as a Flagging Tool for Narrow (MeOH) and Broad-Band (EtOH) Spectral Contamination

The ¹⁷O-excess of MeOH solutions increased linearly with increasing concentrations of MeOH per volume of water, resulting in positive ¹⁷O-excess values (Figure 1A). EtOH solutions resulted in smaller changes in ¹⁷O-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^2 H 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^2 H, 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), δ^{18} O (p < 0.02), and for δ^{2} H (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^2 H 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).

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 δ^{17} O value was sensitive to organics at the lowest concentration (MeOH 0.001%).

3.2 ¹⁷O-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 ¹⁷O-excess (Figure 3). The average soil water ¹⁷O-excess value was 0.56, and -0.28 for the OJP and OBS sites. The highest ¹⁷O-excess was 0.95 at 200 cm depth, at OJP (Figure 3A). The lowest ¹⁷O-excess value was -0.77 at 10 cm depth, at OBS (Figure 3B). Soil water plotted on the meteoric water line (Figure 5).

We identified spectral contamination in xylem samples using the Spectral Fit Residual plot (Figure 4). Spectrally contaminated xylem water showed increases in ¹⁷O-excess values (Figure 4), and narrow-band spectral contamination. The average ¹⁷O-excess value for contaminated xylem was 7.27, and 20.51 for *P. mariana* and P. *bankisiana*, respectively (Figure 4). The average ¹⁷O-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 ¹⁷O-excess of 1.55.

Table 2 shows a comparison of the isotopic composition 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, ¹⁸O and ¹⁷O 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

4.1¹⁷O-excess and Spectral Contamination

The experiments with MeOH and EtOH revealed the sensitivity of ¹⁷O-excess to the presence of organic contaminants when conducting spectral analysis via DVE. The ¹⁷O-excess flagged the presence of both organic compounds, with more sensitivity to MeOH than EtOH. Although there are no previous reports on the effects of organic compounds on the ¹⁷O-excess our results are consistent with previous spectral contamination investigations in the literature with respect to laser spectroscopy. Brand et al ²⁷, West et al²⁶, and Martín-Gómez et al²⁸ have reported pronounced effects from MeOH and EtOH contamination on δ^2 H 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 composition²⁵.

While MeOH produced positive ¹⁷O-excess values, EtOH produced more negative ¹⁷O-excess values. These findings are also in agreement with results found by Schultz et al⁴¹.

While these authors measured neither δ^{17} O nor ¹⁷O-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 δ^{18} O and δ^{2} H 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 δ^{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, only 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.

4.2¹⁷O-excess and the TIWA-45EP OA-ICOS Analyzer Spectral Fit Residual

Plot

TIWA-45EP OA-ICOS offers a quick and reliable method to measure δ^{17} O values compared with IRMS⁴². 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). ¹⁷O was the most sensitive isotope to the presence of organics. The ¹⁷O-excess as an organic contamination detection tool introduces a systematic step to quantitatively inform errors produced during to isotopic analysis. ¹⁷Oexcess 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 unknow 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 ¹⁷O 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 al³⁵). 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 ¹⁷O-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 ¹⁷O-excess values from soil were similar to values produced by distilled water samples. ¹⁷Oexcess 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^2 H and δ^{18} O values) did not show deviations from 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 ¹⁷O-excess. Xylem samples visually identified as contaminated with the Spectral Fit Residual plots showed positive ¹⁷O-excess values. The ¹⁷O-excess values of contaminated samples were above those identified for distilled water, and soil. Spectrally contaminated samples showed more positive δ¹⁸O and δ²H 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 ¹⁸O and ²H in relation to corrected plant water samples⁴⁴, or IRIS analysis⁵⁰.

All contaminated xylem samples showed narrowband spectral contamination. The ¹⁷O-excess value from spectrally contaminated xylem samples correlated to the ¹⁷O-excess values of 0.01 % for MeOH. The strong linear relationship between the concentration of MeOH (narrow-band spectra) and the ¹⁷O-excess values suggests that it may be possible to quantify the concentration of organics in samples utilizing ¹⁷O-excess. This should be further investigated with post-processing correction curves. Taken together, the δ^2 H, δ^{18} O and δ^{17} O values offer a potential tool to assess spectral interference of organics in vapor mode analysis through deviations of ¹⁷O-excess. The δ^{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. Conclusion

Use of δ^{17} O value together with δ^{2} H and δ^{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 δ^{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 plantwater sources. Additional studies are nonetheless required to identify which organics interfere in the ¹⁷O/¹⁶O isotopic ratio measurements, as well as whether other different organic compounds affect the observed δ^{17} O values.

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| | No. total samples | % v/v | δ2Η | sd | Error (‰) | δΟ18 | sd | Error (‰) | δ017 | sd | Error (‰) 17 | O-excess | sd | Error (‰) |
|----------|----------------------|-------|----------|--------|-----------|--------|--------|-----------|--------|--------|--------------|----------|--------|-----------|
| Pure Wat | .€ 5 | 0 | -130.127 | 1.40 | | -16.05 | 0.24 | | -7.51 | 0.09 | | 1.0 | 0 | .19 |
| | 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 |
| MECH | 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 |
| | 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 |
| ELOH | 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 |

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

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Table 2. Xylem samples from black spruce (*P. mariana*), jack pine (*P. banksiana*), and estern 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 current method. Errors were calculated per species.

| Free of Spectra | al Contamina | ation | | | Spectral Contamination | | | | | | | | | | | | | |
|----------------------------|----------------------|--------------------|--------------------|------------------|------------------------|---------|------------------|--------------------------------|-------------------|-----------------------|----------------|----------------|----------------|--------|---------------------------------|---------------|------------------|---------------|
| Species | No. total samples | δ2Η | sd | δ18Ο | sd | δ170 | sd | Species | No. total samples | δ2Η | sd | Error δ2H | δ18Ο | sd | Error δ18O | δ170 | sd | Error δ170 |
| P. mariana P. banksiana | 16 10 | -124.43 -120.15 | ± 17.25 ± 21.43 | -14.00 -13.55 | ± 2.22 ± 2.90 | -6.57 ± | = 1.33 = 1.48 | 3 P. mariana 3 P. banksiana | 14 20 | -114.10 ± -95.77 ± | 23.84 28.87 | 10.33 24.37 | -8.53 -2.28 | ± 5.09 | 5 5.46 7 11.27 | 2.77 19.63 | ± 6.15 ±18.47 | 9.33 26.34 |

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Figure 1. Spectral contamination results on measured isotopic composition of distilled water with different concentrations of MeOH and EtOH. Panel A and B show ¹⁷O-excess (per meg) and the linear relationships for MeOH and EtOH, respectively.

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Figure 2. Dual isotope ratio space results for MeOH (A) and EtOH (B). Black line represents global meteoric water line (GMWL).



Figure 3. Soil ¹⁷O-excess (per meg) from OJP (A) and OBS (B).



Figure 4. Xylem samples in dual isotope ratio space and, respective ¹⁷O-excess (per meg). Panel A shows results for all species. Panel B shows same samples, but distinguishing between spectrally contaminated (grey) identified visually on the 'Spectral Fit Residual' plot, and not contaminated. Note changes in ¹⁷O-excess axis.

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Figure 5. Xylem and soil samples plotted together on 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.

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