CHAPTER 28

STABLE-ISOTOPE TECHNIQUES TO INVESTIGATE SOURCES OF PLANT WATER

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ABSTRACT

Stable isotopologues of water (mainly $^{1}H_2{}^{16}O$, $HD^{16}O$ and $^{1}H_2{}^{18}O$) have been used for decades as tracers of the Earth's water cycle. In this chapter, we briefly describe the theoretical background and state-of-the-art techniques of the use of water stable isotopes to investigate the sources of plant water. We aim to provide the basic understanding of stable isotope fractionation within the Earth's critical zone that is relevant for studies of plant water sources. We then present a practical guide of their most common applications in field studies and the most common and up-to-date laboratory procedures. We finally introduce the existing statistical approaches for estimating the relative contributions of water sources to plant transpiration. By acknowledging the advantages and limitations of each approach, we aim to provide an overview of the current techniques to researchers in the fields of plant ecophysiology, ecohydrology and forest ecology, so that they can make informed decisions when designing their experiments.

1. FRACTIONATION OF WATER STABLE ISOTOPES IN THE EARTH'S CRITICAL ZONE

1.1. Meteoric waters

The number of protons in the atomic nucleus defines each chemical element of the periodic table. Each element generally has several stable or radioactive isotopes, defined by the number of neutrons in the atomic nucleus. Stable isotopes of hydrogen exist with one or two neutrons (^{1}H and ^{2}H) and those for oxygen have 16, 17 or 18 neutrons (^{16}O, ^{17}O and ^{18}O). The most abundant form of water molecules is $^{1}H_2{}^{16}O$ but other forms also exist in relatively high natural abundances, mainly $HD^{16}O$ and $^{1}H_2{}^{18}O$. The difference in mass of these different water isotopologues lead to differential partitioning of heavy and light isotopologues during diffusion or phase changes, called isotopic fractionation (Dawson et al., 2002). Isotopic fractionation during the transfer of water among the various compartments of the water cycle lead to distinct isotopic compositions of the different water pools that can be exploited to trace the origin of water in the landscape and/or within an ecosystem.

During evaporation of oceanic water, light isotopologues tend to evaporate preferentially resulting in a depletion of atmospheric water vapor compared to oceanic water. That is, atmospheric water vapor has lower isotopic ratios ($^{18}O/^{16}O$ and D/H) compared to ocean water. These isotopic ratios are often expressed as a deviation from the Vienna Standard Mean Ocean Water (VSMOW) and noted $\delta^{18}O$ and $\delta^{2}H$. Isotopic fractionations during evaporation and condensation are complex but mass-dependent processes so that the $\delta^{18}O$ and $\delta^{2}H$ atmospheric vapor and meteoric water (precipitation) are linearly related, with a slope of about 8, forming the Global Meteoric Water Line (GMWL) (Craig, 1961). The dynamics of water vapor condensation and precipitation in the atmosphere generate temporal and spatial isotopic variability in meteoric waters, with variations along the GMWL, depending mainly on condensation temperature, latitude, altitude and continentality (Dansgaard, 1964). Indeed atmospheric water masses moving inland from the ocean, or polewards from the tropics, become progressively more depleted as they lose condensates (Gat and Carmi, 1970). For a given location the isotopic composition of meteoric waters varies seasonally along the Local Meteoric Water Line (LMWL) with more depleted values in winter and more enriched ones in summer (Fig. 1). The temperature effect also explains why snow is

usually depleted relative to rain at a given site (Fig. 1). The amount of precipitation also influences the isotopic signal of meteoric water; wetter months have an overall more depleted isotopic signal (Kurita et al., 2009). This amount effect also explains why fog water usually falls on the upper part of the LMWL (Fig. 1), at least in regions where fog originates from the same water body as rain water (Scholl et al., 2011). Seasonal variations in the isotopic composition of meteoric waters lead to distinct isotopic composition of the water pools in the critical zone accessible to plants, and this difference can thus be exploited to trace the origin of plant water.

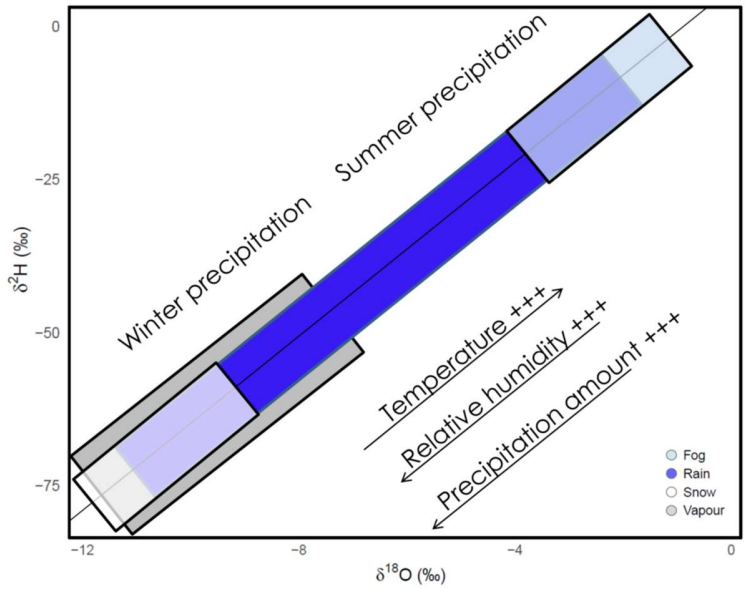

Figure 1. Theoretical dual-isotope plot of meteoric water samples. Different colors indicate different types of precipitation, and the arrows reflect the effect of environmental factors on the isotopic composition. The black line corresponds to the Global Meteoric Water Line (GMWL).

1.2 Soil waters

Plants take up water mostly through their roots, although foliar water uptake can also constitute a substantial water source in some fog-inundated ecosystems (Goldsmith et al., 2013). The signal of meteoric water is modified when the

water is stored underground. Several processes contribute to the isotopic differentiation of underground water pools. Most of these processes lead to isotopic differences that are much larger than the precision of the techniques of isotopic determination, so they are measureable. The two main processes involved are percolation and evaporation. The isotopic signal of underground water pools will partly represent the signal of the precipitated water that percolates through the soil pores. The deviation from meteoric water depends mainly on depth.

Deep pools of groundwater are recharged during seasons in which the soil is close to field capacity, i.e. in wet seasons. Groundwater pools thus represent the average isotopic signal of precipitation during the wet season (Brooks et al., 2010) or in cold seasons with low evapotranspiration and high soil moisture. In addition, the isotopic signal of precipitation can also change during a rain event, with more depleted rain at the end of the event because of the *rainout effect* (Brooks et al., 2010). Deep groundwater pools in confined or perched aquifers created in past geological ages, however, can have a substantially different isotopic composition, indicating the signal of past precipitation (Darling et al., 2003). Signals of stream water can also be relevant in riparian ecosystems, because water accessed by plants and supplying streamflow can belong to the same water pool. Stream water will be more or less seasonally variable depending on its source (snowmelt, storms, old groundwater pools or seasonally recharged groundwater pools). In contrast to deep soil water, groundwater and stream water, the isotopic composition of water in surface soil is more likely to represent the isotopic composition of recent precipitation, regardless of the season, and is affected by soil evaporation.

Evaporative enrichment is another important process contributing to underground isotopic differentiation. Evaporation of soil water produces a progressive enrichment of the water at the soil surface, because lighter isotopes are more easily evaporated. The kinetic fractionation of soil water, however, differs from equilibrium fractionation in the atmosphere, so the slope of the relationship between $\delta^{18}O$ and $\delta^{2}H$ deviates from the GMWL (Horita et al., 1995; Sprenger et al., 2016). This evaporative enrichment creates a isotopic differentiation between enriched surface soil water and non-evaporated, depleted deep soil water or groundwater that can be used to estimate the soil evaporation line (SEL). The depth reached by evaporative enrichment may differ substantially between climatic zones, from 0.2-0.3 m in temperate zones to a maximum of 3 m in arid climates (Sprenger et al., 2016). An isotopic

gradient with progressively more depleted water with depth should be measurable whenever evaporation occurs. Finally, the first few centimeters of the soil can be affected by atmospheric water vapor, which depletes superficial soil and decreases with depth as evaporation dominates the signal. The isotopic signal of plant xylem water could be tracked if the isotopic signal of underground water pools is sufficiently distinct. Importantly, the characterization of potential plant-water sources will critically depend on the correct identification of each isotopically distinct underground water pool. A proper characterization of the isotopic profile with depth is thus recommended before the onset of any field study. A typical distribution of the isotopic composition of underground water pools is depicted in Fig. 2, representing an example of a temperate forest.

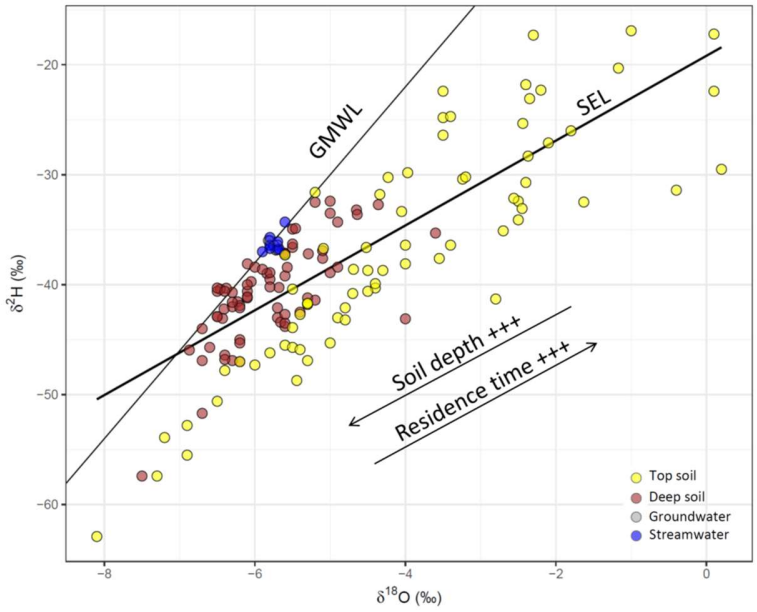

Figure 2. Theoretical dual-isotope plot of belowground water pools. Different colors indicate different water pools, and the arrows reflect the effect of soil depth and residence time. The thin black line corresponds to the Global Meteoric Water Line (GMWL) and the thick black line represent the Soil Evaporation Line (SEL).

Early studies of the stable isotopes of plant and soil water concluded that no fractionation occurred during the uptake of water by roots (Allison et al., 1984; White et al., 1985; Ehleringer and Dawson, 1992). Identifying the spatial and temporal patterns in plant-water sources is therefore possible by simultaneously sampling plant xylem water and its potential sources. The identification of plant-water sources, though, is only possible when at least two underground water pools are isotopically distinct. Xylem water is also very likely to indicate a mixture from different water pools, because many plants have a dimorphic root system that allows them to access more than one water pool at the same time (Dawson and Pate, 1996). In addition, plant-water sources can differ between coexisting species that do not share the same ecohydrological niche (Silvertown et al., 2015), and root development implies that larger and/or older plants may access deeper water pools more than their smaller and/or younger conspecifics (Kerhoulas et al., 2013). These characteristics of plant-water sources require that the sources be investigated individually.

In summary, the sources of isotopic variability of underground water pools are reasonably well understood (Fig. 3). Together with the generally accepted absence of fractionation during the uptake of water by roots (Ehleringer and Dawson, 1992), the theoretical framework for studies of plant-water sources has been straightforward and sound until very recently. The technological development in this discipline, however, has increased the number of studies applying stable isotopes to identify plant-water sources. Larger data sets with higher temporal and spatial resolution have been compiled, and some have challenged the main assumptions of prior studies, for both underground and plant-mediated isotopic fractionation. Even though these data sets do not invalidate previous work, researchers must understand the limitations and uncertainties of these techniques before conducting fieldwork or interpreting their data. We have synthesized the most relevant findings in the following section.

1.3 Soil isotopic heterogeneity and plant-mediated fractionation

Soil-water isotopic signals are governed by precipitation inputs, evaporation and the mixing between new and old water pools in soil pores (Sprenger et al., 2016). Several fractionation processes in the soil, however, must be taken into account to properly track the movement of water to roots. Heavy and light isotopes may interact differently with soil minerals and organic matter. In soils with high

contents of clay minerals the interactions of water with cations can entail isotopic differences between cation-absorbed water and the remaining free water (Oerter et al., 2014). A similar isotopic differentiation has been reported for water in contact with the surface of organic particles (Chen et al., 2016), which would also create isotopically different pools of water in soils with high organic content: one on the surface of organic particles and another formed by free water accessed by plants.

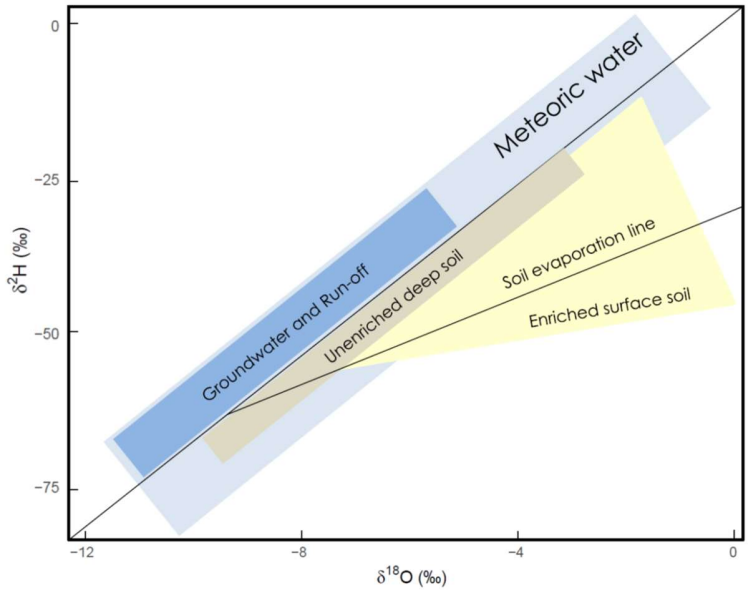

Figure 3. Conceptual scheme depicting the most common distribution of the isotopic composition atmospheric and belowground water pools of an ecosystem.

Roots explore the bedrock in search for water in porous rock and rock fractures (Schwinning, 2010; Barbeta and Peñuelas, 2017). Water infiltrating through rocky layers can be isotopically filtered (Coplen and Hanshaw, 1973), resulting in more depleted rock water (Oshun et al., 2015). The residual solution, i.e. the free water potentially absorbed by plants, is likely to have a more enriched signal. Plants, however, would access structural water from rocks under some environmental conditions (Palacio et al., 2014). These processes can

influence results if the methods used to analyze soil-water isotopes are based on the extraction of bulk soil water, whereas plants do not access all isotopically distinct pools. All these fractionation processes therefore require knowledge of the characteristics of the substrate at the field site. Soil texture and type and depth of bedrock or organic-matter content are particularly relevant characteristics potentially affecting the isotopic composition of underground water pools.

Recent studies have also found that xylem water may not necessarily indicate the source water. Early studies reported that xylem water did not change isotopically until it reached non-suberized twigs and leaves (Ehleringer and Dawson, 1992). The evaporative enrichment of woody stems, however, can occur during periods of low water flow, such as in winter (Bowling et al., 2017) or droughts (Martín-Gómez et al., 2017). Analyzing water from the bottom of the plants (i.e. base of the trunk) less exposed to evaporation is thus preferable following or during winter or droughts, when possible. Finally, a recent pot experiment has reported discrimination during the uptake of water by roots and its dependence on soil texture and soil-water content (Vargas et al., 2017). The mechanisms were not fully resolved by the study, because the fractionation signal did not agree with any known fractionation process in the soil, described in the previous paragraph. The study, however, demonstrated that stable isotopes may not always identify plant-water sources with high precision. The simultaneous analysis of the isotopic composition of xylem and source water, though, is still a valuable tool that works well in most cases. Describing the temporal, spatial and/or species-specific patterns of plant-water uptake is possible following a cautious and informed interpretation of the data.

2. EXISTING PROTOCOLS FOR SAMPLING WATER POOLS IN THE CRITICAL ZONE

2.1 Meteoric waters

Sampling meteoric data is highly recommended for any study aiming at describing the patterns of plant water uptake. These data provide the seasonal variations in the isotope composition input of soil water that will subsequently be modified by soil process dynamics. This allows to derive the local meteoric

water line (LMWL) that can differ slightly from the GMWL. The LMWL is useful to assess possible isotopic deviations of ground and soil water pools. The International Atomic Energy Agency (IAEA), that coordinates the Global Network of Isotopes in Precipitation (GNIP), provide a protocol for installing rain collectors for the analysis of stable isotopes (IAEA/GNIP, 2014). Ensuring a full exposure of the collector to rainwater and a placement away from any source of heat is necessary, and importantly, the design must avoid any evaporation during collection and storage of precipitated water. Rain collectors can be installed at meteorological stations for determining the relationship between precipitation amounts and isotope composition. Precipitation amounts will have consequences in input isotopic signals but also in water infiltration depths. If the establishment of a rain collector is not feasible, the GNIP database can be accessed and searched for the nearest station. Some areas, though, will not be well represented, as differences in altitude and distance to the sea from the nearest GNIP station can strongly affect the isotopic composition of precipitation (Gat and Carmi, 1970). Thus, a site-specific isotopic data for precipitation is recommended, especially for mountainous or remote areas.

Precipitation in the form of snow should be sampled separately, because the accumulation of snow on the collectors and subsequent melting or evaporation could lead to incomplete sampling. A recent study reported an effective and simple method for sampling snow. Bowling and colleagues (2017) buried a plastic bucket leveled to the soil surface immediately before winter to collect melting snow. Snow can also be directly sampled from the snowpack cores (Bowling et al., 2017). A sampling of the entire snowpack profile and over different soil covers will ensure capturing the potential spatial variability in snow-water isotopes.

Plants in regions with frequent fog can also take this water up through leaves or roots from the condensate dripping from the canopy (Limm et al., 2009). Fog water should thus be sampled and its isotopic composition analyzed wherever it may represent a significant part of the plants water input. There are two main types of fog collectors: (1) passive fog collectors that rely on the wind to push air through the collection surface (either a mesh with vertical and horizontal strands, or a harp with only vertical strands), and (2) active fog collectors in which a fan pulls the air through the collection surface (Fisher et al., 2007). Both types of collectors take advantage of the difference in inertia between fog droplets and the surrounding air, leading to the impact of the droplets onto the strands during the deflection of the air stream when passing

through the mesh or harp. Active fog collectors are preferable because they can be more easily protected from precipitation, minimizing potential mixing of fog and rainwater during windy rain events. On the other hand, passive collectors can be installed in remote areas with limited access to electric power. The bottom of the mesh or harp must be connected to a vessel similar to that used in rain collectors, ensuring no evaporation during storage. Alternatively, fog water can be sampled during individual field campaigns in a similar fashion as when sampling water vapor using a cold trap made of a glass coil submerged in a mixture of ethanol and dry ice and connected to a pump that will slowly pump air through the cold trap at a rate that ensures complete solidification (typically below 0.5 liter per minute). This method will exclusively collect water vapor on days without fog. The time required to sample the required amount of water for isotopic analysis will depend on the airflow, temperature and relative humidity (Fig. 4).

Upon collection from the field, all the meteoric water samples must be stored in a refrigerator in airtight glass bottles with a cap covered with Parafilm® to ensure that no vapor escapes during storage, and until stable isotope analysis.

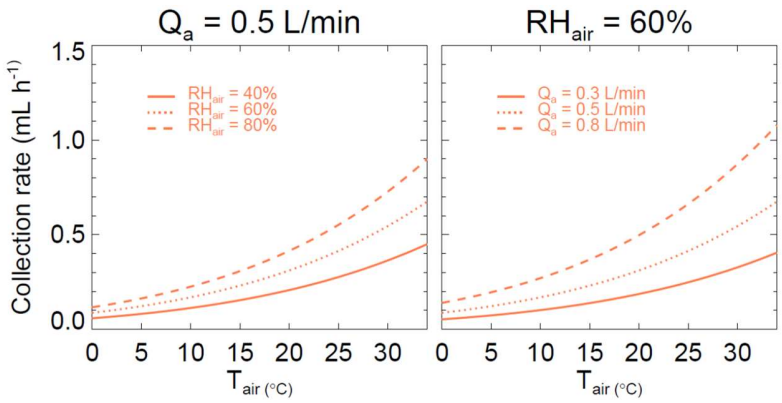

Figure 4. Collection rates of water vapor using a cryogenic trap as a function of temperature, relative humidity and the flow rate of the pump. The left panel is for a set flow of 0.5 L min^{-1} and the right panel is for a constant relative humidity of 60%.

2.2 Soil waters

To make sure that all potential plant water sources are considered, sampling soil water along the entire plant root profile and even below the maximum rooting depth is desirable. Sampling soil water down to the water table (including groundwater) should be sufficient at sites with shallow water tables. The isotopic composition of the water in saprolite, weathered rock or bedrock should at least be obtained at rocky sites where roots do not reach the water table. Because the maximum rooting depth can be difficult, to assess experimentally, a modeling framework for rooting depth (e.g. Fan et al., 2017) can be used to infer the expected maximum rooting depth, as a function of climate, landscape position (valley bottom, hillslope, hilltop) and soil texture. Deep water pools may often not be accessible from the surface with a soil auger, either because the soil is too deep (i.e. > 2-3 m) or too rocky. In such cases, a natural spring or nearby well should provide an isotopic signal comparable to that accessed by deep roots.

The enriched surface soil and the soil below the evaporation front should at least be sampled. The number of depth increments will depend on the isotopic profile. The soil isotopic profiles that will require different sampling strategies are depicted in Fig. 5. In case A, typical of what should be observed after 1-2 days of days without rain, the isotopic change is approximately constant throughout the depth profile. All depths should thus be sampled at increments adapted to the sample processing capacity. In case B, typical of what should be observed after several (>5 days) of days without rain and with high evaporative enrichment, the surface soil and the soil below 0.5 m are clearly differentiated. Characterizing the surface and deep soil would thus be enough. The precision of the estimates of plant-water uptake will also depend on the horizontal variability of the isotopic composition of water in each soil layer. The evaporative enrichment of soil can vary substantially within short distances due to contrasting vegetation cover or orientation exposition to sunlight. The water isotope composition at the surface of the soil will consequently be spatially and temporally more variable than at depth. Obtaining as many replicates as possible is thus recommended, depending on the sample processing capacity. The vials for collecting and storing the soil samples should have caps with septa to avoid water vapor leaks and contamination and a volume of at least 10 mL. Collecting larger soil samples may be necessary at dry sites in order to have enough water to extract for isotopic analysis.

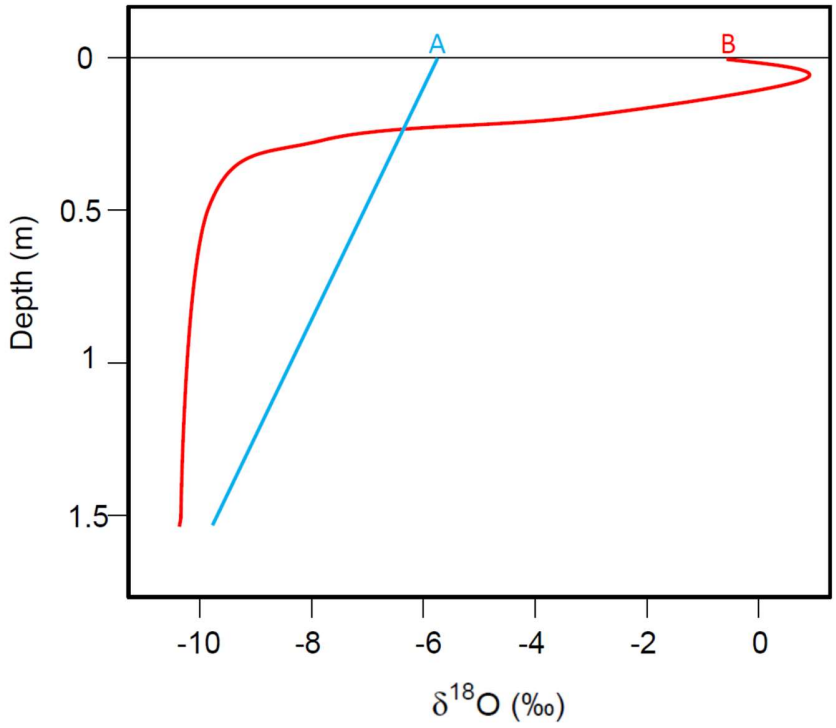

Figure 5. Theoretical isotopic profiles in depth. A corresponds to a steady isotopic depletion with depth, whereas B and C show different cases of evaporation fronts, with more stable isotopic compositions in depth.

In most studies soil water samples are collected from the field and subsequently analyzed in the laboratory (section 3), but new techniques have recently been developed enabling *in situ* measurements of soil water isotopes (Oerter and Bowen, 2017). Briefly, the method is based on the assumption that soil pore water vapor is in isotopic equilibrium with liquid water. Water vapor probes are permanently inserted at different depths within the soil column and connected to a stable isotope water vapor analyzer (Oerter et al., 2017). The main advantage of this method is that it is non-destructive (once the probes are inserted), so that soil water isotopes can be recorded at the exact same location over time. The main disadvantage of the method is that it can only be used to

survey a limited amount of closely related soil profiles. The method also requires main power, which is not always accessible in remote areas.

2.3 Xylem water

Sampling xylem water to determine its isotopic composition in discrete campaigns is relatively straightforward and easy. Xylem water does not generally suffer isotopic fractionation in suberized plant stems and branches (Ehleringer and Dawson, 1992). Evaporative isotopic enrichment of xylem water may occur in transpiring, non-suberized, young branches, especially when the water flow is low (Martín-Gómez et al., 2017). The back-diffusion of evaporatively enriched foliar water can also interfere with the signal from the source water in these terminal branches (Dawson and Ehleringer, 1991). Removing the bark and phloem that may also enrich the signal from foliar water is also recommended when cutting a branch. Alternatively, wood cores could be collected with an increment borer from the base of the trunk or at breast height for large trees with high canopies that are difficult to access. The core would not need to be deep and should only contain sapwood (Ehleringer and Dawson, 1992). The outermost part of the core, corresponding to bark and phloem, should also be removed. Wood cores or pealed branches must be placed immediately (within a couple of minutes maximum) in airtight glass bottles and kept in a cooler until being stored in a laboratory freezer. Plant water sources can change quickly, so each sampling campaign should be conducted as quickly as possible and preferentially at the same time of the day. Likewise, abrupt changes in plant water sources should be monitored after a rain or during extreme climatic events such as heatwaves or droughts.

Similar to what has been done in soils, a new method to continuously monitor *in situ* the isotopic composition of tree xylem water has recently been proposed (Volkmann et al., 2016). Xylem water is diffused through a porous membrane of the probe, pulled by the difference in partial pressure between a vacuum line and the wet xylem. The authors installed more than one probe per trunk and irrigated them with isotopically labeled water. The results were comparable to cryogenically extracted water, but different probes in the same trunk detected slightly different isotopic values. Damage to the xylem caused by probe insertion may therefore have altered the conductive capacity of the xylem, or different parts of the trunk's xylem may have been connected to different parts of the root system. The authors nonetheless recommended the

installation of several probes per tree to obtain a more integrated isotopic signal. The main advantage of this method is the possibility of sampling at a high temporal resolution and thus determining daily cycles in plant water sources that are still poorly understood. This probe-based technique may be more difficult to apply in remote areas without access to power, similar to the soil *in situ* methods.

3. WATER ISOTOPE ANALYSIS FROM SOIL AND XYLEM SAMPLES

3.1 Cryogenic extraction of water from soils and xylems

Compact equipment for monitoring xylem and soil-water isotopes *in situ* has been developed, such as laser-based isotopic analyzers, but most studies of plant-water sources still rely on cryogenic water extraction. This technique consists of a vacuum distillation in which the water contained in a solid sample is evaporated and condensed in a collection tube (West et al., 2006, and citations therein). Detailed descriptions of the design and functioning of cryogenic extraction lines have been provided in previous studies (West et al., 2006; Orlowski et al., 2013). Briefly, (1) the xylem or soil samples and the collection tubes are first frozen with liquid nitrogen and connected to the vacuum line, (2) the xylem or soil samples are then heated (with boiling water, mineral oil or heating blocks) and (3) the water is progressively evaporated from the sample and is trapped by the collection tubes submerged in liquid nitrogen. This extraction technique is a Rayleigh fractionation, because lighter isotopes will evaporate first. A long extraction time, however, will effectively remove all water from a solid sample, thus obtaining a similar isotopic composition of water trapped by the collection tube. The extraction times will depend on the soil and plant types; water in sandy soils will be completely extracted after 30 min, and water in woody stems will be completely extracted after 60-75 min (West et al., 2006). Most of the isotope laboratories extract for 2 h to standardize the time. This technique is widely applied and is consistent for xylem water, but tightly bound water in some types of soils may not be effectively removed during cryogenic extraction (see section 3.2).

3.2 Issues with cryogenic extraction of soil water

Early methodological tests of extraction times of soil water have reported that nearly complete extraction from clay soils requires more time (West et al., 2006), because of a higher fraction of tightly bound water in clayey soils compared to coarser soils. Large effects of the physiochemical properties of soils on the isotopic signal of extracted water have recently been demonstrated (Meißner et al., 2013; Orlowski et al., 2013). In particular, cryogenically extracted water tends to be isotopically depleted relative to input water in re-wetting experiments (Meißner et al., 2013; Orlowski et al., 2013; Orlowski et al., 2016; Newberry et al., 2017). Clay, calcium carbonate and soil-water contents can amplify the isotopic differences between input and extracted water (Meißner et al., 2013; Orlowski et al., 2016; Newberry et al., 2017). The fractionation of water in soil, however, does not imply that tightly bound water in soil micropores or clay or organic particles does not mix with free water (Newberry et al., 2017; Vargas et al., 2017). This isotopic stratification within the soil matrix could be misleading for the study of plant-water sources if water extracted from soils did not represent the total water accessed by plants. Alternatively plants may not access these pools of tightly bound water, even with complete extraction. Testing the effects of various settings of the cryogenic extraction line is recommended for avoiding mismatches between the water extracted from plants and soils. For example, testing various extraction times and temperatures to extract water contents up to the permanent wilting point. Pre-weighing the samples, extracting the water, oven-drying the samples at high temperatures and then re-weighing them to quantify the amount of residual water after the extraction would be helpful. These considerations are more relevant for studies of soils with high clay and/or low soil-moisture contents.

Issues associated with the cryogenic extraction of soil-pore water may be overcome using the recently developed vapor-probe method (Oerter and Bowen, 2017), as mentioned above. This method, however, would be difficult to apply in some cases. Many alternative techniques to cryogenic extraction are available. A recent comparison of techniques, though, found that all produced inconsistent results because the isotopic composition of extracted water always differed from the rehydration water added after oven-drying the soils, particularly for clayey soils with low soil-water contents (Orlowski et al., 2016). Centrifugation (White et al., 1985; Böttcher et al., 1997) and mechanical squeezing (Peters and Yakir, 2008) of the samples performed best (lower deviation from added reference water) and should be the best options for the

precision required in studies of plant-water sources (Orlowski et al., 2016). The time needed for sample processing, however, is longer than for most cryogenic extractions (10 samples per day for mechanical squeezing), and a larger quantity of soil is required. Cryogenic extractions highlight the need for a progressive shift towards alternative extraction techniques, such as centrifugation and mechanical squeezing, for extracting soil-pore water in studies of plant-water sources.

4. ISOTOPIC DETERMINATION OF EXTRACTED WATER

Isotopic determination is the last step in the laboratory after the extraction of water from solid samples. Early and many recent studies of plant water sources have used isotopic ratio mass spectrometry (IRMS), which requires the conversion of water to H_2 or CO or its isotopic equilibration with CO_2 (Gehre et al., 1996; West et al., 2010). Laser-based isotopic analyzers have recently become more popular, because they allow the simultaneous measurement of both the oxygen and hydrogen isotopes of water, reducing the time and cost of the analyses (Lis et al., 2008; Gupta et al., 2010). Off-axis integrated-cavity output spectroscopy (OA-ICOS) (Lis et al., 2008) and wavelength-scanned cavity ring-down spectroscopy (WS-CRDS) (Gupta et al., 2010) are two rather common laser-based techniques. Some studies have found that laser-based instruments can detect non-significantly different isotopic ratios for soil samples compared to IRMS (West et al., 2010; Orlowski et al., 2016), although another study reported discrepancies (Martín-Gómez et al., 2015). Discrepancies for plant water samples (leaves and xylems) have been more consistent with OA-ICOS (Schultz et al., 2011) and WS-CDRS (West et al., 2010) than IRMS.

Organic compounds in plant materials can be distilled together with water during cryogenic extraction and volatilized during analysis into the instrument cavity. These compounds can produce spectral interferences, that can lead to large discrepancies with IRMS results (West et al., 2010; Schultz et al., 2011; Zhao et al., 2011; Martín-Gómez et al., 2015). Because soil and xylem samples may contain amounts of volatile organic compounds large enough to cause spectral interferences, a subset of samples should preferentially be analyzed also by IRMS when using a laser-based instrument. Post-processing softwares for

these laser-based instruments also provide diagnostic tools that indicate if spectral interferences have been detected. These diagnostics can be used to decide how to proceed with the isotopic determination. In addition, post-processing correction methods have been published (Schultz et al., 2011) or are provided by the manufacturers of the laser-based instruments. A step-by-step decision-making scheme is provided in Fig. 6. For OA-ICOS instruments, developing instrument-specific correction curves by analyzing series of dilutions with ethanol and methanol and producing correlations of the broadband and narrowband metrics with the isotopic offset from non-contaminated samples are necessary (Schultz et al., 2011).

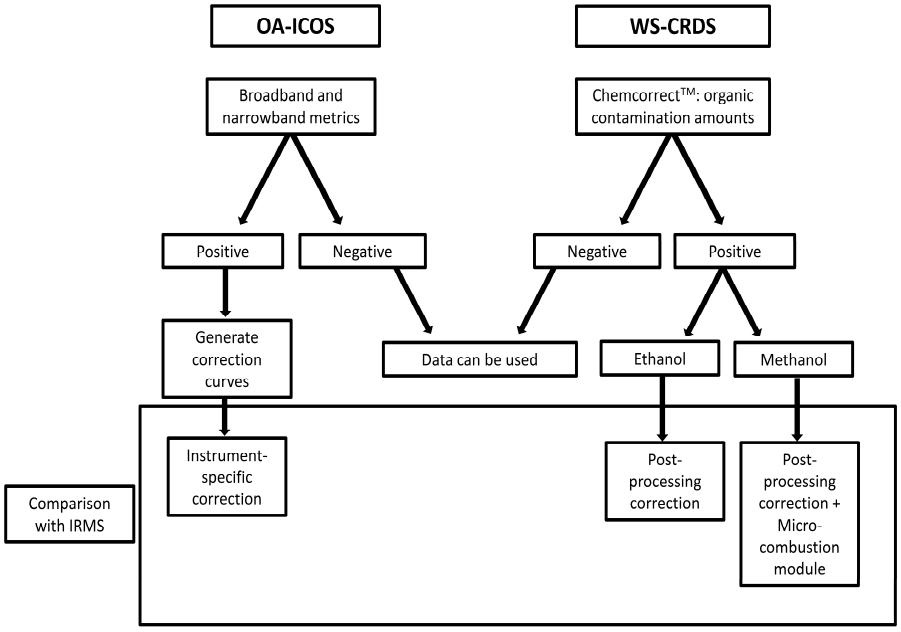

Figure 6. Decision-making scheme for the isotopic determination of plant and soil water samples. For the two main laser-based instruments. After the positive detection of organic contamination by the corresponding post-processing softwares, the comparison of a subset of samples with IRMS results is always mandatory.

5. IDENTIFYING PLANT-WATER SOURCES AND QUANTIFYING THEIR RELATIVE CONTRIBUTIONS

The ultimate aim of the application of stable isotopes described here is to identify the sources of water for plants and how they change spatially, temporally and/or between species or between life forms, plant size and other factors. This identification is achieved by determining the isotopic composition of the source water and xylem samples collected simultaneously. Plant water sources can be identified graphically by plotting isotopic compositions in the dual-isotope space (Fig. 2) and finding the most similar source water for each xylem sample. This method, however, is usually not simple, nor quantitative, because plants may be simultaneously tapping several sources, and the isotopic composition of the xylem water will indicate a mixture of these sources. Xylem water samples would then not match any of the water sources but would lie at the barycenter of their relative contributions. Early studies that used only one water isotope based their identification of plant-water sources on the statistical differences between the isotopic composition of xylem water and the various sources tested (Dawson and Ehleringer, 1991). Conclusions drawn from studies using a dual-isotope approach, however, can differ slightly (Bowling et al., 2017), because samples with the same δ^2H signal may not share the same $\delta^{18}O$ signal. Statistical differences may thus not be adequate if plants are assumed to take up water from more than two sources, which is very likely for deep rooting systems. Various numerical approaches have been developed for these (rather common) cases to estimate the relative contribution of each potential source of plant water. Exhaustive descriptions and comparisons have been provided (Phillips and Gregg, 2001; 2003; Parnell et al., 2013; Ogle et al., 2014; Rothfuss and Javaux, 2016), so we provide here only a synthesis of the most used approaches.

End-member mixing analyses were the first models to be applied to identify plant-water sources (White et al., 1985). These models assume that roots extract water from two sources without isotopic fractionation and that the water mixes completely within the xylem (Rothfuss and Javaux, 2016). Likewise, the contributions of sources *a* and *b* to xylem isotopic composition (δ_p) is expressed as:

$$\delta_p = f_a \, \delta_a + f_b \, \delta_b$$

where δ_a and δ_b are the isotopic compositions of sources *a* and *b*, and *f* is the proportion in volume of water extracted from each source ($f_a + f_b = 1$). This equation can be applied to isotopic compositions with only one isotopologue, and the error associated with the estimation of *x* can also be calculated (Phillips and Gregg, 2001; Rothfuss and Javaux, 2016). The equation, however, can be expanded by adding the other isotopologue (Brunel et al., 1995).

Phillips and Gregg (2003) developed the program *IsoSource* based on a standard linear mixing model for obtaining a combination of source contributions that conserves mass balances for a system of two isotopes (in studies of plant-water sources). Equation (a) will not have a unique solution if a third water source is added, so the program iteratively creates each possible combination of solutions, the predicted isotopic signatures for each combination is then calculated and the predicted mixture signatures are compared with the observed mixing signatures (Phillips and Gregg, 2003). *IsoSource* then provides all feasible source contributions with histograms for each source with the most likely source contribution. *IsoSource* has been extensively used in studies of plant-water sources, but more recent Bayesian mixing models have gained popularity in recent years.

SIAR (Parnell et al., 2010) and *MixSIR* (Moore and Semmens, 2008) *Bayesian isotope mixing models* have been developed more recently, providing statistical uncertainties associated with the estimates of source contribution and an optimal solution rather than a range of feasible solutions (Rothfuss and Javaux, 2016). These two models have been merged into *MixSIAR* (Parnell et al., 2013), which is implemented by an R package (R Core Development Team, 2012). The user prepares three data sets, one with the mean $\square^{18}O$ and $\square^{2}H$ signals and corresponding standard deviations of all potential sources, another with $\square^{18}O$ and $\square^{2}H$ signals of the plants (grouped or not) and the third specifying an "enrichment factor", which should be set to 0 assuming no fractionation during the uptake of water by roots (see section 2). The model uses Monte-Carlo Markov chains to produce a posterior distribution of the relative contribution of each water source. Histograms of the posterior distributions are plotted, allowing a visual representation of the results. The median of the posterior distribution, i.e. the most frequent result of the model, and the corresponding confidence intervals can then be calculated at individual, plot, species or site levels.

End-member and Bayesian mixing models have different routines but require similar data inputs (isotopic compositions of sources and plants). *Bayesian process-based mixing models* (Ogle et al., 2014) also allow the incorporation of prior information about the system (rooting depth, profiles of root density, soil moisture or plant-water potentials) and represent a promising method to improve the precision of plant-sourcing studies. The implementation of process-based models has the advantage of avoiding false positives under some conditions. For example, if the upper soil layers contain too little water to be extracted by roots, a simple linear model would still try to calculate the water's source contribution if it is set as a potential source. In contrast, process-based models can specify that water is not extractable below a certain level of soil moisture, or where roots are not present if information for root profiles was added. The specification of these models, however, is more complex than for *MixSIAR* and *IsoSource*. The additional measurements that should be carried out in the field also require correctly incorporating and specifying this information into a model before it is run, which may require some training in hierarchical Bayesian modeling.

A recent comparative study of simulated data for the uptake of water by roots (Rothfuss and Javaux, 2016) has demonstrated that Bayesian mixing models (Parnell et al., 2010; 2013) perform better than *IsoSource* and previous models (Brunel et al., 1995), but only when the potential sources matched the actual plant-water sources. As mentioned above, the inclusion of sources not accessed by plants and the exclusion of some accessed sources can produce misleading results. In summary, we recommend applying Bayesian mixing models (such as *MixSIAR*) for estimating source contributions for plants. Bayesian process-based models (Ogle et al., 2004; 2014) are a better option if higher precision is required, because they can integrate other ecological data, but are more time-consuming.

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