X Water Worlds and how to investigate them: A review and future perspective on *in situ* measurements of water stable isotopes in soils and plants

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Abstract. The number of ecohydrological studies involving water stable isotopes has been increasing steadily due to technological (i.e. field deployable laser spectroscopy and cheaper instruments) and methodological (i.e. tracer approaches or improvements in root water uptake models) advances in recent years. This enables researchers from a broad scientific background to incorporate water isotope-based methods into their studies.

Several isotope effects are currently not fully understood, but essential when investigating root water uptake depths of vegetation and disentangle isotope processes at the soil-vegetation-atmosphere continuum. In particular different viewpoints exist on i) extraction methods for soil and plant water and methodological artefacts potentially introduced by them; ii) the pools of water measured with those methods and iii) spatiotemporal issues related with water stable isotope research.

In situ methods have been proposed as an innovative and necessary way to address these issues and are required in order to disentangle isotope effects and take them into account when studying root water uptake depths of plants and for studying soil-plant-atmosphere interaction based on water stable isotopes.

Herein, we review the current status of *in situ* measurements of water stable isotopes in soils and plants, point out current issues and highlight potential for future research. Moreover, we put a strong focus and incorporate practical aspects into this review. Finally, we propose an integrated methodology for measuring both soil and plant water isotopes when carrying out studies at the soil-vegetation-atmosphere interface.

For all *in situ* methods, extreme care needs to be taken particularly during set-up in order to obtain reliable data. *In situ* methods for soils are well established. For transpiration, reliable methods also exist but are not common in ecohydrological field studies due to the required effort. Little attention has been payed to *in situ* xylem water isotope measurements. Research needs to focus on improving and further developing those methods.

There is a need for a consistent and combined (soils and plants) methodology for ecohydrological studies. Such systems should be designed and adapted to the environment to be studied. We further conclude that many studies currently might not rely on

in situ methods extensively because of the technical difficulty. Hence, future research needs to aim on developing a simplified approach that provides a reasonable trade-off between practicability and precision/accuracy.

1 Introduction

Since the presentation of the heavily debated 'two water worlds hypothesis' (McDonnell, 2014) the attention of many
35   ecohydrologists – especially those working with water isotopes – has been focussing on what was termed as 'ecohydrological separation'. In the original hypothesis, the authors claim that based on the studies of Brooks et al. (2010) and Goldsmith et al. (2012) plants in some watersheds prefer water which is 'more difficult' for them to access (i.e. soil water with relatively higher matric potential) over 'easier' accessible water resources (i.e. stream water).

The discussion remains controversial, with a number of criticism. Sprenger et al. (2016), for instance, offer a simple and logic
40   explanation for 'ecohydrological separation': "… subsequent mixing of the evaporated soil water with nonfractionated precipitation water could explain the differences in the isotopic signal of water in the top soil and in the xylem of plants on the one hand and groundwater and streamwater on the other hand" (also refer to Fig. 8 in Sprenger et al., 2016). Hence, the authors question "…if ecohydrological separation is actually taking part or if instead the soil water undergoes isotopic changes over space (e.g., depth) and time (e.g., seasonality) leading to distinct isotopic signals between the top soil and subsoil, which will
45   directly affect the isotopic signal of the root water." (Sprenger et al., 2016). Furthermore, plant physiological (rooting depth, water potential of plants) and other aspects (e.g. nutrient availability) were completely neglected in the theory (which the authors themselves admit, McDonnell, 2014). Dubbert, & Werner (2019) state that isotopic differences between soil,plant and groundwater can be fully explained by spatio-temporal dynamics.

Nevertheless – whether one agrees with the theory or not – the hypothesis had a significant impact in terms of i) questioning
50   the comparability of ecohydrological studies because of methodological artifacts (e.g. mobile vs. bound soil water, soil and plant water extraction methods, organic contamination), ii) testing existing and developing novel methods to investigate fundamental processes at the soil-vegetation-atmosphere interface in an integrated manner; and finally, iii) questioning a number of concepts that have been applied since many years but now appear in a new light (e.g. root water uptake studies and the incorporation of isotope effects).

55   Consequently, many researchers have been focussing on these issues since and a number of publications have been pointing out current limitations and ways forward (Berry et al., 2017; Bowling et al., 2017; Brantley et al., 2017; Dubbert et al., 2019b; Penna et al., 2018; Sprenger et al., 2016). One of the most pressing issues identified is the establishment of a consistent, homogenized method for the analysis of water stable isotopes allowing for a solid analysis and interpretation of water isotopes in soils and plants and compare them with each other. Due to partially striking differences in isotopic compositions returned
60   by different extraction methods (Millar et al., 2018; Orlowski et al., 2018b, 2018a), this seems not an easy task. Even the (until recently) commonly accepted cryogenic vaccuum extraction (Koeniger et al., 2011) is being questioned frequently. On the other hand, novel methods based on isotopic equilibrium fractionation (e.g. Hendry, Schmeling, Wassenaar, Barbour, & Pratt,

2015), outperform the extraction methods under certain conditions (Millar et al., 2018). *As a consequence, a big question arises: Are all source water studies wrong?*

1.1 Are ecohydrological source water studies biased? – The need for *in situ* methods

Certainly, not all source water studies are biased. Despite all the controversy on methodological aspects, the systematic evaluations carried out in recent years (Gaj et al., 2017; Millar et al., 2018; N. Orlowski, Frede, Brüggemann, & Breuer, 2013; N. Orlowski et al., 2018; Natalie Orlowski, Breuer, & McDonnell, 2016; Natalie Orlowski, Pratt, & McDonnell, 2016; Thoma, Frentress, Tagliavini, & Scandellari, 2018) it can be stated that in i) soils that contain a high portion of sand (low portion of clay), ii) studies using isotopically labelled tracers (^2H_2O, $H_2^{18}O$), and iii) environments without water stress (low suction tension) the chance of methodological artefacts and the influence of additional isotope effects is the least. In terms of measuring devices, mass spectroscopy remains the standard and most reliable method (West et al., 2010a, 2011).

But, as suggested by recent research, there are a number of isotope effects that clearly complicate the idealized situation where one takes a xylem sample from a tree (=unfractionated mixture of all water sources) in addition to a soil profile (and perhaps groundwater) and subsequently can determine root water uptake depths. An updated view of the isotope effects potentially affecting water sources and consumers, depicted in Fig.1, emphasizes the sheer complexity that now is questioning many water-uptake studies.

Figure 1

In addition to the isotope effects summarized in Fig.1, there might be methodological alteration of water isotope values caused by different extraction methods extracting different water pools and organic contamination causing an offset of isotope values when measured with laser spectroscopy (e.g. Barbeta et al., 2019; Martín-Gómez et al., 2015; Orlowski et al., 2016a).

One of the most important steps - and this is where the community seems to agree (Brantley et al., 2017; Dubbert et al., 2019b; Sprenger et al., 2016; Stumpp et al., 2018; Werner and Dubbert, 2016) - to investigate, disentangle, quantify and incorporate those isotope effects is by increasing the spatiotemporal resolution of water isotope measurements at the soil-vegetation-atmopshere interface and to integrate measurements of sources and consumers into one framework. In other words, we need combined *in situ* systems for measuring both soil and xylem water isotopes in a higher spatiotemporal resolution in order to achieve an integrated analysis of soils and plants using a the same methodology and ultimately, measuring the same water pools (Sprenger et al., 2016). While it might be possible to achieve a high temporal resolution by destructive sampling, a number of disadvantages are associated with that: For instance, the experimental plot is disturbed multiple times, small-scale heterogeneity might bias the outcomes and longer-term studies in a high temporal resolution are basically impossible. For plants, a high frequency of destructive sampling might harm and even kil lthe plant. Lastly, time and costs are associated with destructive sampling and over longer periods this adds up to larger sample amounts. Hence, *in situ* methods are essential for the detection, analysis and interpretation of related isotope effects (see Fig.1).

This review aims on summarizing recent advances in *in situ* water isotope measurement techniques of for soils (depth-dependent and bulk soil) and plants (xylem and transpiration via leaf chambers). We begin with an overview of *in situ* studies

in the compartments soils and vegetation. From thereon, we focus in separate chapters on main issues emerging from the existing sudies, namely i) materials and measurement systems, ii) calibration, standardization and validation and iii) comparability with water extraction studies and measurement of natural abundances of water isotopes. We then conclude and propose ways forward in terms of a combined approach for a consistent, integrated method in order to study processes at the
100   soil-vegetation-atmosphere interface.

2 Review: *In situ* approaches for measuring soil and plant water stable isotopes

2.1 *In situ* soil water isotope depth profiles

A number of early semi- *in situ* studies (pre laser-spectroscopy) exist, where researchers tried to collect soil water vapour. For the sake of completeness and acknowledging these pioneering efforts, those will be summarized briefly. Thoma et al. (1979)
105   directed water from up to 25m through a molecular sieve, vacuum extracted this water and determined δ^2H. The determined isotope ratios agreed well with samples extracted from the soil core. With the similar technique, Saxena and Dressie (1984) analysed δ^{18}O from soil water vapor in profiles up to 4m depth. Allison et al. (1987) extracted soil water vapor in glass jars and extracted it using dry ice and an alcohol bath. Though the shape of the isotope depth profiles for δ^{18}O and δ^2H showed a similar shape, the values did not match with those obtained by cryogenic extraction. Izbicki et al. (2000) used a similar
110   technique and achieved a better agreement compared to distilled core samples.

With the introduction of laser spectroscopy, rapid progress in terms of field deployable systems started. While a gas permeable membrane system was introduced by Munksgaard, Wurster, & Bird (2011) for liquid water samples, the first reported *in situ* measurement in soils was reported by Herbstritt, Gralher, & Weiler (2012). A microporous hydrophobic membrane contactor was combined with an isotope laser spectrometer system and tested for both liquid and soil water isotopes with a soil column
115   experiment. Though not strictly measuring soil water isotopes, this study created the foundation for the membrane-based techniques, which are the method-of-choice at present. The concepts tested in Herbstritt et al. (2012) therefore can be seen as a baseline for all subsequent *in situ* soil water isotope studies. The authors stated that *'in order to be able to observe real-time processes in the liquid phase, one needs to continuously transfer the isotopic signal from liquid water to the vapor phase'* (Herbstritt et al., 2012). They first tested the applicability of the gas permeable membrane with liquid water samples,
120   determined fractionation factors and isotopic equilibrium fractionation factors for a range of temperatures. The latter, being a crucial for any liquid-vapor equilibrium-based isotope measurement, were determined by fitting the empirical factors *a, b and c* to the type-1 model of Clark & Fritz (1997, Eq.1):

$$\alpha = exp^{\frac{a\left(\frac{10^6}{T_k^2}\right)+b\left(\frac{10^3}{T_k}\right)+c}{1000}} \tag{1}$$

where α is the isotopic fractionation factor, T_k is the temperature (in K), and *a, b,* and *c* are empirical parameters. Membrane-
125   induced deviations from Majoube's (1971) prediction ranging from 0.27% to 0.64% for δ^{18}O and from 1.0% to 3.9% for δ^2H

(Majoube, 1971) were reported. In addition, a vapor concentration correction similar to Schmidt et al. (2010) was conducted and recommended by Herbstritt et al. (2012).

In the same year, Soderberg, Good, Wang, & Caylor (2012) presented the first 'real' (i.e. measured in the field) *in situ* data set from a semiarid environment. The authors aimed at investigating the validity of the Craig-and-Gordon (CG) model and
130   incorporating the effect of soil water potential on kinetic fractionation into the model, which they argue improves the model fit for very dry conditions. Their dataset was tested on a single profile of *in situ* measured soil water isotope data in a semiarid environment in Kenya. Soil air was drawn from several depths (5, 10, 20, and 30 cm) and directed to the laser water vapor isotope analyser via buried Teflon tubing, with the final 10 cm of each tube perforated and packed with glass wool. Each depth was measured for 90 s. Soil temperature and soil water potential were measured at several depths. Though the *in situ* field data
135   does not match with the model predicted CG data, the authors support the assumption of liquid–vapor isotopic equilibrium and propose a correction factor to account for dry soils in order to normalize 'free atmosphere' humidity to the soil evaporation. Most likely, the non-matching isotope values reported in the study were due to the dilution with a high amount of ambient air (400 ml/min) causing a contamination of the drawn soil air.

Volkmann & Weiler (2014) presented an automated system for non-destructive, high-resolution monitoring of soil water
140   isotopes. The authors developed specific probes for the purpose of sampling soil water vapor. The main elements of these probes are a microporous membrane (Porex, Aachen, Germany), a mixing chamber and a sampling, dilution and – optional – a throughflow line. The principle of operating the probes is based on drawing soil water vapor into the water isotope analyzer via the sampling line (30 – 35 ml/min). The system can be operated in two modes, advection-dilution sampling (ADS) and diffusion-dilution sampling (DDS). In ADS mode, air is simply drawn into the sampling line, and dry air supplied at a lower
145   rate via the dilution line, which causes i) soil water vapor to actively move into the tube (because of the slightly lower pressure inside the probe) and ii) lowering of the water vapor concentration of the whole system. In DDS mode, a throughflow line ending at the lower end (tip) of the probe is added to the system. This throughflow line allows to supply dry gas (N_2) to the system at a rate that is the difference of water drawn by the sampling line and supplied by the dilution line. Hence, the pressure difference between soil water vapor outside and inside the probe diminishes, and isotopic exchange occurs only via diffusion.
150   Volkmann & Weiler (2014) were also the first to present validated natural isotope soil depth profiles (i.e. via bag equilibration; Hendry et al., 2015; Wassenaar, Hendry, Chostner, & Lis, 2008). An acceptable agreement was achieved with their system. For the soil depth profiles, the authors report 95% limits of agreement of +1 ‰ (upper) and -1 ‰ (lower) for $\delta^{18}O$ and +6 ‰ (upper) and -6 ‰ (lower) for δ^2H, both for ADS and DDS sampling methods compared to destructive laboratory-based isotope measurements. The range of measured isotope values for $\delta^{18}O$ and δ^2H was further in the range of antecedent rainfall isotope
155   ratios.

Rothfuss, Vereecken, & Brüggemann (2013) and Rothfuss et al. (2015) tested precision and accuracy of membrane-based *in situ* in laboratory experiments. Rothfuss et al. (2013) set up an airtight acrylic vessel filled with fine sand, where a custom-made throughflow system with a gas-permeable polypropylene membrane was installed. Synthetic dry air was directed through the system, which during the passage would equilibrate with the water isotopic composition of the surrounding sand. The

160   authors used a mass flow controller to subsequently dilute the sample's water vapor concentration to 17.000 ppm, which eliminates the effect of water concentration dependence (Schmidt et al., 2010). They further investigated the effects of soil temperature, sand water content and dry air flow rate as well as fast changes of isotope ratios on $\delta^2 H_{vap}$ and $\delta^{18}O_{vap}$. Although the measured sand $\delta^2 H_{vap}$ showed a significant enrichment relative to those determined at equilibrium according to (Majoube, 1971) at all tested temperatures, this could be linearly corrected for. Rothfuss et al. (2015) proofed that the gas permeable

165   membranes are capable of delivering reliable isotope data over long time periods under laboratory conditions (in the experiment 290 days). The same group presented another study, where they measured both soil water and atmospheric oxygen isotopologues in carbon dioxide with their membrane-based system (Gangi et al., 2015). It was shown again that the PP membranes used (Accurel PP V8/2HF, Membrana GmbH; 0.2-µm porosity, 0.155 cm wall thickness, 0.55 cm inner diameter, 0.86 cm outer diameter) did not lead to any isotopic fractionation and was suitable for combined measurements of δ^{18}. The

170   experimental results were further modelled using MuSICA (Ogee et al., 2003). The authors proofed that it is possible to simultaneously study oxygen isotope exchange between soil water and CO_2 in natural soils which has an immense potential for constraining the atmospheric CO_2 budget. However, they state explicitly that further testing of the method is required.

The first *in situ* study in a semi-arid environment was published by Gaj et al. (2016). Inspired by the system of Volkmann & Weiler (2014), this study represents a proof-of-concept. In northern Namibia, the authors used commercially available

175   polypropylene membranes (BGL-30, Umweltmesssysteme, Munich) and automated their system for measuring of soil water isotope-depth profiles up to 50 cm depth over multiple campaigns, different land covers (bare soil vs. vegetated) and different climatic conditions (dry and post-rain event) . Further, they are the first to study spatiotemporal differences in isotope depth-profiles with their *in situ* system.The profiles were compared to those obtained by cryogenic vaccuum distillation. While the shape of the isotope depth-profiles were in agreement and the precision of the *in situ* approach was good (0.8 and 2.5‰ for

180   $\delta^{18}O$ and $\delta^2 H$, respectively), there were partially large differences between the *in situ* data and the results based on cryogenic distillation: Between 15 and 50 cm, the RMSE was 3.9‰ for $\delta^{18}O$ and 9.2‰ for $\delta^2 H$. For the shallow depths RMSE was as high as 7.0 ‰ for $\delta^{18}O$ and 43.4‰ for $\delta^2 H$. Gaj et al. (2016) interpret these differences potentially caused by an incomplete soil water extraction, the time-lag between sampling soil water vapor and destructive sampling (day vs. night) , rayleigh fractionation caused by the uptake of air from the *in situ* measurement, natural processes (e.g. hydraulic redistribution), or

185   natural heterogeneity. From today's perspective (author personal comment), it seems very likely that depleted atmospheric water vapor was drawn into the system for the upper soil depths causing the depletion of the *in situ* measurements. Further, condensation inside of the capillaries or contamination with organic substances could explain the observed differences. An important finding of this study is that for the medium sand the authors studied, the standard deviation of for $\delta^{18}O$ and $\delta^2 H$ was inversely correlated to the soil water content, i.e. the lower the water content of the soil, the higher the standard deviation and

190   viceversa.

A study on the effects of materials and methods for *in situ* water isotope measurements was presented by Pratt, Lu, Lee Barbour, & Jim Hendry (2016). While the first part deals with the optimization of the vapor bag sampling method (bag type, tubing, relative humidity) and is not scope of this review, the authors also compared *in situ* analyzed soil water vapor from

depths of up to 180 m. The already existing HDPE tube of two waste sites studied were screened with a 50 mm stainless steel

195   mesh filter and the vapor drawn into the isotope analyzer. The results of the *in situ* part of the study show partially large differences for depth profiles when comparing the field measurements with the ones obtained by the bag sampling method (up to ±30‰ δ^2H and ±10‰ $\delta^{18}O$). In some depths, the agreement is better (±5‰ δ^2H and ±1‰ $\delta^{18}O$). Pratt et al. (2016) conclude that the *in situ* results of their study suggest that establishing natural, stable water isotope depth profiles for pore water in thick unsaturated mine waste is challenging.

200   Another group from the United States developed a system for *in situ* measurements of soils and has applied this methodology since in several studies (Oerter et al., 2017, 2019; Oerter and Bowen, 2017, 2019). Their system – up to date – probably constitutes the most complete in terms of field deployability, calibration and the results reflect that (in particular see Oerter et al., 2017). The authors further present a novel approach for correcting their samples by including water and clay content (see chapter calibration). In a primer, Oerter et al. (2017) used a vapor-permeable membrane technique and measured soil water

205   isotopes *in situ* at four sites in North America and validated the water vapor probe method with a vapor direct equilibration method, and vacuum extraction with liquid water analysis. The authors found that the accuracy of the three compared methods in their study is equivalent, with increased ease of use in its application, and sample throughput rates of 7 samples per hour by using the vapor probes. In fact, RMSE of the vapor probe method for δ^2H_{liq} values is lower than for direct vapor equilibration (bag sampling method) in matching the δ^2H_{liq} values of vacuum-extracted soil water (1.7‰ for δ^2H_{liq} values and 0.62‰ for

210   $\delta^{18}O_{liq}$). Hence, trueness for the vapor probe method in their application was greater than for the bag equilibration method. The analyzed profiles were used to investigate the effect of soil texture and pedogenic soil horizons control the shape of the isotope profiles, which are reflective of local evaporation conditions in the soils.

2.2 Soil and plant chambers for measuring Evaporation (δE) and Transpiration (δT)

2.2.1 Soil chambers for measuring Evaporation water isotopes (δE)

215   Isotopic signatures of evaporated soil water vapor ($\delta^{18}O_E$) can equally be measured *in situ*, using laser spectrometers coupled to chamber systems. There are both flow-through steady-state (Dubbert et al., 2013) and closed chamber systems (Keeling, 1958) plot approach, see Wang et al., 2013). In a closed chamber the amount of water vapor will, upon closure of the chamber, increase over time, while the isotopic composition of water vapor will change due to the ongoing input of evaporated vapor. This method was first applied to measure isotopic signatures of respired CO2 (Keeling, 1958 and later adapted to determine

220   the isotopic signature of water vapor (evaporation or also evapotranspiration, see Walker and Brunel, 1990). The Keeling (1958) plot approach is based on two assumptions. First, the isotopic compositions of the source and background air are constant over the measuring period. Second, there is no loss of water vapor from the ecosystem, e.g. during dewfall despite by turbulent mixing. In an open chamber system, ingoing (ambient background) and outgoing (mixed air inside the chamber) air are measured alternately and the isotopic signature of evaporation (or transpiration or evapotranspiration) can be calculated by

225   a mass-balance equation (von Caemmerer and Farquhar, 1981).

Isotopic signatures of soil evaporated water vapor have been predominantly used to achieve better understanding of the dynamics of hydrological processes (Braud et al., 2005b, 2005a, 2009b, 2009a; Haverd et al., 2011) and mainly to partition ecosystem water fluxes into their components: soil evaporation and plant transpiration (e.g. Dubbert et al., 2014b, 2013; Haverd et al., 2011; Rothfuss et al., 2012, 2010; Williams et al., 2004; Yepez et al., 2007, 2003). Prior to the development of laser

230 spectroscopy there were few studies measuring $\delta^{18}O_E$ directly using cold trapping methods under controlled conditions and - to the best of our knowledge - no observations whatsoever under natural conditions. Instead, researchers relied on the Craig and Gordon model, predicting the isotopic signature of evaporated vapor based on isotopic signatures of source (=soil) water isotopic signatures (e.g. Brunel et al., 1997; Wang and Yakir, 2000).

Therefore, first approaches to combine soil gas-exchange chambers and laser spectrometers, concentrated on conducting

235 sensitivity analysis of the Craig and Gordon model towards its input parameter (relative humidity, soil temperature, soil water isotopic signature, soil water content and atmospheric vapor isotopic signature (Braud et al., 2005b, 2005a, 2009b, 2009a; Dubbert et al., 2013; Haverd et al., 2011; Rothfuss et al., 2010, 2012). Conclusively, the correct estimation of the evaporating front is particularly important. Usually the soil layer with the highest isotopic composition is associated with the location of the evaporation front but therefore the spatial resolution of the soil layers should be as small as possible (preferably layer

240 thickness 2 cm or smaller). Sensitivity analysis revealed that precise parameterization of the environmental conditions at the evaporating front, which may diverge tremendously within a few cm of soil depth (up to 8 ‰ from the soil surface to 5 cm depth), is pivotal for correct predictions of $\delta^{18}O_E$ (Dubbert et al., 2013), particularly in arid regions, where dry periods without any precipitation can last several months and the evaporating front can be located in deeper soil layers (Dubbert et al., 2013; Gaj et al., 2016). This does not only highlight the value of direct *in situ* estimates of $\delta^{18}O_E$, but also the deployment of *in situ*

245 soil water isotope measurement set ups when using the Craig and Gordon model. Only spatially highly resolved continuous *in situ* soil water observations meet the desired requirements necessary to resolve the evaporating front.

Equally important for a correct estimation of $\delta^{18}O_E$ is the choice of formulation for the kinetic fractionation factor α_K. Braud et al. (2009a) modelled α_K-values during long-term laboratory experiments using SiSPAT-Isotope (soil vegetation atmosphere model) and found α_K -values to decrease with soil drying. This is in contrast to the findings of Mathieu and Bariac (1996) who

250 derived an empiric model predicting α_K -values to increase from saturated to unsaturated soil conditions. Similar results were obtained from Rothfuss et al. (2015) during a long-term soil column laboratory experiment. Dubbert et al., (2013) achieved a very good predictability of *in situ* measured $\delta^{18}O_E$ in the field using the Craig and model and the formulation for α_K proposed by Mathieu and Bariac (1996) for bare soil and soils with root ingrowth over an entire growing season.

In any case, direct *in situ* measurements of soil evaporation are mostly limited to laboratory studies conducting sensitivity

255 analysis of the Craig and Gordon model and its input parameters (for a recent paper see Quade et al., 2018). It is often not technically possible to observe $\delta^{18}O_E$ of undisturbed vegetated soil in the field. However, the isotopic signature of evaporated vapor from bare soil patches differs significantly from the isotopic signatures of evaporation from soil with vegetation cover (particularly in grasslands, see Dubbert et al., 2013). Therefore, even today in ecosystem studies aiming i.e. at partitioning evapotranspiration the isotopic signature of evaporation is mostly modelled using the Craig and Gordon equation.

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2.2.2 Plant chambers for measuring Transpiration water isotopes (δT)

Direct estimation of stable isotopic signature of plant water fluxes ($\delta^{18}O_T$) has been difficult prior to the development of laser spectroscopy. Nevertheless, some studies used cold trapping methods to estimate isotopic signatures of plant transpiration (e.g. Harwood et al., 1998) via gas exchange systems and in particular ecosystem evapotranspiration (e.g. Yepez et al., 2007, 2003)
265 via keeling plots (Keeling, 1958). The main limitation of these early studies – similar to those for soil water isotopes – was the spatiotemporal resolution. With the advent of laser technology, studies multiplied coupling laser spectrometer to gas-exchange systems of different scales (i.e leaf to canopy level) to assess the isotopic signature of ecosystem water fluxes (i.e. transpiration, evaporation and evapotranspiration, see Wang et al. (2012) for the first *in situ* observation of isotopic signatures of plant transpiration). Nowadays, open chamber systems are predominantly used to measure the isotopic signature of transpiration *in*
270 *situ* (see chapter soil chambers).

In situ observations of isotopic signatures of transpiration had an immense impact on ecosystem partitioning studies, as they have the advantage of directly measuring the transpiration signature, while destructive sampling techniques observe xylem or leaf isotopic signatures, essentially involving a modelling step to obtain $\delta^{18}O_T$. A number of ecosystem partitioning studies (e.g., Griffis et al., 2010; Williams et al., 2004; Yepez et al., 2003) even simplified by assuming isotopic steady-state (isotope
275 values of xylem=transpiration), although there is growing evidence that plants rarely reach isotopic steady-state throughout the day (Dubbert et al., 2014a, 2017; Simonin et al., 2013). Therefore, assuming isotopic steady-state for the purpose of ET partitioning will largely depend on the desired temporal scale (considering non steady-state definitely necessary at sub-diurnal to diurnal scale but unimportant at larger time scales, i.e. weeks or months). In case non steady-state is likely to occur, $\delta^{18}O$ of transpiration can be modeled using a Dongmann style version of the Craig and Gordon equation (Dongmann et al., 1974).
280 However, this complicates the partitioning approach tremendously in comparison to direct chamber measurements of transpiration water isotope values, as a large number of additional observations are necessary (in particular, stomatal conductance and Transpiration amounts). Another important consideration in regard to the method of choice (*in situ* transpiration measurements vs. modelling) is the possibility to sample unfractionated xylem isotopic signatures. For example, herbaceous and grass or agricultural species do not have suberized stems and destructive sampling would rely on leaf water
285 sampling or sampling the plant culm belowground, which is highly destructive and not possible on normal plot sizes. Moreover, while the majority of studies still provide evidence for an unfractionated uptake and transport of xylem water through plants, there is growing evidence of fractionation of xylem water during times of limited transpiration rate (drought conditions, for deciduous species, see e.g. Martin-Gomez et al. 2017).

Similar to *in situ* soil evaporation isotope observations, *in situ* observations of isotopic signatures of transpiration have further
290 been used to advance our understanding on water isotope fractionation (e.g. Dubbert et al., 2017; Piayda et al., 2017; Simonin et al., 2013; Song et al., 2015a, 2015b, 2013). In particular, the leaf water turn-over time has gained research focus. The leaf water turn-over time can effectively be described by stomatal conductance and leaf water volume, is extremely species-specific

spanning from several minutes to several hours (Song et al., 2015a). As the leaf water turn-over time describes the necessary time for a leaf to reach isotopic steady-state (see Simonin et al., 2013; Song et al., 2015a), isotopic steady-state can either be
295 observed for large parts of the day (e.g. in many herbaceous species) or not at all (e.g. in plant species strongly controlling their stomatal conductance, see Dubbert et al., 2017, 2014a for an overview).

Direct *in situ* estimates of transpiration isotopes have also been used to derive root water uptake proportions (Kühnhammer et al., 2019a; Volkmann et al., 2016b) by assuming isotopic steady state and substituting $\delta^{18}O_X$ (xylem) with $\delta^{18}O_T$. However, given the often very likely violation of steady state assumptions under natural field conditions, this can be recommended only
300 under very tightly controlled laboratory conditions and knowing the leaf water turn-over time.

2.3 *In situ* measurements of plant xylem water isotopes

For the direct measurement of plant xylem water isotopes, only two studies are reported up to date. Volkmann et al. (2016) present field observations of xylem water isotope ratios of two adult field maple trees (*Acer campestre L.*) obtained over several days during a labelled irrigation event using and laser spectroscopy. The obtained *in situ* data was compared against results
305 from destructive sampling with cryogenic distillation and mass spectrometric analysis. Similar to their *in situ* soil measurements, (Volkmann et al., 2016a) used the same membrane system to infer the isotope ratios of xylem water. Several holes were drilled into each of the target trees and the gas permeable membranes inserted into those. In order to prevent the intrusion of atmospheric air the outside was sealed with silicone glue. Similar to the soil studies, dry gas (here N_2) is provided by a throughflow line and directed to the laser spectrometer via the suction of its vacuum pump.
310 **Figure 2**

With the obtained data Volkmann et al. (2016) demonstrated that temporal changes as well as spatial patterns of integration in xylem water isotope composition can be resolved through direct measurement. In both studied trees, diurnal cycles of xylem water isotopes were found. However, the authors could not prove whether this is a true diurnal cycle or introduced through imperfect accounting for temperature-dependent liquid–vapour fractionation at the probe interface. The authors achieved a
315 median precision of 1.1‰ for δ^2H and 0.29‰ for $\delta^{18}O$ values (1σ) for an integration period of 120 s. When comparing the *in situ* measured xylem isotopes with the results obtained by destructive sampling (measured with mass spectrometer) a significant correlation was found for both water isotopes ($\delta^2H_{IRIS}=1.26\times\delta^2H_{IRMS}+14.51$, $r^2=0.86$, P<0.0001, $\delta^{18}O_{IRIS}=0.91\times\delta^{18}O_{IRMS}-4.87$, $r^2=0.46$, P<0.001, robust BSquare-weighted M-regression). However, when taking a closer look at the agreement of *in situ* and destructive data, partially high uncertainties are apparent (see Fig.2, reprinted with permission).
320 In addition, the uncertainty (especially of the *in situ* data) is up to 20 permille for δ^2H and up to 3 permille for $\delta^{18}O$.
Marshall et al. (2019) tested an alternative method for monitoring the isotope values of tree xylem and showed that both natural abundances and highly enriched isotope values (labelling experiment) can be monitored over more than two months *in situ*. Their approach is based on drilling a hole (which the authors refer to as 'stem borehole') laterally through the complete trunk of a tree and connecting both ends with tight fittings to the CRDS-manifold system. The temperatures within the boreholes
325 were monitored using thermocouples and later used for vapor-liquid conversion of the isotope data. The authors tested their

system on two occasions on pine trees: i) in a cut-stem experiment and ii) in a whole-root experiment. They further developed a model to test the feasibility and limits of the borehole method. This included the estimation of the time constants for diffusion of water vapor to and from the borehole wall, and for the flowing airstream in the centre of the borehole (i.e. isotope exchange during the passage of air through the borehole) as well as the prediction of isotope values. For both experiments, Marshall et al. (2019) found close agreement of the source water isotope values provided for the trees, the ones measured in the 'stem boreholes' and the ones predicted by the model. In the cut-stem experiment, it took several hours after a change in water source before this agreement was reached. In the case of the intact-root experiment, it took almost two weeks until source and measured water isotope values agreed. In this experiment, the authors further tested equipping the tree with two stem boreholes. For the bottom borehole, the deviations to source water isotope values were nearly zero, meaning that the derived xylem water isotopic composition agreed with the source water values for both natural abundance ($\delta^{18}O$ = -0.1 $\pm$ 0.6 (SD) ‰, δ^2H = 1.8 $\pm$ 2.3 ‰) and the label phase ($\delta^{18}O$ = -0.25 $\pm$ 0.22 ‰, δ^2H = 0.09 $\pm$ 7.8 ‰). In contrast, the top borehole showed systematic deviations from source water values for both $\delta^{18}O$ and δ^2H. $\delta^{18}O$ xylem values were depleted in ^{18}O in relation to source water by -2.8 $\pm$ 1.5 ‰ and -3.9 $\pm$ 0.3 ‰ for the natural abundance and label phase, respectively. In contrast, δ^2H xylem values were enriched in ^2H as compared to source water by 5.3 $\pm$ 3.0 ‰ and 1.9 $\pm$ 8.5 ‰. Figure 3 shows the results from their intact-root experiment.

Figure 3

With the additional measures taken and the developed model, Marshall et al. (2019) proofed that this deviation was due to non-equilibrium conditions in the borehole due to the small diameter of the upper borehole (relative humidity was 98 $\pm$ 2 % for the bottom borehole and 88 $\pm$ 3 % for the top borehole).

Finally, the authors measured the sap flow velocity, which was 0.97 $\pm$ 0.4 cm/hr based on the sap probes (heat-ratio method). Comparing this to the sap flow estimates derived from the stem borehole isotope measurements yields 1.08 cm/hr on average, which is in very good agreement to the sap flow estimates.

In Supplement 1 information on all reviewed studies, details on the setup main findings as well as advantages and disadvantages of the applied methodologies are compiled.

3 Setup, Calibration and Validation of *in situ* measurements of soil and plant water isotopes

Apparent from the review of studies is that *in situ* measurements are still in development stage; hence, applied methods and approaches vary greatly. In this chapter, we pick out key aspects that need to be considered and propose a way towards more comparable and homogeneous setups. The biggest and most critical issues emerging from the existing studies are i) the materials and approaches used for sampling the air; ii) the calibration of the system iii) the avoidance of condensation and iv) how to validate the *in situ* data compared to other methods and how to interpret it best. We focus in this chapter on methods for obtaining *in situ* depth profiles of soil water isotopes and the measurement of xylem water isotopes due to the fact that

methods for monitoring bulk soil evaporation and transpiration at the leaf level have been reported previously in detail (Soderberg et al., 2012, Song et al., 2015).

3.1 Materials and approaches for sampling soil water vapor

360 Most of the reviewed studies used gas permeable membranes (e.g. Accurel PP V8/2HF, Membrana GmbH; 0.2-µm porosity, 0.155 cm wall thickness, 0.55 cm inner diameter, 0.86 cm outer diameter) with an inlet and outlet (e.g. Gaj et al., 2016; Oerter et al., 2017; Rothfuss et al., 2013; Volkmann and Weiler, 2014). Some groups built the probes themselves (Oerter et al., 2017; Rothfuss et al., 2013) others used factory-made probes (Gaj et al., 2016; Volkmann and Weiler, 2014). The important point is that with all of these membrane systems it was shown that no isotopic fractionation occurs due to the membranes; hence this

365 type of probes is suitable in general. Self-made membranes are much cheaper and can be adjusted to the application (i.e. length of exchange path, number and position of in- and outlets, size of capillary/tubing connected).

The number of in- and outlets of the probes depends on the measurement approach. In general, two of these exist: i) a pull-only system (e.g. Volkman & Weiler, 2014), where water is drawn simply through the gas-permeable membrane by the force of the vaccuum pump of the laser spectrometer. Such a system in fact requires only one capillary and thus is the simplest of

370 the setups. However, it should be considered that a notable amount of air is drawn from the media to be measured (soil/plant) and might cause a dry-out when applied extensively (e.g. measuring one soil depth all the time). This could be especially relvant for applications tree xylem as it might increase the risk of cavitation and hence, damage to the plant. The extracted volume of soil water vapor can be easily calculated by multiplying the flow rate with the measurement time. Because of this, most studies use ii) probes with two capillaries: one in- and one outlet (e.g. Oerter et al., 2017; Rothfuss et al., 2013). This

375 changes the approach drastically, because now dry air is now pushed through the inlet (via a dry gas supply) entering the membrane from one side and leaving it at outlet. During the passage of the dry air, water from the (saturated) soil air diffuses into the membrane and exchanges isotopically through the gas-permeable membrane. Ideally, saturation is reached while the air is passing through the membrane. Only in this case equilibrium fractionation can be assumed. With the push-through method, the system is under overpressure, which has two consequences: First, one needs to get rid of this overpressure before

380 it enters the laser spectrometer to avoid damage. This is commonly achieved by an access tube just before the analyzer inlet. Second, due to this overpressure, the chance of external air entering the stream is excluded, which is a clear advantage over the push-only method, where it needs to be assured that all connections are tight.

The pull-only system can also be operated with an additional inlet capillary/tube connected to a reservoir with drying agent. Doing so, atmospheric or dry air (via passage through a drying agent) is drawn into the gas permeable probe and equilibrated

385 therein during the passage. Flow rates, however, are not adjustable using this approach.

It needs to be carefully decided which approach to use and, ultimately, this depends on the application (e.g. tracer test, measuring natural abundances, long- vs. short-term measurements) A pull-only system is technically much easier to build, install and maintain and also cheaper, but it it is critical to avoid external air to enter the system at any of the connections. The push-through approach is more flexible and flow rates can be adjusted, but it requires more maintenance, connections (for

390   provision and control of dry air at the inlet), and valves. Due to the fact that the system is operated at overpressure, the risk of

drawing outside air (via non-tight connections) into the lines is minimized.

Figure 4 depicts a schematic of an in situ soil water isotope system (reprinted with permission from Oerter and Bowen 2017).

Figure 4

3.2 Saturation of water vapor, condensation and dilution

395   Condensation (or better: avoidance of it) is the most critical practical issue for all *in situ* approaches, regardless if soils or

plants are measured. If condensation occurs inside of the tubing or inside the chamber, the isotope values measured will be

subjected to Raleigh fractionation and hence, do not represent the isotope value of the medium that is subject to measurement.

Hence, it needs to be assured that the water vapor pressure in the sampling line is never exceeding the saturation water vapor

pressure. In the reviewed studies, this issue is resolved in three different ways:

400   i)   Dilution with dry air directly in the membrane system (Volkmann et al., 2016a; Volkmann and Weiler, 2014)

              or shortly after (Oerter et al., 2017; Oerter and Bowen, 2017, 2019; Rothfuss et al., 2013, 2015). This way, the

              water vapor concentration of the system is lowered and condensation less likely.

    ii)   Heating of the tubing (suggested by Gaj et al., 2016). Assuring that the temperature of the transport line is

              always warmer than the temperature at the location where isotopic equilibration occurs will avoid condensation

405   to occur. Even in warm climates this might be necessary as solely the temperature difference between the

              location where water vapor is equilibrated (i.e. inside of the gas permeable probe) and the sampling line is

              decisive if condensation occurs or not (refer to section recommendations for further elabortion on this issue).

    iii)   Flushing the system with dry air prior to the measurement (Kühnhammer et al., 2019, under review; Volkmann

              and Weiler, 2014). Flushing the tubing system with dry air prior to each measurement cycle will remove

410   previously condensed water from the transport line.

Condensation is very likely to occur (whenever temperature outside of the in situ system is higher than inside) and will affect

the water isotope data tremendously. Hence, it is of utmost importance to avoid it at all costs. However, it is not always easy

to identify. For this reason, we present three examples of (raw) isotope measurements which depict i) a 'good' measurement

cycle; ii) a measurement cycle initially influenced by condensation, but then turning into a clean measurement once the

415   condensation dissappears and iii)/iv) bad measurement cycles with condensation affecting the complete data in Fig. 5.

Figure 5

3.3 Calibration protocols

The calibration of water isotope values is a crucial point, and it is more complex and error-prone when measuring water vapor

isotopes *in situ* compared to liquid water samples. It is generally comprised of the following steps: *i) Standard preparation;*

420   *ii) Correction for water vapor concentration; iii) Specific corrections (mineral mediated fractionation, organic contamination,*

carrier gas and biogenic matrix effects); iv) Drift correction; v) Conversion from vapor to liquid values; and finally, vi)

Normalization to VSMOW scale. There is a great variety in the existing studies on how (and even if) each of these steps were addressed in the reviewed papers. The subsequent section highlights the key points based on the reviewed papers in terms of calibration procedures. We then put a special focus on the approaches presented by Oerter et al. (2017) and Oerter and Bowen

425 (2017), who developed a novel, innovative method for the calibration of *in situ* measurements of soils.

3.3.1 Isotope Standards

Ideally, isotope standards are prepared in the same medium that is measured (*personal comment, P. Koeniger*). That means, use soil standards when measuring soil water isotopes, use water standards when measuring liquid water samples. The clear advantage of this is that effects such as mineral-mediated isotope effects will be incorporated into the calibration procedure in

430 a way that the standard will be affected in the same way as the measured sample. The disadvantage is that the preparation of soil standards requires more practical effort. Soil from the site of interest needs to be collected, oven-dried and placed in suitable standard bags or containers. Subsequently this soil needs to be spiked with the isotope standards (Gaj et al., 2016; Oerter et al., 2017; Oerter and Bowen, 2017; Rothfuss et al., 2015). Ideally, soil from different horizons is used for that as well, because the soil texture and, hence, isotope effects might change throughout the soil profile (Oerter et al., 2017; Oerter

435 and Bowen, 2017). In addition, a range of water contents should be covered in the calibration process. This makes the calibration using soil standards labor-intensive and multiplies the number of standards to be measured (different soil horizons x different standards x different water contents). In contrast, using water standards for calibration is rather straightforward, as only different water vapor concentrations need to be injected for the calibration. This can be done either using a system for vapor injection (e.g. a standards delivery module or nebulizer) or simply placing the water standards in bags or containers and

440 measuring the headspace. In the latter case, calibration of water vapor concentrations needs to be controlled via diluting the headspace with dry air to obtain lower H_2O ppm values. The big disadvantage of using water standards is that soil induced isotope effects are not incorporated at all and this can lead to notable errors in the corrected isotope values later on. Hence, for best isotope data we recommend soil standards when measuring soil water isotopes (depth profiles and evaporation) and water standards when measuring *in situ* plant water isotopes (transpiration and xylem) or atmospheric water vapor. In regard to

445 chamber based measurements, correction has mostly been applied liquid injected standards in the past, however, we recommend to, when integrating chambers in a larger in situ framework, to use water equilibration standards instead. Obviously the background dry-gas is of major importance here, as the air matrix of the standard should be the same as that of the sample.

3.3.2 Correction for water vapor concentration

Because of the influence of different water vapor concentrations on measured isotope ratios (Picarro, 2015; Schmidt et al.,

450 2010), a correction needs to be performed. A linear best-fit equation can be fitted if a standard of known isotope composition is measured at different water vapor concentrations. The slope and intercept of the best-fit line through these points are the two values that are used to post-process vapor delta values with variable water concentration (Picarro, 2015).

Schmidt et al. (2010) investigated concentration effects on laser-based $\delta^{18}O$ and δ^2H measurements in detail and showed a positive effect of the water vapor concentration on the isotope ratios. In their study, the authors report a concentration effect
455 of 1.2 to 1.4‰ per 10.000 ppm for $\delta^{18}O$ and 0.6 ‰ per 10.000 ppm for δ^2H. The precision of the CRDS instrument used did not change over the range covered (5000 ppm to 30000 ppm). They proposed to measure isotope standards always with the same water vapor concentration and subsequently (before any other correction) correct the raw values using the following relationships:

$\delta_{ref} = \delta_{obs} + m(\omega_{ref} - \omega_{obs})$

460 where δ_{ref} is the d-value (‰) at the reference concentration, δ_{obs} the observed d-value (‰) at the measured concentration ω_{obs} (ppm), ω_{ref} the reference concentration and m the slope of the concentration dependence (ppm). Schmidt et al. (2010) also provide equations if the slope depends on the δ value (refer to their publication).

Furthermore, the water vapor concentration is affected by the temperature of the media-of-interest but also soil moisture and relative humidity. Indirectly, the flow rate chosen by the user also affects the latter (if flow rates are too high, saturation will
465 not be reached). The interplay of those factors is complex and no trivial to account for (refer to discussion for elaborations on this issue).

3.3.3 Other corrections (mineral mediated fractionation, organic contamination, carrier gas and biogenic matrix effects)

Recent research has shown that especially in clay-rich soils, an offset can be produced due to tightly bound water (Gaj et al.,
470 2017; Newberry et al., 2017; Oerter et al., 2014). This creates a real challenge for any soil water isotope measurements and was discussed heavily (Orlowski et al., 2013, 2016b, 2016a; Sprenger et al., 2016). Up to date, it is not clear how to best handle these additional factors. A stated above, a preparation of isotope standards in the same soil that is to be measured seems to be the most promising approach, and Oerter et al. (2017) provide an innovative procedure to calibrate their data (see section down below).

475 In addition, spectral contamination of laser spectroscopy caused by organic compounds has been discussed frequently and was recognized as a major source of error when extracting water from plant tissues (Barbeta et al., 2019; Brantley et al., 2017; Martín-Gómez et al., 2015; Millar et al., 2018; Newberry et al., 2017; Penna et al., 2018; West et al., 2010; 2011). It is not known up to date, if this plays a role for *in situ* approaches (refer to discussion). Volkmann et al. (2016) speculated in their study that organic contamination might be one of the reasons for the observed discrepancies in their dataset. For liquid water
480 samples, a method for correcting for the influence of organic substances exists (Lin et al., 2019; Schultz et al., 2011; Wu et al., 2013). Thereby, deionized water is spiked with varying amounts of methanol and ethanol to create correction curves for $\delta^{18}O$ and δ^2H. An adaptation of this method is theoretically feasible for water vapor measurements, but has not been tested until today (*personal communication, M. Hofmann, Picarro*). It should be noted, however, that methanol and ethanol are not the only possible contaminants and others might influence the absorption spectra. Generally, it is advisable to perform a check if
485 organic contamination for the particular set of samples is an issue using the pertinent software (e.g. Chemcorrect). If this is the

case, measuring plant samples and samples from the upper soil layers with mass spectrometric analysis or corrections are required.

Finally, the issues of carrier gases and biogenic matrix effects have been raised recently. However, those can be corrected for rather straightforward (Gralher et al., 2016, 2018).

490 **3.3.4 Drift correction**

As for the measurement of liquid water samples, it is recommended to always use a drift standard that can be measured either after each run (e.g. after measuring one soil profile or a set of tree replicates) or after a certain time. A linear correction similar to the regression for water concentration can than be performed.

Conversion of vapor to liquid values

495 All of the presented studies are based on isotopic exchange between the air outside and inside of the gas permeable probe. Ideally, equilibrium fractionation is achieved during the passage of the air through the membrane. The isotope value of water (soil or xylem) can then be calculated applying the well-established equations for equilibrium fractionation (see Clark and Fritz, 1997; Horita and Wesolowski, 1994; Majoube, 1971):

$$\propto {}^2H = \exp^{\frac{\left(a*\left(\frac{10^6}{T^2}\right)+b*\left(\frac{10^3}{T^1}\right)+c\right)}{1000}} \tag{2}$$

$$\propto {}^{18}O = \exp^{\frac{\left(a*\left(\frac{10^6}{T^2}\right)+b*\left(\frac{10^3}{T^1}\right)+c\right)}{1000}} \tag{3}$$

$$\delta {}^2H_{liq} = \propto * (1000 + \delta {}^2H_{vap}) - 1000 \tag{4}$$

$$\delta {}^{18}O_{liq} = \propto * (1000 + \delta {}^{18}O_{vap}) - 1000 \tag{5}$$

where α is the fractionation factor, T is the temperature in Kelvin and δ the isotope ratio of water vapor and liquid water, respectively. The empirical factors a, b and c are tabulated in the above cited literature and commonly used as a= 28.844, b= -

505 76.248, c=52.612 for δ^2H and a=1.137, b=-0.4156, c=2.0667 for δ ^{18}O. As per equations 2 and 3, the temperature is needed for this conversion. Hence, it needs to be measured at the location of exchange (at the gas permeable probe). A conversion of vapor to liquid values is also possible when the water vapor is not saturated (via equal treatment principle of isotope standards), but is not recommended because, for soils, for example, the isotope standards would be needed to be prepared with the exact soil moisture and temperature as the sample to be measured. This becomes very laborious because soil water contents are

510 highly variable with depth and time.

The final step is – similar to liquid water isotope measurements – the Normalization to VSMOW scale: (we spare the equation here is this procedure is widely known and sufficiently documented).

3.4 Validation – comparing apples and pears?

As shown in the succeeding chapter, calibration protocols for addressing the abovementioned steps vary greatly. Not always
515   all the steps are addressed – either because it was not relevant for the particular investigation or because it was simply neglected. Thus, it is necessary to introduce a way of assessing the measurements. Across studies, trueness, precision and reproducibility of *in situ* methods are generally good. For an evaluation of accuracy, the reviewed publications compared the obtained isotope values either with cryogenically extracted samples (Gaj et al., 2016; Soderberg et al., 2012; Volkmann et al., 2016a; Volkmann and Weiler, 2014), results from direct equilibration (bag) methods (Pratt et al., 2016) or both (Oerter et al., 2017). Further,
520   theoretical approaches (mass balance calculations and modelling) have been applied to reproduce the *in situ* measurements (Rothfuss et al., 2013, 2015; Soderberg et al., 2012). The agreement of soil profiles extracted with vacuum extraction at deeper soil layers is generally better. In the upper soil layers, however, partially large differences (> 10 ‰ in δ^2H) are encountered. Possible reasons include contamination with organic compounds (cryogenic distillation) or interference with atmospheric air when using a pull-only system. In the light of recent findings suggesting that water from cryogenic vacuum extraction and *in*
525   *situ* approaches represent different water pools (Orlowski et al., 2016b; Sprenger et al., 2016), this way of validation might not be suitable. Instead, validating *in situ* data with the established direct vapor equilibration method by deploying aluminium or plastic bags and measuring the headspace air, should deliver true means of comparing the data.

The validation of the only *in situ* study of xylem water isotopes so far (Volkmann et al., 2016a) yielded in good results in terms of precision (median of 1.1‰ for δ^2H and 0.29‰ for $\delta^{18}O$) and reproducibility (median of 2.8‰ for δ^2H and 0.33‰ for $\delta^{18}O$).
530   Diurnal variations in both isotopes did not correlate with those of temperature estimates for the different probes; hence, the authors suggest measuring the temperature inside of the probe in the future. They further state that when comparing the values obtained *in situ* with cryogenic extractions and subsequent measurement using IRMS, a significant correlation between the two exists. For a short period of their measurements, they achieved a good agreement and little systematic difference for δ^2H (pre-irrigation, 0.9 ±1.8‰). For $\delta^{18}O$, a clear inter-method bias of -4.3± 0.7‰ was found. The discrepancy in their other data
535   was hypothetically attributed to contamination by volatile organic compounds (VOC's), lateral mixing (through intervessel pits), axial dispersion and the time lag between irrigation water arrival at the twig/crown versus trunk level.

The closest agreement of the reviewed manuscripts when comparing *in situ* derived data with other methods was achieved in the study of Oerter et al. (2017). Both in terms of measurement and data handling, their methodology appears to be the most complete at present. In addition, the authors propose a novel, innovative way of calibrating *in situ* data of soil water isotopes.
540   A reprint of their isotope depth-profiles determined with gas-permeable soil gas probes, direct equilibration and vacuum-extracted profiles are shown in Figure 6.

Figure 6

We propose here an adoption (more general) of the procedure used by the authors:

545   i)   collect samples from each soil depth interval from the site of interest and dry soil in oven, place samples in gastight bags or containers (e.g. 0-10 cm, 10-50 cm, > 50cm)

    ii)   add different amounts of isotope standard with known δ^2H and $\delta^{18}O$ values to obtain a range of water contents (e.g. 5%, 10%, 20% water content x 2 standard solutions x 3 depth intervals = 18 calibration bags)

    iii) add soil temperature sensors to standard bags/containers

550   iv) measure standard preparations under a range of temperatures (e.g. 0 -35°C)

    v)   perform multi-linear regression analysis (e.g. nlme package in R) in order to estimate theoretical liquid water standard values using the parameters *Measured Vapor Isotope Value ($\delta^{18}O_{vap}$ and δ^2H_{vap}), soil moisture content (GWC) and temperature (TEMP);* other parameters such as clay content or water vapor concentration might be added

555   vi) selection of best fit equation for estimation of δ^2H_{liq} and $\delta^{18}O_{liq}$ of the isotope standards (in Oerter et al., 2017: $\delta^{18}O_{liq} = 9.954 - 0.163 \times TEMP + 0.002 \times TEMP^2 + 13.386 \times GWC + 1.051 \times \delta^{18}O_{vap}$; $\delta^2H_{liq} = 120.128 - 1.255 \times TEMP + 0.008 \times TEMP^2 + 1.138 \times \delta^2H_{vap}$)

    vii) statistical analysis: Goodness of estimation? Which parameters explain variation in estimated liquid isotope values best?

560   viii) Application of final equations to dataset, consequent check of isotope standards throughout measurement campaign using derived equations

A procedure like this has several advantages: First, it uses additional information that might have influence on the measurements, such as clay contents, soil temperature and water content. Second, it incorporates these information into one procedure, namely a multi-linear regression. Third, the vapor-liquid conversion that exist in several forms can be avoided. It

565 also might be used as comparison to liquid isotope values calculated via the statistical approach. Finally, the derived relationships can be objectively assessed using goodness-of-fit measures, tested throughout the measurement period, and, if required, adopted later. Thus, we highly recommend this way of calibration and derivation of liquid water isotope values for future studies.

4 Summary and Outlook

570 The goal of this review was to summarize the current state of *in situ* approaches for measuring the isotope ratios of soil water, evaporation and transpiration (in both leaves and xylem) and point out current issues and challenges. Based on this, we propose toward future applications of combined *in situ* investigations at the soil-plant-atmosphere continuum.

In situ measurements are an inevitable step for any holistic study within the critical zone. The current design of many ecohydrological studies is still based on destructive sampling at discrete points in time and space. The number of artefacts

575 (potential isotope effects) and methodological constraints (limited spatiotemporal resolution, issues of measuring different water pools with different extraction methods) associated with that (refer to introduction) is increasingly questioning established methodologies. While certainly - apart from advancements in online methods - new protocols for destructive sampling and analysis are needed in order to account for the findings of the last decade, *in situ* methods provide an elegant way of overcoming a number of current limitations. For instance, the water pools measured in soils and plants using *in situ*

580 methods are ultimately the same, i.e. the mobile fraction that actively takes part in water fluxes and exchange.

Another example is the high temporal resolution that can be achieved with such measurements which resolves the issue of lag time and enables the investigation of non-steady state conditions. Hence, *in situ* methods will be highly useful for any study involving rapid changes of environmental conditions, e.g. root water uptake studies, water partitioning, night-time transpiration, etc. They will also benefit long-term studies, such as monitoring combined reaction of soils and plants to droughts

585   or extreme events. Moreover, high frequency *in situ* monitoring can elevate tracing of the water cycle via isotopic labelling (^2H_2O or $H_2^{18}O$) to a new level and will lead to improved parameterization for a novel generation of models (we spare modelling approaches in this review, but refer to recent developments, e.g. Braud et al., 2013, 2009; Dubbert et al., 2014; Hirl et al., 2019; Kuppel et al., 2018). The same is true for isotopic mixing models. Another aspect that can be studied in much greater detail than before is the process of hydraulic redistribution (Burgess et al., 1998), to name one. Combined with labelling

590   approaches, it might be able to quantify its relevance and impact on a much greater spatiotemporal scale. Let alone these examples, online methods comprise immense potential for future applications.

Having that said, it always should be carefully evaluated, if an *in situ* approach is required for the purpose of study - or if destructive sampling is sufficient. When carrying out *in situ* studies, the aim of the study determines the design of the system to be used and a good starting point would be to clarify the following aspects:

595   i)   Is the particular study a long-term study (weeks to months) or rather short-term (days)?
     ii)   Is the goal to obtain data in a high temporal spatial resolution (or both)?
        This aspects aims to define if the system needs to be portable or rather stationary.

     iii) Is it a tracer experiment or is the goal to obtain natural abundances of soil/plant water isotopes?

The setup of any *in situ* system is neither simple nor easy at present. The complicated technical setup and calibration process

600   might be a reason why only a few research groups have employed *in situ* studies so far. We hope to shed light on some of the technical aspects involved and clarify those through this review.

***In situ* approaches for monitoring depth-dependent soil water isotopes** employing gas permeable probes have advanced tremendously in recent years. It now seems feasible to obtain measurements of natural abundances of soil water isotopes in a high temporal frequency. For **monitoring the isotopic ratios of xylem water continuously**, on the other hand, there is only

605   one existing study employing isotopic labelling (Volkmann et al., 2016a) Future efforts should be directed towards testing and improving the methods suggested therein and develop novel approaches with the ultimate goal to measure natural abundances of plant water isotopes *in situ* (Beyer et al., 2019; Kühnhammer et al., 2019b; Marshall et al., 2019). Subsequently, continuous soil and plant water isotope measures should be combined. In situ monitoring of isotopic signatures of transpired and evaporated water vapor are advanced and have mainly been employed in frameworks focusing on partitioning of ecosystem

610   evapotranspiration (Dubbert et al., 2013, 2014a, 2014b; Rothfuss et al., 2012)or studying isotopic fractionation during soil evaporation (Or et al., 2013)and leaf water isotope signatures (Cernusak et al., 2016; Song et al., 2013, 2015a; Wu et al., 2013). They have also been used in ecohydrological studies tackling questions, such as root water uptake depths (e.g. Volkmann et al., 2016a). However, given the critical and under natural conditions mostly violated assumptions of isotopic steady-state of

transiration (i.e. isotopic signature of transpired vapor not equal to that of xylem water; see e.g. (Dubbert et al., 2014b; Piayda
615   et al., 2017; Simonin et al., 2013)), this can be critical at least under natural ambient conditions.

Despite the great advances in monitoring depth-dependent soil water isotopes *in situ* , there is no generally accepted calibration
protocol existing yet (such as van Geldern and Barth (2012) for water samples). Hence, **homogenization of calibration
protocols and validation** are required. We propose here to make such a development based on the ideas of (Oerter et al.,
2017), which is – in the authors' opinion - the most complete of all currently existing approaches. It also provides an objective
620   way of handling the data (via statistical measures) and is very flexible in including/excluding additional factors that might be
relevant (e.g. mineral-mediated fractionation). In terms of calibration, we further suggest that laboratory standards are provided
using the same media that is to be measured (e.g. use standards prepared and measured in soils when measuring soils *in situ*)
in order to fulfil the assumption of identical treatment principle, which has been violated in a number of studies. We confronted
the developers of the bag equilibration method (Wassenaar et al., 2008) with this question and received the following response:
625   *'We and others have wrestled with this, and you are correct our original publication is technically not fully an identical
treatment. I suppose the real question is how much does either approach matter in practice vs its convenience – are we talking
only 10th's of a permil difference (not an issue) or a lot more (worrisome)?' (L. Wassenaar, personal communication).*
However, the response also states that using soil standards also has pitfalls. *'It is also not identical treatment if you dry and
wet soils or sand with lab standard waters, some soils may more potential for bound residual water or isotope exchange with
630   clay particles, for example, or have soil properties that differ a lot from the field samples.' (L. Wassenaar, personal
communication).* For this reason, an ideal preparation of soil standards is not existing at present. However, running pre-*in situ*
laboratory tests using soil from different depths (e.g. A and B-horizon) from the site to be measured, oven-dry it, spike it with
different water contents and measure it over a range of temperatures and water vapor concentrations will give a sound baseline
for calibrating the on-site data. For the field calibration, soil standards (e.g. two to three) for each soil horizon should then be
635   prepared and measured for each sequence in the field. We further propose to install TDR probes in each of the standard bags
to keep track of the water content and temperature which is needed for the calibration.

For **validation**, it has been shown that a comparison of cryogenically extracted samples, although this has been the standard
method for decades, with equilibration methods is not feasible for soil samples because different water pools are measured
with the two approaches. The same might be true for plant samples. There is an urgent need to develop alternative ideas. For
640   soils, a comparison of *in situ* data with destructive sampling and using the bag equilibration method might be a way. However,
the issue of spatial heterogeneity between the two measures remains. For plants, the bag equilibration method might also be
feasible but has not been tested thoroughly.

For both soils (e.g. the upper soil layers) and plants, the effect of **organic contaminants** (such as volatile organic compounds
- VOC) on *in situ* measurements needs to be evaluated and measures developed to correct for (post-correction). Such might be
645   included into the multi-step procedure suggested by Oerter et al. (2017). As stated, a method for correcting liquid water samples
for the influence of organic substances already exists (Lin et al., 2019; Schultz et al., 2011; Wu et al., 2013) and could be easily

adopted to vapor-phase measurements. However, it needs to be determined before if contamination even plays a role for *in situ* obtained data.

Another point related to calibration we recommend is the establishment of a way of **evaluation if equilibrium conditions**
650 **prevailed** at the site of isotope exchange during the *in situ* measurement (e.g. inside of the gas permeable soil/tree probe). All reviewed studies presented herein use some sort of equilibrium water - vapor conversion (e.g. Horita et al., 2008; Majoube, 1971). Only one of them (Marshall et al., 2019) evaluated if this assumption actually was true for their particular setup (flow rate, exchange length, etc.).

Relative humidity corrected to borehole temperature can be defined as the ratio of "water vapor concentration" (in ppmv)
655 measured by the cavity-ringdown spectrometer compared to the saturated specific humidity at stem temperature T (measured using a thermocouple or PT100 sensor) to obtain an estimate of relative humidity in the borehole. If these two (roughly) match, it is likely that the chosen parameters of the (physical) system are suitable to reach saturation under the given conditions. It also reveals potential condensation under the given environmental conditions. Ideally, relative humidity h should range between 0.9 and 1.0. When h is substantially lower than 0.9, the dry air pushed into the borehole did not approach saturation;
660 hence, the flow rate would need to be adjusted downward. We recommend for any system to check h for evaluating if the defined settings of the physical setup are suitable. This concept is applicable to both push-through and pull-only setups.

One might argue that via equal treatment principal, saturation is theoretically not necessary because it can be calibrated. However, this would require, for instance for soil samples, a preparation of soil standards with the *exact* same conditions as the measured soil depth (water content, temperature), which is practically not feasible.
665 In the final chapter, we propose a combined soil-plant *in situ* monitoring system which - in the authors' opinion - represents a holistic way of investigating ecohydrological processes at the interfaces of soil, vegetation and atmosphere.

One system, one methodology – A call for combined *in situ* studies

The authors of this study have been involved into the development of *in situ* for nearly a decade. As a combined conclusion this literature review and their own experiences, an 'ideal' system is presented in Fig. 7.

670 **Figure 7**

The system depicted in Fig 6. combines measurements of all compartments covered in this review. A setup like this would enable one to monitor the complete cycling of water through the soil-plant system: i) gas permeable probes for measuring depth-dependent soil water isotope ratios (supported by soil moisture/temp. sensors for the equilibrium calculations); ii) soil chambers for monitoring the isotope composition of evaporation; iii) stem probes or stem boreholes (supported by
675 thermocouples for the equilibrium calculations); iv) leaf chambers for monitoring the isotope composition of transpiration and finally the monitoring of atmospheric water vapor. Ideally, all these fluxes are all controlled by one valve/manifold system. Through the inlet of each measurement stream, dry air with the required flow rate (MFC 1) can be directed through the probes/chambers. At the same time, it can be used to flush the systems prior to the measurement sequence with dry air (diving air, synthetic air or N_2 for removal of condensed water in the lines). The equilibrated water vapor then is send back through the
680 manifold and to the water isotope analyser. A second mass flow controller (MFC 2) offers the opportunity to dilute the sampling

air if the water vapor concentrations are too high (less precise values above 30.000 ppm). Any connection is an opportunity for leaks. The system is therefore limited to as little connection pieces as possible (i.e. one piece of Teflon tubing or stainless-steel capillary from the probe/chamber to valve system/manifold). The excess tube avoids the possibility of overpressure at the analyser inlet. The calibration unit consists of a user-selected number of soil standards for the soil measurements and water
685 standards for the plant water isotope measurements. Additional (optional) components might include a higher number of monitored trees and/or soil profiles (heterogeneity), sap flow probes, stem water content sensors and for the soils, matric potential sensors. Though the depicted setup is constructed as push-through system (dry air is pushed through the compartments to be measured and equilibrated therein), it can be operated in pull-only mode as well.

When reading through this explanation, the reader probably gets the impression that this is very complicated and complex.
690 Admittedly, it is. It is, thus, the task of the community to further simplify *in situ* measurements. However, before this happens, several holistic studies need to be carried out and evaluated. Lastly, we encourage the community to carry out and test *in situ* systems. The increased technical effort for setup is often compensated by far with the higher spatial (if using probes as mobile version) and temporal resolution.

Lastly, it needs to be clear to everybody applying *in situ* methods that a higher uncertainty has to be expected when working
695 with such methods. While it should be certainly be decreased as much as possible, it is equally as important to communicate those uncertainties. Many of the 'old' studies are employing a very low number of samples, for instance for plant source water studies. Many of those publications end up with clear statements, but completely neglect the dynamic character of natural systems. Thus, only a part of the story is reported. In order to improve the understanding of ecohydrological processes it is inevitable to develop ready-to-use *in situ* monitoring systems; it is crucial for the community to further develop such methods
700 and make them available for a larger group of researchers and practitioners in the near future.

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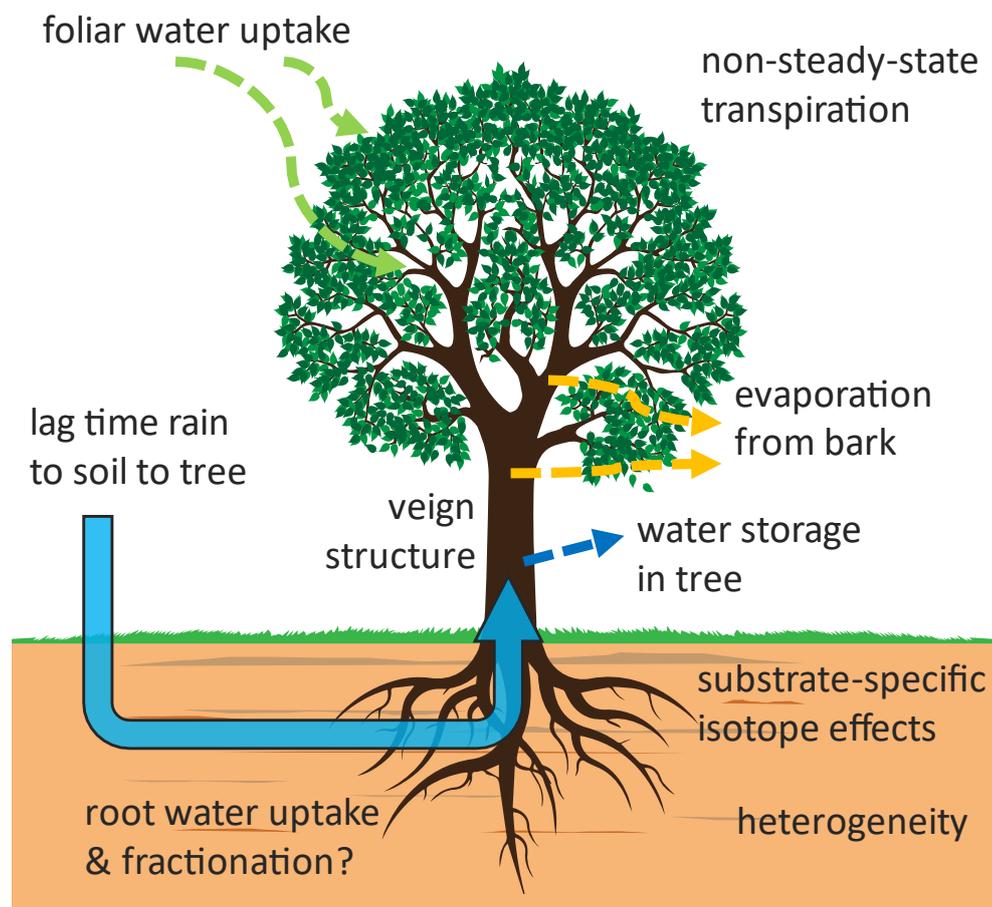
Figure 1: A compilation of isotope effects potentially affecting the soil and plant water isotope ratios
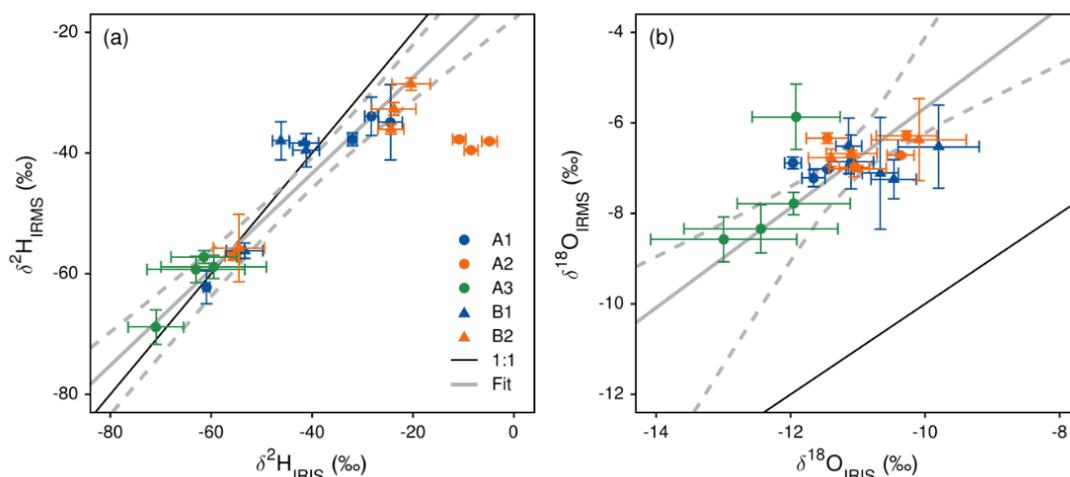
Figure 2: *In situ* measured xylem water isotopes (δ²H$_{IRIS}$) and comparison to results obtained by cryogenic vacuum extraction after destructive sampling and measurement with mass spectrometry (δ²H$_{IRMS}$). Reprinted with permission from Volkmann et al. (2016b)
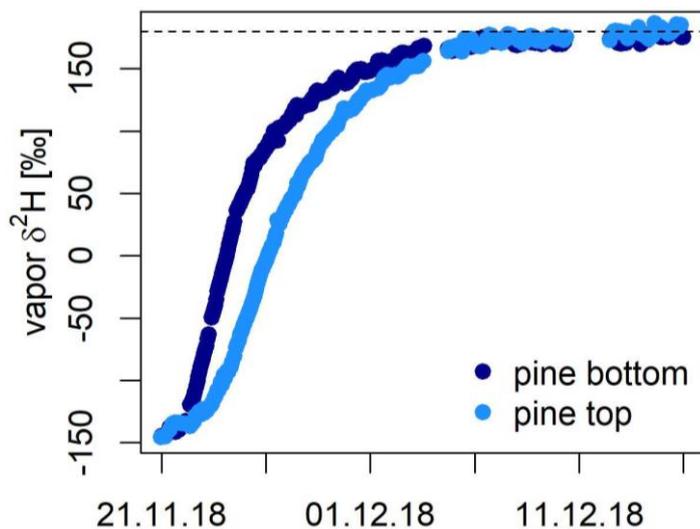
Figure 3: Xylem water δ²H values measured in the stem boreholes during a greenhouse experiment in Freiburg, Germany, on a pine tree (Marshall et al., 2019). Two boreholes were drilled through the stem and their isotope values monitored over a period of two months. For both boreholes a close agreement of δ²H between source water and *in situ* data was achieved.
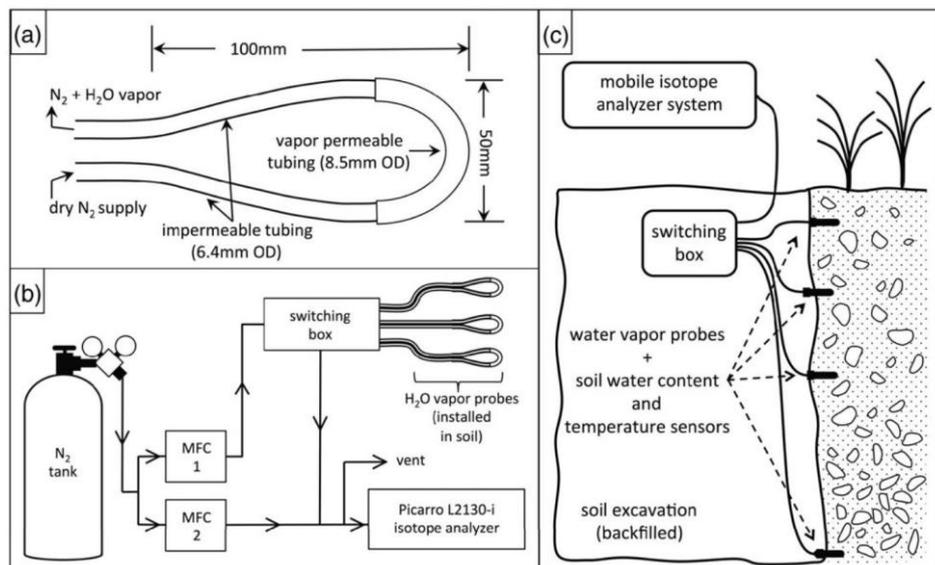
Figure 4: Schematic drawings showing (a) an *in situ* soil probe consisting of a gas-permeable membrane and attached tubing; (b) a concept of the water vapor probe analytical system; (c) the field installation of an in situ system with additional sensors for recording soil moisture and temperature. MFC = mass flow controller. Reprinted with permission from Oerter and Bowen (2017). Note that different probe designs exist, and this is only one example.
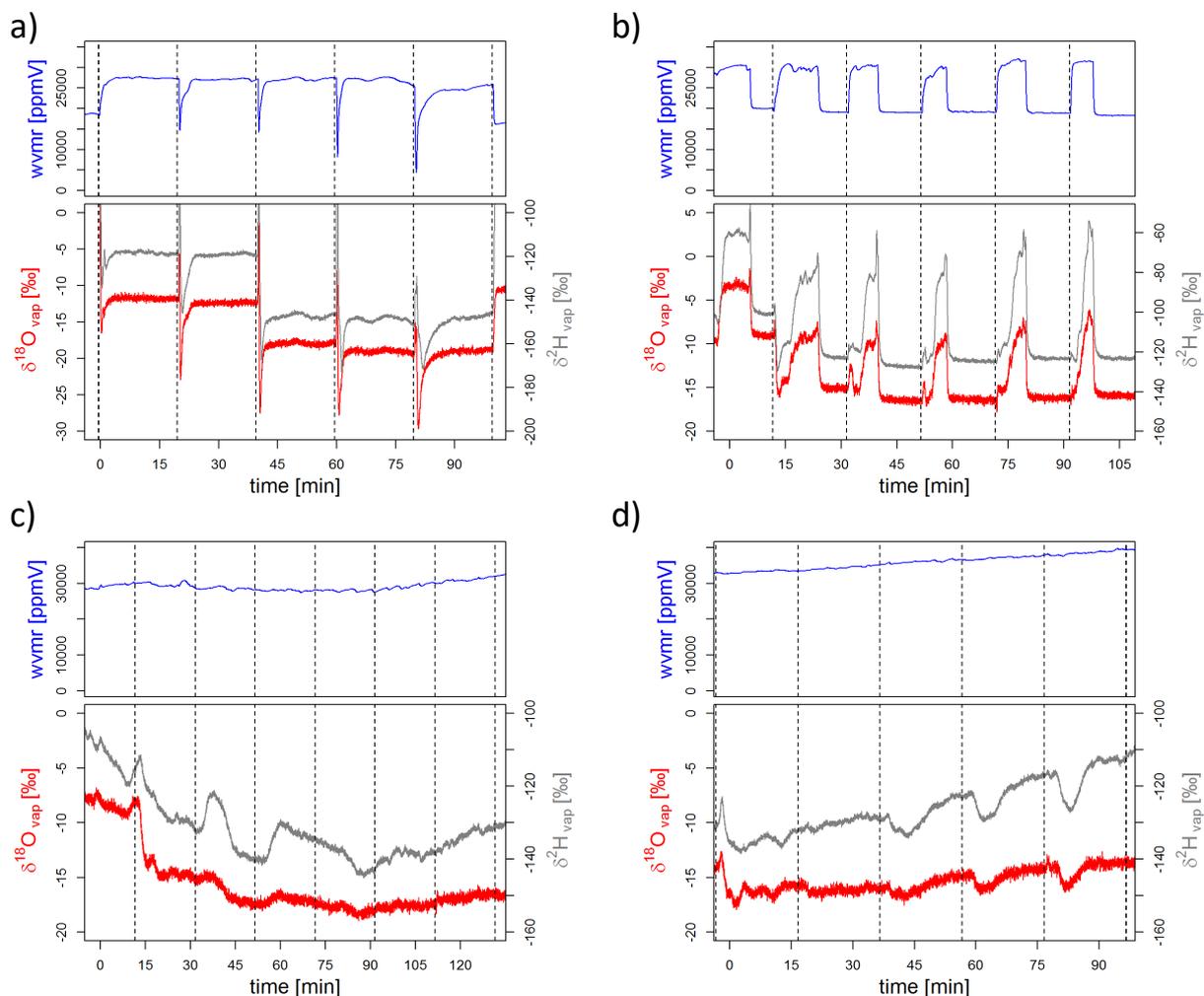
Figure 5: Measurement cycle of an *in situ* system switching through different probes. Shown are water vapor concentration (vwmr in ppm) and the raw vapor isotope values for δ²H and δ¹⁸O in permille. Each probe was measured for 15 minutes, then the manifold switched to the next probe (indicated by dashed vertical lines). The different panels show a) a clean measurement with a stable plateau for the three variables; b) a measurement where small amounts of condensation were present in the system, but then removed during the measurement phase resulting in a stable plateau towards the end of each cycle; c) and d) two examples of erroneous measurements, where condensation (=very high ppm values) does not allow the laser analyzer to reach a stable plateau.

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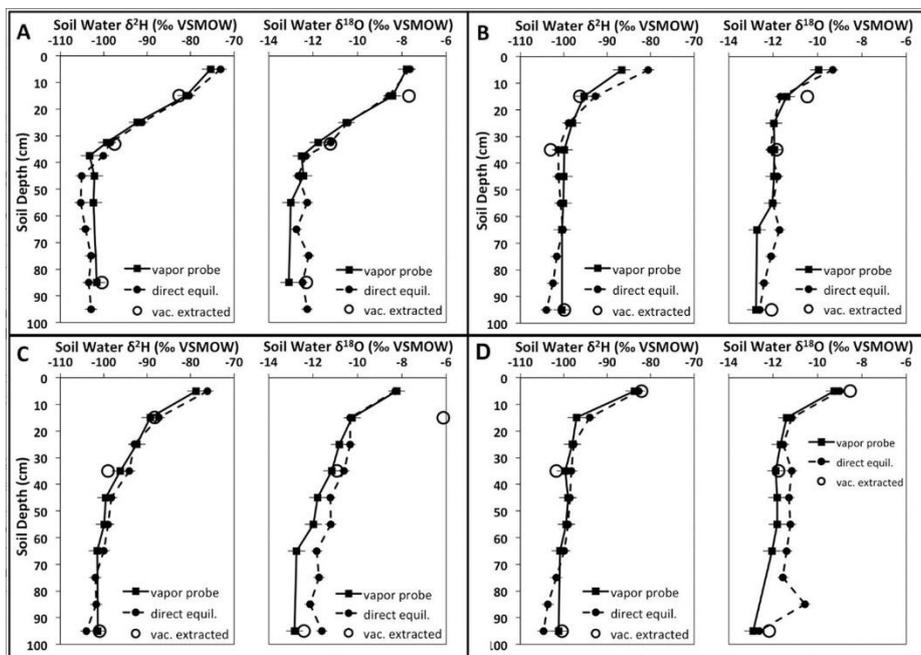
Figure 6: Comparison of soil water δ^2H and $\delta^{18}O$ values determined with the soil probes (solid squares, solid line), direct vapor equilibration (or: bag equilibration, solid circles, dashed line), and vacuum-extracted soil water (empty circles), with soil depth for four different sites. Analytical uncertainty in each vapor measurement methodology is denoted by horizontal whisker marks. Reprinted with permission from (Oerter et al., 2017).
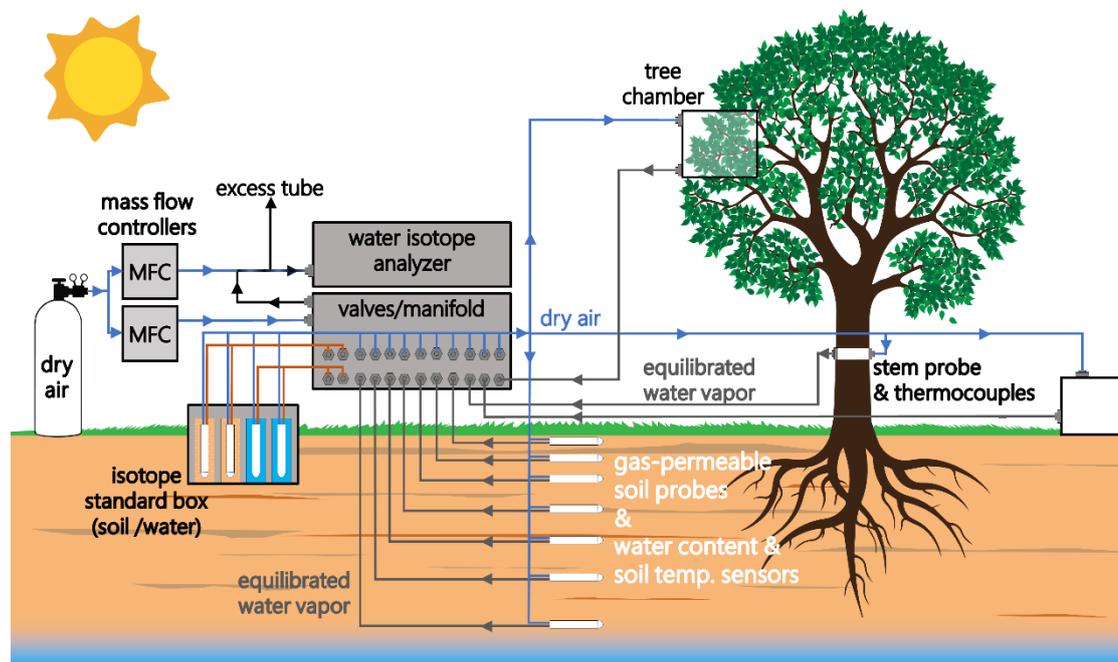
Figure 7: An idealized, yet complicated *in situ* system depicting all relevant components for a complete measurement of water isotopes of soils (depth-dependent and bulk soil) and plants (in tree xylem and at the leaf level).