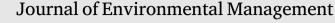
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Review

Using stable isotopes to inform water resource management in forested and agricultural ecosystems

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ABSTRACT

Present and future climatic trends are expected to markedly alter water fluxes and stores in the hydrologic cycle. In addition, water demand continues to grow due to increased human use and a growing population. Sustainably managing water resources requires a thorough understanding of water storage and flow in natural, agricultural, and urban ecosystems. Measurements of stable isotopes of water (hydrogen and oxygen) in the water cycle (atmosphere, soils, plants, surface water, and groundwater) can provide information on the transport pathways, sourcing, dynamics, ages, and storage pools of water that is difficult to obtain with other techniques. However, the potential of these techniques for practical questions has not been fully exploited yet. Here, we outline the benefits and limitations of potential applications of stable isotope methods useful to water managers, farmers, and other stakeholders. We also describe several case studies demonstrating how stable isotopes of water can support water management decision-making. Finally, we propose a workflow that guides users through a sequence of decisions required to apply stable isotope methods to examples of water management issues. We call for ongoing dialogue and a stronger connection between water management stakeholders and water stable isotope practitioners to identify the most pressing issues and develop best-practice guidelines to apply these techniques.

1. Introduction

Water is the molecule of life (Ball, 2017). However, its availability in many parts of the world is becoming more erratic due to unsustainable use and climate change, and future projections suggest increasing difficulties in obtaining and managing water supplies for human activities (Konapala et al., 2020). Developing strategies for adaptation to changing water cycle dynamics requires understanding ecosystem water fluxes and the processes that control them.

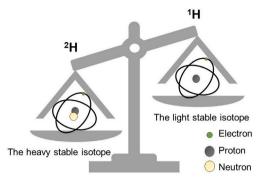
The management of water resources in forested and agricultural ecosystems needs to be based on an understanding beyond just measuring water stores (e.g., in soils and groundwater) and fluxes (e.g., precipitation, evapotranspiration, groundwater recharge, and stream discharge). Environmental tracers include physical properties and chemical constituents that can be used to help in tracking water through the water cycle (Leibundgut et al., 2009), facilitating the assessment of the fate of precipitation (e.g., its partitioning into evapotranspiration versus stream discharge and groundwater recharge; Kirchner and Allen, 2020), the timescales of subsurface storage and transport (Knighton et al., 2020; Jasechko et al., 2016; Jasechko, 2019), as well as the temporal and/or spatial origin of transpiration, groundwater recharge, and stream discharge (Allen et al., 2019a,b). Among the available tracing techniques, stable isotopes of the water molecule have been particularly valuable in advancing hydrological process understanding (Kendall and McDonnell, 2012). Water is formed by two elements, hydrogen and oxygen, each of which exists in different forms known as isotopes, whose characteristics are summarized in Table 1. The general key concept of stable isotope methods is that each water store or water source (e.g., precipitation, plant water, soil water, groundwater, etc.) has its particular isotopic composition or "signature" that can function as a fingerprint of that particular water pool. Because hydrogen and oxygen stable isotopes are intrinsic constituents of the water molecule (Table 1), they are conservative tracers, i.e., they "are the water" rather than some solute dissolved in it. Unlike most chemical tracers, water isotopes react very little with their surroundings, and the mechanisms by which isotope signatures change (primarily evaporation and condensation) are well understood (Hoefs, 2018). Thus, stable isotopes can be particularly useful in determining the relative proportion of different water sources having different stable isotope compositions in a mixture. This separation would be impossible if water fluxes alone were measured.

Over the years, stable isotope analysis has been applied to many research topics and has helped to unravel many scientific questions. For example, stable isotopes have been used to study groundwater recharge rates (Koeniger et al., 2016), soil evaporation (Kleine et al., 2020), water flow through soil columns (Stumpp et al., 2012), hydrograph separation (Uhlenbrook et al., 2002), water pathways of runoff (Mueller et al., 2014), travel times of water in soils (Sprenger et al., 2016b; Muñoz-Villers and McDonnell, 2012), water uptake by plants (Plamboeck et al., 1999; Penna et al., 2021), and the relative importance of sources for plant water uptake (Allen et al., 2019a; Muñoz-Villers et al., 2020; Gessler et al., 2022), water partitioning between transpiration and evaporation (Liebhard et al., 2022), water source mixing in streams and rivers (Lv et al., 2018), and partitioning of precipitation between streamflow and evapotranspiration (Kirchner and Allen, 2020). Data from 27 mountain catchments have shown the correlation of young water fraction with low flow duration and other catchment characteristics (Gentile et al., 2023), opening the way to using stable isotopes for hydroelectric dams' management. Several large stable isotope databases are now available (Table 2) and at the disposal of the community, supported by the efforts of stable isotope researchers who collect and handle samples according to shared protocols (Ceperley et al., 2024).

Due to the pressing water-related issues raised by climate change, stakeholders need multiple tools to support their decisions in an informed way. Until recently, the application of stable isotopes outside scientific research has been limited owing to high costs and other practical limitations. However, recent technological advances and clearer insights into the benefits and limitations (Table 3) have increased the cost-effectiveness and accessibility of using stable isotopes across a broad array of applications (Table 4). While the application of stable isotope techniques is an important emerging tool for sustainable water resources management, more guidelines, communication, and collaboration are required between the scientific community and stakeholders to ensure its effectiveness.

In this paper, we broadly define stakeholders as those who can directly or indirectly benefit from a detailed knowledge of water storage and fluxes in forested and agricultural ecosystems (Table 5). Therefore, to define stakeholders, we first need to identify how they may benefit, which is primarily via decision-making support, as outlined in Table 5. Some stakeholders, i.e., farmers and nursery managers, can have a direct economic benefit in terms of reduced irrigation needs and more efficient water use by crops. Foresters and land managers may need to understand current and future plant water use and water availability before implementing management strategies that may alter water traits. Other water resource managers can directly benefit from a clearer picture of the possible vulnerability of different water bodies under exploitation to ensure uninterrupted water supply. Policymakers and local administrators can identify priorities and needs and be the leading edge





Each molecule of water is formed by two atoms of hydrogen and one of oxygen. Hydrogen has two stable isotopes, ¹H and ²H, while oxygen has three, ¹⁶O, ¹⁷O and ¹⁸O. Scientists analyze the ratio between the heavy (²H and ¹⁸O) and the light isotope (¹H and ¹⁶O) of these elements to track the fate of water. ¹⁷O is very rare, is used only for research, and is not relevant for the topic tackled by this paper. A water molecule therefore can appear in different forms according to how these isotopes combine. In the figure to the right, a few of these part of the water molecule, stable isotopes are considered **conservative tracers.**

Isotopes are forms of chemical elements differing only in the number of neutrons in the nucleus. Isotopes can be stable or radioactive. Stable isotopes do not decay into other elements with time, do not emit radioactivity, and are naturally present in all materials. For example, the chemical element hydrogen is defined by the presence of one proton in the nucleus. The majority of hydrogen atoms have only one proton and they are called hydrogen-1, or ¹H. Fewer atoms can, in addition to the proton, also have a neutron. They are called deuterium, hydrogen-2, or ²H. Hydrogen has also a third isotope, tritium, that has two neutrons in the nucleus. Tritium is unstable and radioactive, therefore it will not be cited further in this paper, although it can also be used for age dating and for tracing water fluxes (Eyrolle et al., 2018)

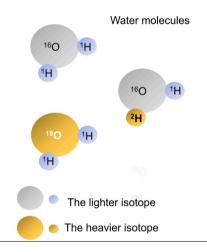


Table 2

List of available water stable isotope databases.

Name of the database	Web address	Type of water isotope data	Ref.
GNIP	https://www.iaea.org/services/ networks/gnip	Precipitation data collected worldwide	International Atomic Energy Agency (IAEA), 2005
GNIR	https://www.iaea.org/services/ networks/gnir	Global database of isotopes in rivers	Halder et al., 2015
SLONIP	https://slonip.ijs.si/	Precipitation data collected in Slovenia	Vreča et al., 2022
The WATSON Isotope Database	https://watson-	Stream water, plants and soils collected in	
-	cost.eu/outputs/databases/	Europe	
		Groundwater recharge studies	
National Ecological Observatory Network	https://www.neonscience.org/	Groundwater, atmospheric water, surface water, precipitation	Thomas et al., 2023
IsoBank Project Home Isobank	https://isobank.tacc.utexas.edu	Stable isotopes from various origin	McDonald, 2021
Waterisotopes Database	https://wateriso.utah.edu/	Precipitation, lakes, streams, soils, snow,	West et al., 2010
	waterisotopes/	tap water, ocean, etc.	
Piso.AI	https:	Globally simulated for precipitation	Nelson et al., 2021
	//isotope.bot.unibas.ch/PisoAI/		

to benefit from these decision-support tools. Overall, stable isotopes of water are essential tools for assessing the effects of climate change and human activities on regional water cycles. This knowledge may support decision-makers in formulating policies to preserve the environment, ecosystem services, and water resources for future generations.

Analogously, we broadly define practitioners as researchers and technicians with a scientific background in the application of stable isotope techniques to hydrological questions and with the skills to interpret analytical data, modeling outputs, and specialist literature. Stable isotope practitioners are commonly employed by universities and research centers, although stable isotope services can also be offered by private companies. The water-related issues faced by stakeholders often require data from the site of interest as well as models supporting data interpretation and decision-making. Practitioners include people with experience in fieldwork and laboratory analysis and people with experience in modeling and programming. Practitioners are not only consultants but they also interact with stakeholders to find a solution to stakeholders' needs based on the integration of specific knowledge and expertise, as outlined in Part 5.

This paper aims to summarize the potential of stable isotope methods for solving practical problems and to establish a template for water isotope applications that can be recommended to a wide group of users. This template can be exemplified by the flowchart shown in Fig. 1 which builds on the mutual exchange of information between stable isotope practitioners and stakeholders. This approach has four objectives: (1) help stakeholders to determine when stable isotopes of



Fig. 1. The flowchart illustrates the application of a stable isotope toolkit to address the needs of stakeholders. The first step can be represented by a list of cases that the stakeholders can compare with the specific issue they are experiencing. Stakeholders can explore the available cases and select the one that is most suitable for their issue. An overview of stakeholders' problems related to water management that can be tackled with the support of water stable isotope analysis can be seen in Table 5. After this initial decision, the toolkit provides the user with information on the advantages and disadvantages of the proposed technique, the timeline, the foreseen outputs and, ideally, an estimate of the cost-benefit evaluation. The process requires the application of specific guidelines that have to be developed and applied through the interaction among stakeholders and service providers (scientists and technicians who have experience in field work, laboratory analysis, and/or modeling) as shown in Fig. 2. Case studies where this flow chart has been applied are summarized in Table 4 and detailed in the Supplementary material.

water may provide information that cannot be obtained through other, more conventional techniques; (2) guide on applying stable isotope methods and interpreting the data; (3) suggest how to communicate the results and implement feedback; (4) provide a framework for (environmental and socio-economic) cost-benefit evaluation. We also present four case studies in agricultural, forested, and urban areas to exemplify our approach.

2. An overview of practical applications of stable isotopes of water

Stable isotope analyses may help many different stakeholders (Table 5) to address diverse questions. Examples include:

- · How can we monitor water fluxes and predict critical situations?
- · How do current management approaches affect water fluxes?
- · Will water be available for the next growing season?
- Will groundwater be recharged sufficiently?

- Which available cultivar has higher resistance and resilience to drought? How much will the current cultivars be affected by climate change?
- What are the long-term effects of climate change on the plant/system?

Stable isotopes are excellent tools for tackling questions like these. The underlying issue in these questions is that agriculture and forested ecosystems are already being affected by climate change and stakeholders are starting to suffer the consequences. Climate change impacts plant physiology with some plant species being positively affected (longer growing season), and others being negatively affected (Gordo and Sanz, 2010). The prolonged drought and unusually high temperatures observed in some parts of the world in 2022 pushed some farmers to plant new crops and implement new types of management (Galindo, 2022). In forests, droughts cause premature browning of leaves and early leaf fall (Klesse et al., 2022; Rukh et al., 2023), which will likely lead to a decrease in forest productivity (Xu et al., 2022). Furthermore,

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Table 3

Analysis of strengths, weaknesses, opportunities, and challenges (SWOC) of a water-stable-isotope-based toolkit.

Strengths	Weaknesses
 Interaction of stakeholders with stable isotope and water practitioners Solid scientific background Incorporation of velocity/transport/fate of water to measured water volumes (e.g., soil moisture, lake or groundwater levels) and fluxes (e.g., stream discharge) New instrument design (laser-based) and methodological advances make water stable isotope analysis more affordable A wide availability of techniques using isotopes for quantitative studies, which makes the method more attractive to practitioners A wide availability of portable instruments with which it is possible to measure soil, plant and atmospheric (vapor and precipitation) water samples extending the portfolio and ranges of disciplines 	 A single analysis quickly provides yields results; however, data interpretation can take time A single analysis can be cheap, but costs may grow if intense sampling or sample pre-treatment are required Analyses results can be available with a time delay due to the availability of laboratory infrastructures and to the delivery of samples It might be difficult to find a common language between all involved players Lack of standardized protocols [ISO norms] In some cases, need to capture data over multiple months or years
Opportunities	Challenges
 Interaction of decision-makers and policymakers to integrate stable isotope analysis into guidelines Can be integrated into user-friendly decision tools Modeling via multi-objective calibration or validation Can integrate with other tracers Incentives for the ecosystem services supported by the stable isotope analysis toolkit 	 Variability of environmental conditions (site-specific results) Lack of knowledge regarding the opportunities provided by the toolkit from policy-makers and decision-makers Diversity of land uses and land fragmentation Forecasting low flows to allow mitigation strategies for sensitive sectors

• International recognition of practitioners through accreditation agencies

wildfires are larger and more numerous than ever before (since systematic recording began in 2006; effis.jrc.ec.europa.eu). Rivers and lakes have reached very low water levels, affecting the supply for irrigation, households, and businesses, as well as the shipping industry. Analyses of water's stable isotopes in water bodies, soils, and vegetation help in understanding these impacts, contribute to projections of long-term changes and support efforts to prevent or mitigate their consequences. Stable isotopes of water allow us to track how rainfall makes its way to streams, typically through a combination of deep infiltration that recharges groundwater and primarily contributes to streamflow during dry periods, and overland or shallow subsurface flow that contributes to streamflow more quickly and transiently (Ansari et al., 2022). These different flow paths also have consequences for the associated transport of nutrients, fertilizers, and other solutes that might have accumulated at the surface or in the soil and can also affect water quality. Below we give some examples of the use of stable isotopes of water in different contexts.

2.1. Applications for water resources users, land managers and policy makers in agricultural settings

The analysis of the stable isotopes of water is a widely applied methodology in agricultural settings. The question of where agricultural crops obtain their water, under both irrigation and natural rainfed conditions, has been addressed in many studies. (e.g., Aguzzoni et al., 2022; Giuliani et al., 2023; Mahindawansha et al., 2018; Muñoz-Villers et al., 2020; Nasta et al., 2023; Xu et al., 2022). In addition, the stable isotopes of water are an excellent tracer for studying plant-plant interactions, such as hydraulic redistribution, in the agricultural context (e.g., Priyadarshini et al., 2016; Hafner et al., 2020; Meunier et al., 2018).

Stable isotopes can support decisions aiming at improving irrigation efficiency by providing information on the fate of irrigation water. For example, through stable isotopes, it is possible to establish how much of the infiltrating water becomes groundwater recharge or river discharge, goes back to the atmosphere via soil evaporation, or is taken up by plants and used for transpiration. The latter is sometimes referred to as "productive green water flow" because it directly supports carbon assimilation (Rockström and Falkenmark, 2000). Stable isotopes can also be used to determine the relative amount of irrigation water that contributes to plant water uptake compared to other water sources such as rain, snowmelt, or groundwater recharge (Penna et al., 2021). Isotopes may further help to define the soil depths where each plant species preferentially takes up water. With this information, irrigation timing and amount can be more precisely determined to ensure that the water and any added fertilizer remain long enough within the rooting zone. In agricultural ecosystems, runoff can have consequences not only for plant nutrient availability but also for water quality, especially in vulnerable areas as, for example, defined by the EU Nitrate Directive (91/676/EEC, 1991).

2.1.1. Case study 1. Water fluxes through agricultural soils

This case study (Table 4), conducted in vineyards in Southwest Germany, was motivated by concerns about the impact of vinicultural management on nitrate leaching into groundwater (Sprenger et al., 2016a). The study therefore aimed at addressing the questions of deep recharge and potentially associated transport of dissolved nitrate towards groundwater. The research involved soil sampling in both recently established and old vineyards with different soil management practices of either seeding grass or keeping the soil bare in between the vine rows. Isotope profiles were taken to a depth of 4 m and the nitrate concentrations were determined across the profile in addition to the measurement of ${}^{2}H/{}^{1}H$ and ${}^{18}O/{}^{16}O$ ratios of the soil water. Precipitation isotopes, along with meteorological data, were collected over several years. All isotope depth profiles in the studied vineyards showed a sinusoidal pattern that reflected very well the isotope dynamics of the infiltrating precipitation with isotopically enriched rainfall during the summer and depleted values during the winter (see Supplementary material for further information). The nitrate concentrations at depths beneath 3.5 m were significantly higher in the soils of the younger vineyards than in the older ones. Especially the young vineyard with bare soil inter-rows had a pronounced nitrate peak between 3.5 and 4.0 m. The isotope depth profiles were used to calibrate a soil physical model (Hydrus-1D) for the different sampling locations. Meteorological data and precipitation isotopes were used as input data, and soil hydraulic parameters, which govern water flow and transport, were determined through inverse modeling, optimizing the fit of the isotope simulations to the observations. The site-specific calibrated model was then applied to track the fate of water and nitrate over time and soil depth. The study revealed that elevated nitrate concentrations at around 3 m depth beneath the young vineyard were a result of nitrate mobilization during the vineyard installation 2.5 years earlier. An effective solution to reduce nitrate leaching into deep soil layers was found to be seeding grass in between the vineyard rows. This combined sampling and modeling approach helped provide insights into the seasonality of isotope ratios in precipitation and their preservation throughout soil profiles. In addition, isotope profiles allowed long-term monitoring of soil moisture, which would have been difficult in those depths.

Table 4

Case studies showing the applicability of water stable isotopes in forested and agricultural ecosystems. More details about each case can be found in the Supplementary material.

Case study number	1. Water fluxes through agricultural soils	2. Water dynamics in wells and streams	3. Aquifer management for domestic supply	4. Urban green areas
The stakeholder	Environment authorities, concerned citizens, farmers	A whisky distillery, other users of private wells and streams	Government authorities	Town administrators of cities and municipalities
The underlying questions	Stakeholders were worried about the effect of agricultural management on the concentration of nitrate in groundwater. The question was whether deep recharge occurred and if so, if it contained nitrate. What could be done to reduce the nitrate leaching into deep soil layers?	Stakeholders noticed changes in water availability. They were worried about the vulnerability of their water supplies to dry periods under climate change and asked what can be done to minimize unpleasant consequences.	Stakeholders wanted detailed information on recharge dynamics of an aquifer before approving its exploitation for domestic water supply. The main questions were whether this deep-seated water was of fossil origin or actively recharged and whether diffusive groundwater recharge could be one source of recharge.	Stakeholders needed efficient decision support tools that can be used not only to estimate the status quo of urban greening's contribution to carbon sequestration, but also to maximize urban greening's climate impact.
The interested area	Vineyards in Southwest Germany	UK upland ~1 km ² headwater catchment.	A large, deep-seated, freshwater-containing aquifer in Namibia/Angola discovered in the late 2000's.	Urban areas of Braunschweig and Brandenburg in Germany.
How the stakeholders' issues were tackled	Soil sampling in young and old vineyards with different soil management.	Sampling of different water sources	Samples from the deep aquifer were obtained by deep drilling. Diffuse groundwater recharge was determined by using deuterated water	Integration of multidisciplinary methods to estimate current carbon storage and carbon storage potential of urban trees in different settings (parks, alleys, single standing trees). At the same time, water relationships of urban trees are under investigation: amount of water used, water use efficiency, groundwater recharge in different settings.
Samples for water stable isotope analysis	Snapshot 4 m deep isotope depth profiles and long-term precipitation isotope sampling	Precipitation and stream water at various locations within the drainage network and wells	Groundwater samples from the deep-seated aquifer Water samples from the shallow aquifer Soil samples Plants	Ground- and surface waters, soils and plants
Ancillary measurements	Nitrate concentration and basic meteorological data	Other water quality tracers and hydrometric monitoring of water balance components	Soil hydraulic parameters Geological profiling Deuterated water to monitor water infiltration Soil moisture sensors for continuous monitoring at different depths over a period of three years Hydrochemical analysis, groundwater dating: ¹⁴ C and ³⁶ Cl isotopes	Tree types, height, diameter at breast height, carbon storage, water use (sap flow), continuous soil moisture, groundwater level, soil and plant water isotopes, stomatal conductance Terrestrial laser scanning, high-resolution satellite imagery (Sentinel), geophysics (ERT)

(continued on next page)

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Case study number	1. Water fluxes through agricultural soils	2. Water dynamics in wells and streams	3. Aquifer management for domestic supply	4. Urban green areas
Timeline of sampling and duration of the survey	One day of soil sampling, several years of precipitation sampling	Varying hydro-climatological conditions across a 20-month period	The deuterium enriched water was applied once and soil sampling was carried out every day for ten days and at the end of the rainy season. The overall research lasted three years	Sampling is done at different temporal scales: geophysical surveys were carried out at the beginning of the project; soil and tree water and water stable isotopes are measured with in-situ techniques every four days; stomatal conductance, leaf water potential and chlorophyll fluorescence are measured every two weeks The project began in 2022 and will last 3 years
Model applied	Hydrus-1D	Endmember mixing modeling	Hydrus-1D	
Data analysis	The isotope depth profiles were used to calibrate a soil physical model for the four different locations. The meteorological data and precipitation isotopes served as input data, while the soil hydraulic parameters describing the water flow and transport along the profile were determined via inverse modeling, by optimizing the fit of the isotope simulations to the observations. Then, the site-specific calibrated model was applied to trace the fate of water and nitrate over time and soil depth.	Endmember mixing analyses complemented by young water fraction analyses for different wells and surface water streams.	A set of potential and reasonable recharge pathways and scenarios were defined and a 2D horizontal steady-state numerical groundwater model (OpenGeoSys) was set up and calibrated.	Variations of the stable isotope of stem water are used to determine water uptake depths every four days.
Specific information provided by stable isotope analysis	Understanding how the seasonality of the isotope ratio in the precipitation was preserved over the depth of the soil profiles	Demonstration of large spatial and temporal variability in relative contributions of old and young water between different wells and streams.	Estimating groundwater recharge through the unsaturated zone and describing soil water movement	High temporal resolution of tree water uptake from different soil depths.
What is the response to stakeholders' questions	The combined sampling and modeling approach revealed that elevated nitrate concentrations at about 3 m depth beneath the young vineyard stem from nitrate mobilization during the vineyard installation 2.5 years earlier. Seeding grass in between the vineyard rows was found to be an effective way to reduce the nitrate leaching into the deep soil layers.	Understanding of catchment dynamics during dry and wet periods. Indication of which wells were most vulnerable, where source water was coming from and thus where to implement management strategies	The aquifer is an additional strategic resource for supply of drinking water. Modeling results and uncertainty analysis for the deep aquifer suggest groundwater recharge originating from the Angolan Highlands. The estimated recharge rates are less than 1% of mean yearly precipitation, which are reasonable for semi-arid regions. Diffusive recharge of the shallow aquifers via precipitation is highly variable, depending on land use and soil type. Highest recharge occurs in the non-vegetated Kalahari sand regions (10%–25% of mean annual precipitation). In the Kalahari woodlands, mean groundwater recharge is around 5% of mean annual precipitation.	This work has just begun therefore no answers have been developed yet. However, it aims at providing baseline information on the status quo of the contribution of urban green spaces to carbon sequestration, and at supporting the development of decision-support tools for maximizing the climate effectiveness of urban green.

2.2. Applications for water resources managers, land managers and policy makers in natural and semi-natural systems

Decisions related to afforestation and forest management also require a deep understanding of the partitioning of recharge, discharge, evaporation, and transpiration to prevent further water limitations,

especially in water-scarce regions (Hoek Van Dijke et al., 2022; Li et al., 2018). For example, smart thinning of forests can be based on tree water uptake depths to reduce competition in soil layers with limited resources. This targeted removal of specific trees, rather than the traditional practice based on the removal of a predetermined number of trees per unit of area, can bring economic benefit. Stable isotopes can

Table 5

Stakeholders' groups	Example	Rational
Water resources users	Farmers and their associations Nurseries Urban garden managers Technicians and consultants Industry Private water (from wells and springs) users	Climate projections foresee long drought periods and an intensification of individual rain events in many regions; in addition, the increasing global temperature will enhance evaporation and transpiration. The combination of these processes will likely decrease water storage in the soil and slow down groundwater recharge. The future of water availability for users depends on more efficient water use and on the introduction of land management techniques that can improve the capture and storage of water Farmers and gardeners are the users mostly and most directly affected by water distribution in the ecosystem. For example, irrigation can be an expensive practice that has to comply with local regulations. In addition, in many parts of the world, climate change is expected to cause changes in precipitation exents. Other users, may indirectly benefit and alter, with their activity, the availability of water. Industries using privately managed wells and springs are beginning to experience a lack of water supply.
Water resources managers	Water management agencies Irrigation facilities Hydroelectric dams' managers Risk managers Land reclamation authorities	The projected changes in water availability due to climate change and increased demand represent a serious threat for the stability of modern society. Water resource managers have a great responsibility to keep water available and safe for all.
Land managers and engineers	Foresters and environmental organizations Water pollution controlling agencies Urban managers Waste managers	Tools are needed to identify temporal and spatial changes in water flows in natural and anthropogenic areas, to evaluate their consequences for ecosystems and water storage facilities, and to plan potential remediation actions.
Policy makers	National and local government authorities and organizations Local departments for agriculture Farmers' insurance companies Farmers' banks	Climate changes, population growth, and land use changes are some of the main challenges of modern society. Public authorities are required to regulate and set thresholds to guarantee a continuous, non-interrupted provision of clean, safe water, but at the same time to preserve the environment, ecosystem functions and resources for future generations.

Description of the stakeholders who can directly or indirectly benefit from the knowledge of water fluxes in forested and agricultural ecosystems provided by a water-stable-isotope-based toolkit either in rural or urban settings.

also play a role in designing new large-scale afforestation to combat global CO_2 rise as requested by the most recent international policy to mitigate climate change effects.

2.2.1. Case study 2. Water dynamics in wells and streams

In many rural areas, industry and households depend on private water supply. In Scotland, numerous households as well as agricultural businesses and distilleries rely on water supply from springs, wells and streams (Table 4). In the past, these sources were mostly reliable, but recent years have experienced increasingly drier summers putting the distilling industry and households at risk (Fennell et al., 2020). Given that such conditions are expected to arise more frequently and more severely in the future (Visser-Quinn et al., 2021) questions were asked about the reliability of different water sources for the whisky industry, and how catchment abstractions could be balanced to meet requirements for both rural households and the environment. Analyses of the stable isotopes of water in precipitation, soil water, groundwater and stream water helped to provide insights into the spatial and temporal variation in water sources to contributing wells and streams (see Supplementary material for further information on the methodology and results). Wells with relatively large young water fractions were most vulnerable to changes in the climate as they were supported by water from relatively recent precipitation via shallow and quick flow paths. In this case, no other analytical technique could have provided this information. Understanding the recharge of different water sources not only helped to understand the potential vulnerability of these to dry conditions; it subsequently also contributed to the design of management strategies that could help to minimize negative effects on water supply (Fennell et al., 2023).

2.2.2. Case study 3. Aquifer management for domestic supply

In 2013, a vast, deep-seated (> 250 m), transboundary freshwatercontaining groundwater aquifer was discovered by the Namibian water authorities and the German Federal Institute of Geosciences (BGR) in the Ohangwena Region, northern Namibia (Lindenmaier et al., 2014). In addition to having potable water quality, the aquifer was under pressure, causing it to rise to 20 m below the ground surface, making pumping relatively cheap (Table 4).

Because Namibia is an extremely water-scarce country, its National News and TV rapidly disseminated the findings. The extent of the aquifer was not known and only rough estimates of the amount of water stored could be made at the time; local and international groundwater experts estimated the aquifer to be able to supply water for domestic use for the whole of Namibia (2.5 million people) for more than 200 years. However, this estimate strictly referred to domestic use; using the aquifer for large-scale irrigation would cause its depletion within 20 years.

Before opening the aquifer to use, and to properly plan its exploitation, the Namibian government commissioned research studies to determine the extent, age, and recharge pathways of this vast water resource. If the aquifer were purely fossil – that is, if the water was stored in the past and disconnected from the current hydrological cycle – and no current recharge pathways existed, then the aquifer should be harvested with extreme care. Alternatively, if the aquifer system were still connected to the hydrological cycle and recharge continued, then the water could represent an even more valuable resource because it would be sustainable.

In the framework of a large consortium project, numerous studies using approaches based on the stable isotope of water have shown that direct recharge to these deep-seated systems is highly unlikely (Beyer et al., 2015, 2016; Gaj et al., 2016). Age dating utilizing the complete portfolio of isotope methods (14 C, 3 H/ 3 He, 36 Cl, 39 Ar, and 81 Kr) and modeling of potential indirect recharge pathways indicated a groundwater age of 30,000 years. Wallner et al. (2017) parametrized the hydrogeological model OpenGeoSys and investigated ten potential recharge pathways, constraining the model with the information from

isotope-based age dating. The results indicated that 1% of the mean annual rainfall contributes to recharging the semi-fossil aquifer, with the indirect recharge source being the Angolan highland in the north, where large amounts of rainfall occur and infiltrate deep into the surface. In addition, the Department of Water Affairs of Namibia and the BGR have carried out explorative deep drilling since then to estimate the extent of the aquifer system, which led to the discovery of even deeper aquifers that extend into Angola, the bordering country (see the Supplementary Material for further information).

The finding that the aquifer is not purely of fossil origin and still receives recharge led the Namibian Water Authorities to open the freshwater aquifer for local drinking water supply, but they take care that total abstraction rates do not exceed the estimated recharge rates. As of 2023, more than 25,000 people in the Ohangwena region now have access to the water, and more townships are being connected. This is a great example of how isotope-based methods can be used hand in hand with stakeholders to develop sustainable strategies for managing vulnerable and precious water resources.

2.3. Applications for water resources managers and policy makers in agricultural and forested systems in urban settings

Water's stable isotope methods have rarely been used in urban settings. Bijoor et al. (2012) studied the sources of water for urban tree transpiration in the Los Angeles metropolitan area. They found that some drought-adapted trees utilize groundwater despite irrigation. However, several irrigated trees - even old ones - had very shallow root systems, which may make them more susceptible to climatic changes and a lack of irrigation. Landgraf et al. (2022) studied dynamic root water uptake depths of urban trees, and Smith et al. (2022) modeled these experiments. Considering the challenges many urban ecosystems are currently facing, the question "Where do city trees obtain their water?" will become increasingly important for city planners and municipalities. The recent prolonged European droughts between 2014 and 2018 have severely damaged city trees. Stable isotope methods might play an important role, for instance, in quantifying the amount of deep water that is used or the effectiveness of current urban tree planting strategies, which are often based on very limited volumes of artificially introduced organic substrate. Another important question in urban environments relates to the availability of water for human consumption. Stable isotopes have been used to explore the spatial and temporal patterns of drinking water availability within urban settings (Ehleringer et al., 2016; Kuhlemann et al., 2020). Scientists mostly used stable isotopes to determine the contribution of different sources to urban water supply. The main advantage of the use of stable isotopes is that they might clarify water sources that cannot be detected otherwise. For instance, a water's stable isotope analysis of the source contributions of groundwater that is used for urban water supply can reveal the most important recharge pathways. Using stable isotopes, it is further possible to estimate transit times and detect contamination from unexpected sources (Ehleringer et al., 2016; Nagode et al., 2021; Heiderscheidt et al., 2022). In such complex systems, stable isotopes are useful for detecting interactions between natural and engineered hydrological systems (Kuhlemann et al., 2020).

In addition to the dynamics of urban water supply, stable isotopes can also help to determine water losses and interactions among water bodies. For example, stable isotopes can quantify the effect of lawn watering (Fillo et al., 2021), the role of green areas in the water cycle in urban environments (Kuhlemann et al., 2020), and their contribution to groundwater recharge and streamflow (Zhang et al., 2020). Such applications can be particularly relevant considering the increasing interest in urban gardening and urban agriculture because stable isotopes can be used to determine the effect of these practices on water dynamics. These activities greatly affect water availability, so this information can be relevant to optimizing the ecosystem services provided by urban gardens and green areas and quantifying their costs and benefits, especially if they have to be irrigated. The application of stable isotopes may contribute to understanding water dynamics in these complex ecosystems and support managers' decisions and planning.

Another useful application of the stable isotopes of water is the analysis of water fluxes within and surrounding landfills and other types of contaminated areas. Water typically enters these systems mainly through precipitation (rain and melting snow) and can flow through the stored material. However, these systems are supposed to be completely encapsulated so that there is no leak from the contaminated site to the surrounding environment (1999/31/EC, 1999). Stable isotope monitoring can be carried out to determine the impact of landfill leachates on the surrounding water fluxes (Andrei et al., 2021; Preziosi et al., 2019). Chemical and biological processes occurring within a landfill alter the composition of the stable isotopes of water, making it distinct from the precipitation water feeding the surrounding streams and aquifers (Hackley et al., 1996).

2.3.1. Case study 4. Urban green areas

In attempting to develop knowledge and tools for climate change mitigation and adaptation, city authorities have involved stable isotope practitioners, among others, to understand the current water status of city trees (Table 4). The investigation involved measuring sap flow and stem water content, stomatal conductance, leaf water potential, chlorophyll fluorescence, and soil moisture at different depths. Terrestrial laser scanning was utilized to estimate the amount of biomass stored in the city trees. Satellite-based approaches were used to delineate, classify, and evaluate the stress status of urban vegetation. This methodological setup aims at better understanding water-carbon relationships of urban trees and identifying leverage points for improved management and adaptation to climatic changes in the future.

Stable isotopes of water were monitored using stem boreholes in tree stems and semi-permeable membranes in soils (Marshall et al., 2020; Kühnhammer et al., 2022, 2023). These minimally invasive in situ methods (Beyer et al., 2020) allow continuous identification of tree water uptake depths. Combined with the soil water content measurements, they offer a temporally resolved image of where city trees obtain their water from and why they obtain water from certain depths. This information is crucial to guide adaptation measures for the future. For example, the preliminary evaluations of the joint dataset for 2023 suggest that urban trees growing in paved surroundings do not take up water from layers deeper than one meter. This could be due to the low water availability and the shallow rooting. Low water availability might be a consequence of the high hydraulic conductivity of the materials used beneath roads, which are optimized to drain, rather than to provide trees with water; shallow rooting might be a consequence of surface irrigation both in nurseries and after planting, which eliminates the need for trees to root deeply.

Valuable lessons that are already transferred to urban tree planning include a new design of planting pits allowing wider volume for root development with a thick organic substrate on top of the drainage layer; more efficient irrigation management from below, rather than from above, to enhance deep rooting; and improved "tree-friendly" street planning with open space/unsealed surfaces to increase the infiltration of water. The quantitative evidence provided by the collected water uptake depths and ecohydrological information for urban settings has been greatly appreciated by the city authorities, landscape planners, and the local population. In particular, the water isotope data delivers crucial information that stakeholders usually do not have and can be an important lever for convincing decision-makers to act. The supplementary material provides further information on this case study.

3. Including stable isotope data into models to increase the ability to represent hydrological processes

The inclusion of stable isotope data in developing and calibrating hydrological models of catchments, soils, and ecosystems has been shown to increase model fidelity (i.e., the ability to represent the dominant processes; Stadnyk and Holmes, 2020). Water resources management in forested and agricultural ecosystems relies on model predictions of hydrological effects of both climate anomalies (i.e., warmer temperatures, more frequent drought and flooding) as well as potential management interventions. However, only when the ecohydrological processes are well represented in a model, simulations of a non-stationary future (e.g., Milly et al., 2008) due to climate change or management will be meaningful. Tracer-aided modeling that includes information on stable isotope composition is an effective way to benchmark model performances. Such isotope-enabled modeling approaches further allow inferring, in addition to soil water volumes (e.g., changes of soil moisture and fluxes), the transport of water and thus, its travel times (Sprenger et al., 2016b). In addition, combining isotope data from soil profiles with xylem water isotope data in soil-hydraulic modeling (Brinkmann et al., 2018) or mixing models (Rothfuss and Javaux, 2017) can track the fate of irrigation or precipitation about plant water uptake for transpiration. These ecohydrological processes can also be modeled on the catchment scale using - in addition to measured volumetric water fluxes - isotope data from stream water and optionally also isotope data from groundwater, soil water, and xvlem water for the calibration (Kuppel et al., 2018; Knighton et al., 2020). Such models can be powerful tools in water resources management in forested (Seeger and Weiler, 2021) and agricultural ecosystems to derive ecohydrological feedback, e.g. under a prolonged drought (Kleine et al., 2021) or derive vegetation traits (Li et al., 2023).

One example of an isotope-enabled modeling study investigating soil water and nitrogen transport was introduced in Section 2.1.1. However, there have been several efforts to include isotope information to parameterize soil physical models to infer water flow characteristics (e.g., Sprenger et al., 2016b; Groh et al., 2018) and new modeling developments enable consideration of isotope fractionation and particle tracking in isotope-enabled soil physical modeling (Zhou et al., 2021). Examples of isotope-enabled catchment scale modeling showed the benefits of considering stable isotopes to assess the spatial variability of "green water" due to land use differences in agriculturally dominated landscapes (Smith et al., 2020, 2021). Adomako et al. (2010), for instance, used soil water isotope measurements in order to parameterize the isotope-enabled numerical soil-plant-atmosphere continuum model Hydrus-1D in order to estimate groundwater recharge in Ghana with clear underlying practical questions such as: How do current water abstractions compare to recharge? How will this change in the future? Post et al. (2022) used the same model to estimate recharge rates on Langeoog Island, Germany. Here, the amount of recharge water under land use change on the dune landscape was of interest.

4. Benefits and limitations of water stable isotope analysis and its application for stakeholders

4.1. Benefits for stakeholders

Stakeholders are unlikely to invest time and money into an analytical technique without a clear idea of the benefits that this technique can bring them (Aarts et al., 2014). Compared to other available techniques, the main strength of using stable isotopes is that, being conservative tracers (Table 1), they can offer a different way of looking at the water cycle (McDonnell, 2017). The analysis of stable isotopes does not substitute for hydrometric techniques (e.g., measurements of stream discharge, groundwater levels, soil moisture, sap flow) but provides additional insights and information (e.g., which water sources contribute to streamflow; which time of the year contributes most to groundwater recharge; which sources contribute to sap flow; travel times of water). Stable isotopes in the water molecule provide information regarding the origins and fates of water in the environment and can pinpoint the most vulnerable aspects of the system and the critical factors threatening future sustainability, especially under changing climatic conditions. In addition, they have no toxicity and are useful for partitioning pools and fluxes even at low background levels; procedures for sampling and storage are relatively straightforward (no samples preservation is needed, just dark and cool place for storage, can be stored for months, if the samples properly sealed), and relatively small volume of sample is required (allowing for low costs for transportation and storage). Hence, analyzing stable isotope ratios as a complement to other techniques can help to inform trade-offs between different water management alternatives. Thus, isotopes represent an important addition for decision-making processes, particularly when multiple processes and compartments (e.g., soils and plants) interact.

4.2. Cost and limitations

Analyzing stable isotopes in water samples is still an evolving technique both in terms of sampling and analytical protocols (Ceperley et al., 2024). In particular, new techniques are being developed to minimize the manipulation of the samples, increase the throughput, and thus decrease the cost of each analysis. In addition, the interpretation of the results is not always straightforward and may require additional information from further sampling or analyses of other parameters in addition to stable isotopes. Despite these drawbacks, costs have been dropping for a decade and stable isotope analysis is now within the reach of many stakeholders. However, when considering whether to use stable isotopes of water, some critical aspects that may limit their application must be considered.

The first limitation is that the water pools under investigation need to have different isotopic values when the purpose is to identify how they mix. For example, rainfall and snowmelt typically have very different isotope signatures, and this allows for the calculation of how much each of these sources contributes to river dynamics (Penna et al., 2016; Kirchner and Allen, 2020). When water bodies have similar isotope signatures, stable isotope techniques cannot provide partitioning information. In such cases, other stable isotopes (¹⁵N and ¹⁸O in nitrate, ¹¹B, ¹³C and others) may provide support to the interpretation of data, but with a substantial cost increase. In many cases, a water's stable isotope practitioner can estimate the likely magnitude of isotope signals, but it is often necessary to obtain preliminary data.

The second limitation is that results are often site-specific. As with any study of environmental processes, transferring results from one site to another, e.g., with different climates, geomorphic settings, or soil, can be challenging. Despite the great effort of many researchers, there is still a lack of data and models that provide the quick answers that decision-makers often need, and data limitations still hamper the development of high-resolution models that make predictions at the fine scales that are often of interest to stakeholders. The ongoing development of datasets and models is important to add robustness to the field application of the stable isotope technique to solve stakeholders' issues (Table 5). For example, one of the objectives of the European network WATer isotopeS in the critical zONe: from groundwater recharge to plant transpiration, WATSON (https://watson-cost.eu/) is to provide a database with stable isotope data collected throughout Europe. This database will make it easier to find existing isotope datasets and collaborators.

A further limitation is that stable isotope analysis comes with uncertainties, as do all other techniques. In stable isotope analysis, the signal-to-noise ratio is in many cases sufficiently good to derive general process understanding. The stable isotope data uncertainty is often several orders of magnitude smaller than the uncertainty associated with other techniques and the application of stable isotope data usually reduces the overall uncertainty related to the process under evaluation.

Despite these limitations, the stable isotope technique, although still largely unexplored in practical applications beyond research studies, is very useful for tracing water fluxes in environmental analyses. It can, and should, be part of the methodological approaches adopted by stakeholders. The ubiquitous presence of water in terrestrial ecosystems,

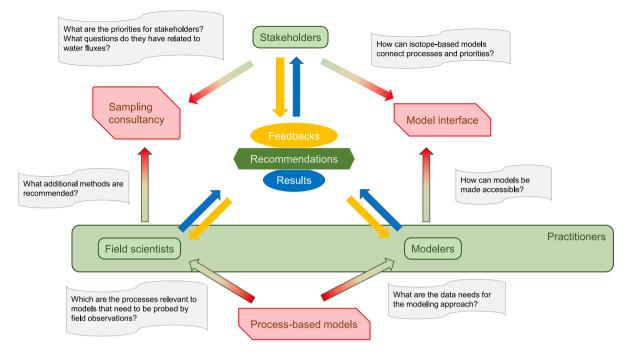


Fig. 2. The flow of information between stakeholders, as defined in Table 5, and service providers (scientists and technicians who have experience in field work, laboratory analysis, and/or modeling) has to be multi-directional and continuous. The players (light green) are interconnected by dedicated tools (red) that can answer specific questions (gray). Pivotal for the success of this process is the transfer of the knowledge derived from the water stable isotope analysis from the service providers to the stakeholders (blue) and the feedback from the stakeholders to the service providers (yellow). This transfer of knowledge is instrumental to develop recommendations (green) to respond to stakeholders' priorities and to improve the toolkit. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and therefore of the isotopes of hydrogen and oxygen in its molecule, allows professionals and managers to apply the isotope technique in different climatic and environmental contexts to address practical needs and requests.

5. Towards a water stable-isotope-based toolkit: a three-directional integration

While examples of successful interactions between water's stable isotope practitioners and wider stakeholders already exist (see e.g. the Case Studies summarized in Table 4 and detailed in the Supplementary Materials), a formal or informal stakeholders' 'toolkit' for using the stable isotopes of water could make an important contribution to increasing the accessibility and number of applications. The details and development of such a toolkit would require involvement and mutual exchange of information between stakeholders, field scientists, and modeling scientists. Here, we outline a first template and simplified design of such a toolkit, as represented by the flowchart in Fig. 1. We propose that the main aim of the toolkit would be to facilitate and guide the interaction between stakeholders and practitioners. Specifically, this interaction should (i) find a balance between responding to the practical needs of stakeholders, (ii) require a minimum burden of fieldwork and therefore limited costs, (iii) take advantage of robust modeling methods for data interpretation and upscaling to various temporal and spatial scenarios, but (iv) also provide data and interpretations available to the public (i.e., open access policy). Ideally, the toolkit would provide interactive help to stakeholders, e.g. to search for a case study, a theoretical example, or a keyword, that relates to their needs and thereby help them to determine whether and how they could benefit from water's stable isotope analysis.

The toolkit could take the form of a web-based application, a decision tree, or a software module to be integrated into an existing decision support system. To be fully operational, a stable isotope toolkit should be integrated into a user-friendly decision support system (DSS). The most proficient way would probably be to integrate water's stable isotope routines into existing DSSs which already deal with water-related decision making. Adding information from stable isotopes would then help to strengthen the decision-making process and provide further background for better-informed decisions.

In practice, the development of such a toolkit requires a process of information exchange between groups of practitioners and stakeholders, based on trust, communication, and solid science (Aarts et al., 2014). In this context, the stakeholders are the people who can benefit from the results of water's stable isotope analysis for a wide range of different purposes, as outlined in Table 5. As such, the development of an interactive water's stable-isotope toolkit requires a three-directional integration of needs, knowledge, and expertise (Fig. 2). In summary, stakeholders highlight priorities, field practitioners identify the relevant ecosystem processes, and modelers predict whether useful isotopic signals are likely to occur and then incorporate new data into their algorithms. In addition, field practitioners can provide the data needed to validate the model, while modelers can guide field scientists in identifying strategic sampling campaigns (Fig. 2). Together, the practitioners communicate the results to stakeholders and receive feedback enabling improvements in the toolkit. Integration of field and lab-based scientists with those developing and using modeling approaches is equally important as communication between practitioners and stakeholders. It is expected that the whole process as outlined in Fig. 2 requires multiple iterations.

The key challenges that might arise in developing a toolkit stem from the three groups involved (stakeholders, field scientists, modelers) typically having different goals and expectations, and typically using different jargon. Efforts should be made on all sides to fruitfully communicate respective needs with realistic expectations on what can be offered. An example of how this aim can be achieved is given by the European network WATSON (https://watson-cost.eu/), which connects scientists, technicians, and stakeholders from many different institutions in the European Union and neighboring countries. This network focuses on summarizing the recent research on stable isotopes in water and developing protocols and methods that could be offered as possible solutions to pressing challenges related to water use, water availability, and water management.

6. Conclusions

In this paper, we reported the strengths, weaknesses, opportunities, and challenges of applying the analysis of stable isotopes of water as a support to decision-making in water resource management. The stable isotopes of water have proven to be informative markers for water flow and mixing processes in environmental systems.

Tools based on the analysis of stable isotopes of water can provide useful information in a wide range of environments including agricultural, natural, semi-natural, and urban settings, as exemplified by the case studies. This work specifically shows that

- isotope-enabled soil physical modeling facilitates the creation of beneficial management practices in an agricultural setting to reduce nitrate leaching to groundwater;
- endmember mixing modeling based on stable isotopes provides insights into the spatial and temporal variation in water sources contribution to wells and streams, highlighting the potential vulnerability to dry conditions and helping to mitigate the risks to water supply;
- isotope techniques allow to monitor water infiltration through the unsaturated zone and quantify the direct recharge of a deepseated aquifer by precipitation percolating through the soil;
- new minimally-invasive in situ approaches allow identifying water uptake depths of the trees at high temporal resolution, thus improving the knowledge base for management of urban vegetation.

Based on our experience in stable isotope research and applications, we propose a first template for a stakeholders' 'toolkit' for using the stable isotopes of water. This could make an important contribution to increasing the accessibility and number of applications. The further development of this toolkit will require mutual exchange of information and needs between stakeholders, field and modeling scientists.

CRediT authorship contribution statement

Francesca Scandellari: Writing - review & editing, Writing - original draft, Supervision, Conceptualization. Taha Attou: Writing - review & editing, Conceptualization. Adrià Barbeta: Writing - review & editing, Conceptualization. Fabian Bernhard: Writing - review & editing, Conceptualization. Concetta D'Amato: Writing - review & editing, Conceptualization. Katya Dimitrova-Petrova: Writing - review & editing, Visualization, Conceptualization. Amanda Donaldson: Writing review & editing, Conceptualization. Oludare Durodola: Writing - review & editing, Conceptualization. Stefano Ferraris: Writing - review & editing, Conceptualization. Marius G. Floriancic: Writing - review & editing, Conceptualization. Gabriela Fontenla-Razzetto: Writing - review & editing, Conceptualization. Malkin Gerchow: Writing review & editing, Conceptualization. Qiong Han: Writing - review & editing, Conceptualization. Isis Khalil: Writing - review & editing, Conceptualization. James W. Kirchner: Writing - review & editing, Conceptualization. Kathrin Kühnhammer: Writing - review & editing, Conceptualization. Qin Liu: Writing - review & editing, Conceptualization. Pilar Llorens: Writing - review & editing, Conceptualization. Ruth-Kristina Magh: Writing – review & editing, Conceptualization. John Marshall: Writing - review & editing, Conceptualization. Katrin Meusburger: Writing - review & editing, Conceptualization. Aline Meyer Oliveira: Writing - review & editing, Conceptualization. Lyssette Muñoz-Villers: Writing - review & editing, Conceptualization. Sabrina Santos Pires: Writing - review & editing, Conceptualization. Diego Todini-Zicavo: Writing - review & editing, Conceptualization. Ilja van Meerveld: Writing - review & editing, Conceptualization. Claudia Voigt: Writing - review & editing, Conceptualization. Luise Wirsig: Writing - review & editing, Conceptualization. Matthias Beyer: Writing - review & editing, Writing - original draft, Supervision, Funding acquisition, Conceptualization. Josie Geris: Writing - review

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data used in this article are those reported in the Case studies. No further data have been used to prepare this work.

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Appendix A. Supplementary data

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