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Water sources for root water uptake: using stable isotopes of hydrogen and oxygen as a research tool in agricultural and agroforestry systems

Daniele Penna[1], Josie Geris[2*], Luisa Hopp[3], Francesca Scandellari[4]

[1]Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence, Italy
[2]School of Geosciences, University of Aberdeen, Aberdeen, United Kingdom
[3]Department of Hydrology, University of Bayreuth, Bayreuth, Germany
[4]Isotracers S.R.L., Bologna, Italy

*Correspondence to: St Mary’s Building, University of Aberdeen, Elphinstone Road, Aberdeen, AB24 3UF, UK. email: j.geris@abdn.ac.uk

Abstract

Understanding water sources for crop water uptake in agricultural and agroforestry systems is an essential step to develop more efficient and sustainable water management strategies, which is increasingly important in the light of current world population growth, changing climatic conditions and consequent growing pressures on agricultural- and agroforestry production. Stable isotopes of hydrogen and oxygen in the water molecule are powerful and, nowadays, affordable tracers that can help to define the proportion of water sources accessed by plants. Yet, contrary to natural environments, their application is still relatively limited in agricultural and agroforestry research. In this work, we synthesize the advantages and the current knowledge deriving from the use of the stable isotopes of hydrogen and oxygen, in support of more traditional techniques, to understand root water uptake dynamics in agricultural and agroforestry systems. We also underline the practical implications related to the application of this technique for management purposes, and provide a vision for new challenges and future research opportunities in exploring crop and plant water use based on isotopic data.

Keywords: stable water isotopes, root water uptake, water sources, water resources management, irrigation, agriculture and agroforestry systems

1. Introduction

Water is considered as the most critical resource for sustainable agricultural development worldwide. Today, 70 % of total water withdrawals from surface water and groundwater resources are used in the agricultural sector for irrigation (World Bank, 2017). Around 20 % of the total agricultural land worldwide is irrigated, with the majority of existing irrigation systems still having low efficiency (FAO, 2019). As such, there is an urgent need for the management of water resources in agricultural and agroforestry systems to deliver freshwater more efficiently to plants. The global population is projected to grow to almost 10 billion by 2050 (UN, 2019). Worldwide there is an enormous challenge to double food production between today and the middle of the century (World Resources Institute, 2019). This will, in all likelihood, have to be achieved with less water, as a growing population and increasing urbanisation and industrialisation, especially in developing countries, compete for water (WWAP, 2019). These pressures on global freshwater resources are exacerbated by the effects of climate change. While water availability is becoming more challenging, irrigation is regarded as one of the main means to increase crop yields. Therefore, it is imperative to improve water governance and irrigation systems and, just as importantly, to expand the knowledge about demand and uptake patterns of crops so that irrigation can either be avoided or can be applied more efficiently, allowing a sustainable intensification of agriculture.

Key water sources for crops and agroforestry plants include soil moisture, groundwater and, where needed, water applied by irrigation. Crop water requirement has been studied extensively in the past decades (e.g., Shalhevet and Bielorai, 1978; Beyazgül et al., 2000; Kahlown et al., 2005; Senay and Verdin, 2014; please also refer to the review by Pereira and Alves, 2013). Whereas total crop water requirement may be known for individual plants, the respective proportions of the different water sources that plants use over the growing period are known only for relatively few species in agricultural and agroforestry systems (see examples and references in Section 2). Practical questions on root water uptake arise here in the context of water resources management: Which sources do crops use? How much water from each source do they take up? And how do water proportions change over the growing season, as a function of developmental stage, weather or environmental conditions? And also, how much of any irrigation water is actually being taken up by crops? These might look like trivial questions for some, as plants might eventually take up any water that is accessible, no matter what the source is. However, answering these questions would greatly help to predict adaptability to changes in crops or water availability, or improve and adjust the timing and amount of irrigation water being applied, and thus conserve freshwater resources and lower financial expenses.

With this opinion paper, we aim to demonstrate that the use of stable isotopes of water ($^{2}H/^{1}H$, $^{18}O/^{18}O$) can be a very effective tool in agriculture and agroforestry systems to better understand the temporal and spatial patterns of root water uptake (RWU) and, thus, to tackle water management-related questions as stated above. We synthesize the current status of the application of stable isotopes in agricultural and agroforestry research and provide a perspective on opportunities and challenges for isotope-related research.

2. Isotopes in agricultural and agroforestry systems

Plants predominantly absorb water from the soil through their root system (Fig. 1). Water held by the soil is mostly derived from precipitation and, in many agroecosystems, irrigation, but also infiltration from nearby surface water and the capillary rise of groundwater. Moreover, water contained in deeper soil layers can be absorbed and redistributed to shallower soil by deep roots. The various water sources may have different isotopic composition according to their past history (Sprenger et al., 2019) which makes isotopes excellent tracers to investigate root water uptake (Dawson et al., 2002; Barbour, 2007). In addition to the robust and well tested traditional isotope mass spectrometer, recent technical advances have led to the commercialization of new, relatively cheap and transportable instruments, which can measure the stable isotope composition of different waters, including transpired and leaf water (Cernusak et al., 2016). As a result, analyzing stable isotopes in water has become quick, relatively easy, and inexpensive, while results are reproducible and highly informative. For these reasons, analyzing stable isotopes has become the method of choice for many environmental researchers, such as, e.g., hydrologists, forest scientists, and ecophysiologists; yet, in comparison to more natural environments, this technique is still relatively sparsely applied in agricultural and agroforestry research to address RWU.

The rationale is that the water in tree vessels is derived, assuming without fractionation (Dawson et al, 2002), from roots after uptake and that the contributing water sources completely mix inside the plant. This implies that it should be possible to estimate RWU by comparing the isotope values of the potential water sources with that of the water extracted from the plant xylem (Fig. 1). Several methods, recently summarized by Rothfuss and Javaux (2017) and Wang et al. (2019), have been developed to this aim. The simplest method consists of estimating the mean RWU depth by directly comparing the isotope value of xylem with the isotope value of water extracted from different soil depths. It is also possible to estimate the proportion of potential water sources to xylem, e.g., by applying end-member mixing analysis (EMMA, Christophersen and Hooper, 1992). One limiting factor to successfully applying an EMMA is that the difference in isotope composition of potential water sources must be considerably larger than the precision of the isotopic measurements. When there are only two potential water sources, their relative contribution can be estimated by applying a linear model based on classical statistics. For more complex situations with several potential water sources, models based on Bayesian statistics have been developed (Parnell et al., 2013).

Stable isotopes of oxygen and hydrogen in the water molecule have been used in managed ecosystems for different purposes. Typical applications include the identification of flow pathways and the quantification of runoff components in agricultural catchments (Merot et al., 1995; Tekleab et al., 2014; Tweed et al., 2016; Zajíček et al., 2016), the assessment of the seasonal role of precipitation and irrigation water in recharging groundwater (Kaown et al., 2009; Brown et al., 2011), the computation of residence time (Tekleab et al., 2014), the partitioning of evapotranspiration fluxes in soil evaporation and plant transpiration components (Yepez et al., 2003; Williams et al., 2004; Wen et al., 2016; Lu et al., 2017; Wu et al., 2017), the determination and characterization of contaminant sources to rivers (Briand et al., 2017), the movement of fertilizers from soil to streams and groundwater (Parra Suarez et al., 2019), and to a limited extent, the determination of RWU from different water sources.

2.1. Water sources for plant water uptake

A large variability of RWU patterns and water sources can exist, even within a single species. This variability is typically a function of growing stage, seasonality, soil moisture, phenological stage, tree size, transpiration rate, and irrigation and fertilization regimes (Schwendenmann et al., 2015; Ma and Song, 2016; Bargués Tobella et al., 2017; Hardanto et al., 2017; Liu et al., 2019). Specifically, most of the research so far has focused on identifying the depth from which soil water is absorbed by plants and its variability during the growing periods. For example, maize (*Zea mays*), across different countries and various climatic and agronomic conditions, was typically found to access shallow soil water, mainly in the first 20 cm of the soil and particularly during the seedling and jointing stage (Asbjornsen et al., 2007 and 2008; Wang et al., 2010; Ma and Song, 2016; Zhao et al., 2016; Liao et al., 2018). However, the primary soil water sources for maize normally vary with the growth stage, as more water from deeper layers is used as the plants grow, and soil depths of 50 cm (Wang et al., 2010;

Ma and Song, 2016), 60 cm (Peng et al., 2017; Liu et al., 2018;), 80 cm (Zhang et al., 2011b), and, as recently found, even 120 cm (Zhao et al., 2018a; Zhang et al., 2019) are reached.

Variability in RWU patterns as a function of the growing stage has also been reported for other crops, both herbaceous and trees. For instance, the primary soil depths of RWU for winter wheat (*Triticum aestivum*) were found to be between 0 and 50 cm for the majority of the growing period (Zhang et al., 2011a; Wang et al., 2016; Yang et al., 2018), and especially in the 0-20 cm soil layers during the wintering and seedling phases (Zhao et al., 2018a), and increasing depths were observed during the end of the growing period (Cheng and Liu, 2017; Ma and Song, 2018) and up to 220 cm in the filling stage (Guo et al., 2016). Similar trends were found for cotton (Li et al., 2017). Among trees, walnut tree (*Juglans regia*) was reported to source water mainly in the 0-20 cm soil layer at the sprouting and leaf expansion stages, and the 20-40 cm soil layer at blossoming and fruit-bearing, fruit expansion, and fruit maturity stages (Liu et al., 2019), whereas cherry trees (*Prunus avium*) was found to take up soil water from 20-50 cm at the fruit growth stage, then mainly 0-20 cm and 50-100 cm at the end of the of growth period (Cao et al., 2018).

Switches between different soil water sources, especially for trees, can also occur as a function of seasonality or different meteorological forcing, mostly in terms of dry vs. wet periods and high vs. low soil moisture. Indeed, isotope-based observations in agro- and agroforestry ecosystems showed that most trees are characterized by a plastic behaviour in water uptake depths, extracting water from both shallow and deep soil layers but with a general tendency to access water in the first 50 or 60 cm of the soil during wet conditions and switching to deeper soil water sources during dry periods when the water content of shallow soil layers is low. This pattern was observed for walnut trees (Sun et al., 2011), jujube trees (*Ziziphus jujube*) (Gao et al., 2018), rubber trees (*Hevea brasiliensis*) (Liu et al., 2014; Wu et al., 2016; Hardanto et al., 2017; Wu et al., 2017), and indigenous West-African trees (Smith et al., 1998; Bargués Tobella et al., 2017).

Variability in soil RWU depths and dynamics was also observed for crops of different age and, especially for trees, variable size. Isotope studies reported different soil depths of RWU for 5-, 10-, 15-, and 22-year-old apple trees (*Malus sp.*) in China, with soil depths contributions varying between 10 and 300 cm, and 22-year old apple trees mainly using water from the whole soil profile down to 160 cm during all growing stages (Wang et al., 2018; Zheng et al., 2018; 2019). Similarly, another study carried out in China revealed that 4-year-old jujube trees mainly used soil water from 0-20 cm and 20-60 cm layers, 8-year-old trees shifted flexibly their water sources between 0-20 cm, 20-60 cm, and 60-200 cm layers, whereas 15- and 22-year-old trees often ignored moisture in shallow layers and primarily used water from the deeper soils, especially when precipitation was low (Huo et al., 2018). As for tree size, a clear relationship between stem diameter and soil water uptake depth was found for rubber trees in cultivations in Sumatra, with bigger trees that tended to take up soil water closer to the soil surface (Hardanto et al., 2017), although contrasting findings were obtained for native trees (*Vitellaria paradoxa*) in West-Africa where the switch to deeper water sources was especially pronounced for younger, smaller trees (Bargués Tobella et al., 2017).

Other factors leading to different soil sources for RWU are related to phenological stage and transpiration rate, as showed by a study conducted in Panama that revealed a higher proportion of deep water uptake being associated with a higher percentage foliage cover in the dry season, higher sap flux densities, and water use rates (Schwendenmann et al., 2015). In case of rice, different RWU patterns are typically related to the field management, e.g., continuous flooding versus alternate flooding and drying (Shen et al., 2015; Mahindawansha et al., 2018).

Although precipitation-fed soil water is by far the primary source for plant transpiration, there are cases in agricultural and agroforestry systems where isotopes helped to identify and quantify other water sources, such as groundwater and irrigation. Groundwater is typically directly accessed only by trees (Smith et al., 1997) and in some cases represents the dominant water source (e.g., the Australian *Casuarina glauca*, Cramer et al., 1999). Most often, trees use soil water during the wet periods and switch to groundwater only during the dry season (e.g., tagasaste tree, *Chamaecytisus proliferus*, Lefroy et al., 2001, and the already mentioned *Vitellaria paradoxa*, Bargués Tobella et al., 2017). Irrigation was identified and quantified using stable isotopes as water source for plant transpiration in two cherry tree orchards in China with contributions declining below 11 % from the postharvest stage in one case (Chao et al., 2018) and, conversely, being the highest (up to 22 %) during the drought period in spring in another case (Li et al., 2019).

2.2. RWU under different irrigation and fertilization conditions

Irrigation and fertilization can largely affect RWU by cultivated plants, leading crops to access distinct proportions of water sources or different proportions of water from various soil depths, especially during dry periods (Goebel et al., 2015; Du et al., 2018). For instance, for maize, different depths of RWU were observed under the application of distinct irrigation treatments, such as furrow irrigation, border irrigation, and alternate furrow irrigation (Wu et al 2018a). In the study by Ma and Song (2016) maize was fertilized with different nitrogen application rates from zero to 368 kg N ha^{-1}, resulting in clear differences in the proportional contribution of soil water at different depths. Similarly, fertilization with 105 kg N ha^{-1} or irrigation of 20 mm during the greening-jointing stage significantly promoted soil water uptake of winter wheat at 70-150 cm and 150-200 cm whereas other treatments led to the much shallower RWU depths of 0-20 cm (Ma and Song, 2018).

Isotope-based analysis of distinct RWU patterns under different irrigation regimes and fertilization treatments can thus be useful to provide insight and develop recommendations for fertilization and irrigation management in agricultural and agroforestry systems. Observations of the seasonal and growth stage variability of soil water sources used by maize and winter wheat in a rotation system in Northern China suggested that irrigation events should be applied at a specific stage of the winter wheat growth (Zhao et al., 2018a). Similarly, considerations of the RWU patterns of cherry trees in an irrigated Chinese field led to the identification of the optimal water regulation treatment, resulting in different irrigation quota at the end of the fruit growth stage, at the postharvest stage and during the final part of the growth period (Cao et al., 2018). Later isotope work in the same area showed that the drip irrigation mode demonstrated higher irrigation water use efficiency for cherry trees compared to the surface irrigation mode (Li et al., 2019). In this context, isotopes can help to clarify the relationship between root growth and irrigation method, and give practical, scientific-based indications to apply ad-hoc irrigation strategies (e.g, alternate furrow irrigation, deficit irrigation) or irrigation methods (e.g., pit irrigation systems) that contribute to plant water use efficiency and water saving mechanisms (Wang et al., 2010; Wu et al., 2018a,b; Zheng et al., 2019).

2.3. Effects of co-cultivated or coexisting plants

Stable isotopes of hydrogen and oxygen are also useful tools to assess the possible competitive or complementary RWU patterns of co-cultivated or coexisting plants. Evaluating the water use strategies of co-cultivated or coexisting crops or trees is indeed useful to plan water management interventions and agricultural practices. Various studies reported isotope-based evidence of similar (and therefore competitive) or alternative (and therefore complementary) water use strategies in different cultivates species. For example, complementary use of soil water resources through vertical partitioning of water uptake was found in an agroforest stand with cocoa trees (*Theobroma cacao*) and gliricidia trees (*Gliricidia sepium*) in Sulawesi, Indonesia (Schwendenmann et al., 2010). This behaviour, in combination with acclimation, may help cocoa trees to cope with drought conditions. Walnut tree (*Juglans regia* and *Juglans nigra*) was also reported to extract water from different soil layers compared to other species: from deeper soils compared to the Italian alder (*Alnus cordata*) in a mixed plantation in central Italy (Lauteri et al., 2006) but from shallower soils compared to a hybrid poplar (*Populus deltoids* X *Populus nigra* clone) in an agroforestry system in Ontario, Canada (Link et al., 2015). Poplar was also shown to be plastic in the depth of RWU and to have complementary water use strategies compared to maize in an agroecosystem in Northern China (Liu et al., 2018). Other complementary relations were found for the jujube tree with respect to annual and perennial crops (Gao et al., 2018), for native trees in mixed species plots in Panama (Schwendenmann et al., 2015), and for the rubber tree with tea, coffee and cocoa plants in Chinese agroforestry systems (Wu et al., 2016; 2017). However, rubber tree was reported to have competitive displacement of RWU in a plantation in Sumatra (Hartanto et al., 2017), indicating that water use strategies are not only species-specific and might be also driven by local conditions. Furthermore, pronounced competitive RWU patterns were observed in acacias (*Acacia nilotica* and *Acacia holosericea*) as they extracted large quantities of water through lateral roots, and the competition was particularly severe at locations where trees could not access groundwater (Smith et al., 1998). Competition was found for herbaceous crops as well: an example from Northern Namibia in a pearl millet (*Pennisetum glaucum*) plantation intercropped with cowpea (*Vigna unguiculata*) showed that the greater ability of cowpea to acquire existing soil water forced pearl millet to develop deeper roots and shift to recently supplied water (Zegada-Lizarazu et al., 2006). Other studies have reported low degrees of competition for water use between herbaceous species and shrub and tree species due to the plasticity in RWU depths of the latter (Asbjornsen et al., 2008). Some studies even reported contrasting findings, such as an alteration of fine root distribution patterns of cocoa trees due to interspecific competition but, at the same time, a not-significant limitation in their productivity (Rajab et al., 2018).

3. Opportunities and challenges

Despite the potential of using water isotopes for RWU analysis, some remaining methodological questions still need to be considered. For example, this includes the validity of the assumption that no fractionation occurs either during root water uptake (Barbeta et al., 2019; Poca et al., 2019) or elsewhere in the subsurface, due, for example, to the interactions with soil clay content (Gaj et al., 2017a; Meißner et al., 2014), to the effect of root exudates in the rhizosphere (Schwartz et al., 2015), or to the activity of arbuscular mycorrhizal fungi (Coomans de Brachène, 2018). Furthermore, much recent water isotope-based work has shown that there is an apparent distinction between ‘tightly-bound’ and more mobile soil water (e.g. Oerter et al., 2019a; Sprenger et al., 2016), and that plants might favour one over the other (McDonnell, 2014) or that differences reflect variations in spatio-temporal patterns of soil water processes (Dubbert et al., 2019; Oerter et al., 2019b). This implies that it is important to sample potential soil source water that is held across the variability of soil water tensions and at multiple times. There are further challenges associated with the need for complete extraction to avoid artificial effects of fractionation when soil and xylem water are extracted in the laboratory for isotope analyses (Gaj et al., 2017b; Orlowski et al., 2018).

Another aspect that requires consideration is that isotope signatures in soil and xylem water may be the product of any combination of source variation, mixing, and fractionation effects so that isotopic values may not be exclusive to specific origins and processes (Benettin et al., 2018; Penna et al., 2018). Such non-uniqueness can be largely addressed, for example via the use of Bayesian mixing models that explore the uncertainties in mixing of various plant water sources (Rothfuss and Javaux, 2017; Wang et al., 2019). Limitations can be further overcome by also considering nutrient availability to further understand plant water and nutrient resource uptake and how this varies in time (Dubbert et al., 2019). In this context, a critical point is the quantification of the uncertainty in the estimates of sources for RWU that is missing from most of the studies so far conducted in agro- and agroforestry ecosystems but that is of high importance in order to provide more realistic scenarios of RUW dynamics and more reliable implementation of irrigation and fertilization treatments.

The key contribution that the analyses of stable isotopes of hydrogen and oxygen in agricultural and agroforestry systems could make is to provide insights into the origin and the proportional water use of different sources. Hence, the use of stable water isotopes to address questions on RWU is complementary to and cannot fully replace the more traditional techniques that explore quantitative water fluxes, nor can they fully complete our ecophysiological understanding (including, for example, water use efficiency and other aspects of plant production). For a more complete picture of water-plant-environment interactions and utilization, a combination with other available tools may be employed. These include other stable isotopes, particularly ^{13}C for understanding utilisation of water resources (e.g. Cernusak et al., 2013; Rumman et al., 2018) and ^{15}N for nutrient uptake (e.g. Kulmatiski et al., 2017). Nevertheless, addressing RWU questions with knowledge on proportional source water use can have important practical implications for the management of agricultural and agroforestry systems. These could include the improvement of local to regional farming practices such as irrigation or fertilization treatment schemes (e.g. Du et al., 2018; Ma and Song, 2018). Strategic planning at longer timescales would also benefit from insights into the ability of plants to access different sources, especially under changing hydro-climatological conditions such as extreme weather events, droughts or altered flow regimes (e.g. Sun et al., 2011; Gao et al., 2018), or whether co-existent species have competitive or complementary water use strategies (e.g. Muñoz-Villers et al., 2019). Especially for agricultural and agroforestry regions which are sustained at the natural limits of water availability, understanding the implications of climate or management change could be essential, for example in terms of evaluating the need for irrigation, which in turn is vital for designing and implementing efficient and equitable farming programs.

Most of the current isotope-based studies in agricultural and agroforestry regions involve RWU in maize. Moreover, a high proportion of these studies were carried out in the semi-arid regions of North China. These involve applications where RWU questions are clearly pressing and where irrigation forms an important water source. However, many opportunities lay in extending these isotope-based RWU studies to other geographic and climatic regions and a wider range of crops, especially as climate change may affect the ratio between rain-fed and irrigation-supported agriculture. RWU patterns and responses are known to vary in space and time (e.g., Geris et al., 2017) and between vegetation types (e.g., Zegada-Lizarazu and Iijima, 2004; Wu et al., 2018). Hence, beyond practical local applications, increased understanding of RWU sources across a broader range of conditions will also enable new scientific insights into the wider functioning of plants (including plasticity or adaptability to change) which are typical for agricultural and agroforestry environments, especially as previous research has focussed mainly on natural systems. As such, extending our knowledge on RWU across different ecosystems will provide new opportunities for addressing fundamental ecohydrological questions with implications of global relevance.

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Figures

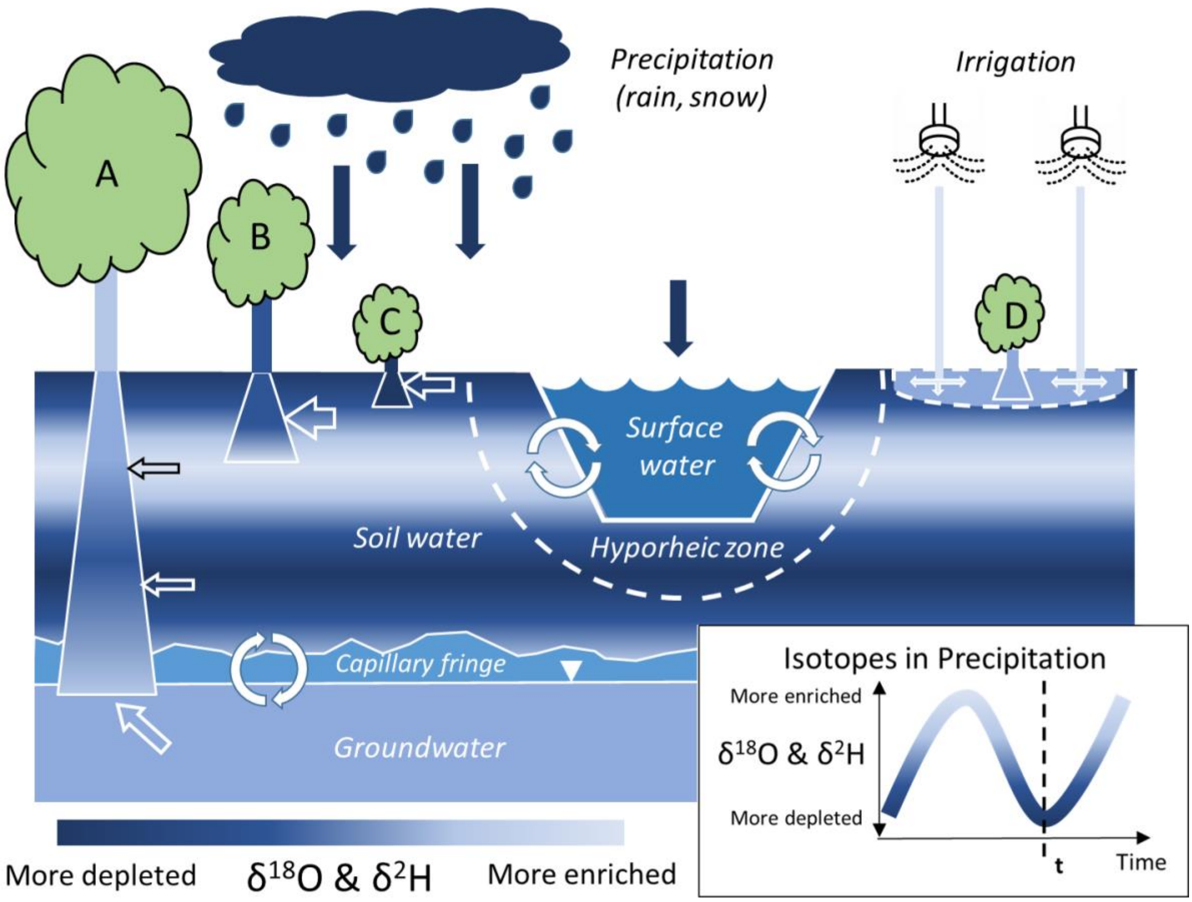

Fig. 1. Conceptual diagram of stable water isotope signatures in different root water sources (precipitation, soil water at different depths, surface- and groundwater, and irrigation water) at time t. The lower right inset shows that precipitation isotopic signatures change with time, e.g. seasonally (after Allen et al., 2019). The isotopic composition of water sources and crop water are indicated by colours, with more enriched values in light and more depleted values in dark blue. Subsurface arrows indicate from where crops might take up water, with arrow thickness reflecting the proportion of each water source; white circular arrows refer to active hydrological mixing zones. Depending on different factors (see text at Section 2.1), the crop roots access different water sources, and the isotopic value of various crops (e.g., herbaceous, shrubs, and trees, A-D) can be different at one specific moment in time.