35

HYDROGEN AND OXYGEN ISOTOPE TRACERS IN ECOHYDROLOGY

Natalie Orlowski, Cody Millar, Jeffrey J. McDonnell, Magali F. Nehemy, Youri Rothfuss, Dörthe Tetzlaff and Hongxiu Wang

35.1 Hydrogen and oxygen stable isotopes in ecohydrology

35.1.1 Isotope-aided ecohydrological research

The stable isotopes of hydrogen (^{2}H, ^{1}H) and oxygen (^{18}O, ^{17}O, ^{16}O) are powerful tools to trace the movement, mixing, and storage of water through the soil-plant-atmosphere-continuum (SPAC). The isotopic analyses of samples from precipitation (rain, throughfall, stemflow), ground water, surface waters (streams, lakes, oceans), soil, and plant water (xylem, leaves, roots), as well as atmospheric moisture (fog, dew), enables the quantification/ partitioning of fluxes, water ages, and source contributions. This can enable crucial insights into ecosystem functioning, the hydrological cycle, and informs our ability to optimize vegetation water use (i.e., irrigation practices), and vegetation's resilience to environmental change (i.e., climate and land use). Isotope records in ice cores, lake and ocean sediments, and plant material also inform paleoclimate reconstructions.

This chapter outlines sampling practices for ecohydrological investigations, water extraction, and *in situ* techniques to access the water bound within samples. It also covers the analytical methods for H and O isotopes, isotopic data interpretation and its value, and current challenges in isotope-aided ecohydrological modeling.

35.1.2 What are stable isotopes?

Isotopes are variations of the same element differing in neutron number (1) and thus, differentiated by mass number, for example, Hydrogen-1 (^{1}H, protium) or Hydrogen-2 (^{2}H, deuterium), Oxygen-16 (^{16}O), or Oxygen-18 (^{18}O). A *stable isotope* does not undergo radioactive decay over time. Mass differences cause *isotopic fractionation*—the sorting of isotopes during physical or chemical processes (1). Ecohydrologists typically employ the stable isotopes of hydrogen and oxygen, though others (C, N, S) may also be used. Different isotopic variations of the water molecule are known as isotopologues (i.e., $^{1}H^{2}H^{18}O$, $^{1}H_{2}{}^{16}O$, $D_{2}{}^{17}O$, etc.).

The application of stable isotopes as ecological tracers relies on some **fundamental concepts.** Isotopes have skewed distributions resulting in **natural relative abundances**. For H and O, the lighter isotope is more abundant while the heavier are less abundant (rare) (1). The relative

DOI: 10.4324/9781003383918-41

abundances of the stable H isotopes are 99.984% (^{1}H) and 0.016% (^{2}H) and relative abundances for the O isotopes are 99.762 (^{16}O), 0.038% (^{17}O), and 0.200% (^{18}O) (1).

Measured **isotopic ratios** within ecological samples are expressed as the ratio of the less abundant isotope to the more abundant isotope, that is, $^{2}H/^{1}H$, $^{18}O/^{16}O$, these being *absolute ratios*. Isotopic ratios are reported as *relative ratios* using delta (δ) notation (i.e., $\delta^{2}H$: $^{2}H/^{1}H$, $\delta^{18}O$: $^{18}O/^{16}O$, $\delta^{17}O$: $^{18}O/^{17}O$) (1, 2), reported as a difference relative to an international water standard VSMOW (Vienna Standard Mean Ocean Water) in per mille (‰, per thousand). This allows for intercomparison of data sets from many different locations.

Fractionation occurs via equilibrium and kinetic fractionation (see Chapters 2 and 3 in (1), and Section 1.2 in (3)) creating unique isotopic signatures in different ecohydrological compartments that trace their origin and history (Figure 35.1). During **equilibrium fractionation** isotopes distribute between phases (e.g., liquid–vapor) according to their thermodynamic properties (i.e., vibrational frequencies). During evaporation, the lighter isotopes (^{1}H, ^{16}O) are preferentially partitioned into the vapor phase, leading to enrichment of the heavier isotopes (^{2}H, ^{18}O) in the liquid phase. Temperature and isotopic mass differences further affect equilibrium fractionation. **Kinetic fractionation** arises from differing reaction rates due to mass differences, with heavier isotopes tending to react slower (due to higher reaction activation energies). Again, this can lead to *enrichment* (or depletion) of isotopes in one phase relative to another, depending on the system's kinetic constraints. Both equilibrium and kinetic fractionation can affect ecosystem processes like evaporation. In this regard, the Craig-Gordon model (4) represents a fundamental framework that explains the combined effect of equilibrium and kinetic isotopic fractionation during the evaporation of water from different reservoirs (revised by (5, 6)).

35.1.3 The transformation of the isotope signal through the SPAC

Climate, geography, and hydrological processes drive spatial and temporal variations in H and O isotope ratios. For example, precipitation isotopic composition varies with temperature, latitude, altitude, and distance from moisture sources (7). The isotopic composition of local meteoric waters (1) exhibits seasonal variation along the so-called *Local Meteoric Water Line* (LMWL, the linear relationship between $\delta^{18}O$ and $\delta^{2}H$ of meteoric waters (1)), characterized by lower (more depleted) values in winter and higher (more enriched) values in summer (1); and globally precipitation plots along the *global meteoric water line* (GMWL). In the global water cycle, there are two main processes that can cause $\delta^{18}O$ and $\delta^{2}H$ variations: (i) evaporation from water bodies, soils, or vegetation surfaces and (ii) the progressive rain-out of the water vapor masses as they move toward regions with lower temperatures (e.g., higher altitudes and latitudes) (8).

As water evaporates from the ocean and forms clouds, oceanic air masses travel inland with successive precipitation events leading to a continuous decrease in isotope ratios (depletion). Less abundant, and heavier isotopologues, such as $^{2}H^{1}HO$ and $^{1}H_{2}{}^{18}O$, tend to condense and fall out of the atmosphere preferentially compared to $^{1}H_{2}{}^{16}O$. This preferential removal causes the remaining moisture in clouds to become isotopically "lighter" as the system progresses across the continent.

Precipitation falling onto the vegetation canopy is partitioned into throughfall, interception, and stemflow components, with stemflow and throughfall eventually infiltrating into the soil. Canopy processes further modify the isotopic composition of this water. Throughfall was generally reported to be enriched in heavy isotopes relative to open rainfall (9). In non-rainfall, dryland, coastal, or cloud-affected ecosystems, fog and dew supply critical amounts of water to the ecosystem (10). It is assumed that fog undergoes equilibrium fractionation (11); however, dew formation

might be dominated by kinetic fractionation (12). When fog and rainfall are produced by different climatic mechanisms (e.g., regions with wet and dry seasons), fog is isotopically enriched compared with rainfall from the same area (9).

Soil water isotopic composition reflects inputs from precipitation, throughfall, and stemflow modulated by root hydraulic redistribution, evaporation, and subsurface mixing (13, 14). Evaporation of soil water preferentially removes the lighter isotopes (^{1}H, ^{16}O) causing enrichment of heavy isotopes in the remaining water, while new precipitation shifts soil's δ-values depending on its isotopic input (15, 16).

Water available in the subsurface is absorbed by plant roots as a result of water potential differences along the SPAC, with water uptake and transport influenced by plants' vascular system and soil texture (17). During root water uptake, it is generally assumed that no isotopic fractionation occurs (18, 19), but it can occur in very dry or saline environments (20) or in association with arbuscular mycorrhizas (21). Plants in fog- or dew-inundated ecosystems can additionally take up condensation water through their leaves; or roots take up water dripping from the canopy (22).

Water that enters the xylem is transported from roots to leaves and is transpired once it reaches the stomata. The xylem isotopic composition can change as a result of preferential transfer of lighter isotopes to the phloem during periods of water stress (23), which may change diurnally (24), or as a result of bark evaporative enrichment and/or kinetic effect during cellulose biosynthesis (25). Along the flow path, where plant tissue is not suberized (i.e., green stems, leaves), evaporative fractionation can also occur. Differences in isotopic composition along the tree's water flow path can reflect variable flow velocities (26), with the tree's base reflecting more recent precipitation, and the canopy older events (27). Still little is known about water mixing within tree trunks (28) and plant tissues (29). When water reaches the leaves, it undergoes fractionation in the stomata due to site-specific preferential evaporation of lighter isotopes, with the remaining water being enriched in heavy isotopes. The magnitude of this fractionation is dependent on environmental conditions and leaf characteristics (30). However, relative plant water sources can still be computed (31). Figure 35.1 highlights the water flow pathways through the SPAC and their isotopic variations.

35.2 Sampled materials in isotope ecohydrology

To obtain interpretable isotopic data from ecosystem samples, a multi-step process chain is followed: sampling and processing, transport and storage, water extraction, pre-analysis processing, isotopic analysis, and data post-processing and correction (34, 35).

35.2.1 Research design, types of samples, and ancillary data

Various research design factors influence the collection of ecological samples for isotopic analysis (reviewed in (35)). Common samples include atmospheric waters (precipitation, fog, dew); terrestrial waters (streams, rivers, lakes, oceans); groundwater; vegetation water/vapor samples; soil solid/ water/ vapor; and bedrock water. Study questions determine what samples to collect. For vegetation water sourcing, concurrent soil, vegetation, atmospheric, and terrestrial waters are collected with replication at multiple points in time. Ancillary data should be collected (and reported on) detailing the bio-geo-physical characteristics of the site, as these impact isotopic composition (see Table 1 in (35*, 29*)). Isotopic investigations should be accompanied by additional plant hydraulic and physiological measurements that can provide important information on plant response to environmental conditions and aid isotope interpretations.

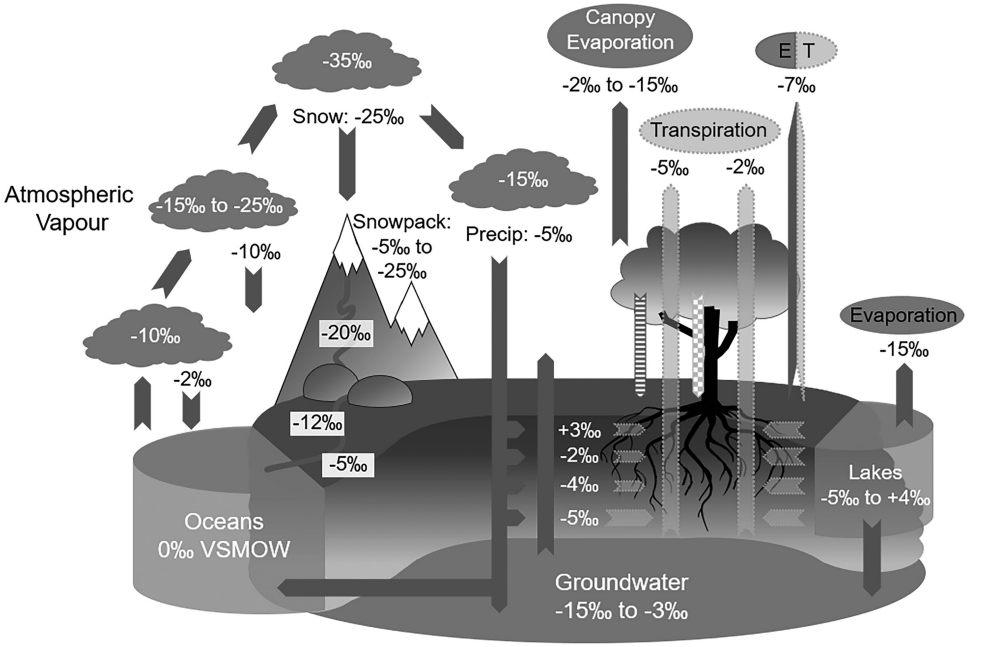

Figure 35.1 Schematic of δ^{18O} values (‰) as water moves through the SPAC (sourced from (32, 33)). Arrows indicate abiotic (blue) water fluxes (solid fill, solid border), biotic (green) water fluxes (transparent fill, dashed border) throughfall (striped fill), and stemflow (checkered fill). Factors such as geography (latitude, elevation, distance from source), precipitation and evapotranspiration amount, seasonality, and biotic controls change isotope values as water moves through each compartment. Two transpiration scenarios are provided: one where water is sourced mainly from a deeper soil layer; or where water is sourced across the soil profile (uptake arrow size indicates amount). Changes to the isotope values of fog and dew in mountainous settings and under various rain-out scenarios can be seen in (9).

35.2.2 Sampling and storage strategies

Sampling strategy depends on the study design, sampling location, sample type, intended water extraction method, and storage logistics (35). Best practices and comprehensive guidance for liquid, soil, and vegetation samples are provided in (35, 36). Briefly, sufficient sample material must be collected for isotopic and other desired analyses; with replication for statistical validity; to adequately represent meteorological conditions; to ensure the possibility of re-analysis; and to represent the research site's spatio-temporal isotopic variation. This is simple for liquid samples, but for vegetation and soil samples, where water volumes depend on plant water status or soil water content, pre-testing of water contents is advised. For isotopic analyses 1–2 ml of water is sufficient (35), though more is needed if re-analysis, or additional analyses like organic compound contents are required.

Liquid water is grab-sampled from various types of collectors (i.e., precipitation/ fog/ dew collectors, passive lysimeters, ice corers), or directly from the source (streams, lakes, etc.) using appropriate vials and limiting headspace. Automated samplers support event-based sampling (37) to aid computation of isotope composition weighted means (36). Liquid water samples can directly move to the analysis step (Section 35.4). For vegetation and soil samples, destructive sampling or

in situ methods may be applied. For soil samples, site variability (i.e., topography, etc.) is characterized by collecting multiple replicated samples from various depths and several boreholes/ pits/ *in situ* probes (35). Vegetation samples may be collected for a variety of ecohydrological purposes, one such example is to identify plant water sources. These are collected with the aim of obtaining unenriched xylem sap water/ vapor, typically from stems, twigs, roots, or tree trunks in the case of *in situ* methods.

Upon collection, sample water may begin to evaporate unless prevented by environmental conditions (37), so sample processing (e.g., bark removal, etc.) should occur under controlled conditions when sampling in hot, dry environments. Evaporation during sampling, storage, and transport can cause significant errors in isotopic composition; up to 20‰ (δ^2H) and 6‰ ($\delta^{18}O$) (37). Immediate storage in appropriate media following sample collection helps minimize fractionation risks (35).

Storage media type depends on the sample type and the intended extraction/ analysis method (see (34, 35)). For liquid samples, glass vials and thick-walled High-Density Polyethylene (HDPE) and Perfluoroalkoxy alkane (PFA) containers with screw caps containing polymer septa are appropriate (34, 36). For soil and vegetation samples, storage media compatibility with the intended extraction method reduces transfer needs and minimizes sample fractionation risks.

Storage temperatures are interrelated with evaporative fractionation risks (37); in-storage soil respiration issues (38); soil microstructure damage (39); enhanced organic contamination issues from vegetation tissue damage (40); and degradative impacts on samples during storage (41). Short-term field storage can be managed with coolers, while longer-term storage requires cool (4–6 °C) or frozen (< 0 °C) conditions, though freezing is not appropriate for all samples (35).

35.3 Accessing matrix-bound water

Following collection, water is extracted from soil and vegetation samples for isotopic analysis (34). *In situ* methods bypass the extraction step via combination with isotope analyzers in the field (or lab). These methods are briefly described here, with detailed reviews in the literature (34, 35). During extraction of water from vegetation, clays, and organic-matter-rich soils, co-extracted volatile organic compounds (VOCs) can cause isotopic analysis errors (discussed in (34, 35, 42)).

35.3.1 Types of extraction methods

Extraction methods vary in type, mechanism, benefits, and limitations. They can be categorized by their extraction mechanism:

i Mechanical force methods: high-pressure mechanical squeezing (HPMS) (43); centrifugation (44) and cavitron extraction (vegetation only) (45); Scholander-type pressure chambers (SPC; vegetation only) (46); and passive or suction lysimeters (soil only) (47).

ii Phase-change methods: cryogenic vacuum distillation (CVD) (48, 49); vapor equilibration methods (some in-line) (50, 51); microwave in-line (52) or chemical distillation approaches (53). In-line methods are connected to isotope analyzers, combining the extraction and analysis steps. Phase change from liquid to water for vapor equilibration methods typically occurs at ambient temperatures, whereas the other phase change approaches use heat/ cold to induce the transition. Phase change methods will co-extract present VOCs which can be volatilized under the given extraction conditions.

iii *In situ* methods: collect water through equilibration processes, often enabling continuous measurements via connection with an isotope analyzer (54–58). Suction cups, wick samplers, and passive lysimeters (47), also fall under this category, but collection, storage, and transport of the water is required for later analyses.

Method selection balances scientific considerations with practical concerns, such as resource (funding, time, labor) and method availability. Review of the characteristics, limitations, expected error, and benefits of the various methods are available (see Table 2 in both (34) and (35)). Key selection factors include: suitability for a given sample type (e.g., vegetation); water pool(s) of interest for the given study (e.g., low tension soil water, xylem water); number of samples to process; and the risk of method-induced artifacts (e.g., via CVD, VOC co-extraction).

35.3.2 Assessing ecohydrological pools of water

Our research questions may require us to study isotopically different soil or vegetation pools of water (45, 59), or perhaps the isotopic composition of proxy materials like cellulose for paleoclimate reconstruction. In plant water sourcing studies, our interest is typically the xylem sap water/vapor, which participates in transpiration (see (34, 45, 59) for descriptions of these water pools). Applied extraction methods and parameters (temperature, duration, pressure) can impact which water pools are accessed (45, 59) and thus plant water sourcing interpretations (60). Debates persist on whether bulk stem water appropriately reflects vegetation water sources (see (45, 61) for contrasting findings).

For soils, mechanical techniques apply variable pressures targeting water held at different tensions (35, 59), preferentially removing only a specific water pool; whereas a phase change method like CVD requires removal of >98% of a sample's water to avoid isotopic fractionation (see Box 1 in (35)), thus accessing the bulk sample water.

For vegetation, modified mechanical approaches (e.g., cavitron, SPC) can isolate specific water pools like xylem sap, which may differ isotopically from bulk water extracted via CVD (45, 46). Conversely, mechanical methods tend to extract bulk plant water due to pre-extraction maceration before centrifugation, or by crushing of the vegetation tissues (via HPMS) which may lead to enhanced VOC co-extraction (40), though not in all cases (62). Phase change methods like CVD will target bulk water and are sensitive to incomplete extraction, though pre-processing (e.g., bark removal) can help isolate specific tissues and therefore water pools.

In situ methods may also target specific water pools depending on the placement of probes / borehole and their operational parameters, with these procedures still being refined. *In situ* and equilibration methods are also prone to VOC-induced errors, as their mechanism of operation relies on collection of vapor and some organic compounds may concentrate in the vapor phase (63). *In situ* vapor analysis was less sensitive to co-equilibrated VOCs than CVD or vapor equilibration (head-space method) for some vegetable species (42).

There are a number of issues involved in the extraction of matrix-bound waters, some of which can affect their isotopic composition or cause erroneous values (34, 35, 42). Key challenges include a lack of internationally standardized operating protocols; variation in applied extraction parameters (60) and sample physicochemical properties (64) potentially causing extraction artifacts; and co-extraction of VOCs that cause errors during isotopic analyses (65–68). Factors such as vegetation species, soil content, sample type, and sampling timing affect the amounts of co-extractable VOCs.

35.4 Isotope ratio analysis methods

35.4.1 Isotope ratio analysis systems: mass spectrometry and laser spectroscopy

Isotope ratio mass spectrometry (IRMS) and isotope ratio infrared spectroscopy (IRIS) analyze the stable isotopic composition of H and O in water samples, each with distinct operational approaches and benefits. IRMS and IRIS are colloquially known as mass spectrometry and laser spectroscopy, respectively. IRIS uses laser-based absorption techniques to measure the photo-absorption of H_2O isotopologues (i.e., $^1H_2{}^{16}O$, $^1H_2H^{16}O$, and $^1H_2{}^{18}O$) (67). Two different types of IRIS analyzers exist: off-axis integrated cavity output spectroscopy (OA-ICOS, ABB Ltd.) and wavelength-scanned cavity ring-down spectroscopy (WS-CRDS, Picarro Inc.). Both allow the simultaneous measurement of the abundance ratio of water isotopologues with precision similar to IRMS, especially for pure water analysis (69). IRMS measures isotopic ratios by ionizing the sample, separating ions based on their mass-to-charge ratio, detecting these ions, and quantifying the relative abundance of different isotopes.

Both IRMS and IRIS analyzers measure isotopic ratios in the vapor phase requiring liquid (extracted) water samples to be converted into vapor prior to analysis (67). There are two categories of the vaporizing method: combustion of water at a high temperature and equilibration of water and soil/ vegetation samples with headspace vapor. For combustion, a syringe withdraws a volume of liquid water from the sample vial and injects the water into a vaporizer with a temperature varying from 70 °C to 1350 °C (66). For equilibration, liquid water, soil, and vegetation samples are placed in a container, and dry gas is injected into the headspace of the container. Dry synthetic air and N_2 (nitrogen gas) are commonly used as carrier gas for IRIS analysis (39); and H_2 and CO_2 gases for IRMS analysis (67).

IRIS is currently the most widely used method (69), which can be attributed to several advantages compared to IRMS: IRIS is more cost-effective regarding instrument purchase and consumables; no prior chemical equilibration or conversion into elemental constituents is required; operation procedure is user-friendly; analysis via coupling with automatic samplers for liquid (70) and gaseous water phases (e.g. (54, 55)) is possible; simultaneous measurements of different isotopologues, easy transportation and operation in power-guaranteed environments for fieldwork feasible.

35.4.2 Standards for calibration and accuracy tracing

Measured isotopic ratios need to be normalized to the Vienna Standard Mean Ocean Water and Standard Light Antarctic Precipitation (VSMOW-SLAP) scale (71), standards provided by the International Atomic Energy Agency (IAEA) (see Chapter 2 in (1) for reference standards). To achieve normalization, a minimum of two calibration standards and one validation standard with known true isotopic composition are analyzed alongside the samples (72). The measured isotopic values of samples are then converted to true values using the relationship between the measured values and true values of the standards. Labs can establish their own in-house standards, but cross-calibration using IAEA standards is required. To ensure the accuracy of the analysis, another standard should be measured during analysis runs serving as the quality assurance and quality control (QA/QC) standard (73). Analysis accuracy and precision are determined through QA/QC measurement (see (69) for discussion of accuracy and error propagation tracking).

35.4.3 Current challenges with organic contamination

Volatile or soluble organic compounds in water (and vapor) samples from vegetation and soils can cause inaccurate isotopic data by interfering with the absorption spectra during IRIS analyses (see references in (34, 35)). These spectral interferences are caused by methanol, ethanol, and other biogenic VOCs with hydroxyl groups similar to water (65). Such naturally occurring, ubiquitous VOCs, originating from living organisms or decaying organic matter, can be extracted alongside water during laboratory or *in situ* extraction and analysis. However, few studies have published contaminant concentration data alongside isotopic data (with some exceptions, e.g. (40)). Contamination is influenced by vegetation functional categories and species, vegetation component sampled, phenological state, habitat, soil type, extraction method applied, and climate (35).

Methods for detecting and removing co-extracted VOCs prior to or concurrent with IRIS analyses are available (reviewed in (34)), and decision-making flow charts, which provide guidance on handling spectral interference during IRIS analysis (35, 74). The following methods are used to best address organic contamination-related spectral interference: (i) if soil and vegetation water extracts are suspected of being contaminated, solely use IRMS; otherwise, run a representative sample subset on both IRMS and IRIS; (ii) use filtration or combustion methods to remove organic compounds from the water prior to analysis; and (iii) develop in-house correction protocols (42), or use manufacturer-supplied post-run software/protocols (35, 74). Developing protocols to minimize VOC co-extraction or implementing data corrections are crucial steps for mitigating VOC impacts on IRIS measurements.

35.5 Interpreting isotope data: the value and challenges of ecohydrological models

Standard interpretation of isotope data involves (i) single isotope plot regression (δ vs a non-isotopic variable or parameter) such as the "Keeling plot" for determining ecosystem water vapor isotopic composition, (ii) dual isotope plots ($\delta^{18}O$ vs $\delta^{2}H$) to investigate water sources and differentiate their origin or the prevalence of evaporation in meteoric waters, and (iii) two to multi-source statistical mixing models (e.g., Bayesian models such as MixSIAR) to separate evaporation and transpiration fluxes or to quantify the relative contributions of sources to vegetation water use (75, 76).

Incorporating stable isotope data into process-based ecohydrological models can be considered as a step further to using the above statistical tools as they include processes that are not represented (e.g., hydraulic lift in MixSIAR) and go beyond some of their simplifying hypotheses (e.g., two single water sources). In addition, tracer-aided models allow water and isotopes to be tracked through different landscape co-existing compartments, thus, they are not compartment-specific. Finally, isotope-aided model parametrization, informs model structure, and reduces uncertainty through multi-criteria calibration (e.g. (76)).

Some of the current questions and challenges for interpreting isotope data include:

I Spatial and temporal data coverage: despite technological advances, continuous isotope data remain scarce (especially when compared to hydrometric data), with trade-offs between high temporal and spatial resolution (77). High temporal measurements via *in situ* techniques are commonly applied on one site, while spatially distributed sampling (mostly via destructive methods) is usually only conducted at coarse temporal resolutions. However, modeling requires the integration of sparse coverage of isotope data and much higher resolution data of climate and hydrology (78). One challenge identified by the community is to adapt sampling strategies

to disentangle temporal dynamics in isotopic composition from natural spatial variability for hypothesis testing (35).

II Uncertainties in data and model parametrization and structure: all data carry inherent uncertainty, and isotopes are no exception—indeed, their integrative nature may amplify this uncertainty. Uncertainties stem from monitoring in (and transport from) the field (e.g., through evaporation effects, condensation in sample containers, etc.) followed by uncertainty in laboratory analyses (e.g., VOC issues, appropriate standardization, etc. (35)). All related scientific publications should account for data uncertainty through provision of standard deviations and equipment precisions (34). Kinetic effects greatly impact isotopic composition in surface water bodies (e.g., lake, soil water), but an appropriate parametrization of its associated fractionation factor in models is still missing (79).

III Under-representation of processes in models: To this day, the processes by which vegetation physiology affects isotopic temporal dynamics and distribution inside the different (conductive vs. non-conductive) plant organs are generally missing in small-scale soil-vegetation-atmosphere transport models. Recent work questions the assumption of fractionation-free root uptake (23, 29). Others acknowledge that existing root water uptake models are limited, both in their structure and by their (high) data need, and further propose to combine different approaches (conceptual, hydrodynamic, optimality principle, statistical) revolving around isotopic information to study vegetation water use (80). Most recently, research tested the implication of considering potential soil water tension effects on the isotopic composition of soil water in vegetation water use estimation (81).

IV Upscaling and emerging processes: as processes scale from the catchment to ecosystem level, water sources and mixing processes become more complex and heterogeneous. Therefore, damping of the isotope signals in stream water—compared to the highly variable precipitation input signals—increases and thus, small-scale hydrological processes are less reflected at larger scale modeling (82). This, so far, has hindered the upscaling of tracer-aided modeling from intensively monitored small-scale to larger catchments.

References

1 Z. Sharp, *Principles of Stable Isotope Geochemistry*, 2nd Edition. *Open Textbooks* (2017).

2 C. R. McKinney, J. M. McCrea, S. Epstein, H. A. Allen, H. C. Urey, Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. *Rev Sci Instrum* **21**, 724–730 (1950).

3 S. Jasechko, Global isotope hydrogeology—Review. *Reviews of Geophysics* **57**, 835–965 (2019).

4 H. Craig, L. I. Gordon, "Stable isotopes in oceanographic studies and paleotemperatures: Spoleto 1965" E. Tongiorgi, Università di Pisa, Italy, Eds. (Consiglio nazionale delle ricerche, Laboratorio di geologia nucleare, Spoleto, Italy, 1965), pp. 9–130.

5 J. Horita, K. Rozanski, S. Cohen, Isotope effects in the evaporation of water: A status report of the Craig-Gordon model. *Isotopes in Env. & Hlth. Stud.* **44**, 23–49 (2008).

6 R. Gonfiantini, L. I. Wassenaar, L. Araguas-Araguas, P. K. Aggarwal, A unified Craig-Gordon isotope model of stable hydrogen and oxygen isotope fractionation during fresh or saltwater evaporation. *Geochim. Cosmochim. Acta* **235**, 224–236 (2018).

7 Y. Vystavna, I. Matiatos, L. I. Wassenaar, Temperature and precipitation effects on the isotopic composition of global precipitation reveal long-term climate dynamics. *Sci Rep* **11**, 18503 (2021).

8 J. R. Gat, Oxygen and hydrogen isotopes in the hydrologic cycle. *Annu. Rev. Earth Planet. Sci.* **24**, 225–262 (1996).

9 M. Scholl, W. Eugster, R. Burkard, Understanding the role of fog in forest hydrology: Stable isotopes as tools for determining input and partitioning of cloud water in montane forests. *Hydrol. Process.* **25**, 353–366 (2011).

10 K. F. Kaseke, L. Wang, M. K. Seely, Nonrainfall water origins and formation mechanisms. *Sci. Adv.* **3**, e1603131 (2017).

11 R. Gonfiantini, A. Longinelli, Oxygen isotopic composition of fogs and rains from the North Atlantic. *Experientia* **18**, 222–223 (1962).
12 R. D. Deshpande, A. S. Maurya, B. Kumar, A. Sarkar, S. K. Gupta, Kinetic fractionation of water isotopes during liquid condensation under super-saturated condition. *Geochim. Cosmochim. Acta* **100**, 60–72 (2013).
13 A. H. Halbritter, H. J. De Boeck, A. E. Eycott, S. Reinsch, D. A. Robinson, S. Vicca, B. Berauer, C. T. Christiansen, M. Estiarte, J. M. Grünzweig, R. Gya, K. Hansen, A. Jentsch, H. Lee, S. Linder, J. Marshall, J. Peñuelas, I. Kappel Schmidt, E. Stuart-Haëntjens, P. Wilfahrt, the C. W. Group, V. Vandvik, The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx). *Methods Ecol Evol.* **11**, 22–37 (2020).
14 M. Sprenger, H. Leistert, K. Gimbel, M. Weiler, Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes. *Rev. Geophys.*, **54** (2), 674–704 (2016).
15 J. R. Ehleringer, R. K. Monson, *Plant Ecology in a Changing World* (CRC Press, Boca Raton, 2025).
16 S. T. Allen, M. Sprenger, G. J. Bowen, J. R. Brooks, Spatial and temporal variations in plant source water: O and H isotope ratios from precipitation to xylem water in *Stable Isotopes in Tree Rings: Inferring Physiological, Climatic and Environmental Responses*, R. T. W. Siegwolf, J. R. Brooks, J. Roden, M. Saurer, Eds. (Springer International Publishing, Cham, 2022) *Tree Physiology*, pp. 501–535.
17 C. Bachofen, S. J. Tumber-Dávila, D. S. Mackay, N. G. McDowell, A. Carminati, T. Klein, B. D. Stocker, M. Mencuccini, C. Grossiord, Tree water uptake patterns across the globe. *New Phytol.* **242**, 1891–1910 (2024).
18 T. E. Dawson, J. R. Ehleringer, Streamside trees that do not use stream water. *Nature* **350**, 335–337 (1991).
19 A. I. Vargas, B. Schaffer, L. Yuhong, L. da S. L. Sternberg, Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. *New Phytol* **215**, 582–594 (2017).
20 L. Zhao, L. Wang, L. A. Cernusak, X. Liu, H. Xiao, M. Zhou, S. Zhang, Significant difference in hydrogen isotope composition between xylem and tissue water in populus euphratica. *Plant Cell Environ.* **39**, 1848–1857 (2016).
21 M. Poca, O. Coomans, C. Urcelay, S. R. Zeballos, S. Bodé, P. Boeckx, Isotope fractionation during root water uptake by Acacia caven is enhanced by arbuscular mycorrhizas. *Plant Soil*, doi: 10.1007/s11104-019-04139-1 (2019).
22 E. B. Limm, K. A. Simonin, A. G. Bothman, T. E. Dawson, Foliar water uptake: A common water acquisition strategy for plants of the redwood forest. *Oecologia* **161**, 449–459 (2009).
23 M. F. Nehemy, P. Benettin, S. T. Allen, K. Steppe, A. Rinaldo, M. M. Lehmann, J. J. McDonnell, Phloem water isotopically different to xylem water: Potential causes and implications for ecohydrological tracing. *Ecohydrology* **15**, e2417 (2022).
24 A. Vega Grau, J. Herbohn, S. Schmidt, J. McDonnell, Bark water affects the isotopic composition of xylem water in tropical rainforest trees. *Front. For. Glob. Change* **7** (2025). https://doi.org/10.3389/ffgc.2024.1457522
25 A. Montemagno, R. F. Keim, V. López-Días, V. Bense, A. J. Teuling, C. Hissler, Progressive enrichment in ^{18}O and ^{2}H in xylem water along sap flow paths in Fagus sylvatica trees. *Ecohydrology* **16**, e2582 (2023).
26 M. F. Nehemy, J. Maillet, N. Perron, C. Pappas, O. Sonnentag, J. L. Baltzer, C. P. Laroque, J. J. McDonnell, Snowmelt water use at transpiration onset: Phenology, isotope tracing and tree water transit time. *Water Resour. Res* **58**, e2022WR032344 (2022).
27 F. Bernhard, M. G. Floriancic, K. Treydte, A. Gessler, J. W. Kirchner, K. Meusburger, Tree- and stand-scale variability of xylem water stable isotope signatures in mature beech, oak and spruce. *Ecohydrology* **17**, e2614 (2024).
28 J. Knighton, S. Kuppel, A. Smith, C. Soulsby, M. Sprenger, D. Tetzlaff, Using isotopes to incorporate tree water storage and mixing dynamics into a distributed ecohydrologic modelling framework. *Ecohydrology* **13**, e2201 (2020).
29 J. de la Casa, A. Barbeta, A. Rodríguez-Uña, L. Wingate, J. Ogée, T. E. Gimeno, Isotopic offsets between bulk plant water and its sources are larger in cool and wet environments. *Hydrol. Earth Syst. Sci.* **26**, 4125–4146 (2022).
30 L. A. Cernusak, M. M. Barbour, S. K. Arndt, A. W. Cheesman, N. B. English, T. S. Feild, B. R. Helliker, M. M. Holloway-Phillips, J. A. M. Holtum, A. Kahmen, F. A. McInerney, N. C. Munksgaard, K. A. Simonin, X. Song, H. Stuart-Williams, J. B. West, G. D. Farquhar, Stable isotopes in leaf water of terrestrial plants. *Plant Cell Environ.* **39**, 1087–1102 (2016).
31 P. Benettin, M. F. Nehemy, L. A. Cernusak, A. Kahmen, J. J. McDonnell, On the use of leaf water to determine plant water source: A proof of concept. *Hydrol. Process.* **35**, e14073 (2021).

32 C. Werner, M. Dubbert, Resolving rapid dynamics of soil–plant–atmosphere interactions. *New Phytol.* **210**, 767–769 (2016).

33 K. G. Sjögren, T. D. Price, A complex Neolithic economy: Isotope evidence for the circulation of cattle and sheep in the TRB of western Sweden. *J. Archaeol. Sci.* **40**, 690–704 (2013).

34 C. Millar, K. Janzen, M. F. Nehemy, G. Koehler, P. Hervé-Fernández, H. Wang, N. Orlowski, A. Barbeta, J. J. McDonnell, On the urgent need for standardization in isotope-based ecohydrological investigations. *Hydrol. Process.* **36**, e14698 (2022).

35 N. Ceperley, T. E. Gimeno, S. R. Jacobs, M. Beyer, M. Dubbert, B. Fischer, J. Geris, L. Holko, A. Kübert, S. Le Gall, M. M. Lehmann, P. Llorens, C. Millar, D. Penna, I. Prieto, J. Radolinski, F. Scandellari, M. Stockinger, C. Stumpp, D. Tetzlaff, I. van Meerveld, C. Werner, O. Yildiz, G. Zuecco, A. Barbeta, N. Orlowski, Y. Rothfuss, Toward a common methodological framework for the sampling, extraction, and isotopic analysis of water in the Critical Zone to study vegetation water use. *WIREs Water* **11**, e1727 (2024).

36 IAEA (International Atomic Energy Agency), "Sampling and Isotope Analysis of Agricultural Pollutants in Water" (text, IAEA, Vienna, Austria, 2018); https://www.iaea.org/publications/12374/sampling-and-isotope-analysis-of-agricultural-pollutants-in-water.

37 B. M. C. Fischer, J. Frentress, S. Manzoni, S. A. O. Cousins, G. Hugelius, M. Greger, R. H. Smittenberg, S. W. Lyon, Mojito, Anyone? An exploration of low-tech plant water extraction methods for isotopic analysis using locally-sourced materials. *Front. Earth Sci.* **7** (2019). https://doi.org/10.3389/feart.2019.00150

38 B. Gralher, B. Herbstritt, M. Weiler, L. I. Wassenaar, C. Stumpp, Correcting laser-based water stable isotope readings biased by carrier gas changes. *Environ. Sci. Technol.* **50**, 7074–7081 (2016).

39 B. Gralher, B. Herbstritt, M. Weiler, Technical note: Unresolved aspects of the direct vapor equilibration method for stable isotope analysis ($\delta^{18}O$, $\delta^{2}H$) of matrix-bound water: Unifying protocols through empirical and mathematical scrutiny. *Hydrol. Earth Syst. Sci.* **25**, 5219–5235 (2021).

40 C. Millar, D. Pratt, D. J. Schneider, J. J. McDonnell, A comparison of extraction systems for plant water stable isotope analysis. *Rapid Commun. Mass Spectrom.* **32**, 1031–1044 (2018).

41 M. A. Teece, M. L. Fogel, Preparation of ecological and biochemical samples for isotope analysis in Pier A. de Groot (ed.), *Handbook of Stable Isotope Analytical Techniques* (Elsevier, 2004), pp. 177–202. https://www.sciencedirect.com/book/edited-volume/9780444511140/handbook-of-stable-isotope-analytical-techniques#editors

42 B. Herbstritt, L. Wengeler, N. Orlowski, Coping with spectral interferences when measuring water stable isotopes of vegetables. *Rapid Commun. Mass Spectrom.* **38**, e9907 (2024).

43 G. Böttcher, H.-J. Brumsack, H. Heinrichs, M. Pohlmann, A new high-pressure squeezing technique for pore fluid extraction from terrestrial soils. *Water Air Soil Pollut* **94**, 289–296 (1997).

44 L. I. Peters, D. Yakir, A direct and rapid leaf water extraction method for isotopic analysis. *Rapid Commun. Mass Spectrom.* **22**, 2929–2936 (2008).

45 A. Barbeta, R. Burlett, P. Martín-Gómez, B. Fréjaville, N. Devert, L. Wingate, J.-C. Domec, J. Ogée, Evidence for distinct isotopic compositions of sap and tissue water in tree stems: Consequences for plant water source identification. *New Phytol* **233**, 1121–1132 (2022).

46 G. Zuecco, A. Amin, J. Frentress, M. Engel, C. Marchina, T. Anfodillo, M. Borga, V. Carraro, F. Scandellari, M. Tagliavini, D. Zanotelli, F. Comiti, D. Penna, A comparative study of plant water extraction methods for isotopic analyses: Scholander-type pressure chamber vs. cryogenic vacuum distillation. *Hydrol. Earth Syst. Sci.* **26**, 3673–3689 (2022).

47 L. Weihermüller, J. Siemens, M. Deurer, S. Knoblauch, H. Rupp, A. Göttlein, T. Pütz, In situ Soil Water Extraction: A Review. *J. Environ. Qual.* **36**, 1735 (2007).

48 P. Koeniger, J. D. Marshall, T. Link, A. Mulch, An inexpensive, fast, and reliable method for vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry. *Rapid Commun. Mass Spectrom.* **25**, 3041–3048 (2011).

49 N. Orlowski, H.-G. Frede, N. Brüggemann, L. Breuer, Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis. *J. Sens. Sens. Syst.* **2**, 179–193 (2013).

50 M. J. Hendry, B. Richman, L. I. Wassenaar, Correcting for methane interferences on $\delta^{2}H$ and $\delta^{18}O$ measurements in pore water using H_2O (liquid)-H_2O (vapor) equilibration laser spectroscopy. *Anal. Chem.* **83**, 5789–5796 (2011).

51 A. Kulmatiski, L. E. Forero, Bagging: A cheaper, faster, non-destructive transpiration water sampling method for tracer studies. *Plant Soil*, doi: 10.1007/s11104-021-04844-w (2021).

52 N. C. Munksgaard, A. W. Cheesman, C. M. Wurster, L. A. Cernusak, M. I. Bird, Microwave extraction–isotope ratio infrared spectroscopy (ME-IRIS): a novel technique for rapid extraction and in-line analysis of $\delta^{18}O$ and $\delta^{2}H$ values of water in plants, soils and insects. *Rapid Commun. Mass Spectrom.* **28**, 2151–2161 (2014).
53 K. Revesz, P. H. Woods, A method to extract soil water for stable isotope analysis. *J. Hydrol.* **115**, 397–406 (1990).
54 Y. Rothfuss, H. Vereecken, N. Brüggemann, Monitoring water stable isotopic composition in soils using gas-permeable tubing and infrared laser absorption spectroscopy. *Water Resour. Res* **49**, 1–9 (2013).
55 T. H. M. Volkmann, M. Weiler, Continual in situ monitoring of pore water stable isotopes in the subsurface. *Hydrol. Earth Syst. Sci.* **18**, 1819–1833 (2014).
56 T. H. M. Volkmann, K. Kühnhammer, B. Herbstritt, A. Gessler, M. Weiler, A method for in situ monitoring of the isotope composition of tree xylem water using laser spectroscopy. *Plant Cell Environ.* **39**, 2055–2063 (2016).
57 R. K. Magh, B. Gralher, B. Herbstritt, A. Kübert, H. Lim, T. Lundmark, J. Marshall, Technical note: Conservative storage of water vapour – practical in situ sampling of stable isotopes in tree stems. *Hydrol. Earth Syst. Sci.* **26**, 3573–3587 (2022).
58 J. D. Marshall, M. Cuntz, M. Beyer, M. Dubbert, K. Kuehnhammer, Borehole equilibration: Testing a new method to monitor the isotopic composition of tree xylem water in situ. *Front. Plant Sci.* **11** (2020).
59 W. H. Bowers, J. J. Mercer, M. S. Pleasants, D. G. Williams, A combination of soil water extraction methods quantifies the isotopic mixing of waters held at separate tensions in soil. *Hydrol. Earth Syst. Sci.* **24**, 4045–4060 (2020).
60 S. E. Younger, J. Blake, C. R. Jackson, D. P. Aubrey, $\delta^{2}H$ isotopic offsets in xylem water measurements under cryogenic vacuum distillation: Quantifying and correcting wood-water hydrogen exchange influences. *Ecohydrology* **17**, e2640 (2024).
61 K. Kühnhammer, A. Dahlmann, A. Iraheta, M. Gerchow, C. Birkel, J. D. Marshall, M. Beyer, Continuous in situ measurements of water stable isotopes in soils, tree trunk and root xylem: Field approval. *Rapid Commun. Mass Spectrom.* **36**, e9232 (2022).
62 R. Sánchez-Murillo, D. Todini-Zicavo, M. Poca, C. Birkel, G. Esquivel-Hernández, M. M. Chavarría, G. Zuecco, D. Penna, Dry season plant water sourcing in contrasting tropical ecosystems of Costa Rica. *Ecohydrology* **16**, e2541 (2023).
63 C. Millar, K. Janzen, M. F. Nehemy, G. Koehler, P. Hervé-Fernández, J. J. McDonnell, Organic contamination detection for isotopic analysis of water by laser spectroscopy. *Rapid Commun. Mass Spectrom.* **35**, e9118 (2021).
64 M. Wen, B. Si, Y. Lu, H. Wang, Water recovery rate and isotopic signature of cryogenic vacuum extracted spiked soil water following oven-drying at different temperatures. *Hydrol. Process.* **35**, e14248 (2021).
65 P. Martín-Gómez, A. Barbeta, J. Voltas, J. Peñuelas, K. Dennis, S. Palacio, T. E. Dawson, J. P. Ferrio, Isotope-ratio infrared spectroscopy: A reliable tool for the investigation of plant-water sources? *New Phytol* **207**, 914–927 (2015).
66 W. A. Brand, H. Geilmann, E. R. Crosson, C. W. Rella, Cavity ring-down spectroscopy versus high-temperature conversion isotope ratio mass spectrometry; a case study on $\delta^{2}H$ and $\delta^{18}O$ of pure water samples and alcohol/water mixtures. *Rapid Commun Mass Spectrom* **23**, 1879–1884 (2009).
67 A. G. West, G. R. Goldsmith, P. D. Brooks, T. E. Dawson, Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. *Rapid Commun. Mass Spectrom.* **24**, 1948–1954 (2010).
68 J. E. Johnson, L. Hamann, D. L. Dettman, D. Kim-Hak, S. W. Leavitt, R. K. Monson, S. A. Papuga, Performance of induction module cavity ring-down spectroscopy (IM-CRDS) for measuring $\delta^{18}O$ and $\delta^{2}H$ values of soil, stem, and leaf waters. *Rapid Commun. Mass Spectrom.* **31**, 547–560 (2017).
69 L. Wassenaar, S. Terzer-Wassmuth, C. Douence, Progress and challenges in dual- and triple-isotope ($\delta^{18}O$, $\delta^{2}H$, $\Delta17O$) analyses of environmental waters: An international assessment of laboratory performance. *Rapid Commun. Mass Spectrom.* **35**, e9193 (2021).
70 B. Herbstritt, B. Gralher, M. Weiler, Continuous in situ measurements of stable isotopes in liquid water. *Water Resour. Res.* **48**, W03601 (2012).
71 R. Gonfiantini, Standards for stable isotope measurements in natural compounds. *Nature* **271**, 534–536 (1978).
72 G. Lis, L. I. Wassenaar, M. J. Hendry, High-precision laser spectroscopy D/H and $^{18}O/^{16}O$ measurements of microliter natural water samples. *Anal. Chem.* **80**, 287–293 (2008).

73 T. B. Coplen, L. I. Wassenaar, LIMS for Lasers 2015 for achieving long-term accuracy and precision of $\delta^{2}H$, $\delta^{17}O$, and $\delta^{18}O$ of waters using laser absorption spectrometry. *Rapid Commun. Mass Spectrom.* **29**, 2122–2130 (2015).

74 A. G. West, G. R. Goldsmith, I. Matimati, T. E. Dawson, Spectral analysis software improves confidence in plant and soil water stable isotope analyses performed by isotope ratio infrared spectroscopy (IRIS). *Rapid Commun. Mass Spectrom.* **25**, 2268–2274 (2011).

75 Y. Rothfuss, M. Javaux, Reviews and syntheses: Isotopic approaches to quantify root water uptake: A review and comparison of methods. *Biogeosciences* **14**, 2199–2224 (2017).

76 S. Wu, D. Tetzlaff, X. Yang, A. Smith, C. Soulsby, Integrating tracers and soft data into multi-criteria calibration: Implications from distributed modeling in a Riparian Wetland. *Water Resour. Res.* **59**, e2023WR035509 (2023).

77 M. Beyer, D. Penna, On the spatio-temporal under-representation of isotopic data in ecohydrological studies. *Front. Water* **3** (2021). https://doi.org/10.3389/frwa.2021.643013

78 C. Birkel, C. Soulsby, Advancing tracer-aided rainfall–runoff modelling: A review of progress, problems and unrealised potential. *Hydrol. Process.* **29**, 5227–5240 (2015).

79 Y. Rothfuss, M. Quade, N. Brüggemann, A. Graf, H. Vereecken, M. Dubbert, Reviews and syntheses: Gaining insights into evapotranspiration partitioning with novel isotopic monitoring methods. *Biogeosciences* **18**, 3701–3732 (2021).

80 M. Dubbert, V. Couvreur, A. Kübert, C. Werner, Plant water uptake modelling: Added value of cross-disciplinary approaches. *Plant Biol.* **25** (2023).

81 T. Zhou, J. Šimůnek, P. Nasta, G. Brunetti, M. Gaj, C. Neukum, V. Post, The impact of soil tension on isotope fractionation, transport, and interpretations of the root water uptake origin. *Water Resour. Res.* **59**, e2022WR034023 (2023).

82 X. Yang, D. Tetzlaff, C. Müller, K. Knöller, D. Borchardt, C. Soulsby, Upscaling tracer-aided ecohydrological modeling to larger catchments: Implications for process representation and heterogeneity in landscape organization. *Water Resour. Res.* **59**, e2022WR033033 (2023).