Tracing ecosystem water fluxes using hydrogen and oxygen stable isotopes: challenges and opportunities from an interdisciplinary perspective

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Abstract. In this commentary, we build on discussions that emerged during the workshop "Isotope-based studies of water 35 partitioning and plant-soil interactions in forested and agricultural environments" held in San Casciano Val di Pesa, Italy, in September 2017. Quantifying and understanding how water cycles through the Earth's critical zone is important to provide society and policy makers with the scientific background to manage water resources sustainably, especially considering the ever-increasing worldwide concern about water scarcity. Stable isotopes of hydrogen and oxygen in water have proven to be

a powerful tool to track water fluxes in the critical zone. However, both mechanistic complexities (e.g., mixing and fractionation processes, heterogeneity of natural systems) and practical methodological issues (e.g., lack of standard protocols to sample specific compartments, such as soil water and xylem water) limit the application of stable water isotopes in critical zone science. In this commentary, we examine some of the opportunities and critical challenges of using isotope-based
5   ecohydrological applications, and outline new perspectives focused on interdisciplinary research opportunities for this important tool in water and environmental science.

1 Understanding water availability in the environment

Understanding water fluxes in the critical zone, the thin dynamic skin of the Earth that extends from the top of the vegetation canopy, through the soil, down to groundwater (Brooks et al., 2015), is becoming increasingly important as the climate
10   changes, the human population grows, and water supplies become increasingly constrained (OECD, 2012, WWAP, 2015, and 2030 WRG, 2009). Although human water use often relies on rivers or aquifers, these resources are maintained by critical zone processes that determine the routing of water downward to groundwater, lakes and streams ("blue water"), or upward to the atmosphere via evapotranspiration ("green water"). A better understanding of the factors that control the availability and the fate of water in the critical zone is vital to maintain ecosystem services in a changing world (Grant and Dietrich, 2017). A
15   more detailed mechanistic understanding of water fluxes in the critical zone would serve at least two important purposes: first, it would enable hydrological and climate models to better predict changes in green and blue water quantity; second, it would support management and conservation strategies that promote long-term sustainability of water resources and linked ecosystem functions.

20   Given the variety of intertwined processes at work in the critical zone, understanding water movement through ecosystems is inherently interdisciplinary. Critical zone processes have often been examined separately within different disciplines, such as hydrology, soil physics, forest and landscape ecology, agroecology, biogeochemistry, and plant physiology. Stable isotopes of hydrogen (^{2}H) and oxygen (^{18}O) are an effective tool for tracing water through soils, aquifers, streams, plants, and the atmosphere, and therefore can connect the disciplines mentioned above. Stable isotopes of hydrogen and oxygen have been
25   used as hydrological and ecophysiological tracers for more than five decades (Kendall and McDonnell, 1998; Vitvar et al, 2005; Dawson et al., 2002; Werner et al., 2012), and new advancements in isotope-based tools and methods (e.g., Volkmann et al., 2014, 2016a; von Freyberg et al., 2017) have recently contributed to interdisciplinary research on critical zone water movement. A search in any literature database will reveal that there has been a sharp increase in the number of papers published on these topics in the last 10-15 years, corresponding to the introduction of commercially available and affordable laser
30   spectroscopy systems for simultaneous analysis of hydrogen and oxygen isotopes. This has made it easier to analyse isotopic data in dual-isotope space (δ^{18}O vs. δ^2H) instead of considering only one of these isotopes. Consequently, recent studies have

revealed problems in the simplifying assumptions that underlie past investigations, especially those related to steady-state and well-mixed conditions.

5   These topics were intensively discussed at the recent workshop on "Isotope-based studies of water partitioning and plant-soil interactions in forested and agricultural environments" held at Villa Montepaldi, San Casciano in Val di Pesa, Florence, Italy on 27-29 September 2017. The workshop brought together scientists from 12 countries who use stable isotopes of hydrogen and oxygen to study water movement across the critical zone. The objective was to share perspectives on major obstacles (and their potential solutions) in applying isotope analyses in critical-zone studies. This paper reports the main outcomes of the workshop, summarizing perspectives on several urgent challenges and future research opportunities.

10   2 Stable isotopes of hydrogen and oxygen: a versatile and interdisciplinary methodological tool

Stable isotopes of hydrogen and oxygen are naturally present in the water molecule, allowing for effective tracing of water as it moves through the critical zone. In general, we have good theoretical knowledge about individual chemical, physical and biological processes that control the isotopic composition of water (Gat and Gonfiantini, 1981; Kendall and McDonnell, 1998). Specifically, the interactions between the vapour, liquid, and solid phases of water explain most of their isotope variability. 15   Applying this theoretical knowledge to real-world conditions, models have been developed to explain the isotopic composition of (liquid) precipitation condensing from cloud vapour (Dansgaard, 1964; Gat, 1980; Clark and Fritz, 1997). Although these models were shown to yield reliable predictions at annual time scales, predicting the isotopic composition of water in the atmosphere on shorter time scales remains difficult due to its short residence time (around 9 days; van der Ent and Tuinenburg, 2017) and non-uniform atmospheric mixing. Other models are available to explain the isotopic fractionation that occurs during 20   evaporation from water bodies (Craig and Gordon, 1965) or from the upper part of the soil profile (e.g., Barnes and Allison, 1988) and plant canopies (Cernusak et al., 2016). Predicting fractionation from water bodies is relatively easy because they are reasonably well mixed near the surface, while predicting the isotopic composition of water in soils and canopies remains difficult (Sprenger et al., 2016).

25   In soils, difficulties arise because soil water content, soil texture, mineral composition, and the content of organic matter are spatially heterogeneous and strongly influence how soils interact with water molecules (e.g., Barnes and Allison, 1983; Oerter et al., 2014; Oshun et al., 2016; Gaj et al., 2017a). Also the origin of soil water is diverse, comprising a mixture of precipitation events from different times, sources (air masses), and types (e.g., rainfall, snow, hail); it may also include groundwater and, in the case of agricultural fields, irrigation water derived from groundwater, lakes, or rivers.

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It is usually assumed that root water uptake does not alter the isotopic composition of the water in the roots or the stem, and therefore xylem water samples from plant tissue should reflect the isotopic composition of the source water (Dawson et al.,

2002, and references cited therein). However, studies have suggested that plants, particularly halophytes and xerophytes, may fractionate the water they are using (Ellsworth and Williams, 2007), which results in an enrichment of heavy isotopes in the surrounding soil water under certain conditions (Vargas et al., 2017). At the leaf level, evaporation during transpiration can cause strong isotopic enrichment of the heavier isotopes ^{2}H and ^{18}O in the leaf water that remains (Dawson and Ehleringer,

5 1998). In addition, other mechanisms, such as the exchange of hydrogen and oxygen between H_2O and CO_2, can affect the degree of isotopic fractionation of leaf water, especially when transpiration rates are low, although the effect of such processes on the isotopic composition of leaf water is still not well understood.

Recent technological advancements can help gather isotope measurements at higher temporal or spatial resolution. The oldest,

10 but still most common device for analysing stable isotopes of light elements is the isotope-ratio mass spectrometer (IRMS) coupled with different peripherals allowing different sample media to be processed. In the past 10-15 years, new types of isotope analysers have become widely available, based on the use of tuneable diode lasers that can scan across a range of frequencies (off-axis integrated cavity output spectroscopy, OA-ICOS, and cavity ring-down spectroscopy, CRDS). The repeatability and reproducibility of these instruments are comparable to IRMS (Penna et al., 2010; 2012); they are substantially

15 cheaper and can be installed directly in the field for continuous, automatic measurements of liquid or water vapour samples (e.g., Berman et al., 2009; Pangle et al., 2013; Oerter et al., 2017; von Freyberg et al., 2017). One limitation of laser instruments is their sensitivity to interference by organic substances that are often present in plant and soil water samples, and recent efforts have been directed towards identifying the interfering molecules and providing sample preparation protocols and software tools to avoid or correct for such interferences (e.g., West et al., 2011; Martín-Gómez et al., 2015). Recently, new *in-situ*

20 sampling techniques have been developed to analyse components of the water cycle that have eluded researchers until now. For example, new sampling probes have been developed to quasi-continuously extract water vapour from tree stems or soils for real-time isotope analysis (Volkmann et al., 2016b; Oerter and Bowen, 2017) and to analyse gas exchange at the leaf level (Dubbert et al., 2014; Volkmann et al., 2016a). These technical advances allow for continuous and unattended isotope measurements and hold promise for advancing our understanding of water storage dynamics, flow pathways and exchange

25 processes in the critical zone.

3 Limitations and challenges

At the workshop, several key challenges in isotope-based studies of water flow pathways and plant-soil interactions in the critical zone were identified and discussed. Here, we summarize these challenges in three main themes: methodological and conceptual limitations; heterogeneity in catchments and ecosystems; and scaling issues.

3.1 Methodological and conceptual limitations: sampling the right water pool

Questions such as "Where do plants get their water from?" and "How do plants select their water source?" are central to understand water fluxes in the critical zone, and they have been explored in many stable isotope studies (e.g., White et al., 1985; Dawson and Ehleringer, 1991; Stahl et al., 2013; Bowling et al., 2017; Evaristo and McDonnell, 2017). While isotope

5 measurements have become more accurate over the years and progress has been made in quantifying the proportions of different water sources by using Bayesian-based mixing models (such as SIAR, MixSIAR; see, for example, Evaristo et al., 2017), many conceptual and methodological challenges remain.

For example, some studies have found that the isotopic signature of xylem water sampled from trees does not match the

10 signatures of potential soil water sources (e.g., Brooks et al., 2010; Evaristo et al., 2015; McCutcheon et al., 2017). This suggests that either source-water sampling was incomplete, that fractionation processes modified the isotope composition of the water taken up by plants before or during uptake itself, or that other methodological issues may limit the utility of stable isotopes in tracing ecosystem water fluxes. Vital here is the issue of how to appropriately sample and extract water from soil and plant tissues. Several studies have shown that water from the same soil or xylem sample can have different isotopic

15 compositions when extracted with different techniques. For example, soil water extracted with tension lysimeters may be isotopically different from that extracted with cryogenic distillation (Landon et al., 1999; Koeniger et al., 2011; Orlowski et al., 2016b; Gaj et al., 2017b; Thoma et al., 2018) and plant water extracted cryogenically may differ from water directly sampled from the xylem (e.g., Volkmann et al., 2016b). Isotope ratios may also differ with different extraction times (West et al., 2006). It has been suggested that these differing signatures may represent different fractions of the total soil- or plant-water

20 reservoir. What is lacking, but urgently needed, is to (i) develop well-tested and standardized sampling, extraction and isotope analysis protocols; and (ii) verify whether these extraction methods return the water pool we actually aim to analyse.

Another issue that needs to be addressed is the pore-scale variation in soil water isotopic composition. Currently, extraction of bulk water from soil is done on relatively large soil sample volumes (50 cm^3 and above; McCutcheon et al., 2017). Differences

25 in soil water isotopic composition depend on the soil water potential in different pore spaces (often referred to as tightly bound versus mobile water) (Brantley et al., 2017). In contrast, McCutcheon et al. (2017) did not find any indication of changes in soil water isotopic composition among different pore spaces. Experimental designs and methods to target the isotopic composition of water that is bound with different potentials and distributed in different pore sizes on scales below 50 cm^3 are needed to test whether such small-scale differences exist and to subsequently represent them in mixing models. If soil water

30 isotopic composition cannot explain the observed isotopic signature in plant (xylem) water, then other factors (e.g., plant physiological processes such as fractionation at the soil-root interface, uptake of dew and fog via leaves and bark, or mixing of xylem and phloem water) may explain these patterns (Eller et al., 2013; Berry et al., 2014; Sprenger et al., 2016; McCutcheon et al., 2017; Lehmann et al., 2018; Dawson and Goldsmith, in press).

Similar conceptual constraints relate to measuring the isotopic composition of water in plant tissue. For example, what are the implications of transport time and water storage within plants? It can take hours to days for water absorbed by tree roots to reach the leaves (Dye et al., 1992; Ubierna et al., 2009). Further, water can be stored in the sapwood and outside the water

5   transport pathway for days, particularly in conifers (Waring and Running 1978, Meinzer et al., 2006). Thus, the isotopic composition of xylem water may not reflect the current soil water source but may instead be influenced by soil water taken up days or even months beforehand (Brandes et al., 2007; Treydte et al., 2014). Recent experimental studies have revealed that xylem isotopic signatures also vary on short, sub-daily time scales (Volkmann et al., 2016a). So far, most studies have assumed –not explicitly but often implicitly– some kind of steady-state conditions when trying to determine the uptake pattern. In

10 addition, considerable spatial variation in xylem isotopic signatures within trees has been observed with values differing around and along tree stems (Cernusak et al., 2005; Volkmann et al., 2016a,b) and between stem and branch water (Dawson and Ehleringer 1993; Cernusak et al., 2005; Ellsworth & Williams, 2007; Zhao et al., 2016). How these temporal and spatial variations in plant isotopic signature can inform end-member determination and how they can be integrated into mixing models remain unsolved issues at present.

The proposed "two water worlds hypothesis" (McDonnell, 2014) has challenged the assumption of complete subsurface mixing that underlies many catchment models (Pfister and Kirchner, 2017). This hypothesis postulates that more mobile soil water contributes to groundwater recharge and streamflow whereas more tightly bound water tends to be used by plants (McDonnell, 2014; Evaristo et al., 2015). This conjecture has stimulated new interpretations of ecohydrological data and new research

20 questions to investigate water flow pathways in catchments (McDonnell, 2014). However, there seems to be a trend to focus the interpretation of recent data on just confirming or rejecting this one hypothesis. As outlined in Berry et al. (2017) and Sprenger et al. (2016), alternative hypotheses need to be developed and tested to improve our current understanding. Because water held in the soil or moving through the soil and other subsurface layers is a continuum, where water transport is driven by gradients, and not separate "worlds", we see the necessity to move from the simplistic "two water worlds hypothesis" to an

25 "*n* water worlds" concept, where multiple water reservoirs and flow pathways are invoked and parameterized, doing justice to the properties of the different substrate types and sites.

3.2 Heterogeneity in catchments and ecosystems

Given that the natural environment is heterogeneous on all scales, that sampling is always partial and incomplete, and that the analytical process adds random errors (and, often, systematic biases), isotopic data are inherently subject to uncertainties.

30 While many of our research questions are specifically focussed on exploring heterogeneous patterns across different domains (e.g., different climatological conditions, soil types or vegetation types), there is also considerable variability within each domain. Observed differences in the isotopic composition of water in various compartments of a catchment or an ecosystem

are the result of many, often simultaneous, processes. The isotopic composition of water samples is often shaped by mixing as well as fractionation processes; both mixing and fractionation can occur in different compartments (e.g., soils, plants, atmosphere) either simultaneously or at different times as water passes through the system. Because we still lack a thorough understanding of the underlying mixing and fractionation processes and of the spatial and temporal scales at which they

5   operate, difficulties remain in interpreting the isotopic compositions that we measure in our environmental samples. While early isotope applications in catchment studies demonstrated the importance of considering temporal variability in precipitation and runoff (Kendall and McDonnell, 1993), less attention was paid to spatial heterogeneity under the assumption that streamflow inherently integrates over spatial variations in the upslope contributing area. In contrast, applications in ecohydrology often require spatially explicit characterizations of soil water, groundwater, and plant water, which do not

10   necessarily integrate across large spatial areas and thus reflect local heterogeneous processes. In the following, we highlight the main sources of heterogeneity that characterize different water pools relevant to ecohydrological studies.

Precipitation represents a major source of spatio-temporal heterogeneity that results in variations in all subsequent biological and hydrological compartments. The combined effects of variability in atmospheric parameters such as humidity, temperature

15   and solar radiation influence the isotopic composition of precipitation, manifesting in temporal (Dansgaard, 1964; Rozanski et al., 2013; Coplen et al., 2008; Coplen et al., 2015) and spatial (Ingraham, 1998; Fischer et al., 2017; Allen et al., 2018) variability at multiple scales. However, at least at the plot scale (i.e., tens of meters) and in the absence of significant altitude variations, the spatial variability of precipitation is usually of minor importance. Precipitation is often collected by tipping buckets with a ~200 mm diameter (Fig. 1), a scale that is assumed to integrate small-scale variations and be representative of

20   larger areas. At larger scales (e.g. > hundreds of meters), we might observe systematic variations that are functionally relevant and should be characterised. However, an atmospheric vapour sample collected from an eddy covariance tower might represent a footprint area of several thousand square meters (Fig. 1). Conversely, temporal variability can be very pronounced, even during a single storm event, as demonstrated by quasi-continuous precipitation sampling (Munksgaard et al., 2012; Pangle et al., 2013; von Freyberg et al., 2017).

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Canopy interception of liquid water involves flow through a small storage with short mean residence time, largely resulting in throughfall and stemflow having a similar pattern of temporal variability as initial precipitation inputs (e.g., Ikawa et al., 2011). Nevertheless, storage and subsequent evaporation of intercepted precipitation can result in below-canopy inputs to soil that differ from open precipitation by more than 2‰ in $\delta^{18}O$ for single events and over longer periods (Allen et al., 2017). There

30   can also be spatial variations of several ‰ in $\delta^{18}O$ because stemflow and throughfall dripping points involve longer residence times (Allen et al., 2014) and thus have a distinct isotopic composition.

In places where snowfall is an important component of precipitation, snow accumulation dynamics can significantly modify the spatio-temporal patterns of precipitation isotopic composition. Snowpack depth and density are known to be very irregular,

following complex compaction and redistribution dynamics that are influenced by topography, wind and vegetation (e.g., Trujillo et al., 2009). As a consequence, snowmelt is very heterogeneous and its flow pathways change through time as the snowpack evolves. Due to these dynamics, the spatial variability in the isotopic composition of snowpacks and snowmelt can be very large (Rücker et al., 2019; Webb et al., 2018). Moreover, melt-and-refreeze dynamics during water percolation through

5   the snowpack cause heterogeneous and time-variable isotopic fractionation (Taylor et al., 2001). Canopy-intercepted snow can have longer residence time than liquid water and, because fractionation due to sublimation is greater, especially at lower temperatures, it may contribute to larger isotopic changes (Koeniger et al., 2008).

Water flow and transport through heterogeneous porous media are complex processes that still represent a hot topic for the

10   vadose zone and groundwater communities (Kitanidis, 2015). However, not only is subsurface flow always heterogeneous (Gehrels et al., 1998; McDonnell et al., 2007; Troch et al., 2009), but also fractionation processes in the subsurface vary in both time and space. Indeed, evaporation, which is largely controlled by surface energy variations, is a major contributor to isotopic fractionation, especially at shallow soil depths. In soils and groundwater, isotopic heterogeneity results from differences in the inputs (precipitation, throughfall, snowmelt), differences in the temporal integration of previous precipitation

15   events, and differences in the subsequent fractionation from evaporation and transpiration (Benettin et al., 2018). As a general rule, smaller storage-to-output ratios with short residence times generally lead to higher temporal variability. Conversely, larger storages with longer residence times are likely to lead to a more dampened signal that integrates over longer periods of time (e.g., Zhang et al., 2016; Benettin et al., 2017). Moreover, in soils, the interaction with soil particle surfaces (Lin et al., 2018), soil organic matter (Orlowski et al., 2016a), microorganisms (Blake et al., 1997; Kool et al., 2007) and plants (Vargas

20   et al., 2017) may introduce additional isotopic heterogeneity. With increasing soil depth and down to groundwater, we generally expect that dispersive transport will lead to increasingly damped spatio-temporal variations around the average input composition. Nevertheless, we commonly characterize groundwater with wells that receive water from a variety of depths that may have isotopically distinct waters, but this variability will be obscured in mixed samples (Jasechko et al., 2017). While it is generally assumed that groundwater integrates inputs over longer times, this may not apply when distinguishing shallow

25   groundwater or perched water tables from soil water (Uhlenbrook and Hoeg, 2003). Smaller-scale spatial variations in groundwater isotopic composition have generally not been well characterized, and ecohydrological applications often assume spatial homogeneity to simplify the analysis.

Plant xylem water reflects the spatial and temporal heterogeneity of water that functional plant roots access (Ehleringer and

30   Dawson, 1992). Due to differences in rooting among species and individuals, plant water isotopic composition is often distinctly different among different species (Bertrand et al., 2014; Schwendenmann et al., 2015; Volkmann et al., 2016a). However, heterogeneity in soils and other subsurface compartments with respect to texture, structure, and water content (water filled pore space) could also result in differences in xylem water isotopic composition across trees with similar rooting patterns. Within-plant variations in xylem water composition occur because travel times can increase with within-plant path length, so

temporal variations in source isotopic composition must result in vertical variations in xylem isotopic signatures (Dawson and Ehleringer, 1993; Cernusak et al., 2005; Zhao et al., 2016). Lateral or radial variations in the trunk occur due to radial variations in the source water composition or potentially in water transport rates (and thus water age), depending on the degree of sectoriality of a plant's xylem transport (Steppe et al., 2015; Volkmann et al., 2016b). Even among individual vessels, we

5   should expect variations, given that many trees have individual branches that are seemingly plumbed to specific roots, with little evidence of dispersion across these xylem flow pathways (Zimmerman and Brown, 1971; Kline et al., 1976). Once water in the xylem reaches the stomatal aperture of the leaves, the vapour pressure deficit between the ambient air and the intercellular cavities results in isotopic fractionation that significantly alters the source signal (Dongmann et al., 1974). While these fractionation effects are spatio-temporally variable, this heterogeneity is often neglected because wood and other tissues are

10   likely to be temporal and spatial integrators of heterogeneous processes in leaves (Gessler et al., 2014).

3.3 Scaling issues

Many of the issues raised in the above sections relate to small-scale processes of water flow and transport, as research to date has often been performed at the level of soil patches or individual plants. Less attention has been directed towards determining how small-scale ecohydrological processes can be used to understand catchment- or landscape-scale phenomena. For example,

15   Bertrand et al. (2012) found that trees used different water sources depending on their location within an alluvial system. Such differences in the depths and types of water trees take up may have a critical impact on streamflow and hydrochemistry (Brantley et al., 2017), both of which generally depend on travel times and flow pathways (Rinaldo et al., 2015; van der Velde, 2015). Studies that systematically monitor ecohydrological processes across environmental conditions, soils, and vegetation types within landscapes will certainly be needed in the future, but a critical question today is how representative such individual

20   tree/plant studies are for larger-scale systems. Answering this question will require us to understand both the heterogeneity in ecohydrological processes and the resulting heterogeneity in the isotopic composition of water when moving up in scale. As we move from, e.g., the individual to stand or hillslope scale, systematic sampling approaches that account for this heterogeneity within a landscape element of interest will have to be tested. For example, these approaches might identify a sample size that is statistically representative and integrates over the main sources of variation within a given system.

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If such an approach exists for a given process of interest, it could inform sampling protocols (Fig. 2). As we move further up in scale of, e.g., catchments or an entire landscape, we need to develop approaches that appropriately represent this heterogeneity in hydrological and ecological models. Here, much may be learned from work that has been carried out in soil science (Lark, 2012a,b) on how to investigate spatial patterns and scaling related to isotopic studies in ecohydrological systems.

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Figure 2 further highlights the gap between the scale at which we typically apply our observational isotope techniques and the range of spatial and temporal scales across which we draw ecohydrological inferences. Repeated observations have provided insights into the heterogeneity of hydrological and ecological processes at these larger scales, but they have also revealed the

limitations in our current sampling strategies. Inevitably, our ability to observe the variability within a sampling event is a product of the duration and size of that sampling (Fig. 2A). In practice, we often rely on a few samples to characterize a much larger heterogeneous domain (Fig. 2). For example, small samples of xylem tissue are commonly used to characterize an entire forest or a few soil cores are meant to represent the entire range of spatial variation in soils. Temporally variable processes are

5   often sampled in a few short measurement campaigns, yet they are assumed to be representative of much longer (and perhaps variable) periods. Furthermore, investigators should consider whether concurrently sampled components of the hydrologic cycle are representative of the same time (e.g. leaf water may be sourced by water accessed weeks or days prior to the day of sampling). Inadequately sampling heterogeneous domains with respect to their mean properties and/or dispersion can lead to interpretation errors. This problem is exacerbated when analysis methods do not properly reflect the statistical properties or

10   uncertainty of the sample pool. For example, single mean values are often used as end-members in mixing models, which exaggerates the precision of the source partitioning (Phillips, 2001). We recommend that researchers adopt sampling strategies to deliberately integrate across the smaller-scale variability so that heterogeneity across the ecohydrological domains of interest can be appropriately characterised (Fig. 2B). The magnitude of heterogeneity, and therefore the most appropriate sampling strategy, will vary markedly between different water pools (Fig. 2C).

15   **4 New perspectives and research opportunities**

The current constraints and knowledge gaps we have presented above can also be seen as opportunities for new ecohydrological research. In this section, we outline future directions for research into water fluxes and partitioning using stable isotopes.

First of all – and perhaps most urgently – we call for systematic comparisons and methodological reviews of techniques for

20   extracting water from xylem, soil and other rooting media in the subsurface, followed by the development of standard protocols. Recent experimental work has attempted to evaluate the cryogenic vacuum distillation method for soil and plant water extraction and critically discussed its suitability as a standard method for plant-water investigations (Orlowski et al., 2013 and 2018a, b; Newberry et al., 2017; Thoma et al., 2018). Some of these studies have shown that the extraction method can have a significant effect on the isotopic value obtained from the analysis of pore water, depending also on the soil type

25   and organic matter content. It has been suggested that future work should examine how the full range of cryogenic extraction conditions (extraction time, temperature, vacuum threshold) as well as physicochemical soil properties affect the isotopic composition of extracted water (Orlowski et al., 2016 a, b; 2018; Gaj et al., 2017b). For instance, recent results (e.g., Gaj et al., 2017a) suggest that water from different soil types should be extracted with different temperatures to extract the same water pool, but investigations on the range of needed temperatures for each soil type are still needed. Comprehensive

30   intercomparisons of both soil water sampling and extraction methods (including, for instance, techniques such as suction cups, high pressure mechanical squeezing, centrifugation, direct vapour equilibration, microwave extraction, and cryogenic vacuum distillation) and xylem water extraction methods (such as wood cores, pressure vacuum, centrifugation, Scholander-type

pressure chambers as well as direct vapour equilibration and cryogenic vacuum distillation) are missing and urgently required to develop standardised sampling and extraction protocols.

More specifically, we believe it is critical to set up experiments that will allow us to understand whether the observed
5   differences in isotopic composition of extracted waters reflect isotopic variations in the real world or are instead associated with sampling and/or analytical artefacts. We need to work towards a better understanding of how to extract the particular water from soils and plant tissue that is relevant to answer our specific research questions. How do we extract the soil water that takes part in water flow processes? From which plant tissue should we extract water? This calls for a more detailed analysis of which water pool each method is able to access, because different methods can sample different waters in both soils and
10   plant tissues, therefore leading to potential differences in isotopic composition. For instance, cryogenic vacuum distillation can extract nearly all water from soil samples, even water held at tensions so high that plants cannot access it (although the volumes of this very tightly bound water are likely to be very small relative the volumes plants require and use). In contrast, tension lysimeters typically sample water held at <200 kPa (Geris et al., 2015), and thus do not collect all the water that plants can access and take up. For plant samples, cryogenic vacuum distillation normally extracts all water from plant tissue, including
15   intra-cellular water that is not part of the advective flow system. In contrast, other techniques (e.g., Scholander-type pressure chamber, vapour equilibration) are able to extract water from xylem vessels only (Volkmann et al., 2016b). In addition to the above-mentioned aspects, the extraction and analytical methods used (e.g., extraction technique, temperature, time, number of replicates, laser or mass spectrometer used) need to be documented and reported thoroughly.

20   Secondly, we call for more high-resolution monitoring and extensive labelling experiments with known boundary conditions (e.g., Kulmatiski et al., 2010; Grossiord et al. 2014; Beyer et al., 2016; Priyadarshini et al., 2016). These would facilitate more rigorous observations of physiological and ecohydrological processes and a more detailed characterization of the spatial heterogeneity and temporal dynamics of isotopic composition in different compartments of the critical zone. At the same time, limitations that might not be possible to overcome using natural abundances (e.g., the differentiation of isotopically similar
25   water sources) can be addressed using isotopic labelling (Koeniger et al., 2010). The usefulness of labelling studies has been acknowledged for decades, also coupled to modelling approaches (e.g., Stahl et al., 2013), but the combination of high-resolution monitoring with labelling leads to a new dimension of research opportunities. Indeed, labelling and high-resolution monitoring experiments have the potential to provide new insight into the size and speed of water flow pathways in both soils and plants. While high-frequency measurements of isotopes in soil water have been often reported, *in-situ* measurements of
30   xylem water isotopes remain challenging (Martín-Gómez et al., 2015; Volkmann et al., 2016b). Resolving this limitation would be a major step towards broadening the range of time scales that can be investigated. This also requires a thorough examination of how organic compounds in plant waters may distort laser spectroscopy measurements of water isotopes (West et al., 2010; 2011). There is also great potential for studies using two or more tracers simultaneously ("dual-labelling"). For instance, different soil layers might be labelled with different tracers (e.g., "high" deuterium label on the surface, "low" oxygen label at

depth) to explore which water pool plants preferentially access under variable conditions. In this regard, research on the often-raised issue of water vs. nutrient availability could improve our current understanding of ecohydrological feedbacks (Bakhshandeh et al., 2016).

5   The third main aspect highlighted during the workshop's discussions is the need to incorporate knowledge regarding fractionation effects (e.g., Dawson and Ehleringer, 1993) into the models that are used to interpret isotope data. For example, process-based models may help interpreting observations and experimental data (Benettin et al., 2018). Clarifications are needed on which parameters to include in a model and on when it might be possible to ignore their influence. This knowledge is still lacking, which may lead to incorrect interpretations of data and development of unnecessarily complex models. This

10   knowledge will also produce better estimates of the uncertainties associated with isotope data and better methods to propagate them. Uncertainties, also related to fractionation effects, should also be applied to Bayesian mixing models, which are used to quantify the proportional contributions of various sources to a mixture (Davis et al., 2015; Evaristo et al., 2017). Additional tracers can be helpful to support and strengthen the observations obtained by using stable isotopes. Using other types of tracers, such as fluorobenzoic acids, dissolved ions, and isotopic ratios of other elements such as radium or strontium, will introduce

15   further complexity to the system due to potential interactions of these tracers with soil, roots and the water itself. Even the use of labelled water can produce artefacts, for example masking fractionation processes that in turn can influence the results. Therefore, there is a need to understand the conditions that limit the use of stable isotopes as tracers in ecohydrological applications and to pinpoint the processes for which they may not be the best tracers. By carefully matching the methods with the research objectives, we can assess the reliability of stable isotopes of hydrogen and oxygen and determine whether

20   integrating isotopes with additional tracers would be helpful.

We strongly recommend designing studies that are not overly sensitive to the intrinsic uncertainty of the domain of interest and that represent heterogeneity in a way that costs (i.e., labour) and benefits are balanced. Potential solutions include the use of highly controlled settings, using tracer injections to amplify the signal, constraining the spatial or temporal domain of a

25   study, determining whether end-members are sufficiently (and consistently) distinguishable, asking coarser questions, or simply anticipating the higher costs associated with collecting more samples than are conventionally used. While we often do not quantify variations within samples, the uncertainties associated with the (hypothetical) effects of within-sample variations should also be considered more consistently in analyses and interpretations.

30   Finally, the ubiquitous presence of hydrogen and oxygen isotopes in different compartments of the critical zone (atmospheric water, subsurface and surface waters, plant tissues) and the close linkages between physical processes in the biosphere, lithosphere, atmosphere and hydrosphere inherently call for new interdisciplinary isotope-based investigations. Posing research questions from an interdisciplinary perspective can help to achieve a more comprehensive interpretation of data and results, and a more detailed understanding of physical processes involved. We encourage collaborations among ecologists,

plant physiologists, hydrologists, hydrogeologists and soil scientists to achieve a broader perspective from different points of view on water fluxes in the critical zone. We particularly advocate for new interdisciplinary studies into controls on spatial and temporal patterns of ecohydrological fluxes for different plant species, in different landscapes, and under different climatic forcing.

5 Concluding remarks

The workshop on "Isotope-based studies of water partitioning and plant-soil interactions in forested and agricultural environments", held in Italy in September 2017, offered scientists with different backgrounds the opportunity to meet and share ideas, experiences, and perspectives on studies of water fluxes in the critical zone based on stable isotopes of hydrogen and oxygen. The past decade has seen the emergence of new instruments and new insights, oftentimes questioning the simplifications we were forced to make earlier, but at the same time opening our eyes to new and important sources of variation. Although the need to re-evaluate our methods was a consistent theme, the opportunities provided by continuous measurements are very promising. Within the workshop and the scope of this paper, our effort has been to convert these identified knowledge gaps into new interdisciplinary research opportunities that can pave the way towards a better understanding of the physical processes governing water movement in natural and anthropogenic environments. We believe that interdisciplinary discussion of these themes is useful for the entire ecohydrological community to foster collaborations and to develop suitable methods to take full advantage of the stable isotopes of hydrogen and oxygen as an effective tool to investigate the fate, availability and the distribution of water in the environment.

Author contribution

DP, LP, and FS organized the workshop, wrote the outline and the first draft of the manuscript, supervised the whole writing process by integrating the corrections and comments of the other authors and critically revised each draft version. STA, PB, MB, JG, JK, JDM, LS, THMV, and JvF wrote specific sections of the manuscript. AA, NC, ME, JJBF, YG, JJM, and GZ provided further specific comments and literature references. PL, RTWZ, TED, and JWK led the discussion groups during the workshop, contributed to reach uniformity and coherence throughout the text, and improved the language stylistically and grammatically.

Competing interests

The authors declare that no competing interests are present

Acknowledgements

The authors thank Marialaura Bancheri, Michele Bottazzi, Roman Cibulka, Massimo Esposito, Alba Gallo, Cesar D. Jimenez-Rodriguez, Angelika Kuebert, Ruth Magh, Stefania Mambelli, Alessia Nannoni, Paolo Nasta, Vladimir Rosko, Andrea Rücker, Noelia Saavedra Berlanga, Martin Šanda, and Anna Scaini for their contributions during the discussion at the workshop

5   "Isotope-based studies of water partitioning and plant-soil interactions in forested and agricultural environments". The authors also thank "Villa Montepaldi" and the University of Florence for the access to the workshop location, and the municipality of San Casciano in Val di Pesa for logistical support.

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Figures
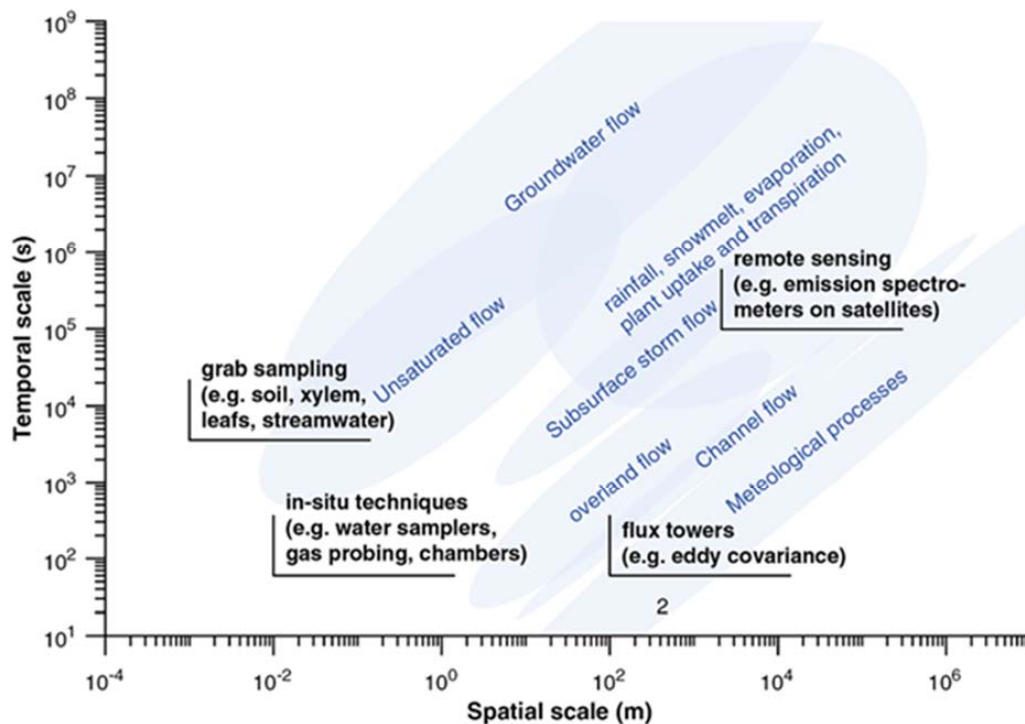
Figure 1: Simplified representation of spatial and temporal scales of ecohydrological processes (based on Blöschl and Sivapalan, 1995) and of isotope observational techniques frequently used to characterize these processes. Method scales represent typical minima that may be expanded through multiple observations, and processes scales approximate characteristic scales of variation. While recent developments in sample acquisition and analytical techniques have increased sample throughput and pushed the limits of observational capacity, a lack of ability to immediately characterize heterogeneous hydrological and ecological processes at typical study scales is glaringly apparent. As a consequence, large uncertainty and interpretation errors can result in isotopic studies, and open questions exist regarding appropriate sampling strategies and frequently.
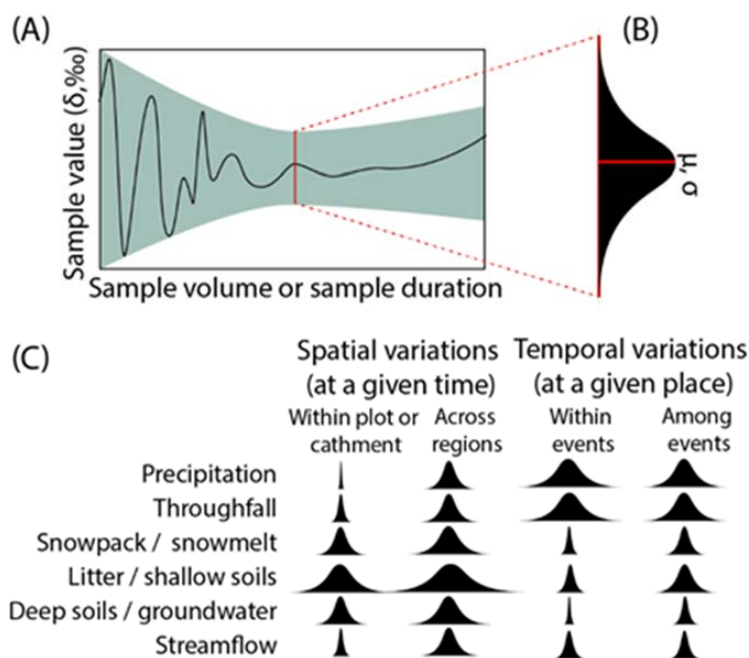
Figure 2: Measurements are made to characterize a heterogeneous domain (e.g., pore, plot, catchment, continent). (A) There is always variability within and among samples. Isotopic values of small samples are often erratic and not relevant to studying larger scale critical zone behaviour. Larger samples may average across fundamentally different domains, and not be useful for studying smaller scale behaviour.
5   There is always heterogeneity within and among samples; these trade-offs are unavoidable. (B) For an ideal sample size that integrates across micro-scale heterogeneity, there will still be macroscale heterogeneity; this heterogeneity is generally of interest in environmental isotope studies. (C) The magnitude of heterogeneity, as sampled by conventional approaches at common scales, hypothetically differs substantially among pools as a function of the processes that contribute to their isotopic compositions.