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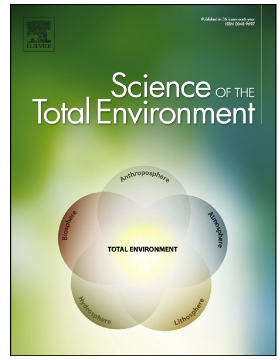

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Challenges in studying water fluxes within the soil-plant-atmosphere continuum: A tracer-based perspective on pathways to progress

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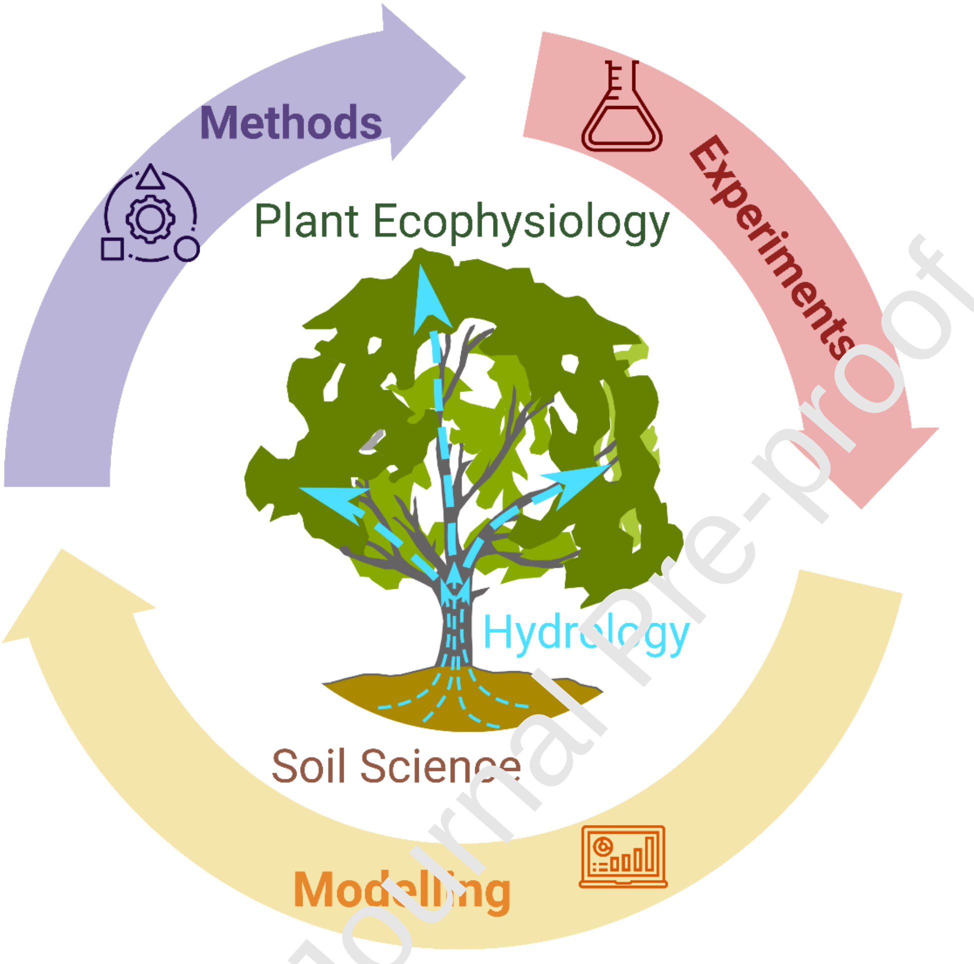
Highlights

- There is substantial overlap in experimental approaches, methodologies and models across hydrology, plant ecophysiology and soil science
- Comprehensive investigation of water fluxes in the soil-plant-atmosphere continuum through interdisciplinary research is needed
- Water stable isotopes are an ideal tool to connect the disciplines
- Ideas for new interdisciplinary collaborations are provided to address climate-induced changes in water fluxes through ecosystems

Graphical abstract



Tracing water fluxes through the soil-plant-atmosphere continuum (SPAC) by combining methods, experiments and modelling in an interdisciplinary framework.

Abstract. Tracing and quantifying water fluxes in the hydrological cycle is crucial for understanding the current state of ecohydrological systems and their vulnerability to environmental change. Especially the interface between ecosystems and the atmosphere that is strongly mediated by plants is important to meaningfully describe ecohydrological system functioning. Many of the dynamic interactions generated by water fluxes between soil, plant and the atmosphere are not well understood, which is partly due to a lack of interdisciplinary research. This opinion paper reflects the outcome of a discussion among hydrologists, plant ecophysiologists and soil scientists on open questions and new opportunities for collaborative research on the topic "water fluxes in the soil-plant-atmosphere continuum" especially focusing on environmental and artificial tracers. We emphasize the need for a multi-scale experimental approach, where a hypothesis is tested at multiple spatial scales and under diverse environmental conditions to better describe the small-scale processes (i.e., causes) that lead to large-scale patterns of ecosystem functioning (i.e., consequences). Novel in-situ, high-frequency measurement techniques offer the opportunity to sample data at a high spatial and temporal resolution needed to understand the underlying processes. We advocate for a combination of long-term natural abundance measurements and event-based approaches. Multiple environmental and artificial tracers, such as stable isotopes, and a suite of experimental and analytical approaches should be combined to complement information gained by different methods. Virtual experiments using process-based models should be used to inform sampling campaigns and field experiments, e.g., to improve experimental designs and to simulate experimental outcomes. On the other hand, experimental data are a pre-requisite to improve our currently incomplete models. Interdisciplinary collaboration will help to overcome research gaps that overlap across different earth system science fields and help to generate a more holistic view of water fluxes between soil, plant and atmosphere in diverse ecosystems.

Keywords: hydrology, plant ecophysiology, soil science, isotope tracers, interdisciplinary perspectives, water fluxes, ecosystem research

1 Introduction

The study of water fluxes between soil, plant and atmosphere is critical for understanding terrestrial ecosystem functioning and its development in a changing climate. Under terrestrial ecosystem, we consider the composition of and interaction between organisms (here limited to plants) in a specific terrestrial landscape characterized by its soil, geology, topography, climatic setting etc. Plants play a pivotal role in the hydrological cycle by mediating and influencing most terrestrial water fluxes, including canopy throughfall, stemflow, soil infiltration, subsurface flow and the amount of groundwater recharge. Plants globally account for 50-90 % of terrestrial evapotranspiration (ET) (Coenders-Gerrits et al., 2014; Jasechko et al., 2013; Wei et al., 2017). As the climate changes, the water and carbon fluxes together with the associated energy fluxes (i.e., latent heat flux) are likewise undergoing significant changes. We expect shifts in hydroclimatic conditions to lead to spatial and temporal changes in precipitation regimes, soil moisture distribution and vapor pressure deficits in the atmosphere (IPCC, 2022). These changes will not only affect the long term average climatic conditions but also their variability and extremes (e.g., severity and frequency of droughts) (IPCC, 2022). Water fluxes in soils and plants in many parts of the world will be affected by more frequent and more extended drought conditions leading to reduced transpiration rates and dependency on deeper seated groundwater resources. Ecohydrological fluxes occur via many interconnected pathways that different disciplines, such as plant ecophysiology, soil science and hydrology, have been studying at different spatio-temporal scales and each with their own disciplinary focus, methods and approaches.

The driving motivations for investigating water fluxes in the soil-plant-atmosphere continuum (SPAC) are to better understand how ecosystems respond to changes in water, nutrient, energy supply and to identify species-specific adaptation strategies and resilience capacity. We want to better understand the role of vegetation and soil in runoff generation processes and modulation of water and carbon fluxes in the critical zone. We seek to identify the water storage "pools" from which plants draw water and nutrients, how these pools are depleted or replenished and what ecological strategies plants develop to persevere despite obstacles. We are interested in the detailed processes at the small scale but also on how small-scale interactions manifest in larger scale patterns such as runoff response, species composition or soil development. The final goal is to discern the organizing principles and the key drivers of catchment and ecosystem functioning, which lay the foundation for modelling and thus prediction of natural systems to change; a prediction with for-reaching implications for all dimension of life on our planet including human societal well-being. This change eventually is of high societal relevance as it determines human living conditions and wellbeing.

Tracers, such as water stable isotopes (^{2}H, ^{18}O and ^{17}O), that are the focus of this article, have shown to be powerful tools to characterize and quantify ecohydrological fluxes. Ecohydrology as defined by Nuttle (2002) studies the interactions between biotic and abiotic processes on various water cycle components as well as the distribution, structure, and function of ecosystems.

In hydrology, water stable isotopes have been used extensively to partition runoff into event- and pre-event water (e.g., review by Klaus and McDonnell (2013)) and precipitation fate into discharge and ET (Kirchner and Allen, 2020) or to estimate residence- and transit times of hydrological systems from lysimeter- to catchment-scales (Benettin et al., 2022; Hrachowitz et al., 2021; Małoszewski and Zuber, 1982; McGuire and McDonnell, 2006; Sprenger et al., 2019). Water stable isotopes together with biogeochemical tracers (e.g., major ions, dissolved organic carbon) have also been used to quantify hydrochemical parameters of soils using breakthrough curve experiments at the scale of soil cores to field plots (Benettin et al., 2019; Koeniger et al., 2010; Liu et al., 2017; McCarter et al., 2019; Queloz et al., 2015). A wide range of studies used water isotopes to describe detailed infiltration and flow mechanisms at small scales under natural conditions or in fully controlled labelling experiments (Benettin et al., 2021; Mennekes et al., 2021; Rinderer et al., 2021). Other studies identified runoff generation processes at larger, i.e., at catchment- and river basin-scales (e.g., Seibert et al., 2003; Fenicia et al., 2008; Hrachowitz et al., 2013; Birkel et al., 2010, 2011; Knighton et al., 2019; Soulsby et al., 2015), taking advantage of the source-specific isotopic composition of different water fluxes. In soil science, deuterated water (enriched in ^{2}H) applications have been used to quantify the hydrochemical parameters (such as dispersivity or anion exclusion) of complex organic media (McCarter et al., 2019), to trace soil water movement in the unsaturated zone (Koeniger et al., 2016), macropore flow and water flow processes in laboratory column experiments (Rothfuss et al., 2015) and at the field scale (Koeniger et al., 2010), while tritium has been used to understand the impacts of drainage on wetlands (Knigh et al., 1972; Zimmermann et al., 1966). Examples of ecosystem physiological research encompass water isotope tracer applications for identifying plant mediated water fluxes as well as plant water sources; and a review by Rothfuss and Javaux (2017)). Moreover, the evaporative enrichment of leaf water and the assimilates formed within it have been used to assess stomatal responses of plants to changes in water availability (Dubbert et al., 2017; Ferrio et al., 2009; Keitel et al., 2003), as well as on leaf hydraulic functioning (e.g., Hommel et al., 2014; Ferrio et al., 2012). Plants control via their stomata transpiration actively and are thus “the living part” of the water cycle (Wang-Erlandsson et al., 2022). Especially under reduced water supply, plants may also actively change the hydraulic properties of the cells which are part of the plant water pathway. In roots as well as in leaves, changes in the activity and expression of aquaporins which facilitate transmembrane transport have been observed (Javot et al., 2003; Kaldenhoff et al., 2008; Sakurai-Ishikawa et al., 2011; Wong et al., 2022) and might be an additional factor in controlling water uptake by roots on the one hand and water loss in leaves on the other. Species interaction by below- and above-ground niche complementarity and by facilitation (e.g., hydraulic lift and redistribution; Hafner et al. (2021); Cardon et al. (2013)) can affect the water pool that is available for the vegetation and thus also affect whole ecosystem regulation of transpiration and - on the larger scale - catchment water storage and runoff. Knowledge on such vegetation related regulation mechanisms that can strongly affect water fluxes need to be included into an interdisciplinary view. Finally, it should not go unnoticed that sampling of tree rings and subsequent isotopic analysis of, e.g., ^{2}H in the cellulose is a powerful and long-term tracer of the interaction between plants and the water cycle to assess, e.g., drought impacts (Libby et al., 1976), drought occurrence (Büntgen et al., 2021), and stomatal conductance throughout the life of a tree (Scheidegger et al., 2000), as well as identify past soil water sources assessed by trees (Brinkmann et al., 2019; Marshall and Monserud, 2006).

Despite methodological progress, further development in experimental design and improvement in modelling, a comprehensive description of water fluxes in the SPAC is still missing. A reason for the status quo is the fact that the potential of interdisciplinary research activities has not as efficiently been used as it could (Mazzocchi, 2019). But different research disciplines frequently have different perspectives and spatio-temporal scales of investigation, which may partly be due to differences in their historic roots (Breshears, 2005; Bruin and Morgan, 2019). Different disciplines also often focus on one specific aspect that is relevant to their field such as quantifying a specific component of the water cycle but not further exploring the relevance of their finding for e.g., the C- and N-cycle that are linked to it, as illustrated in the first panel of Figure 1. As a consequence, significant knowledge gaps remain that cannot be answered with research initiatives isolated in individual disciplines (Bhaskar et al., 2017; Ridde et al., 2019). The current status is particularly unfortunate as all disciplines use methods, experimental approaches and models that would complement each other when brought together in a coordinated research effort (Cocozza and Penna, 2022). Interdisciplinary approaches allow us to study the same element or process from different points of view (i.e., "triangulation", Nightingale (2009)) and analyze more systematically the interrelated and interacting physical and ecophysiological processes in the bedrock, in the soil, in plants, and above the canopy from a holistic perspective (Cocozza and Penna, 2022). Combining different disciplines' research strategies (e.g., from ecosystem ecology, plant ecophysiology, soil science and hydrology) can help to gain a better mechanistic understanding of fundamental processes in ecosystems, as illustrated in the second and third panels of Figure 1.

We call for a process that moves us from intradisciplinary or multidisciplinary research where researchers only work together with colleagues from their field (as in the first panel of Figure 1, today no longer a dominant case) or from multiple fields i.e., at a shared field site or by sharing data or models, but draw their disciplinary conclusions, to creating an interdisciplinary dialogue that will start to synthesize our approaches for an eventual transdisciplinary framework that is more relevant and drives the innovation of future methods, experiments, and models of our fields (final panel of **Error! Reference source not found.**). Following Stember (1991), we define "intradisciplinary" as collaborations of researches within their disciplinary field, "multidisciplinary" as a collaboration between researchers from different disciplines, each drawing on their disciplinary knowledge, "interdisciplinary" when the researchers integrate knowledge and methods from different disciplines, using a real synthesis of approaches and "transdisciplinary" when researches create a unity of intellectual frameworks beyond the disciplinary perspectives (e.g., optimality principle).

Several past studies and commentaries have called for interdisciplinary ecosystem research (most recently Cocozza and Penna (2022)) that can overcome the "insular focus" of specific disciplines (Bruin and Morgan, 2019). However, in our opinion only a few cases exist today where the idea of interdisciplinary collaboration has been a fundamental component of all stages of a project beginning by formulating research questions, designing an experimental concept, obtaining, analyzing and interpreting data and drawing conclusions (e.g., Lang et al. (2021); Werner et al. (2021)). In Chapter 3 we will describe one of these research initiatives in more detail.

This paper summarizes the discussion during and after the workshop on "Water and nutrient fluxes in ecosystems under a changing climate - a tracer-based perspective" on the 12th and 13th of October 2021 organized by N. Orlowski, M. Rinderer, J. Krueger and M. Dubbert. The workshop was designed to bring together scientists from hydrology, plant ecophysiology and soil science to facilitate an interdisciplinary discussion to reach a more holistic view on water fluxes through the SPAC. In this context we discuss new methodological perspectives, ideas on interdisciplinary experimental approaches and challenges in modelling with a focus on water stable isotopes. The aim of this discussion paper is to foster interdisciplinary studies in the future that can provide a more holistic view on water fluxes in the SPAC (also see Figure 2).

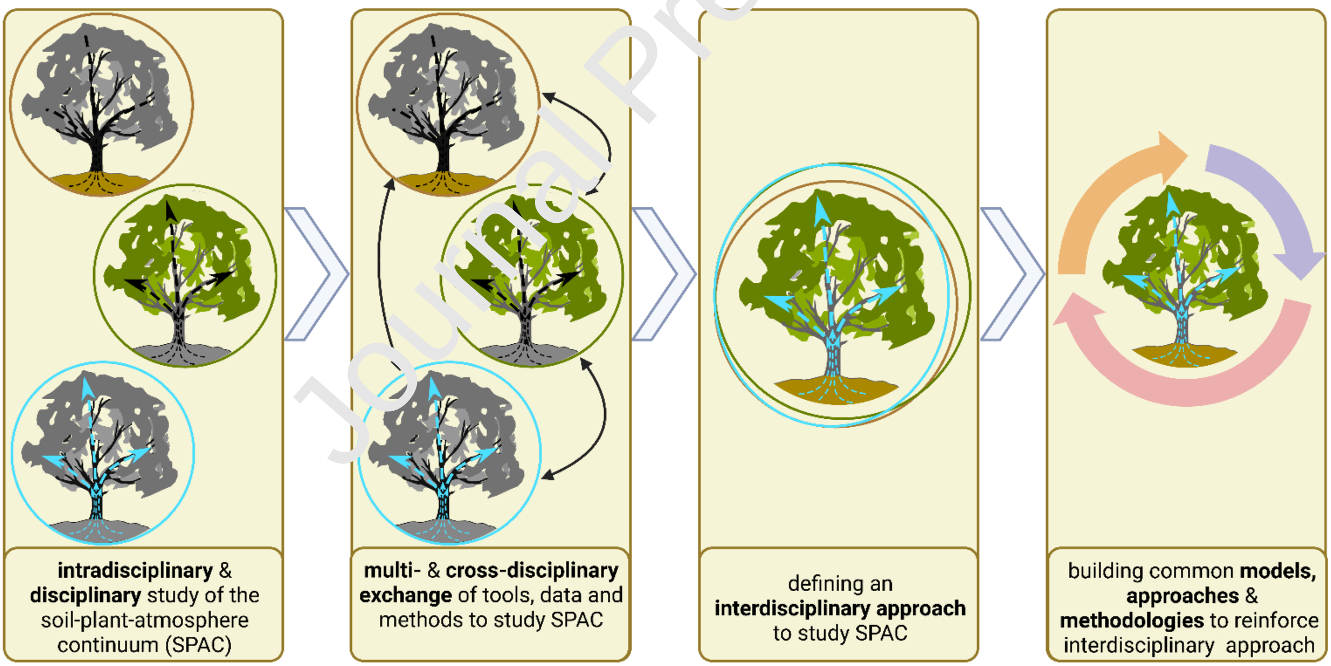


Figure 1. To improve research on the water fluxes in the SPAC (represented with the tree icon with fluxes and soil), we need to move from intradisciplinary and disciplinary studies (left panel) of soil (brown accented icon), plant ecophysiology (green accented icon) and hydrology (cyan accented icon) to exchange of tools, data and methods between disciplines (center left panel, represented as black double edged arrows) to defining an interdisciplinary approach (center right panel, represented as tree icon with all color highlights and all

disciplinary rings) to building common models, approaches and methodologies (represented as grey arrows) to reinforce and accelerate that interdisciplinary approach (right panel, represented as tree icon surrounded by arrows). The panels are connected with simple arrows indicating our recommended shift from one way of practicing to the next.

2 New methodological perspectives

Highlights:

- In-situ isotope measurements allow for high resolution assessment of plant source water dynamics, constraining plant ecophysiological processes, partitioning water fluxes on the ecosystem scale, and integration of new emerging processes into models.
- Integration of remote sensing techniques to resolve 'upscaling' limitations.
- Through the combination of methods, approaches and knowledge from plant ecophysiology, soil science, atmospheric science and remote sensing with isotope ecohydrology, a highly complete and mechanism-based picture of water fluxes in ecosystems is in sight.

The availability and improvement of a number of technologies, such as field-deployable water stable isotope analyzers (Berman et al., 2009; Lee et al., 2005), approaches for direct measurement of water isotope fluxes between the surface and the atmosphere (Wahl et al., 2021), new isotope-based ET quantification approaches (Rothfuss et al., 2021) and high-resolution remote sensing technologies for monitoring vegetation water status (Al-Ali et al., 2020; Reddy, 2021), have significantly improved our ability to integrate and model data across scales (Asbjornsen et al., 2011).

Water stable isotopes are a powerful tool for facilitating and enhancing interdisciplinary research (Penna et al., 2018) and have become a widely used tool to trace water fluxes and stores, as well as to study ecosystem processes in soil, groundwater, streams, plants, and the atmosphere. Information from such studies can be used to constrain process-based models (e.g., Kuppel et al., 2020) and help to inform about the source and age of water in storage and in flux (Sprenger et al., 2019). In ecohydrological research, the water stable isotopic composition of soils and plants has in the past commonly been measured after destructive sampling and subsequent water extraction (e.g., via cryogenic vacuum extraction (CVE) (Orlowski et al., 2013)). Destructive water extraction methods from soils and plants fall short of producing consistent water isotopic compositions because soil parameters (e.g., organic matter content) and co-extracted organic contaminants (e.g., methanol and ethanol) can influence isotopic composition (Araguás-Araguás et al., 1995; Gaj et al., 2017a; Gaj et al., 2017b; Millar et al., 2018; Orlowski et al., 2018, 2016a, 2016b; Walker et al., 1994). Additionally, the destructive sampling in itself eliminates the possibility for true repeated measurements of the same sample and location, severely reducing the spatio-temporal resolution of isotope data and subsequently limiting ecohydrological process understanding (Kübert et al., 2020; Mennekes et al., 2021). Moreover, water extraction in the laboratory is time-consuming and labor-intensive (Kübert et al., 2020; Orlowski et al., 2016b). In-situ measurement techniques are increasingly used to avoid these compounding problems within the ecohydrological community (Gaj et al., 2016; Kübert et al., 2022; Marshall et al., 2020; Oerter et al., 2017; Rothfuss et al., 2013; Volkmann et al., 2016b; Volkmann and Weiler, 2014). These in-situ water stable isotope methods now allow measurements at finer spatial (10^0-10^1cm scale; (Deseano Diaz et al., 2022a; Volkmann et al., 2016b; Volkmann and Weiler, 2014) and higher temporal resolution (daily to sub daily scale e.g., Mennekes et al., 2021; Kübert et al., 2020; Piayda et al., 2017; Volkmann et al., 2016b) for relatively low monitoring costs with high accuracy (Volkmann and Weiler, 2014). Beyer et al. (2020) provided a broad review of in-situ measuring methods for water stable isotopes. Studies applying environmental tracer methods are now tackling complex interactions between soil, plant ecophysiological and hydrological processes. As such, methodological advances and modifications are evolving rapidly and impact process understanding and interpretation significantly (Mennekes et al., 2021). These methods have a high potential to unravel temporally dynamic processes occurring in the SPAC, such as fluctuations of water storage in and travel time through plants. This further opens opportunities to study feedback processes with higher detail than previously possible (e.g., hydraulic redistribution and preferential flow in soils, plant water uptake responses to wet and dry cycles). Through the co-investigation of other non-isotopic soil (e.g., soil water content, soil matric potential, soil temperature), plant (e.g., sap flow, tree water deficit) and atmospheric (e.g., air temperature and vapour pressure deficit) variables at high temporal resolution, ecosystem dynamics, short- as well as long-term interactions between ecosystem compartments will eventually become decipherable (e.g., Gessler et al., 2022).

Current methodological issues

A thorough comparison of methods has begun, particularly between in-situ (e.g., borehole or soil/xylem water isotope probe methods; e.g., Marshall et al. (2020); Volkmann et al. (2016b); Volkmann and Weiler (2014)) and widely used lab-based methods such as CVE or the water-vapor equilibration technique by Wassenaar et al. (2008) (see Gessler et al., 2022; Kübert et al., 2020; Mennekes et al., 2021; Kühnhammer et al., 2021), but careful comparison among these new in-situ methods to measure soil and plant water isotopic compositions has still not been done. Despite the challenges posed by the diversity of these methods, we argue that community attempts must be pursued, beginning by unifying protocols for single methods (e.g., the most commonly used CVE, see suggestions by Orlowski et al. (2018) and Millar et al. (2022)). Furthermore, solutions to overcome effects of co-extracted/-equilibrated organic compounds on isotope ratio infrared spectroscopy measurements (see e.g., West et al. (2010); Chang et al. (2016); West et al. (2011); Brand et al. (2009); Hendry et al. (2011)) are still lacking for a wider range of applications (i.e., for vapor equilibration-based techniques including in-situ methods) and no unified protocol exists (Millar et al., 2022). So far, post-processing functions must be developed for each individual isotope analyzer (Barbeta et al., 2019). Finding solutions to the above-mentioned issues and research questions would improve the comparability of studies' results and widen the range of applications.

Thus far, in-situ methods have only been applied to determine the water isotopic composition of soils and a limited range of tree species in mainly temperate (forest) ecosystems. There are still several open research questions with regard to in-situ water isotope measurements in soils and plants:

1. Do gas-permeable membranes used for in-situ isotope probes cause isotopic effects and how do those compare with isotopic effects due to mixing and diffusion processes in soils and plants?

2. How spatially (and temporally) representative are in-situ isotope measurements and how do they compare with those of destructive methods?

3. How long (months to years?) can we conduct in-situ tree xylem water isotope measurements (e.g., with respect to tree wound reactions)?

Furthermore, the following issues regarding both in-situ and laboratory-based isotope methods remain:

4. Is the vegetation's internal water storage fully mixed (at any time) or which plant internal water pools are we sampling (e.g., influence of stem water storage or bark evaporation)?

5. How do we address the spatial variation in xylem water transport velocities in trees? How do we represent it in isotope-based root water uptake and transport models?

6. How are different tree physiologies affecting in-situ isotope measurements (e.g., ring porous vs. diffuse porous tree species)?

7. Do we sample all relevant sources and at representative locations with our sampling designs/strategies?

8. To what extent do fractionation in the plant xylem and during water uptake by the plant, sampling procedures and the subsequent choice of analytical methods (in-situ vs. laboratory-based) influence the obtained isotopic composition?

While most sampling techniques have been designed for woody species, many difficulties related to sampling different parts of the xylem (Barbeta et al., 2022) still exist. The chosen sampling technique for xylem water considerably affects the xylem water isotopic composition and consequently water transport velocities derived from isotope-based plant water uptake and transport models (Seeger and Weiler, 2023). Also, sampling from non-woody species (e.g., grasses) remains challenging. Volkmann et al. (2016b) for example applied a closed chamber technique to assess the isotopic composition of grass' transpiration that should be equivalent to the water taken up when isotopic steady state is assumed. However, isotopic non-steady state conditions were encountered that limited measurements in the morning and evening. Indeed, the prevalence of isotopic non-steady state during most parts of the day (Dubbert et al., 2017, 2014; Kübert et al., 2022) has been a hindrance to utilize chamber measurements coupled to laser spectrometers in the field to infer xylem isotopic composition in high temporal resolution (Deseano Diaz et al., 2022a; Kühnhammer et al., 2020). Nevertheless, a recent study clearly demonstrated that integrated to daily resolution, in-situ chamber measurements of transpiration are very well suited to reflect steady state and hence dynamics in the xylem isotopic composition of both woody and non-woody species (Kübert et al., 2022). Concerning the potential fractionation between soil and xylem water that mainly concerns the hydrogen isotopologues, new approaches to quantify potential isotopic offsets have been applied. Only recently, Barbeta et al. (2019) and Li et al. (2021) used the concept of the line-conditioned excess to examine the δ^2H offsets between xylem samples and their corresponding soil water lines in the ^{2}H-^{18}O space.

Future directions

Future studies need to consider interactions between individual plants (e.g., Hafner et al., 2021) and ecosystem heterogeneity (e.g., spatial variation, plants interactions with mycorrhizae) (see suggestions by Beyer et al. (2020)). Moreover, the impacts of plant species diversity on water uptake dynamics in ecosystems need to be addressed (e.g., Guderle et al., 2018). Applications that encompass a range of different plant species (including non-woody species), ecosystems, functional diversity levels with different vessel and wood anatomies leading to different hydraulic traits and applications of in-situ measurements over longer time periods (> months to years) are needed to e.g., assess species ecohydrological niche or complementarity (e.g., Fresne et al., 2023) and to assess feedback mechanisms from individual plants to ecosystems. Figure 2 highlights future research directions and shows interdisciplinary potential in conducting experiments, applying methods and models.

Isotope labelling

Currently, isotope labelling techniques are applied in many ecosystem studies to quantify and describe water fluxes and mixing processes, their related travel and residence times, as well as water ages (Sprenger et al., 2019). Most often, labelling with deuterated water is conducted since it is not radioactive nor toxic during both labelling and measurement (Becker and Coplen, 2001) and more affordable than ^{18}O or ^{17}O labelling. The deuterium tracer provides a distinct label, which enables the estimation of ecosystem response and transit times of water in the subsurface flow pathways and during plant water uptake (Kübert et al., 2020; Kühnhammer et al., 2022; Marshall et al., 2020; Mennekes et al., 2021) more precisely (e.g., in terms of the differentiation of isotopically similar water sources) than with the natural variation of water stable isotopes (Penna et al., 2018). However, analyzing variations of natural abundances of source water (depending on the origin and time) allow to continuously track temporal (e.g., winter vs. summer precipitation, Allen et al. (2019)) or spatial origins of water (Brinkmann et al., 2018; Gessler et al., 2022) over long times but often to the cost of a lower temporal resolution. Combining the advantages of long-term natural abundance measurements with labelling approaches can help to disentangle water fluxes through the soil-plant-compartments. Such combined approaches could especially be valuable when studying extremes such as vegetation's drought response and recovery (Gessler et al., 2022; Werner et al., 2021), a key question that crosses disciplines and requires exchange between disciplines (Figure 1, panels 2 and 3).

However, main open questions are what the best label strategy is for the soil-plant compartments (from profile to ecosystem scales) and which tracer applications are best to be used or be combined. We suggest carefully determining the necessary label amount (see spreadsheet to calculate mixing of isotope labels: https://web.gps.caltech.edu/~als/resources/), best timing (dependent on fractionation and/or decay processes) and soil depth/area of application beforehand. This could be done via hydrological model simulations e.g., via Hydrus-1D (Zhou et al., 2021), Soil Water Isotope Simulator (Sprenger et al., 2018), LWFBrook90.jl (Schmidt-Walter et al., 2020) or SiSPAT-Isotope for plants (Rothfuss et al., 2012) if the necessary input data is already available for the respective research site/experiment. Ideally, the deuterated or ^{18}O-modified tracer application onto the soil should lead to a significant change in soil water isotope composition in comparison to natural abundance concentration but not to a significant change in soil water content at the point of labelling (unless it is part of the respective research question/s) or exceed analytical limits that induce a sustained analytical memory effect. In most experimental setups the goal is to set the prerequisite isotopic conditions for the partitioning among plant water sources (i.e., by setting of an informative soil water isotopic profile) without affecting the plant from a physiological standpoint. This is best achieved by using the natural capillary forces to distribute the tracer and by avoiding tracer spreading via preferential flow paths. The cheaper ^{2}H tracer could be applied in combination with the more expensive ^{18}O tracer at specific locations (e.g., soil depths, height in the tree trunk) to disentangle dynamics of water uptake and water distribution, mixing and storage within trees. However, in the soil, the $\delta^{2}H$ and $\delta^{18}O$ profiles should be decorrelated to add interpretation power: soil water isotopic data should not fall onto one evaporation line but rather onto an evaporation surface in a dual isotope plot ($\delta^{18}O$ vs. $\delta^{2}H$). Measurements of soil water content and matric potential are recommended simultaneously with isotopic monitoring to follow the applied label in terms of soil water status changes (ideally via multiple soil profile measurements). The combined use of isotopically labelled water (^{2}H and ^{18}O) and nutrient isotopes (i.e., ^{15}N or ^{13}C) provides an opportunity to increase understanding of coupled ecosystem fluxes, a goal that is at the intersection of multiple disciplines (Werner et al., 2021).

Applying interdisciplinary methods

Future research should combine comprehensive monitoring of multi-source information through a variety of different monitoring techniques (Xia et al., 2021), as illustrated in Figure 2. For instance, isotope studies dealing with plant response to drought and soil-plant interactions could utilize plant-specific parameters and traits like fine root distribution, leaf area index (LAI), sap flow (transpiration rates), stem water storage, leaf water potential, leaf temperature, and leaf solar radiation (see e.g., Nehemy et al. (2021)). In addition, for trees, point dendrometer measurements allow high-temporal resolution information on growth and tree water deficit (Zweifel et al., 2021). It is known that the mere presence of roots in a given soil depth is not a good indicator for actual water uptake (Volkmann et al., 2016a). Specific root traits such as root hydraulic conductance or aquaporin abundance and their dependence on changes in soil water availability might help to better understand the impact of roots system acclimation on the water uptake potential.

There is also significant cross-scale potential for complementing field-based measurements with remote sensing approaches. For instance, the use of vegetation and stress indices such as the NDVI (Normalized Difference Vegetation Index), EVI (Enhanced Vegetation Index) or LMA (leaf dry mass per leaf area). According to Lambers and Poorter (1992), the LMA is a crucial characteristic of plant growth and an important predictor of plant strategies (Westoby et al., 2002). The NDVI is an index which provides information on vegetation coverage and plant health. The EVI is a proxy for canopy photosynthetic capacity and gross primary production (Huete et al., 2006; Hunt et al., 2013). It is derived from multispectral imagery and has become common in plant health assessments (Baluja et al., 2012; Leinonen and Jones, 2004). It can be obtained in different resolutions (down to <1cm) and on different spatial scales depending on the platform and sensors used (e.g., satellite-, airplane- or unmanned aerial vehicles (UAV)-based). Apart from plant water stress and health detection, certain vegetation indices can be used for an estimation of model input parameters. For instance, the normalized difference red-edge index (Xie et al., 2018) can be applied to derive LAI (Gong et al., 2021). The photochemical reflectance vegetation index (Gamon et al., 1992) can be used to infer relative leaf water content (Sun et al., 2014) and CO_2 uptake (Peñuelas et al., 2011). Of particular interest are methods to quantify important, and otherwise laborious-to-measure parameters such as stomatal conductance (Ellsäßer et al., 2020; Leinonen et al., 2006), sap flux (Ellsäßer et al., 2020) and transpiration

(Marzahn et al., 2020). These approaches use UAV-borne thermal-infrared information (i.e., leaf and surface temperatures) on different scales and resolutions and offer great potential for obtaining a greater spatial coverage than point measurements. Ellsäßer et al. (2021) further showed that drone-based thermography together with energy balance modeling is a reliable method for ET studies and can provide additional information for spatially explicit research. Potential benefits of implementing these methods include the ability to capture heterogeneity across stands/sites and even single trees (sun- vs. shade-exposed leaves). The information can further be used to be tested against or compared with single-tree measurements and, in a next step, used for upscaling the measured parameters. This is a difficult and yet unsolved task. However, using satellite- and UAV-borne imagery for tree species segmentation (e.g., to identify the canopy shape of particular trees) and classification seems a promising way forward in this concern (Brandt et al., 2020; Oldeland et al., 2017; Onishi and Ise, 2021). Testing, improving and applying these methods would be invaluable for obtaining results which are representative for larger areas (forest, catchment) rather than a small number of trees or a single species. However, often ground data is necessary to test, evaluate and calibrate image data (ground-truthing), such as required absolute leaf temperatures, and thus make sensor calibration challenging (Ludovisi et al., 2017). Even without extensive calibration, remote sensing data can still help to obtain a "bigger picture" (e.g., is the studied tree species relevant for the overall ecosystem functioning?).

When investigating canopy-atmosphere exchange processes, parameters including air temperature, rainfall, and net radiation along with the isotopic composition of xylem, leaf and atmospheric water can provide further insights. A systematic comparison between the isotopic compositions of xylem water (measured either destructively or non-destructively) and of plant transpired water vapor (e.g., measured in gas-exchange chambers) would allow for identification of isotopic transient/non-equilibrium conditions or of isotopic storage processes in plant tissues (Cernusak et al., 2016). Isotopic measurements in water vapor have been used to separate transpiration and evaporation in the overall ET flux (e.g., Wang and Yakir, 2000), using a wide range of methods – destructive sampling of soil and plant material, non-destructive collection of water vapor within (e.g., with gas-exchange chambers (Dubbert et al., 2013)) and above the canopy (e.g., with micrometeorological masts (Good et al., 2014)) and models – from simple linear regression relationships to process-based models (e.g., Craig and Gordon, 1965). However, the outcome, i.e., transpiration to ET ratio values, is generally limited with respect to the spatial scale (up to the plot~ 100 m^2) (Rothfuss et al., 2021). The widely used eddy covariance technique, applied by Griffis et al. (2010) (see also Braden-Behrens et al. (2019)), may fill the gap by providing continuous and field-scale (up to several ha) isotopic ET time series. This provides ground for a field-scale partitioning of ET into and to its two component fluxes. Moreover, uptake of water vapor by leaves might be important for a partial rehydration of the canopy (e.g., during fog events) independent from root water uptake (RWU) (Limm et al., 2009). Application of ^{18}O or ^{2}H labelled water allows us to quantify the importance of such uptake and provides a link to assimilation (Lehmann et al., 2020).

Untapped opportunities exist to study soil water movement and mixing processes in the field, such as soil property measurements like the analysis of the soil organic carbon content or soil microbial activities. Mycorrhizal fungi, for instance, have been shown to potentially affect water and nutrient transport (Allen, 2007; Kattge, 2022; Weigelt et al., 2021), increase the drought resistance of plants (Burke, 2006) and potentially alter the isotopic composition of plant water (Poca et al., 2019). These aspects provide excellent opportunities for interdisciplinary approaches. Incorporating soil electrical conductivity (or other chemical measures) covering the vertical and horizontal heterogeneity of the subsurface, or subsurface resistivity variation (by repeated geophysical surveys) using electrical resistivity tomography (ERT) can further help to find influencing parameters on soil water movement patterns. This can provide a broader overview of the soil physico-chemical conditions at the respective research site, thus moving understanding of the water-soil interface from Figure 1, panel 2 to 3. Furthermore, investigating soil water movement, as well as determining how precipitation reaches the soil as stemflow or throughfall can be important when studying subsurface processes and runoff generation mechanisms in forested areas. Recently, there has been a renewed interest towards the determination of stemflow infiltration area by using direct observations, which are generally rare (e.g., Carlyle-Moses et al., 2020; Llorens et al., 2022; Van Stan and Allen, 2020). Direct observations of stemflow infiltration area are usually made by the application of dye tracers, which have been proven to be useful for testing the double-funneling hypothesis (Gonzalez-Ollauri et al., 2020; Spencer and van Meerveld, 2016). However, dye tracer experiments generally require extensive excavation, and therefore, are destructive and non-repeatable. An alternative to such destructive methods is represented by geophysical methods, such as ERT which has been used successfully for the assessment of the temporal dynamics and spatial distribution of soil moisture and the routing of stemflow (e.g., Dick et al., 2018; Guo et al., 2020). Recently, Zuo et al. (2021) combined ERT with a dye experiment to show the role of roots on infiltration processes and soil water distribution. These recent studies based on ERT suggest that future experiments, aiming at the investigation of stemflow infiltration area on soil water movement processes, could rely on time-lapse ERT surveys used to trace the infiltration of salt water applied as simulated stemflow. The variation in soil resistivity quantified for different time steps can provide a direct observation of infiltration rate, area and volume, as well as information on the hydraulic behavior of soil layers and subsurface frozen layers (e.g., Pavoni et al., 2022). Such experiments could provide valuable data for developing new models reproducing subsurface flow generation or simulating stemflow and infiltration at the tree, as well as the plot scale and are a clear example of how synergized interdisciplinary methods would drive process understanding as illustrated in Figure 1 (panel 4).

3 An interdisciplinary view on experimental approaches

Highlights:

- Cross-scale experimental designs enable investigating relationships, feedback processes, and the impact of ecosystem variability.
- Hypotheses are preferably tested at multiple scales using interdisciplinary methods.
- Sampling frequency is a function of specific research questions, processes of interest, and applied tracers and should be correspondingly adapted.

New progress in observation techniques and analytical methods alone do not necessarily lead to scientific advancement per se. The experimental approach and the design of how we apply these new tracing techniques and analytical methods significantly determines the success of answering open questions, emphasizing the importance of moving into an interdisciplinary framework surrounded by synergetic methods (Figure 1 panel 4). In the following we sketch a few ideas on multidisciplinary experimental approaches in research on water fluxes through the SPAC.

From small-scale to large-scale experimental approaches

Cross-scale experimental design has been identified as a crucial element for future research initiatives. We need both, small-scale experiments in the laboratory or field (e.g., soil column or lysimeter) as well as intermediate scale (e.g., mesocosm, climate chamber or greenhouse) experiments to improve our mechanistic understanding and large-scale experiments (e.g., hillslope- and watershed experiments) to study interactions, feedback mechanisms and the influence of heterogeneity. Laboratory experiments can be used to test and validate the experimental design before the main study begins, e.g., by testing a new method or sampling technique in the laboratory or greenhouse before their application in the field. These laboratory experiments could further help to simplify processes, limit boundary conditions (e.g., in terms of lateral water flow) and study isolated processes. Yet, the outcome of laboratory experiments might then be difficult to translate to field conditions. Greenhouse experiments may neglect the effect of feedbacks, competition and emergent properties that are relevant in understanding processes under complex natural conditions. Small-scale experiments in the laboratory might therefore lead to a limited insight in terms of natural ecosystem functioning. A scale of intermediate complexity might be offered by macro- or mesocosm experiments, where interaction among plants and between plants and soil (micro)organisms can occur in intact soil but where environmental conditions are strictly controlled. Some examples are the Montpellier Ecotron (Guderle et al., 2018; Milcu et al., 2016), the Biosphere 2 (Kim et al., 2022; Werner et al., 2021), the MODOEK (Didion- Gency et al., 2021) and SPRUCE (Hanson et al., 2016). Even though long-term ecosystem legacy effects might not be fully captured and fluxes between different ecosystems cannot be simulated in such settings, ecosystem level mechanisms can be targeted by varying single biotic or abiotic drivers. Alternatively, hillslope or ecosystem scale experiments can provide a critical link between laboratory experiments and field conditions (Orlowski et al., 2019). However, they often do not cross multiple environmental boundaries, such as ecotones (defined as transition area between two biological communities (Palladino, 1974)), that may limit their applicability to derive universal (eco)system functioning. Large-scale experiments that study water or other solute fluxes within an entire watershed are rare because these are time-, cost- and labor intensive. Some examples encompass the Hubbard Brook Ecosystem Study (Campbell et al., 2021; Likens, 2013), studies from the H.J. Andrews Experimental Forest (Johnson et al., 2021), the Marcell Experimental Forest (Kolka et al., 2011), the Alptal Experimental Watersheds (Meerveld et al., 2018; Stähli et al., 2021), the East River Watershed (Hubbard et al., 2018), the Weierbach Experimental Catchment (Hissler et al., 2021), the Wüstebach Experimental Catchment (Hrachowitz et al., 2021; Stockinger et al., 2017) or the IISD Experimental Lakes Area (Blanchfield et al., 2022; Harris et al., 2007; Hintelmann et al., 2002). Even if all these limitations could be overcome, large-scale experiments alone do not guarantee success (e.g., due to low tracer recovery, various processes that are not necessarily independent and might show unknown interactions that concurrently affect the target variables). Nevertheless, since the stream water at the catchment outlet provides an integrated signal of the processes taking place across the investigated catchment, isotope mass balance analyses based on endmember splitting (Kirchner and Allen, 2020) can provide a new perspective into ET sources (e.g., snow vs. rain, (Sprenger et al., 2022)) on the catchment scale. Moving forward, we need to build on the "lessons learned" from such large scale experiments. We think that ideally, a multi-scale approach should be employed in future experiments to leverage the strengths of small-scale, intermediate-scale and large-scale experimental designs and minimize the weaknesses of each different experimental scale. The idea behind this multi-scale experimental approach is to test a hypothesis at multiple scales. By this, we mean to perform experiments with the same or with complementary approaches at the small and at the large scale to develop a more holistic picture of how small-scale processes result in large-scale patterns of ecosystem functioning (Lang et al., 2016). For instance, monitoring the change in isotopic composition of soil water in different depth of a soil profile during an artificial sprinkling experiment on a hillslope can inform about the small-scale infiltration and soil water flow and storage processes (Rinderer et al., 2021; Seeger and Weiler, 2021). The observed dynamic of the subsurface flow and tracer breakthrough at a trench on the bottom of the same experimental hillslope (hillslope scale) allows to learn how the small-scale processes lead to large scale functioning through complex process interactions on that hillslope. With such an approach, it is more likely that we identify the processes that are relevant for the patterns or functioning we observe at different scales.

Laboratory experiments can be used to test new hypotheses and answer specific questions. For instance, artificial soils can be used to test different water extraction methods for isotope analysis and the occurrence of isotopic fractionation during soil water transport and plant water uptake (Amin et al., 2021; Millar et al., 2018; Orlowski et al., 2016b). The processes can then be further analyzed under more complex field conditions. Mesocosms or large-scale pot/lysimeter experiments provide a link between highly controlled laboratory experiments (e.g., climate chamber) and field studies (Deseano Diaz et al., 2022a; Marshall et al., 2020; Mennekes et al., 2021). They allow to study process interactions under semi-controlled conditions. This will lead to a more mechanistic understanding of the small-scale processes (causes) while considering the process interactions, feedbacks and heterogeneity (consequences) emerging at larger scales.

Similarly, recent progress in quantifying subsurface water volumes available and accessible to vegetation has allowed increasingly robust aggregate estimates of root depths that characterize root systems at catchment-scales without the need for direct root depth observations of individual plants (Bouaziz et al., 2020; de Boer-Euser et al., 2016; Gao et al., 2014; Gentine et al., 2012; Kleidon, 2004; McCormick et al., 2021; Stocker et al., 2023). This has opened opportunities towards more reliable process-based descriptions of catchment-scale soil-plant-atmosphere interactions together with their evolution over time as function of a changing climate and/or land cover (Bouaziz et al., 2022; Nijzink et al., 2016). In combination with isotope data, there are first steps made towards describing how the changes in these interactions then affect the catchment-scale partitioning of water fluxes as well as the structure of transpiration and drainage transit times and soil water residence times (Hrachowitz et al., 2021).

Temporal and spatial resolution

Crucial aspects of a successful experimental design are the temporal duration and resolution of the investigation. Since we cannot measure "everything everywhere" all the time, we usually aim for measuring "only what is needed" (Brantley et al., 2017). Yet, what is needed will depend on the research question. A long-term monitoring over several years (e.g., Aubert et al. (2013)) at a coarse temporal resolution might be appropriate for capturing seasonal or annual variability or trends of change. In different settings when the short-term variability (e.g., the change in tracer concentration during an event or experiment) is of interest, an event-based experimental design (natural or controlled) or snapshot-sampling campaigns might be the best choice (e.g., Pinos et al., 2020). For research aiming at short-term rainfall-runoff investigations, continuous, high frequent monitoring might be a pre-requisite (e.g., von Freyberg et al. (2017)). Depending on the measuring or sampling frequency, tracer data will either appear as noise that is hard to interpret or as pattern that is easier explained by a natural process. Therefore, the sampling frequency needs to be adapted to the process we study, the research question we try to answer and the tracer we apply (Rode et al., 2016; Torres et al., 2022). In this concern, in-situ isotope measuring systems in soils and tree xylem have substantially improved our ability to measure short-term ecosystem dynamics that with traditional soil or xylem core sampling would not have been as easy to capture. Partly the lack of high-frequency data has led to false assumptions and mis-conceptualizations of the underlying processes (e.g., for stream water Chappell et al., 2017).

The spatial resolution of monitoring or the number of replicates in an experimental design are important in terms of yielding representative measurements. Challenges in terms of tracer application, measuring and sampling methods have been discussed in Chapter 2. Some need to be kept in mind, when choosing an appropriate spatial resolution of an experimental design. In terms of tracing water fluxes across the SPAC, adverse effects of the labelling procedure due to horizontal and vertical spatial heterogeneity in soils and related differences in tracer abundance can lead to misinterpretation of data. For instance, spatial heterogeneity of applied label water might translate into biased isotope measurement in the plant xylem water (Allen and Kirchner, 2022; Barbeta et al., 2022; Chen et al., 2020; Zhao et al., 2016). Injection of tracers into tree stems might influence the tracer distribution within the tree trunk (Treydte et al., 2021). On the other hand, depth-specific or spatial differences in the tracer composition of soil water is the pre-requisite for attributing sampled xylem water to different natural or artificial water sources. We therefore reiterate that for an ideal tracer recovery, the number of sampling locations or replicates, the applied tracer concentration, the tracer application, the timing and place of sampling and the sampling method are crucial to derive meaningful interpretation from the experimental data.

Good-practice examples of interdisciplinary experimental approaches

In the Special Priority Program (SPP 1689) on Ecosystem Nutrition (Lang et al., 2016), a cohort of soil scientists, ecophysiologists, hydrologists, microbiologists, geologists, geochemists and computer sciences aimed at a better understanding of ecosystem nutrition strategies of European beech forests. Nutrient availability has been highlighted as the decisive factor of forest ecosystem productivity in more than 90 forest sites across the globe (Fernández-Martínez et al., 2014). Therefore, the aim of this research initiative was to identify the processes, controls and organizing principles of phosphorus and nitrogen acquiring or recycling from the molecular scale (Ganta et al., 2020) to the catchment scale (Sohrt et al., 2019). To do so, a suite of experimental approaches has been applied. Here, we want to highlight a series of isotope tracer-based hillslope sprinkling experiments to investigate infiltration processes, subsurface flow and associated nutrient leaching at individual soil profiles (Makowski et al., 2020) at a lysimeter, at a hillslope trench and in a nearby stream (Rinderer et al., 2021). It could be shown that predominantly old water that was stored in the soil before the sprinkling experiment was discharged from the forest stands. Significant phosphorus flushing with a systematic time delay at deeper soil depth could be observed followed by chemostatic transport conditions during the rest of the 12 h sprinkling experiments. This suggests that phosphorus replenishment was in the order of minutes to hours in these European beech stands. However, the translocation of phosphorus from the forest floor to the mineral soil might be of high relevance at sites where the forest floor is the dominant source for the phosphorus nutrition of trees.

During the same sprinkling experiments, the isotopic tracer (deuterated water) was also monitored at various depths in soil profiles and in the stem of mature trees at multiple heights. The high-frequency data that was collected during the experiments using in-situ isotope probes (Volkmann and Weiler, 2014) showed a distinct isotopic profile in the soils of the hillslope and clearly identifiable breakthrough curves with a delay of multiple days to weeks with increasing stem height (Seeger and Weiler, 2021). The findings suggest that a direct link between the instant isotopic signature measured in a tree stem and the isotopic signature in the soil profile without considering temporal delays is likely to lead to wrong identification of trees' source waters.

This is only one good-practice example of interdisciplinary experimental approaches covering many of the methods highlighted in Figure 2. Other good examples that are still ongoing and will soon yield interesting insights into ecohydrological processes and functioning are e.g., the EU COST (European Cooperation in Science and Technology) action WATSON (WATer isotopeS in the critical zONe, https://watson-cost.eu/) in which tracer hydrologists, soil scientists and modelers aim at advancing the understanding of water mixing in the critical zone by investigating ecohydrological processes of water exchange between vegetation, surface and subsurface water compartments. Another good-practice example is the

ISODRONES (https://www.isodrones.com/) project which aims to develop a framework that quantifies the importance of deep roots for the water balance across various environments. Finally, the DFG project CONFOR (Project number: 501530203) focuses on a quantification of the contribution of tree use of distinct deep soil water for tree health and forest stability for key forest species across Europe, a goal leveraging impressive interdisciplinary collaborations.

4 Challenges in modelling water transport processes in ecosystems

One of the current challenges of ecohydrological modelling is predicting system responses to variability in e.g., climate or land use. Here, we summarize the potential of tracer aided models for cross-scale approaches and interdisciplinary research questions.

Highlights:

- Mechanistic models informed by isotope tracers are a way to transfer process understanding in space (across biomes, climates, etc.) and time (predictions).
- Virtual experiments combined with field observations/experiments forming a feedback loop of iterative adaptation/modification can help to improve both the representation of the real world within models as well as the overall system understanding.
- Building new hybrid models and using machine learning to identify dominant drivers of isotopic dynamics in the SPAC helps to gain cross-disciplinary understanding.

Challenge to predict responses across scales

We need to improve our ability to predict responses of the different compartments of the SPAC to hydro-climatic variability, vegetation patterns and dynamics (Breshears, 2005). One of the challenges is that our experimental studies predominantly cover the plot to catchment scale, while most societal questions are related to basin or continental scales. Emerging properties and processes that are relevant for one but irrelevant for another scale include, for instance, effective soil hydraulic conductivity and preferential flow. Scale-dependent properties such as hydraulic conductivity or dispersivity are prime examples of model parameters that need to be adjusted from measurements at small-scale experimental sites to the larger hillslope or catchment scale. At other times, the temporal and spatial resolutions of measurements to train models are too coarse to meaningfully capture most of the natural variability. This is problematic for several reasons: i) small spatio-temporal scale process dynamics remain insufficiently understood, ii) there is a significant lack of hierarchical understanding (e.g., emergence of driving processes across scales), due to strong limits in linking the impact of structural features (such as soil type; land-use, species identity and abundance) and biophysical functioning of plant communities on e.g., soil-plant-atmosphere feedbacks across scales. Together this significantly hampers our ability to successfully predict long-term and large-scale impacts of climate change related perturbations and associated legacy effects on the resilience of ecosystems (see Silva and Lambers, 2021).

Model-data connection

Mechanistic modelling of water fluxes and their drivers across spatio-temporal scales requires a strong connection between data and model approaches. Therefore, we should aim for the development of modelling frameworks that are able to identify the emergence of dominant processes across scales. We need to constrain levels of model complexity and devise sampling strategies specific to different spatio-temporal scales to train models at sufficient data frequency and spatial coverage. Virtual experiments carried out with process-based models (i.e., Price et al., 2010) bear the potential to (i) point us towards specific processes and potential interactions that cannot be directly observed at our field sites, (ii) reveal data needs to better constrain the model's parameters and structure and (iii) allow us to assess which tracers to apply where and at what concentration (see Chapter 2). This is particularly necessary for large scale experiments where potential interactions, feedbacks and thresholds within the hydrologic cycle are hard to foresee (Ketcheson et al., 2017; Price et al., 2010; Sutton and Price, 2022). Models can also be a means of upscaling from a hillslope to an entire catchment. Therefore, we need case-specific combinations of experimental and modelling frameworks that feed back to each other to advance our understanding of complex systems. This is a crucial step to gain enough in-depth process understanding to finally create transferrable data-model integration frameworks. Without a process-based model it becomes increasingly difficult to predict interactions, thresholds and feedbacks within the observed system that eventually lead to consequences on larger scales. For example, based on observations alone it is quite difficult to confidently predict how changes of one element/variable within the catchment (e.g., cutting down one specific tree species) will affect the hydrologic cycle of the entire catchment. This is because there are countless processes and interactions that turn the cause (clearance of one tree species) into the eventual effect (more/less ET, changes in the water balance) (see examples of research at Hubbard Brook e.g., Campbell et al. (2021);

Federer (1990); Holmes and Likens (2016)). A sample campaign driven by a spatially-distributed model can point to locations and times where data is most informative. This said, it is imperative to maintain a continuous exchange between field work and model development to evaluate whether the model as representation of the real-world system is consistent with observations and whether or not there are processes (or parameterizations thereof) that are relevant but not (yet) included in the models. This allows for substantial improvements of the understanding of relevant natural or managed ecosystem processes.

Benefits of integrating tracers into process-based models

Tracer-aided models are versatile objects; they range from (i) non-isotopic models constrained (or simply challenged) externally by isotopic data (Sutanto et al., 2014), (ii) primarily non-isotopic models, later extended to simulate time series in isotopic composition of a particular water flux (e.g., RWU, Couvreur et al. (2020), and (iii) "isotopic core" models, where isotopic information is used as variable inputs/parameters and isotopic outputs are produced (Rothfuss et al., 2012; Sprenger et al., 2018; Zhou et al., 2022, 2021). Still, there is not (yet) a consensus on how to handle certain aspects of water partitioning relevant to the transport and distribution of tracers in process-based models. One example is the representation of green and blue water, the former becoming ET and the latter becoming runoff or groundwater recharge (McDonnell, 2014). In process-based models one can use dual or multi soil domains having different permeability and porosity values in order to model preferential flow within fractures and macropores. The exchange between the domains can be governed by advection, diffusion or both (Ho, 2000), which, on the one hand, can be a problem since it bears the risk of causing very different propagation behaviours of the isotope tracer. However, on the other hand, this also means that tracers can be used to select the appropriate mode of water partitioning employed in the model and this will ultimately help differentiating between water that is held in small pores in the soil and water that percolates rapidly to deeper aquifers and streams. Similarly, recent developments demonstrate and highlight the value of the "StorAge-Selection" function concept as a coherent analytical framework describing non-uniform sampling of water fluxes from different water pools in a system (Benettin and Bertuzzo, 2018; Rinaldo et al., 2015). At the same time, plant water uptake is often still simulated as a simple mechanistic process extracting water from one domain governed by plant and climatic conditions. Such a simplified representation can lead to modelled tracer concentrations that do not match measurements. Experiments labelling soil water of different depths with isotopic tracers in order to learn more about RWU can help to add some more complexity and realism to the models. It is increasingly accepted that mechanisms controlling plasticity in RWU are highly complex with strong interlinkages between water uptake, carbon sequestration and allocation as well as nutrient uptake and cycling (Deseano Diaz et al., 2022a). There are two families of RWU process-based models completing each other in the literature (Javaux et al., 2013): (i) the macroscopic, effective models (featuring e.g., a stress factor, LAI) that are compatible with large-scale simulations because they are little demanding in parameter estimations and (ii) microscopic, descriptive models (e.g., R-SWMS (Javaux et al., 2008); Cplantbox (Zhou et al., 2020)), far more demanding in data for their parametrization but explicitly taking into account e.g., the role of root-soil and xylem resistance as well as stomatal aperture. However, novel in-situ high-frequency water isotope data sets offer a unique opportunity to test, validate or further develop the representation of small-scale process feedbacks of water cycling into existing models . High-frequency water stable isotope in-situ data allow for (i) the incorporation of vapor exchange between soil evaporation, transpiration and the atmosphere (Rothfuss et al., 2021), (ii) the testing of underlying hypotheses to the evaporation process (e.g., the location of the evaporation front, Rothfuss et al. (2015)), (iii) the investigation of the process of RWU (e.g., how root hydraulic properties are distributed across the root system; Meunier et al. (2017)), its spatio-temporal plasticity (Kühnhammer et al., 2022, 2020) and (iv) the incorporation of preferential uptake of different soil water sources (i.e., held at different tensions) in response to water stress conditions (Kühnhammer et al., 2020) into model structures. Recently, machine learning and artificial intelligence methods have been utilized in the eddy-covariance and, to a lesser degree, gas-exchange chamber flux community. Starting with large and high resolution eddy co-variance data (Guevara-Escobar et al., 2021; Zhu et al., 2022), there have been recent developments to use e.g., random forest or support vector machine learning approaches to gap-fill and predict automatic chamber-based gas-exchange datasets that commonly have less data availability and larger singular gaps (Dahlmann et al., 2022). While this is still largely unexplored in isotope-enabled modelling, the high-frequency of stable isotope in-situ approaches provides a large data amount that would likely be able to train machine learning algorithms.

Interdisciplinarity in modelling approaches

As highlighted above in relation to the potential of interdisciplinary experimental approaches, different research disciplines frequently have different perspectives and resolutions of investigation, which may partly be due to differences in their historic roots. Considering RWU, plant ecophysiologists focus on the role of water uptake for plant productivity and hydrologists focus on the role of RWU to subsoil water cycling (e.g., Cocozza & Penna, 2021; Breshears, 2005; Bruin and Morgan, 2019). To achieve a holistic system understanding, it is crucial to not only look at, e.g., RWU through disciplinary glasses but to also include ecological dynamics (e.g., phenology; water-carbon-nutrient cycling), optimality principles and the associated feedbacks with the environment into the analysis (Breshears, 2005; Gentine et al., 2019; Stephens et al., 2021, Dubbert et al., 2022). For example, stomatal control, water uptake and transpiration are not only related to soil moisture and atmospheric humidity but also to other factors: different plant species show different degrees of their leaf water potential controls, placing them in different positions within the gradient between isohydry (strong regulation of leaf water potential by stomatal conductance) and anisohydry (less strong regulation) (Haberstroh et al., 2022; Martínez-Vilalta et al., 2014). Thus, the same water availability might lead to different transpiration fluxes depending on the species. It has also been observed that carbon allocation within the soil (and thus the energy and substrate available to produce new roots that allow additional water uptake) is affected by water availability. It can differ among species (Hommel et al., 2016; Joseph et al., 2021) additionally contributing to the above-mentioned species-specific effects on water fluxes, albeit on longer time scales. Moreover, the root system is developed foremost to optimize efficient nutrient uptake (Carvalho and Foulkes, 2018), hence trade-offs between nutrient and water acquisition particularly under drought need more attention. This can be achieved through building cross-disciplinary modelling frameworks that move away from 'simple' statistical mixing model approaches (as per Parnell (2008)) for e.g., RWU estimations, towards process-based models (see Rothfuss and Javaux (2017)) or creating new hybrid models based on current dominant hydrological modelling approaches of RWU (Dubbert et al., 2022). These modelling frameworks are another example of the fourth panel of Figure 1, where modelling, is represented by the third arrow reinforcing the interdisciplinary framework together with approaches and methodologies. In a recent review, Dubbert et al. (2022) summarize how the creation of hybrid models such as through i) the integration of physiological process feedbacks into Bayesian approaches (water potential gradients, root distributions, water-nutrient uptake feedbacks, travel time estimates; e.g., Seeger and Weiler (2021); Kühnhammer et al. (2020)) or ii) the integration of Bayesian statistics into hydrodynamic approaches (e.g., De Deurwaerder et al. (2020)) might help to overcome current limitations in predicting spatio-temporal dynamics in RWU under non-stationary conditions.

Concluding remarks

We hope that our shared thoughts and ideas will inspire new interdisciplinary collaborations, as they are essential to address climate-induced changes in water fluxes through ecosystems. Expertise across disciplines, such as hydrology, plant ecophysiology and soil science (and other disciplines), is urgently needed in order to help solve the myriad of pressing societal issues. Again, we think that a multidisciplinary approach needs to be followed in future studies to overcome limitations of our currently disciplinary separated research activities because what seems to be an artefact or an unexpected result in the eyes of one discipline, might have a logical explanation in the view of another. What one discipline considers a boundary condition might be the study object of other disciplines (e.g., Staudinger et al. (2019); see Figure 1). Other researchers have already highlighted the need to investigate interfaces across which water is exchanged between system compartments, but this requires us to leave the conceptualization of simple system boundaries and enable cross-compartmental approaches (see commentary by Bishop and Eklöf (2022)). The different points of view from colleagues from neighbouring disciplines can help to solve via triangulation a common problem and help to better understand different processes and the scales at which they are relevant. Cross-disciplinary research needs to focus on solutions to preserve, restore and protect natural ecosystems against climate change impacts and develop adequate adaptation strategies. For example, we advocate synchronous observations of water, nutrient and CO_2 cycling to close knowledge gaps related to the tight links between water, carbon and nutrient cycling particularly under drought. In any case, a certain degree of openness to knowledge bases of unfamiliar disciplines is required to leave the “disciplinary” boundaries (see strategies by Woiwode and Froese (2021) for scholars). Here, community approaches such as the COST action WATSON are extremely valuable when addressing key environmental problems linked to the sustainable management of water resources. Water stable isotopes are used as a common tool across disciplines to study water movement and partitioning from the vegetation canopy to the groundwater. Such research collectives are the perfect breeding ground for future interdisciplinary research initiatives that integrate field-, laboratory- and modelling approaches to study water flux dynamics under changing environmental conditions.

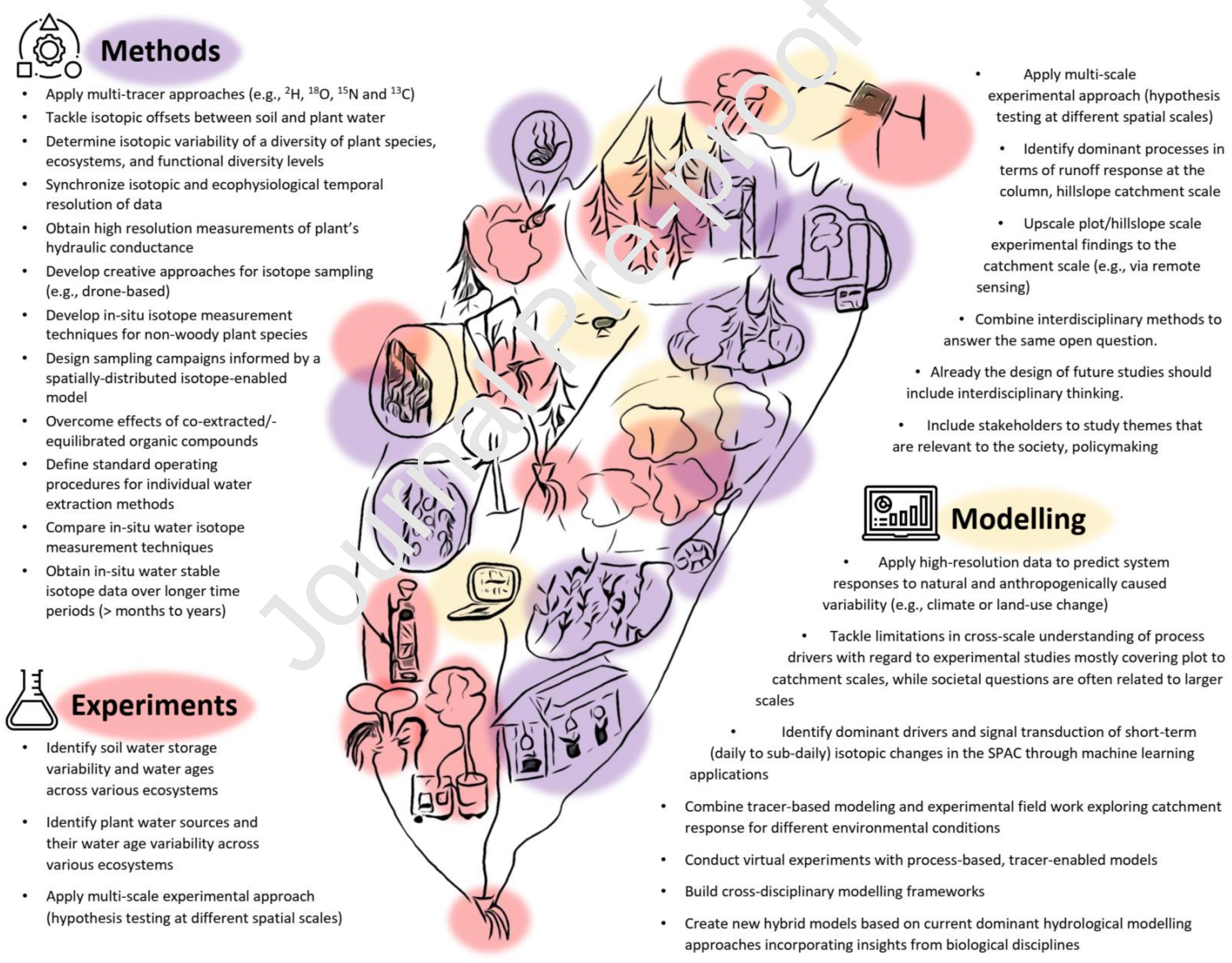


Figure 2. A summary of future ways forward in terms of methods, experiments and modelling in an interdisciplinary framework to trace water fluxes through the SPAC: We suggest developing standardized sampling procedures and comparison of in-situ-measuring techniques for high resolution tracer tests for a diversity of plant species and ecosystems. Already the design of experiments should involve experts from several disciplines and hypothesis should be tested at the scale of soil columns, microcosms in the lab, hillslopes and forest stands as (depicted by small icons). Tracer-based modelling, experimental fieldwork and innovative sampling techniques such as drones and satellites (shown as small icons) should be combined to upscale our process understanding to the watershed- or landscape-scale where scientific advancement has an impact on societal questions.

We advocate for future and more widespread interdisciplinary work leveraging the cross-cutting methodological, experimental, and modelling approaches highlighted in this paper (Figure 2) and furthermore, future reflections about not only *what* tools are used to create these interdisciplinary frameworks but also about *how* interdisciplinary teams come together and succeed in terms of initiation, communication, conceptual exchange and subsequent conclusions and output resulting in impacts across multiple disciplines. Figure 2 summarizes promising future directions in terms of methods, experimental approaches and modelling in an interdisciplinary framework to trace water fluxes through the SPAC.

Data availability

No data are associated with this manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Author contributions

Funding acquisition: NO, MR, MD, Jaane Krüger. NO, MR, MD drafted the manuscript. NC, MR and NO drafted the figures. All authors contributed to and reviewed the manuscript.

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