Technical Report

A method for in situ monitoring of the isotope composition of tree xylem water using laser spectroscopy

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ABSTRACT

Field studies analyzing the stable isotope composition of xylem water are providing important information on ecosystem water relations. However, the capacity of stable isotopes to characterize the functioning of plants in their environment has not been fully explored because of methodological constraints on the extent and resolution at which samples could be collected and analysed. Here, we introduce an in situ method offering the potential to continuously monitor the stable isotope composition of tree xylem water via its vapour phase using a commercial laser-based isotope analyser and compact microporous probes installed into the xylem. Our technique enables efficient high-frequency measurement with intervals of only a few minutes per sample while eliminating the need for costly and cumbersome destructive collection of plant material and laboratory-based processing. We present field observations of xylem water hydrogen and oxygen isotope compositions obtained over several days including a labelled irrigation event and compare them against results from concurrent destructive sampling with cryogenic distillation and mass spectrometric analysis. The data demonstrate that temporal changes as well as spatial patterns of integration in xylem water isotope composition can be resolved through direct measurement. The new technique can therefore present a valuable tool to study the hydraulic architecture and water utilization of trees.

Key-words: hydraulic integration; isotope ratio infrared spectroscopy (IRIS); isotopic ratio mass spectrometry (IRMS); nutrients; stable isotopes; water relations; xylem transport.

INTRODUCTION

Analysis of the stable isotope composition of hydrogen and oxygen in xylem water (δ2H and δ18O) is now being widely employed in ecology, hydrology and related disciplines. Applications have allowed researchers to study plant water sources (Dawson & Ehleringer 1991), water use patterns (Schwinning et al. 2002), competitive interactions (Ehleringer et al. 1991; Meißner et al. 2012), hydraulic lift (Caldwell & Richards 1989), evapotranspiration partitioning (Brunel et al. 1997) and plant hydraulic architecture (Drake & Franks, 2003). As the number and spatiotemporal scope of environmental isotope studies continue to expand, the limitations of available indirect observational techniques form a bottleneck (West et al. 2006). This study presents a step forward by introducing a technique for direct in situ quantification of the xylem water isotope composition in trees.

Conventionally, measurements of tree xylem water isotope composition are obtained through manual collection of sapwood and subsequent water extraction for isotope ratio mass spectrometry (IRMS) analysis (Ehleringer et al. 2000; West et al. 2006). IRMS instrumentation allows high measurement precision (Horita & Kendall 2004), but is expensive and laboratory-bound, and the required sample pre-treatment is highly time-consuming (Kerstel & Gianfrani 2008). Through significant recent technological advances, instruments based on isotope ratio infrared spectroscopy (IRIS) have become commercially available that allow direct, continuous and simultaneous measurements of δ2H and δ18O of water vapour at high frequency and provide a precision and accuracy comparable to IRMS (Berden et al. 2000; Kerstel & Gianfrani 2008; Gupta et al. 2009). These instruments also facilitate broader usage of xylem water stable isotope analysis by allowing measurement of extracted liquid samples with minimal sample preparation, higher throughput rates and lower cost (West et al. 2010; Martin-Gómez et al. 2015). Still, to date, the need for destructive harvesting of plant material and water extraction restricts the number of samples that can be obtained over time and in space and makes continuous and high-frequency measurements difficult to sustain or simply infeasible. It has thus remained challenging to cope with the variability exhibited by natural ecological systems in isotope-based studies.

Importantly, however, IRIS instruments are also portable and field-deployable. This has stimulated recent development of in situ methods for direct estimation of, for instance, the isotope composition of transpired water using leaf chambers (Wang et al. 2012; Dubbert et al. 2014) or whole-plant chambers (Volkmann et al. 2016). These developments represent important progress toward a more continuous isotope-based characterization of plant water relations. Unlike xylem water,
the transpiration isotope composition is, however, influenced by complex interactions between ambient water vapour, water at the evaporation site and environmental conditions (Farquhar et al. 2007; Dubbert et al. 2014). In addition, specific integral measurements for larger plants as well as representative automated long-term measurements are difficult and/or costly to implement.

Here, we present a novel approach to overcome previous methodological limitations by monitoring the xylem water isotope composition of trees directly in the field. Such methodology could provide for a less expensive, faster and higher-resolved alternative to existing indirect approaches. We therefore employ a diffusion–dilution sampling technique (Volkmann & Weiler 2014) using gas-permeable probes installed into the sapwood of the tree stem to continually deliver xylem water vapour for laser spectroscopic $\delta^2$H and $\delta^{18}$O analysis. The technique is based on the idea that vapour exchanged into and with an artificial cavity within the xylem will equilibrate isotopically with the surrounding liquid xylem water and that direct analysis of the former will hence allow inference of the latter. We tested the capacity of the method to monitor spatiotemporal isotope dynamics in the xylem of two adult field maple trees (Acer campestre L.) in response to isotopically labelled irrigation and compared the direct in situ IRIS measurements with the common indirect technique (e.g. West et al. 2006) using cryogenic vacuum distillation of sapwood tissue and IRMS.

**MATERIALS AND METHODS**

**Set-up for direct in situ xylem water isotope monitoring**

A schematic diagram of our sampling set-up is presented in Fig. 1. A central set of devices includes an isotope ratio infrared spectrometer (L2120-i wavelength-scanned cavity ring-down spectrometer, Picarro Inc., Santa Clara, CA, USA) and a nitrogen gas ($N_2$) supply installation, housed in a vented trailer with pressure regulator (2), isotope analyser (3), vacuum pump (4), mass flow controller (5), valve manifold (6) and xylem water isotope probe (7). Black arrow heads indicate direction of gas flow.

**Figure 1.** Schematic of the set-up for direct in situ monitoring of xylem water stable isotope composition. Labels indicate $N_2$ gas bottle (1), pressure regulator (2), isotope analyser (3), vacuum pump (4), mass flow controller (5), valve manifold (6) and xylem water isotope probe (7). Black arrow heads indicate direction of gas flow.

The flexible dilution functionality is used in a typical probing sequence, which comprises two distinct phases: During the initial flushing phase, a high dilution rate is used to clear the sample path from unwanted residual vapour. Then, during the sampling phase, the dilution is reduced to a rate that allows sufficiently precise laser spectroscopic measurements yet prevents condensation of sampled vapour. Results of vapour isotope analysis acquired during the sampling phase after the signal has stabilized can then be statistically summarized, corrected for instrument-specific biases and deviations from the

**Principle of operation for direct in situ xylem water isotope monitoring**

To conduct a measurement, $N_2$ is supplied via the throughflow line to the tip of the probing head while a vacuum pump imposes an outflow of 30–35 mL min $^{-1}$ through the sample line toward the IRIS instrument. Because of a partial pressure gradient, water molecules diffuse from the wet plant xylem through the porous wall of the probing head to mix with the through-flowing carrier gas. Before the resulting gas mixture enters the sample line, it passes through the mixing chamber where a second $N_2$ supply line (the dilution line) allows controlled dilution to lower vapour concentrations. This prevents potential condensation of vapour along the entire sample path while avoiding heating of the sample line. The total inflow rate of $N_2$ is set equal to the outflow rate through the sample line to minimize differences in total pressure across the porous material. The relative flow rates through dilution and throughflow line can, however, be varied to adjust the vapour concentration in the sample air.

The three main gas transport lines are distributed to each of a number of probing locations via arrays of two-way solenoid valves (Clippard Minimatic, Cincinnati, OH, USA) mounted on manifold blocks made of polyvinylidene fluoride. The valves are controlled by a programmable microprocessor switching unit to subsequently connect individual probing locations to the central devices. The tubing used consists of largely inert and low-permeable fluorinated ethylene propylene, has low dead-volume (inner diameter of 1 mm for sample lines) and is protected by mesh hoses.

A xylem water isotope probe (XWIP, Fig. 2) has a rigid gas-permeable head consisting of microporous hydrophobic polyethylene (Porex Technologies, Aachen, Germany) with 50 mm length and 10 mm outer diameter. The head is connected to a central element that contains a mixing chamber and receives one branch of each of the three gas transport lines for sample extraction and $N_2$ supply, protected outwards by a robust attachable shaft and a shrink hose. The probe is installed into a horizontal hole pre-drilled into the main trunk sapwood of the tree to be investigated. The hole corresponds in dimensions to the probe head to achieve close contact. To prevent intrusion of ambient air that would contaminate samples and lead to pressure loss in the sapwood, the contact area between probe and stem is sealed using commercial silicone.
reference scale, and ultimately related to liquid xylem water isotope composition through temperature-dependent liquid–vapour fractionation.

Study site and experimental design

The in situ technique was tested with two adult deciduous field maple trees (Acer campestre L.; 9 m tall, 0.2 m in diameter at breast height and 30 m$^2$ of crown projected area) located on a lawn-covered green space in urban Freiburg, southwest Germany (273 m above sea level; 47°59′58.15″N and 7°50′52.75″E; Fig. 3). Mean annual temperature is 11 °C, and mean annual rainfall is 930 mm. The soil is characterized as loamy sand, with increasing rock content toward greater depth.

For each tree (henceforth trees A and B), three XWIPs were installed at 1.2 m height with radial orientation at approximate azimuths north (probes A1 and B1), south-east (A2 and B2) and south-west (A3 and B3). The length of the sample paths (from probe to analyser) was 12 m.

Direct and indirect (destructive) xylem water isotope sampling was conducted during a period of 11 d in September and October 2014. To investigate if changes in the isotope composition of xylem sap can be tracked in situ, we imposed a change in soil source water isotope composition on the second day of the experiment by applying a 30 mm irrigation pulse of tap water enriched in deuterium ($\delta^2$H of 308.8‰). The $\delta^2$H value of irrigation water was thus approximately 366‰ and 341‰ more positive compared with annual average (37.3‰) and recent summer (32.6‰) precipitation, respectively. The $\delta^{18}$O value of irrigation water was −9.3‰ and thus slightly more negative compared with annual (−8.3‰) and summer (−5.1‰) precipitation. A drip irrigation system was used to reduce evaporative effects on the irrigation water. The irrigated area generally corresponded to the crown projected area, but a small section was omitted, which caused spatially variable soil water isotope compositions (Fig. 3).

Procedure for direct in situ xylem water isotope sampling

The XWIPs were continuously operated during daytime hours except for the day of irrigation and day 10 of the experiment. The probing interval was 600 s per probe (cf. Fig. 4), with a 120 s flushing and a 480 s sampling phase using one-third dilution. Raw sample values of vapour concentration, $\delta^2$H and $\delta^{18}$O were obtained as arithmetic mean over the last 120 s of the sampling phase, and measurement precision was estimated as the standard deviation (σ) over this period. Subsequently, the raw isotopic values were drift-corrected and calibrated to the Vienna Standard Mean Ocean Water (VSMOW) reference scale (e.g. Gröning 2011) based on direct-equilibration measurements (Wassenaar et al. 2008) of three laboratory water standards spanning the range of encountered abundances before and after the experiments. The data were then corrected for analyser-specific vapour concentration dependent bias (e.g. Schmidt et al. 2010) relative to a reference concentration. Least-squares regressions were derived based on discrete aliquots of laboratory water standards of varied volume injected into a vaporizer using a liquid auto sampler with N$_2$ as carrier gas. Finally, the liquid xylem water $\delta^2$H and $\delta^{18}$O values were estimated using the equations for free water liquid–vapour equilibrium fractionation by Majoube (1971) along with estimated temperatures of xylem water. Therefore, an empirical relation was established between the measured vapour concentrations from probe B2 and temperatures measured with a temperature probe in the sapwood at 35 mm depth (10 min

Figure 2. Schematic of a xylem water isotope probe installed into the sapwood of a tree trunk. Labels indicate microporous tube (1), central element (2), mixing chamber (3), line retainer (4), sample line (5), dilution line (6), throughflow line (7), insertion and protection shaft (8), protective shrink hose (9), and sealant (10).

Figure 3. Schematic map of the experimental green space. Shown are the two sampled maple trees (A and B) along with the orientation of the installed xylem water isotope probes (A1–A3 and B1–B3) as well as the crown projected and irrigated areas.
coupled with a high-temperature pyrolysis analyser (TC/EA, Thermo Fisher Scientific). The results are reported on the VSMOW scale. The reproducibility of IRMS-based measurements was obtained as the standard deviation of delta values from collocated samples collected on a given day.

RESULTS AND DISCUSSION

Acquisition and quality of direct xylem water isotope data

The probing system allowed rapid and stable measurement of isotope composition and concentration of vapour directly extracted from the xylem into the laser spectroscopic analyser (Fig. 4). The resulting time course of xylem water δ2H and δ18O observed in situ is shown in Fig. 5. No measurements were obtained for probe B3 because of occurrence of water droplets in the sample line on the first day of measurements. The likely cause was a crack in the porous tube found when removing the probe from the tree. This can be avoided in future designs by using a robust protective mantle. All other probes allowed continual data generation over the duration of the experiment with a resolution of <1 h for the five active probing locations. Optimization of analysis time and fewer sampling locations could further reduce the sampling intervals if higher temporal resolution is required.

The isotopic signal typically levelled off within only 60 to 120 s of sampling (Fig. 4). The quick signal stabilization can be attributed to the low dead volume and low water sorption along the sample path. Over the experiment, a median precision of 1.1‰ for δ2H and 0.29‰ for δ18O values (1σ) was achieved for the integration period of 120 s (Fig. 6a,b). This is reasonably close to the vapour analysis performance specified for the IRIS instrument (<1.0‰ for δ2H and <0.2‰ for δ18O; Picarro Inc. 2012) and about two to three times less precise than typically attained with water sample analysis using IRIS or IRMS under laboratory conditions (Horita & Kendall 2004; Kerstel & Gianfrani 2008; Munksgaard et al. 2011). The precision of δ2H measurements deteriorated toward lower sample vapour concentrations (Spearman’s ρ = –0.62, P < 0.0001; Fig. 7a) diverging from optimal conditions for the analyser (specified 17 to 23 × 10⁵ ppmv). By contrast, δ18O precision deteriorated toward higher vapour concentrations (ρ = 0.46, P < 0.0001; Fig. 7b). These findings agree with those of Aemisegger et al. (2012) using a similar IRIS instrument and may be attributed largely to decreasing instrument signal-to-noise ratios at low and optical saturation at high vapour concentrations. No obvious relation was found, however, with the standard deviation of sample vapour concentrations (ρ < 0.09, P > 0.05), precluding major effects of probing instability. Improvements in measurement precision may thus be achieved through enhanced analyzing instruments and by optimized adjustment of vapour concentrations (e.g. through adaptation of the dilution rate to varying ambient temperature).

Data collected on the first and the five last days of the experiment (i.e. before and several days after labelled irrigation, when temporal trends in actual soil and xylem water composition may be assumed small) indicate median

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reproducibility of 2.8‰ for $\delta^2$H and 0.33‰ for $\delta^{18}$O sample values (1σ) over operational periods of c. 7 h (Figs 5, 6c,d). Variability may be introduced through imperfect accounting for temperature-dependent liquid–vapour fractionation at the probing interface. However, the diurnal variations in $\delta^2$H or $\delta^{18}$O did not usually correlate with those of temperature estimates for the different probes (for A3 and $\delta^{18}$O: $\rho = 0.80$, $P < 0.05$; for the remainder: $\rho < 0.60$, $P > 0.05$). Nevertheless, strong temperature gradients within tree stems (e.g., Derby & Gates 1966) complicate the determination of the average temperature at the phase exchange and thus of fractionation factors, and single or even multiple measurements of temperature directly within the probe could likely improve data quality in future designs. On the other hand, diurnal variations in actual xylem sap isotope composition cannot be precluded. In fact, the daily reproducibility of direct measurements did not differ substantially (neither for $\delta^2$H nor $\delta^{18}$O; Wilcoxon signed-rank test, $P > 0.05$) from that of indirect measurements (1.9‰ for $\delta^2$H and 0.47‰ for $\delta^{18}$O; Fig. 6c,d).

![Figure 5](image1.png) **Figure 5.** Xylem water isotope dynamics obtained through direct in situ measurement over several days for two maple trees (A and B) using a total of five probes (A1–A3 and B1–B2) installed at varied azimuths (different colours) in the trunks. Data shown are individual measurements (small pale symbols) of $\delta^2$H (a,b) and $\delta^{18}$O (c,d) values (<1 h resolution during daytime hours) along with daily means and standard deviations (large symbols and error bars). Dashed vertical lines indicate application of a deuterated irrigation pulse.

![Figure 6](image2.png) **Figure 6.** Relative frequency distributions (gray bars) of precision (a, b; 1σ for 120 s integrations) and reproducibility (c,d; 1σ for sampling periods of 7 h on the first and the five last days of the experiment) for direct in situ measurements of xylem water $\delta^2$H and $\delta^{18}$O values using IRIS. Solid black lines represent associated Gaussian kernel density estimates (scaled). Dashed black lines in (c,d) represent kernel density estimates of daily reproducibility for destructive sampling with cryogenic distillation and IRMS analysis.

![Figure 7](image3.png) **Figure 7.** Precision (1σ for 120 s integrations) of direct in situ measurements of xylem water $\delta^2$H (a) and $\delta^{18}$O (b) values in relation to sample water vapour concentrations.
Direct field observations of xylem water isotope dynamics

The probes show a clear positive response in xylem water $\delta^2$H (mean enrichment of $29 \pm 17\%$ over all probes, Fig. 5) to the application of deuterated irrigation water on day 2 of the experiment (Wilcoxon signed-rank test on maximum response versus day 1, one-sided, $P < 0.05$). Changes in the hydrogen isotope composition were most pronounced during day 4 to 6 (second to fourth day after irrigation), after which more steady values were observed with probe-specific-maxima on day 8 to 9 (sixth to seventh day after irrigation). Noticeable negative but much smaller responses were observed for $\delta^{18}$O (mean of $-1.1 \pm 0.2\%$; $P < 0.05$). This is consistent with expectations, because irrigation water was strongly enriched in $^2$H and only slightly depleted in $^{18}$O compared with the annual average and recent summer rainfall at the experimental site, and thus with the putative soil water isotope signature.

While the recorded temporal dynamics in xylem water composition are similar among the probes, the differences in the response magnitude for $\delta^2$H with differing azimuth in a given tree are striking (Fig. 5). These differences can clearly be associated with the heterogeneous irrigation pattern (Fig. 3). That is, probes installed at a side of a tree with more complete coverage by labelled irrigation show larger responses and vice versa. This suggests that water (and nutrients) taken up by different compartments of the field maple root system follows highly radially sectored trajectories with limited lateral mixing and integration on its way through the trunk xylem. Such strong sectoriality of transport is consistent with previous studies (Sperry 1995; Orians et al. 2004) on species of the maple genus (*Acer rubrum*, *A. saccharum* and *A. grandidentatum*) and may be associated with a low density of intervessel pit pairs of adjacent xylem vessel elements (i.e. vascular constraints).

Comparison of direct and indirect observations of xylem water isotopes

The $\delta^{18}$O and $\delta^2$H values obtained by IRIS are compared against results obtained with the well-established destructive method using cryogenic distillation and IRMS in Fig. 8. The comparison shows that the two methods give highly significantly correlated data ($\delta^2$H$_{IRIS} = 1.26 \times \delta^2$H$_{IRMS} + 14.51$, $r^2 = 0.86$, $P < 0.0001$, $\delta^{18}$O$_{IRIS} = 0.91 \times \delta^{18}$O$_{IRMS} - 4.87$, $r^2 = 0.46$, $P < 0.001$, robust bisquare-weighted M-regression).

The $\delta^2$H data obtained with both methods prior to irrigation are in good agreement and show little systematic difference ($0.9 \pm 1.8\%$; Fig. 8a). The strong response to irrigation and the radial sectoriality pattern of transport trajectories inferred from the *in situ* data are also generally confirmed by the IRMS data. Overall, the $\delta^2$H data across locations were not substantially different for the two methods on the days with concurrent sampling (Wilcoxon signed-rank test, $P > 0.05$). Still, it is noticeable that maximal irrigation-induced changes in $\delta^2$H based on IRMS tended to be relatively smaller (by 28%) and less variable (by 42%, based on variation coefficients), and that results obtained from particular locations (e.g. A2 in Fig. 8a) diverged more strongly from the regression line. We hypothesize that a potential disparity could be due to progressed lateral mixing (through intervessel pits), axial dispersion and delay of irrigation water arrival at the twig/crown versus trunk level. That is, the strong and variable response associated with the non-uniformly applied irrigation (including e.g. the large response at probe A2 on the side of tree A with the most complete irrigation coverage) may have been attenuated with increased transport distance between heights of *in situ* (i.e. at 1.2 m in the trunk) and destructive (i.e. at ~2.5 m in the lower crown) sampling. A gradually changing, more integrated and overlapping connectivity of plant channels (or stem xylem) with different root sectors along the plant height has also been indicated for Norway maple (*Acer platanoides*) based on sap flux measurements during branch severing.

![Figure 8](image-url)  
*Figure 8.* Comparison of xylem water isotope composition obtained with the direct *in situ* IRIS and the destructive (cryogenic distillation) IRMS method. Data shown are daily means and standard deviations of $\delta^2$H (a) and $\delta^{18}$O (b) values obtained for two maple trees (A and B; different symbols) using a total of five probes (A1–A3 and B1–B2) installed at varied azimuths (different colours) in the trunks. Solid and dashed gray lines are robust linear regression fits with confidence intervals. The identity line is shown in solid black.
In summary, we have introduced a novel technique that allows monitoring of the isotope composition of xylem water in trees directly, continuously, and in high resolution. The set-up is inexpensive, efficient, automatable and suitable for field application. The obtained data are of promising quality, and the technique is demonstrated to facilitate resolution of short-term temporal dynamics of xylem water isotope composition in response to changes in source water composition as well as of patterns of radial sectoriality within individual stem cross sections. Potential future work should focus foremost on (i) influences of VOCs and xylem sap constituents and suitable post-processing corrections; (ii) integrated temperature sensing and improved fractionation estimation; (iii) a more robust probe design; and (iv) expansion to longer time periods and varied tree functional types. We believe that the presented method will enhance our capacity to address a wide spectrum of important questions in plant physiological and ecohydrological research, regarding the uptake of water and nutrients by plants, the processes and

General considerations for applying direct xylem water isotope probing

We would like to note several aspects that may potentially be considered relevant when using direct xylem water isotope probing on short and/or long (e.g. multiple seasons) experimental time scales. Similar to sap flow sensors (Moore et al. 2010), the installation of XWIPs unavoidably severs tracheids or vessel elements, which may alter xylem function in the immediate vicinity to the degree that water transport is increasingly diverted or restricted over time. In addition, wound response may lead to filling of xylem elements with air or exudates (Kramer & Boyer 1995) or may induce callus or tumour formation (Taiz & Zeiger 1991). In general, the xylem of woody plants is not uniform, and sapwood properties that may not be obvious based on outside visual inspection can disrupt flow and create non-conductive regions of the tissue, rendering a local measurement unrepresentative. Therefore, and because of potential sectoriality of xylem flow, multiple probing locations per tree should be considered (note this holds also for conventional sampling). Independent of such effects, different sapwood parts of the stem or differently bound xylem water pools may contribute variably to isotope compositions measured with different methods, which could cause variations and discrepancies (see e.g. Zhao et al. 2011). Further, although the employed free water liquid–vapour fractionation equations (Majoube 1971) seem to have accounted for much of the isotopic equilibration process and its temperature dependence at the probing interface, consideration of additional factors such as plant water potential or solute concentrations may yield improvements to precision and accuracy of the in situ method. Finally, continued extraction of depleted vapour alters the isotope composition of the surrounding xylem water, but given an effective liquid volume of ~3 μL per sample, this would only be of concern when xylem transport related water renewal is very slow and the sampling frequency is very high (Vollmann & Weiler 2014). For context, a volume of 3 μL would flow hourly through a cross-sectional area equivalent in size to the probing head (i.e. 5 cm × 1 cm orthogonal to the stem axis) if the sap flux density was as low as 6 × 10⁻¹⁵ cm h⁻¹, which is typically exceeded by several orders of magnitude in trees during daytime hours (e.g. Gessler et al. 2001; Meinzer et al. 2013).

Summary and outlook

Spreading of fluxes with increasing tree height could be promoted by tangential drift of conducting vessels around the stem axis (Tyree & Zimmermann 2002; Kitin et al. 2004). In addition, axially aligned measurement locations along a tree may not necessarily be associated with the same root compartment because of spiralling or twisting of plant vessels within the stem, which is found in most tree species (Zimmerman & Brown 1971; Orians et al. 2004).

The recorded dynamics in δ¹⁸O values are also similar for the two methods (Fig. 8b). However, a clear inter-method bias of −4.3 ± 0.7‰ persisted throughout the experimental period (Wilcoxon signed-rank test, P < 0.0001). We suspect that this discrepancy may be related to analytical interference caused by plant-produced volatile organic compounds (VOCs). Organics are often found with plant water, and some (e.g. methanol and ethanol) can result in pronounced negative deviations (up to ~8‰ or more for δ¹⁸O) and limited correlation between xylem water samples analysed using IRIS and IRMS methods (West et al. 2010; Zhao et al. 2011; Martín-Gómez et al. 2015). To evaluate whether the observed inter-method deviation could potentially be explained by presence of organic compounds, we analysed the relation of the difference between delta values obtained using IRIS and IRMS prior to irrigation (up to _2006; Alañón et al. 2011). The empirical relation

\[ \delta_{IRIS} = \delta_{IRMS} - RCS \times \delta_{IRMS} \]

where \( \delta_{IRIS} \) is the IRIS-derived δ¹⁸O value, \( \delta_{IRMS} \) is the IRMS-derived δ¹⁸O value, and \( RCS \) is the relative contamination factor. The slope of the regression analysis shows that inter-method differences correlated strongly with \( RCS \) (\( r² = 0.48, P < 0.0001 \)) but not for \( \delta_{IRMS} \) (\( r² = 0.01, P > 0.05 \)). Such isotope-specific effects may be related to, for example volatile benzene derivatives (M. Palmer, Piccaro Inc., personal communication, 2015) such as certain phenols found in various tree species (Fernández de Simón et al. 2006; Alaño et al. 2011). The empirical relation obtained for δ¹⁸O (δ¹⁸OIRIS − δ¹⁸OIRMS = −5.1 × RCS − 0.6) thereby suggests that substantial negative deviation of several per mil between IRIS and IRMS measurements may be explained by spectral contamination (mean RCS of 0.7 ppb cm⁻¹ over the experiment). Additional factors related to the probing and calibration process may have further played a role. However, it is likely that specific post-processing corrections (e.g. Martín-Gómez et al. 2015; Schmidt et al. 2012; West et al. 2011) can reduce or remove the discrepancies observed in this study and should thus be considered in future applications.
topology of vascular transport from roots to shoots, and the partitioning of evaporative transpiration as driven by varying environmental conditions.

**ACKNOWLEDGMENTS**

This work is part of the project ‘Coupled soil-plant water dynamics – Environmental drivers and species effects’ funded by the Deutsche Forschungsgemeinschaft (DFG; contract numbers GE 1090/10-1 and WE 4598/2-1). We are grateful to R. Siegwolf and M. Sauer from the Paul-Scherrer-Institute (Villigen, Switzerland) for facilitating the IRMS analysis. We further thank G. Thirerkez and two anonymous referees for their effort spent helping to improve this paper.

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Figure S1. Difference of xylem water $\delta^2$H (a) and $\delta^{18}$O (b) values obtained using the in situ isotope ratio infrared spectroscopy (IRIS) method to the respective mean values based on the indirect isotope ratio mass spectrometry (IRMS) method in relation to the root mean square residual ($R_s$) of the spectral model fits to the actual absorption spectra recorded by the IRIS instrument. Larger residuals are potentially related to specific organic molecules that absorb in the wavelength range of the target absorption features of water isotopologues but that are not considered in the spectral model employed by the software of the IRIS instrument. Data were obtained on two days from a total of five locations at varied azimuths in two field maple (Acer campestre L.) trees. Solid and dashed gray lines represent the best fit and confidence intervals from linear regression analysis. The relation is highly significant for $\delta^{18}$O ($\delta^{18}$O_{IRIS} - $\delta^{18}$O_{IRMS} = -5.1 \times R_s - 0.6, $r^2 = 0.48$, $P < 0.0001$) but not for $\delta^2$H ($\delta^2$H_{IRIS} - $\delta^2$H_{IRMS} = 3.2 \times R_s + 1.0, $r^2 = 0.01$, $P > 0.05$). Gray shading indicates the range of delta values from IRMS measurements about their mean.