Isotopic approaches to quantify root water uptake: a review and comparison of methods

Youri Rothfuss[1], Mathieu Javaux[1,2,3]

[1]Institute of Bio- and Geosciences, IBG-3 Agrosphere, Forschungszentrum Jülich GmbH, Jülich, 52425, Germany
[2]Earth and Life Institute, Environnemental Sciences, Université catholique de Louvain (UCL), Louvain-la-Neuve, 1348, Belgium
[3]Department of Land, Air and Water Resources, University of California Davis, Davis, California, 95616, USA.

Correspondence to: Youri Rothfuss (y.rothfuss@fz-juelich.de)

Abstract. Plant root water uptake (RWU) has been documented for the past five decades from water stable isotopic analysis. By comparing the (hydrogen or oxygen) stable isotopic composition of plant xylem water to those of potential contributive water sources (e.g., water from different soil layers, groundwater, water from recent precipitation or from a nearby stream) studies could determine the relative contributions of these water sources to RWU.

In this paper, the different methods used for locating / quantifying relative contributions of water sources to RWU (i.e., graphical inference, statistical (e.g., Bayesian) multi-source linear mixing models) are reviewed with emphasis on their respective advantages and drawbacks. The graphical and statistical methods are tested against a physically based analytical RWU model during a series of virtual experiments differing in the depth of the groundwater table, the soil surface water status, and the plant transpiration rate value. The benchmarking of these methods illustrates the limitations of the graphical and statistical methods while it underlines the performance of one Bayesian mixing model, but only when the number of considered water sources in the soil is the highest to closely reflect the vertical distribution of the soil water isotopic composition. The simplest two end-member mixing model is also successfully tested when all possible sources in the soil can be identified to define the two end-members and compute their isotopic compositions. Finally, future challenges in studying RWU with stable isotopic analysis are evocated with focus on new isotopic monitoring methods and sampling strategies, and on the implementation of isotope transport in physically based RWU models.

Keywords

Root water uptake; hydraulic redistribution; hydraulic lift; water stable isotopologues; isotope mixing model; physically based root water uptake model

1 INTRODUCTION

Root water uptake (RWU) is defined as the amount of water abstracted by a root system from soil over a certain period of time. Understanding the relation between the distributions of soil water, roots, RWU location and magnitude, and root hydraulic properties is important for managing soil water and plant water status (e.g., by irrigation), developing new plant genotypes more tolerant to drought or tackling ecological questions in water-limited ecosystems, such as the competition for soil water by different plants (Javaux et al., 2013).

RWU is principally driven by transpiration flux taking place in the leaves. Its magnitude depends on the atmospheric evaporative demand and stomatal opening. The latter depends amongst others on leaf water status and stress hormonal signals from the roots transported to the leaves (e.g., Huber et al., 2015; Tardieu and Davies, 1993). Leaf water status and hormonal signals are related to the soil water potential distribution and to the plant hydraulic architecture (Huber et al., 2015). The distribution of RWU is very variable in time and space, depends on the presence of roots and their ability to extract water. This ability is a function of radial conductivity but axial conductance may also limit water flow in younger roots or when cavitation occurs. The flux of water depends also on soil water availability, i.e., the ability of the soil to provide water at the plant imposed rate (Couvreur et al., 2014): a highly conductive root segment will not be able to extract water from a dry soil. Locally, this is the difference of water potential between the root and the soil which drives RWU, and its magnitude is controlled by the radial hydraulic resistances in the rhizosphere, at the soil root interface and in the root system (Steudle and Peterson, 1998). The actual RWU profile is thus a combination of different aspects: the root ability to extract water (characterized by the amount of roots and their hydraulic properties), the ability of the soil to fulfill the plant water demand, and the water potential difference between soil and root (Couvreur et al., 2014).

Plants have numerous mechanisms to cope with heterogeneous soil water distribution, and adapt their RWU rate distribution: adaptive root growth, adaptive root conductivity (Javaux et al., 2013), exudation (Carminati et al., 2016). A particular process, which has attracted the attention of plant breeders and ecologists is the ability of plants to extract water from non or less water limited soil areas with potentially low root lengths densities (RLD [$L\ L^{-3}$], usually expressed in cm root per cm^3 soil), known as root water uptake compensation (Heinen, 2014). To describe the RWU rate in soils, we will use the root water uptake flow per volume of soil, defined as S [$L^3\ L^{-3}\ T^{-1}$] in reference to the sink term of the Richards equation. According to Couvreur et al. (2012), root compensation is defined in the present article as the process that decreases or increases RWU at a certain location compared to the water uptake from that location when the soil water potential would be uniform in the root zone. Thus, the distribution of the $S(x,y,z)$ is a sum of two spatially distributed components:

$$S(x, y, z) = S_{uniH}(x, y, z) + S_{comp}(x, y, z) \tag{1}$$

where x, y and z are the 3-D spatial coordinates, S_{uniH} is a term proportional to the root distribution and S_{comp} the compensatory part of the RWU distribution. The first term on the right-hand side of Eq. (1) is always positive while the second one can be either positive or negative. Figure 1 illustrates how this equation affects S distribution in a one-dimensional (1D) space. When there is no compensation ($S_{comp}(x,y,z) = 0$), the RWU distribution follows the root distribution

(i.e., highest at the surface and lowest in the deepest layer, Fig. 1a). When $S_{comp}(x,y,z) < 0$ but its absolute value is lower than $S_{uniH}(x,y,z)$, then $S(x,y,z)$ is positive and different from the root vertical distribution. In case $S_{uniH}(x,y,z)$ is small, as in Fig. 1c, $S_{comp}(x,y,z)$ can locally be higher in absolute value and $S(x,y,z)$ can be locally negative which implies that there is a water efflux out of the root (called "Hydraulic redistribution" of "Hydraulic lift" in this particular case, Caldwell and Richards, 1989; Dawson, 1993; Kurz-Besson et al., 2006).

Despite its importance, there is a lack of measurements of RWU, related to the difficulty of measuring root and soil water fluxes. Often soil water content change is used as a proxy for RWU. Yet, as change of soil water content with time is not due to root extraction only (i.e., soil water redistribution can also occur), the assessment of RWU based on water content distribution alone is not possible in conductive soils (Musters and Bouten, 2000). Rather, the full soil water flow equation accounting for root uptake and soil water redistribution must be solved in an inverse mode, and, with an accurate knowledge of soil and root properties RWU distribution can be inferred (Guderle and Hildebrandt, 2015; Hupet et al., 2002; Musters and Bouten, 1999; Vandoorne et al., 2012). Nuclear magnetic resonance (NMR) imaging has been suggested as an adequate technique to measure water flow velocity in xylem vessels but no application exists yet on living roots in soils (Scheenen et al., 2000). More recently, Zarebanadkouki et al. (2012) could measure for the first time RWU in porous media by combining a tracer experiment (i.e., deuterated water) monitored by neutron tomography with inverse modelling of a transport equation. Yet, this was done under controlled conditions while there is no standard method to monitor three dimensional water uptake distribution of growing roots *in situ*. In woody plants, in which roots are thick enough, Nadezhdina et al. (2010; 2012; 2015) used sap flow measurements in roots to quantify hydraulic redistribution.

Since the seminal work of Zimmermann et al. (1967) which reported that RWU of *Tradescantia fluminensis* occurred in the absence of fractionation against water oxygen stable isotope, water stable isotopologues ($^1H^2H^{16}O$ and $^1H_2^{18}O$) have been frequently used to identify and quantify root water uptake in soils through the measurements of their natural (and artificial) isotopic abundances. Methods include simple graphical inference to more sophisticated statistical methods, i.e., two-end members and multi-source linear mixing models. While the former attempts to locate the "mean root water uptake" in the soil, the latter category of methods provides profiles of relative contributions to transpiration flux across a number of defined soil layers.

This present paper (i) aims at reviewing these methods (i.e., graphical inference and statistical multi-source linear mixing models) and (ii) proposes to compare them against each other during a series of virtual experiments differing in the water and isotopic statuses in the soil and the plant. Prior to the review and inter-comparison, the paper reports on the mechanisms at the origin of the spatiotemporal dynamics of natural isotopic abundances in soil and on the background knowledge of isotopic transfer of soil water to and from roots. Finally, we address future challenges to be undertaken such as the dynamic isotopic assessment of HR. We also evoke opportunities offered by novel isotopic monitoring tools which provide unpreceded high frequency isotopic measurements.

2 FLOW OF ISOTOPOLOGUES IN THE SOIL-PLANT SYSTEM

In a study that laid the basis for future work in isotopic ecohydrology, Zimmermann et al. (1967) provided a steady-state analytical solution for soil water isotopic composition (δ_S, expressed in ‰ relative to the Vienna Standard Median Ocean Water international (VSMOW) isotope reference scale, Gonfiantini, 1978) in a water-saturated isothermal bare sand profile
5   from which water evaporated at a constant rate. Under these steady-state and isothermal conditions, the upward (convective) liquid flux of isotopologues, triggered by evaporation (E) and rising from deeper layers equals the downward (diffusive) isotopic flux from the evaporating surface which is enriched in the heavy stable isotopologues due to evaporation. Furthermore, by conservation of mass, the isotopic composition of evaporation equals that of its source (e.g., groundwater), i.e., $\delta_E = \delta_{source}$. A profile is obtained (Fig. 2a, dark blue line) whose exponential shape depends on boundary conditions, i.e.,
10   the source water and surface water isotopic compositions (δ_{source} and δ_{surf}), the diffusion coefficient of the isotopologues in water, and of a soil "tortuosity factor", conceptually defined as the ratio of the geometrical to actual water transport distance. Barnes and Allison (1983) extended this formulation to a non-saturated sand column evaporating at isotopic steady state. In this case, the evaporating surface (i.e., the liquid-vapor interface) can be located below the soil surface and splits the profile into two regions where isotopic transport predominantly occurs either in the vapor phase above or in the liquid phase below
15   it. In the "vapor region", relative humidity generally is still close to unity for sand total water potential below 15 bars. At isotopic steady state, the maximal isotopic enrichment is at the evaporation front (δ_{EF} at soil depth z_{EF}) and can be simulated with the Craig and Gordon (1965) model. The isotopic composition of the soil residual adsorbed water in the "vapor region" above the evaporation front can be obtained by assuming thermodynamic equilibrium conditions and by applying Fick's law, and is shown to decrease linearly towards the value of the liquid water at the soil surface which is at thermodynamic
20   equilibrium with the ambient atmospheric water vapor (Fig. 2a, light blue line). Finally, note that at transient state ($\delta_E \neq \delta_{source}$), the maximal isotopic enrichment in the soil profile does not point to the location of the evaporation front as was demonstrated by Rothfuss et al. (2015). Instead, the depth where the steepest gradient in the isotopic profile is observed corresponds to the evaporation front.

Above the evaporation front and at isotopic steady-state, soil liquid water is in equilibrium with a mixture of atmospheric
25   water vapor ($\delta^{18}O$-δ^2H slope ~8) and evaporated soil water vapor rising from the evaporation front ($2 < \delta^{18}O$-δ^2H slope < 6) (Sprenger et al., 2016). As a result, an intermediate value for the slope is expected, depending on the mixing ratio of atmospheric water vapor to evaporated soil vapor at a given soil depth. Finally, under natural conditions, the δ_S profile is not solely a result of isotopic fractionation but is as well highly impacted both spatially and temporally by input precipitation isotopic composition through modification of the upper boundary condition (δ_{surf}).

30   As opposed to the removal of water vapor by evaporation, RWU has been described in a number of studies and over a wide variety of plant species not to be associated with (kinetic) isotopic fractionation (Bariac et al., 1994; Dawson and Ehleringer, 1993; Thorburn et al., 1993; Walker and Richardson, 1991; Washburn and Smith, 1934; White et al., 1985; Zimmermann et al., 1967). Consequently, for plants growing in homogeneous external conditions, e.g., in hydroponic solution, root xylem

sap water and external water have the same isotopic compositions. In natural soils where the liquid phase is not homogeneous and a vertical gradient of isotopic composition due to evaporation exists, the root system takes up water at different depths having thus different isotopic compositions.

Assuming that water transport time in roots is negligible, the isotopic concentration of the xylem sap water at the root tiller (C_{Ti} [M L^{-3}]) can be modeled as the weighted average of the product of the soil water isotopic concentration (C_S [M L^{-3}]) and $S(x,y,z)$:

$$C_{Ti} = \frac{\int\limits_{x,y,z} C_S(x,y,z) \cdot S(x,y,z) \cdot dx \cdot dy \cdot dz}{\int\limits_{x,y,z} S(x,y,z) \cdot dx \cdot dy \cdot dz} = \frac{\int\limits_{x,y,z} C_S(x,y,z) \cdot S(x,y,z) \cdot dx \cdot dy \cdot dz}{J_{Ti}} \tag{2}$$

with J_{Ti} [L^3 T^{-1}] the xylem sap flux at the root tiller. Following Braud et al. (2005):

$$C = \rho \cdot R_{ref} \frac{M_i}{M_w}(\delta+1) \tag{3}$$

with ρ [M L^{-3}] the volumetric mass of water, R_{ref} [-] the Vienna-Standard Mean Ocean Water (V-SMOW) hydrogen or oxygen isotopic ratio, M_w and M_i [M L^{-3}] the molar masses of $^1H_2^{16}O$ and isotopologue ($^1H^2H^{16}O$ or $^1H_2^{18}O$), respectively, the xylem sap water isotopic composition at the root tiller δ_{Ti} [-, expressed in ‰] can be expressed as:

$$\delta_{Ti} = \frac{\int\limits_{x,y,z} \delta_S(x,y,z) \cdot S(x,y,z).dx \cdot dy \cdot dz}{J_{Ti}} \tag{4a}$$

with $\delta_S(x,y,z)$ [-, expressed in ‰] the isotopic compositions of soil water at coordinates (x,y,z). Mostly, a one dimensional description of root water uptake is used assuming that δ_S and RWU do not vary in the horizontal direction and δ_S is obtained for discrete soil layers of depths z_j ($j \in [1,n]$) and thickness $\Delta z_j = z_{j+1} - z_j$. It is usually further hypothesized that J_{Ti} equals the transpiration flux T [L^3 T^{-1}] (low to no plant capacitance or phloem-xylem contact):

$$\delta_{Ti} = \frac{\sum\limits_{j=1,n} \delta_S(z_j) \cdot S(z_j) \cdot \Delta z_j}{\sum\limits_{j=1,n} S(z_j) \cdot \Delta z_j} = \frac{\sum\limits_{j=1,n} \delta_S(z_j) \cdot S(z_j) \cdot \Delta z_j}{q_{Ti}} \tag{4b}$$

where $q_{Ti} = J_{Ti}/(\Delta x \Delta y) = T/(\Delta x \Delta y)$ represents the sap flow rate in the root tiller per unit surface area [L T^{-1}].

δ_{Ti} can be accessed at different locations in the plant depending on the species, but the sampling location should not be affected by evaporative enrichment in heavier isotopologues or back-diffusion of the isotopic excess accumulated at the sites of transpiration (stomatal chambers) in the leaf. For grasses and nonwoody plants, this is done by sampling the root crown (e.g., Leroux et al., 1995), the aerial nodal roots (e.g., Asbjornsen et al., 2007), the meristematic petiole, or else the collars (e.g., tillers) at the base of the plant (e.g., Dawson and Pate, 1996; Sánchez-Perez et al., 2008). In the case of ligneous plants the fully suberized stem (Asbjornsen et al., 2007) or sapwood (e.g., White et al., 1985) is sampled. On the other hand, δ_S is

usually measured by sampling soil profiles destructively. Finally, water from plant and soil is predominantly extracted by cryogenic vacuum distillation (Araguás-Araguás et al., 1995; Ingraham and Shadel, 1992; Koeniger et al., 2011; Orlowski et al., 2013; West et al., 2006).

Lin and Sternberg (1992) and Ellsworth and Williams (2007), amongst other authors, reported however that for some

5   xerophyte (plants adapted to arid environments, e.g., *Prosopis velutina* Woot.) and halophytes species (plants adapted to saline environments, e.g., *Conocarpus erecta* L.), and mangrove species (e.g., *Laguncularia racemosa* Gaert.), RWU led to fractionation of water hydrogen isotopologues. For mangrove species, it was hypothesized that the highly developed Casparian strip of the root endodermis would force water moving symplastically (i.e., inside the cells) and therefore crossing cell membranes (Ellsworth and Williams, 2007). Water aggregates are then dissociated into single molecules to move across

10   these membranes. This demands more energy for $^1H^2H^{16}O$ than for $^1H_2^{16}O$ and $^1H_2^{18}O$, thus preferentially affects $^1H^2H^{16}O$ tranport and leads to a situation where xylem sap water is depleted in this isotopologue with respect to source water. Meanwhile, this affects to a much lesser extent $^1H_2^{18}O$ transport, so that no detectable isotopic fractionation of water oxygen isotopologues is observed. It can be concluded that, for the majority of the studied plant species, either RWU does not lead to isotopic fractionation or its magnitude is too low to be observable.

15   Finally, plant water samples will, similarly to soil water samples, also fall onto an "evaporation line" of a slope lower than eight in a two-dimensional ($\delta^{18}O$, δ^2H) space (Javaux et al., 2016).

3 LITERATURE REVIEW

By entering the search terms (("root water uptake" or ("water source" and root) or "water uptake") and isotop*) into the ISI Web of Science search engine (webofknowledge.com), 159 studies published in the last 32 years were finally identified (see

20   a listing of all studies in the supplementary materials). Cumulative number of articles as a function of publication year follows a typical exponential law: in average over the period 1985-2014, number of publications per year increased for about 0.3 and reached 8 (2014). In both years 2015 and 2016, the isotopic method for locating or partitioning water sources to RWU gained significantly more attention with 20 publications per year.

When sorting plant species simply by their form and height, it appears that trees are the most studied group of plants (present

25   in about 60 % of the studies), followed by annual and perennial grasses (21 %) and shrubs (e.g., desert and mangrove species, 21 %). Only 13 % of the publications study RWU in agricultural systems (e.g., maize, wheat, millet, rice), which is reflected by the small portion of peer-reviewed journals of which the category is listed under "Agronomy and Crop Science" (8 %) by Scimago Journal & Country Rank (www.scimagojr.com). "Soil Science" is also a relatively underrepresented category with 8 % as well. This is corroborated by the fact that 27 % of the studies do not report any information about soils

30   (e.g., texture, FAO class, structure, particle size distribution, or physical properties). In comparison, the "Ecology" and

"Ecology, Evolution, Behavior and Systematics" categories are significantly more represented with 22 % of the studies altogether.

Four classes of methods for root water uptake analysis on basis of isotopic information emerged from the analysis. In a first one, representing 46 % of the studies, root water uptake is either located precisely in the soil using the method of "direct inference" (Brunel et al., 1995) or much generally in some water pool (or water "source", not to be mistaken with the concept of water source defined in the previous section), e.g., groundwater, soil water, or rainwater (Andrade et al., 2005; Beyer et al., 2016; Roupsard et al., 1999). In a second class (18 % of the studies) relative contributions of two particular water sources (e.g., water in two distinct soil layers, or groundwater versus recent precipitation etc.) to RWU are calculated "by hand" with a two end-member linear mixing model (Araki and Iijima, 2005; Dawson and Pate, 1996; Schwendenmann et al., 2015). In a third class (32 % of the studies) relative contributions of at least three water sources to RWU are determined using multi-source mixing models (e.g., MixSir and MixSIAR, Moore and Semmens, 2008, representing 2 % of the studies; SIAR, Parnell et al., 2013, 5 %; IsoSource, Phillips and Gregg, 2003, 21 %). Note that classes two and three are both based on end-member mixing analysis (EMMA) (Barthold et al., 2011; Christophersen and Hooper, 1992) and will be further pooled into "statistical approach" in section 3.2.

In a fourth class, only accounting for 4 % of the studies, assessments are provided using physically based analytical (Boujamlaoui et al., 2005; Ogle et al., 2014; Ogle et al., 2004) or numerical (i.e., SiSPAT-Isotope, Rothfuss et al., 2012; HYDRUS-1D, Stumpp et al., 2012; Sutanto et al., 2012) models.

Note that all methods have in common to use an inverse modelling approach: the RWU distribution is obtained by optimizing model input parameters until the simulated δ_{Ti} and/or the simulated δ_S profiles fit to the isotopic measurements. One important feature of the three first classes of methods is that they consider soil water isotopic transport flow to be negligible for the duration of the experiment. Numerical models such as HYDRUS-1D and SiSPAT-Isotope on the other hand take this into account in the computation of RWU profiles.

61 % of the studies based their estimation of location or quantification of relative contributions on measurement of either δ^2H or $\delta^{18}O$, i.e., in a single isotope framework, while 25 % used both δ^2H and $\delta^{18}O$ (i.e., in a dual isotope framework). In the remaining studies, both isotopic compositions were measured and used to provide two separate estimates of relative contribution distributions even though δ^2H or $\delta^{18}O$ distributions were strongly linked (see section 2). This last approach is in the present study referred as "double single" (see supplementary materials). The vast majority of the studies (82 %) took advantage of natural isotopic abundances while the rest (18 %) applied labelling pulses to the soil (either in the profile or at the soil boundaries, e.g., the soil surface and groundwater) to infer RWU from uptake of labeled water.

Table 1 summarizes 21 particular isotopic studies that use either one of the first three classes of methods (i.e., accounting for about 96% of the published studies), while class four (physically based RWU models) will be treated separately in section 4. These 21 studies were chosen according to either the number of citations and contribution importance (for studies published before 2015) or to the novelty of the publications (publication year $\geq$ 2015).

3.1 Graphical inference (GI)

This straightforward approach first proposed by Brunel et al. (1995) and applied by, e.g., Leroux et al. (1995), Weltzin and McPherson (1997) (Table 1) and else by Midwood et al. (1998), Armas et al. (2012), and Isaac et al. (2014) (see supplementary materials) defines the "mean root water uptake depth" $\overline{z}$, as the depth where $\delta_S = \delta_{Ti}$. $\overline{z}$ conceptually indicates to the soil depth where the plant root system, represented as one unique root, would extract water from.

There are cases where $\overline{z}$ cannot be unambiguously identified (e.g., $\overline{z}_1$ and $\overline{z}_2$ of case 2, Fig. 2b) due to the non-monotonic character of the δ_S profile (shown in black dashed line, case 2 of Fig. 2b). In order to define a mean RWU depth for such a case one can derive a monotonously decreasing δ_S profile by smoothing the profile (shown as symbols in Fig. 2b), e.g., by averaging δ_S in a number of layers using the following mass balance:

$$\delta_{S,J} = \frac{\sum\limits_{j \le J} \delta_S(z_j) \cdot \theta(z_j) \cdot \Delta z_j}{\sum\limits_{j \le J} \theta(z_j) \cdot \Delta z_j} \tag{5}$$

where J represents the set of depths that belong to the Jth soil layer, with θ [L^3 L^{-3}] and Δz_j [L] the soil volumetric water content and thickness of the soil layer centered around depth z_j. Due to this smoothing, the vertical resolution may be drastically reduced. In the example presented Fig. 2b where a uniform θ profile is assumed, the $\delta_{S,J}$ profile intersects with the vertical line of value δ_{Ti} deeper than for the initially non-monotonic δ_S profile, i.e., $\overline{z}$ (case 2, integrated δ_S profile) $< \overline{z}_2 < \overline{z}_1$.

Some authors rule out solutions in case of multiple mean root water uptake depths, e.g., by excluding the $\overline{z}$ solutions where soil water content was low and/or soil water potential was high in absolute value (e.g., Li et al., 2007; see Table 1).

Note that while Eq. (5) provides a representative value for the isotopic composition that would be measured in soil layer J as a function of those of the water in the set of depths, $\delta_{S,J}$ is however equivalent to the isotopic composition "sensed by the plant" only if the root profile is homogeneous, i.e., when RLD is constant with depth in that particular soil layer J.

The method of graphical inference may not only provide $\overline{z}$ but also its uncertainty caused by the uncertainty in measuring δ_{Ti} (e.g., based on the precision of the isotopic analysis and/or sampling natural variability, shown as gray stripe in Fig. 2b). The steeper the soil water isotopic profile, the larger the uncertainty in determining $\overline{z}$ is. Figure 2b illustrates this with estimated minimum and maximum $\overline{z}$ for the monotonic δ_S profile and for the vertically averaged profile. In the latter case, the possible range of $\overline{z}$ is the largest. These ranges give first quantitative indication of variance around $\overline{z}$. Finally, for a complete "graphical assessment" of the variance of $\overline{z}$, one should also consider the uncertainty associated with measurements of the δ_S profile (not shown here; for a complete assessment of errors associated with determination of δ_S, see Sprenger et al., 2015).

3.2 Statistical approaches

3.2.1 Two end-member (TM) mixing model

The TM method is a particular case of end-member mixing analysis (EMMA) and is based on the concept that (i) a plant extracts water from two predominant water sources A and B (e.g., water in distinct upper and lower soil layers, or groundwater and recent precipitation water etc.) in given proportions, (ii) there is no isotopic fractionation during water uptake, and (iii) there is a complete mixing inside the plant of the contributing water sources A and B to RWU. The mass conservation for isotopologues gives:

$$J_{Ti}^{i} = J_{i}^{A} + J_{i}^{B} \tag{6a}$$

$$C_{Ti} \cdot J_{Ti} = C_A \cdot J_A + C_B \cdot J_B \tag{6b}$$

with J_A, J_B, and J_{Ti}, [L^3 T^{-1}] (respectively J_i^A, J_i^B, and J_{Ti}^i [M T^{-1}]) the fluxes of water (respectively isotopologues) originating from water sources A and B, and at the plant tiller. C_A, C_B, and C_{Ti} [M L^{-3}] are the water sources A and B, and xylem sap water measured isotopic concentrations. By introducing $x = J_A / J_{Ti}$ and following Eq. (3), Eq. (6b) becomes:

$$\delta_{Ti} = x \cdot \delta_A + (1-x) \cdot \delta_B \tag{7}$$

In this approach, δ_{Ti} is therefore defined as the mean value of the isotopic compositions of water sources A and B (δ_A and δ_B) weighted by the proportions to J_{Ti} of water volume extracted by the plant from water sources A and B, i.e., x and $(1 - x)$, respectively. The error associated with the estimation of x (σ_x [-, expressed in ‰]) can be calculated following Phillips and Gregg (2001):

$$\sigma_x^2 = \left(\frac{\partial x}{\partial(\delta_A)}\right)^2 \cdot \sigma_{\delta_A}^2 + \left(\frac{\partial x}{\partial(\delta_B)}\right)^2 \cdot \sigma_{\delta_B}^2 + \left(\frac{\partial x}{\partial(\delta_{xyl})}\right)^2 \cdot \sigma_{\delta_{Ti}}^2 \tag{8a}$$

$$\sigma_x = \sqrt{\sigma_x^2} = \frac{1}{(\delta_A - \delta_B)} \sqrt{\left(\sigma_{\delta_{Ti}}^2 + x^2 \cdot \sigma_{\delta_A}^2 + (1-x)^2 \cdot \sigma_{\delta_B}^2\right)} \tag{8b}$$

with σ_{δ_A}, σ_{δ_B}, and $\sigma_{\delta_{Ti}}$ the standard errors associated with the measurements of δ_A, δ_B, and δ_{Ti}, respectively. The sensitivity of Eq. (8b) to different values of σ_{δ_A}, σ_{δ_B}, and $\sigma_{\delta_{Ti}}$ can be tested by considering either minimal possible errors, i.e., the analytical precision of the isotopic analyser (e.g., isotope ratio mass spectrometer, laser-based spectrometer), or by taking into account additional errors involved with sampling procedure and vacuum distillation technique (see e.g., Rothfuss et al., 2010). Equation (8b) also shows that, independently of the values considered for σ_{δ_A}, σ_{δ_B}, or $\sigma_{\delta_{Ti}}$, σ_x is inversely proportional to $1/(\delta_A - \delta_B)$, indicating that the two end-members should have as much as possible distinct isotopic compositions for a low standard error of x. Therefore, it is especially important, e.g., for partitioning between water from an upper and lower portion of the soil profile, to properly define the thickness of these layers, so that they have distinct isotopic compositions, and that the difference is considerably larger than the precision of the isotopic measurements. Figure 3 shows

for example that when (i) x is evaluated at 10 % and (ii) σ_{δ_A}, σ_{δ_B}, and $\sigma_{\delta_{Ti}}$ are estimated being equal to 0.02 ‰ (dark blue solid line), $(\delta_A - \delta_B)$ should be greater than 0.75 ‰ (in absolute term) in order to reach a σ_x value lower than 5 %, i.e., more than 37 times the error made on δ_A, δ_B, and δ_{Ti}. To obtain the same standard error for x in case of a higher standard error on the estimation of δ_A, δ_B, and δ_{Ti} (e.g., σ_{δ_A}, σ_{δ_B}, and $\sigma_{\delta_{Ti}} = 0.1‰$), $(\delta_A - \delta_B)$ should be greater than 3.00 ‰ (in absolute

5 term). This difference becomes much greater for σ_{δ_A}, σ_{δ_B}, and $\sigma_{\delta_{Ti}} = 1.00‰$ and reaches 42 ‰ (not shown in Figure 3). This certainly highlights the advantage of artificially labelling soil water with water enriched (or depleted) in heavy isotopologues for a more precise assessment of the relative contribution of soil water sources to RWU, as mentioned by Moreira et al. (2000). In another study, Bachmann et al. (2015) labelled the upper and lower portion of the soil profile in a natural temperate grassland with ^{18}O-enriched and ^{2}H-enriched water, respectively. They defined two distinct (upper and lower) soil

10 water sources, for which they calculated the corresponding δ^2H or δ^{18}O on the basis of measured soil water isotopic profiles and using Eq. (5). They could find evidence against the so-called hypothesis of "niche complementarity" regarding plant water use, which states that RWU of competitive plant species is spatially and temporally distinct, and that this distinction is stronger at high species richness. Figure 3 illustrates also that for given $(\delta_A - \delta_B)$, σ_{δ_A}, σ_{δ_B}, and $\sigma_{\delta_{Ti}}$ values, the "optimal x value" for a low σ_x is 50% (showed by the orange lines).

15 Table 1 displays a sample of studies that used the two end-member mixing approach. Authors could distinguish between uptake of irrigation and precipitation water (Goebel et al., 2015), precipitation and groundwater (White et al., 1985), soil water and groundwater (McCole and Stern, 2007), or else between stream water and soil water (Dawson and Ehleringer, 1991; McDonnell, 2014). Thorburn and Ehleringer (1995) could for instance locate the dominant source for RWU, i.e., groundwater for their mountain and floodplain test-site and water from the soil between 0.3 and 0.4 meters depth for their

20 cold desert test-site. Other studies (e.g., Brunel et al., 1995) combined two mixing equations, i.e., one for each isotopologue, into a single one or else, calculated the ratio of geometrical distances between δ_{Ti} and δ_A and between δ_{Ti} and δ_B in a dual isotope (δ^{18}O and δ^2H) space (Bijoor et al., 2012; Feikema et al., 2010; Gaines et al., 2016). As infrared laser-based spectrometry now enables simultaneous measurements of δ^{18}O and δ^2H at lower cost, we believe that this dual-isotope approach (referred as "D" in Table 1) will or should gain in importance in isotopic studies. This is especially useful when (i)

25 under natural conditions the δ^{18}O-δ^2H slope is not constant over depth (Sprenger et al., 2016) or (ii) in the context of pulse labelling experiments, which can artificially change the value of the δ^{18}O-δ^2H slope at given locations in the soil profile. In these cases, two independent mixing equations are obtained, one for each isotopologue.

3.2.2 Multi-source (MS) mixing models

When there are more than two identified plant water sources contributing to RWU, e.g., water from different layers j ($j \in$ [1,

30 N]) in soil the profile, Eq. (7) becomes:

$$\delta_{\text{Ti}} = \sum_{j=1}^{N} x_j \cdot \delta_{S,j} \qquad\qquad (9)$$

with N the number of plant water sources (e.g., soil layers) and $\sum_{j=1}^{N} x_j = 1$. As there are more water sources than (number of

mixing equations + 1), there is not a unique solution but an infinite range of possible solutions. However, some of these solutions are not likely or possible based on background information or knowledge. A range of solutions that is most likely

5   based on prior information can be obtained using Bayesian methods. In the method proposed by Phillips and Gregg (2003), the isotopic composition calculated for each considered x_j combination (δ_{Ti}) is compared with the measured value ($\delta_{\text{Ti,m}}$). The number of combinations depends on the value of contribution increment (i, %, typically 5 or 10 %) and the combinations for which δ_{Ti} meets the following requirement are selected:

$$\delta_{\text{Ti}} \leq \left| \delta_{\text{Ti,m}} \pm \tau \right| \qquad\qquad (10)$$

10   where τ [-, expressed in ‰], standing for "tolerance", usually accounts for precision of the isotopic measurements or possible errors during sampling and vacuum distillation steps. This multi-source mixing model approach strongly depends on τ and i, which therefore should be carefully chosen by the user. A smaller i also refines the analysis. For this, the program "IsoSource" (https://www.epa.gov/sites/production/files/2015-11/isosourcev1_3_1.zip) is available (Phillips et al., 2005). Wang et al. (2010) compared the outcome of the GI and MS approaches and came to the conclusion that even though the

15   latter did not solve the non-uniqueness problem and provided diffuse patterns of frequency that were difficult to interpret in some cases (e.g., in case of a non-monotonic isotopic profile), it had the advantage over the former method of providing a systematic and quantitative assessment of ranges of relative contributions. Romero-Saltos et al. (2005) extended the model of Phillips and Gregg (2003) by constraining RWU to follow a normal distribution within a delimited 50-cm soil vertical segment of one centimeter vertical resolution and centered around $\bar{z}$, the mean root water uptake depth. The location of this

20   section and thus $\bar{z}$ is as well obtained by mass balance from inverse modelling similarly to by IsoSource (see applications of Grossiord et al., 2014; Rossatto et al., 2013; Stahl et al., 2013).

Parnell et al. (2010) proposed to overcome two limitations of the approach of Phillips and Gregg (2003), i.e., its inability to (i) account for uncertainty in the estimations of δ_{Ti} and of the water sources isotopic compositions $\delta_{S,j}$, and (ii) provide a optimal solution rather than ranges of feasible solutions. For this they use a Bayesian framework (for details see also Erhardt

25   and Bedrick, 2013; Moore and Semmens, 2008; Parnell et al., 2013), which allows uncertainty in the x_j proportions and incorporates a residual error term ε_j (normally distributed with mean equal to zero and variance σ^2):

$$\delta_{\text{Ti}} = \sum_{j=1}^{N} x_j \cdot \delta_{S,j} + \varepsilon_j \qquad\qquad (9')$$

Note that the terms of (i) trophic enrichment factor (TEF [-, expressed in ‰], see, e.g., meta-analysis of Vanderklift and Ponsard, 2003) and (ii) isotope concentration dependency (Koch and Phillips, 2002; Phillips and Koch, 2002) originally

incorporated in the formulation of Parnell et al. (2010) for other applications are not present in Eq. (9') since (i) no isotopic fractionation during RWU is assumed and (ii) isotope concentration dependency applies only for situations where isotopic compositions of different elements are measured and available.

Parnell et al. (2010) developed the program "Stable Isotope Analysis in R" (SIAR, https://cran.r-project.org/src/contrib/siar_4.2.tar.gz) in which the initial (*a priori*) x_j distribution is by default the Dirichlet distribution, of which information can be partly specified by the user. *A posteriori* x_j distribution is obtained by fitting the linear model to data via a Metropolis-Hasting (Hastings, 1970; Metropolis et al., 1953) Markov Chain Monte Carlo algorithm.

Prechsl et al. (2015) apply both graphical and Bayesian approaches to evaluate the shift in $\bar{z}$ and change of RWU profile following drought treatments (approx. 20 to 40 % precipitation reduction with transparent rainout shelters) in both extensively and intensively managed grasslands. From both approaches it appeared that a shift in $\bar{z}$ was inexistent or not observable from isotopic analyses. Another recent application of the Bayesian approach was performed by Volkmann et al. (2016b), who took advantage of a newly developed soil isotopic monitoring method to confront high frequency δ_S profiles time series to time series of δ_{Ti} (indirectly obtained from the isotopic measurement of the transpired water vapor and assuming isotopic steady state, i.e., $\delta_{Ti} = \delta_T$) following a labelling pulse (see Table 1 for details on the study).

4 INTER-COMPARISON OF METHODS

We tested and compared the different methods (GI, TM, MS) during a series of virtual experiments in a single isotope framework ($\delta^{18}O$) and at natural isotopic abundance. Mean RWU depths (provided by the GI method) and x_j distribution (provided by the two end-member and multi-source mixing models) were determined from soil and xylem water oxygen isotopic composition distributions. While the former information was prescribed to the different methods, the latter was calculated with the physically based analytical RWU model (referred to as "Couv") of Couvreur et al. (2012). The inter-comparison of models was performed using a single isotope (^{18}O) approach as the focus here was the differences of outcomes rather than the impact of the input isotopic data on these results. The reader can refer to Appendix B1 for a description of the model of Couvreur et al. (2012) and to Appendix B2 on how it was run for the inter-comparison.

4.1 Methodology

4.1.1 Scenario definition

We developed eight virtual plausible scenarios of soil-plant systems under different environmental conditions. This synthetic information was based on (i) experimental data and (ii) expert-knowledge. Each environmental condition was defined as a combination of different total soil water potential distributions (resulting from the location of the groundwater table and weather conditions), soil water oxygen isotopic composition (δ_S) profiles, and actual transpiration rate (T). The groundwater table was either shallow at -1.25 m depth (prefix "Sh") or deep at -6 m depth (prefix "De"); the soil water potential was

considered to be at static equilibrium below the groundwater level; the soil surface was either dry under evaporative conditions (suffix "Dr"), or wet, e.g., shortly after a rain event (suffix "We"); the transpiration rate was either low (e.g., relevant at night, $T = 0.01$ mm h^{-1}, suffix "_lT") or high ($T = 0.30$ mm h^{-1}, suffix "_hT"). They all relied on a common measured root length density vertical distribution of *Festuca arundinacea*. Table 2 reports the input data. Note that, as hypothetized in Eq. (4b), transpiration and sap flow rates (i.e., per unit of surface area [L T^{-1}]) were considered as equal.

4.1.2 Setup of the models

The two end-member mixing approach (TM) was tested against the isotopic data for two different cases: (i) two conjoint soil layers spreading from 0 – 0.225 m and 0.225 – 2.00 m and (ii) two disjoint soil layers spreading from 0 – 0.225 m and 1.75 – 2.00 m. The latter case was designed to evaluate the impact of lacunar soil isotopic information on the calculation of x, i.e., when not all potential water sources are properly identified. Representative values of water oxygen isotopic compositions for these soil layers ($\delta_{S,J}$, $J \in$ [I,II]) were obtained from the mass balance (Eq. (5)) after interpolation of the measured soil water content and δ_S profiles at a 0.01 m vertical resolution.

For the multi-source mixing models of Phillips and Gregg (2003) (IsoSource) and Parnell et al. (2010) (SIAR), the number of potential water sources was initially fixed to three, i.e., water from the soil layers I (0.000-0.050 m), II (0.050-0.225 m), and III (0.225-2.000 m). Upper and lower boundaries of these layers were defined to reflect the exponentially shaped (monotonic) δ_S profiles (experiments ShDr and DeDr) or to smooth the non-monotonic δ_S profiles observed during experiments ShWe and DeWe. IsoSource and SIAR were also tested for eight soil layers (i.e., as many layers as measurement points, I: 0.000-0.020, II: 0.020-0.050, III: 0.050-0.110, IV: 0.110-0.225, V: 0.225-0.400; VI: 0.400-0.750, VII: 0.750-1.500, and VIII: 1.500-2.000 m). Increment and tolerance in IsoSource were fixed at 10 % and 0.25 ‰, respectively. Similarly to the TM approach, profiles of $\delta_{S,J}$ ($J \in$ [I,III] or [I,VIII]) were obtained from the mass balance (Eq. (5)) after interpolation of the measured soil water content and δ_S profiles at a 0.01 m vertical resolution.

Finally for the SIAR model, uncertainty associated with δ_S measurements was set to 0.2 ‰ and the number of iterations was fixed to 500000 and number of iterations to be discarded to 50000.

For a detailed description of the inter-comparison methodology, refer to Appendix C.

4.2 Results and discussion

Figure 4 displays x_{Couv}, the simulated $\dfrac{S(z)dz}{T/(\Delta x \cdot \Delta y)}$ ratios (solid colored lines) simulated by the analytical model of (Couvreur et al., 2012) for the eight scenarios together with uncertainty (shaded areas) and the corresponding $\delta_{\text{Ti\_Couv}}$ ($\pm$1sd) (for a description on how uncertainty was assessed, refer to Appendix C). In general, at high T the compensation was negligible and the S profile was mainly proportional to the *RLD* profile (Fig. 4b, d, f, and h). The only exception was a soil with deep groundwater table and dry surface, where this dry layer limited root water uptake (DeDr_hT). At lower

transpiration demand, the S profile predicted by the Couvreur et al. (2012) model generally differed from the RLD profile (Fig.4a, c , e, and g) due to the fact that the second term of Eq. (1) (i.e., S_{comp}, see also Eq. (B4) and (B4') in Appendix B) was proportionally larger. Water uptake from the upper layer was always more than proportional to the RLD, when this layer was wetter, and vice versa. Water release to the soil (i.e., HR) was observed only for the soil with the deep groundwater table

5 and dry upper layer (DeDr_hT, Fig. 4e). From the graphical method GI, either a single or two distinct solutions for $\bar{z}$ (displayed as gray-shaded horizontal stripes) could be retrieved, depending on the monotonic/non-monotonic character of the δ_S profile, and ranged between –0.02 and –0.95 m.

Figure 5a displays the relative contribution to T of the upmost layer $0 - 0.225$ m in case of two conjoint soil layers as computed with the TM approach and a comparison with the results of the analytical model. Except for the very last two

10 virtual experiments with the deep groundwater table and wet upper layer at both low and high transpiration rates (i.e., DeWe_lT and DeWe_hT), there was a very good agreement between the analytical model and the two end-member mixing model: absolute difference between x_{Couv} and x_{TM} ranged between 1.5 % (ShDr_lT) and 6.3 % (ShDr_hT). For the experiment with the deep groundwater table and dry upper layer at low transpiration rate (DeDr_lT), the TM approach estimated that x was equal to 12.3 % while the analytical model simulated hydraulic redistribution, i.e., excluded the layer 0

15 – 0.225 m as potential source. The significant difference between results of the two models during experiments DeWe_lT and DeWe_hT and the higher standard error associated with x_{TM} (σ_x, displayed in the form of error bars in Figure 5) were due to the small difference between the isotopic compositions of the defined soil water sources $\delta_{S,I}$ (–6.0 ‰) and $\delta_{S,II}$ (–5.3 ‰) as illustrated in section §3.2.1. Figure 5b gives the relative contribution to T of the layer 1.75 –2.00 m in case of two disjoint soil layers, i.e., when not all potential water sources are accounted for into the calculation of $\delta_{S,I}$ and $\delta_{S,II}$. In this case there

20 were important disparities between x_{TM} and x_{Couv}. The mean absolute difference between these two estimates was equal to 43,5 ($\pm$17.8) %. Omitting some of the potential water sources contributing to T had in this second case the consequence of artificially overestimate the contribution of the lowest layer. We therefore suggest to always attempting to fully characterize the soil isotopic profile before aggregating the isotopic information when defining the two water sources.

Figure 6 gives the relative contributions from soil layers I, II, and III (upper, middle, and lower panel, respectively) to T

25 provided by IsoSource, the multi-source mixing model of Phillips and Gregg (2003) (x_{IsoS}, in %, displayed in the form of gray histograms) and by SIAR, the Bayesian method of Parnell et al. (2010) (x_{SIAR}, in %, gray probability density curves). The colored vertical lines are x_{I_Couv}, x_{II_Couv}, and x_{III_Couv}, the simulated $\dfrac{S(z)dz}{T/(\Delta x \cdot \Delta y)}$ ratios from layers I, II, and III. The color-shaded areas associated with x_{I_Couv}, x_{II_Couv}, and x_{III_Couv} refer to their uncertainty by accounting for the uncertainty of the input data. As for Fig. 5, δ_{Ti_Couv} is reported above each plot along with its standard deviation. x_{J_IsoS} probability

30 distribution was observed to be either narrow (e.g., for the experiment with the deep groundwater table and dry upper layer at low transpiration rate – DeDr_lT / layer I, Fig. 5m) or broad (e.g., for the experiment with the deep groundwater table and wet upper layer at high transpiration rate – DeWe_hT / layer I), i.e., the range of the possible solutions for x_{J_IsoS} was

relatively small or large (10 and 100 % respectively for these two examples). In general, both IsoSource and SIAR results were in good agreement: the x_{SIAR} most frequent value (MFV, at the peak of the density distribution curve) was in most cases either located near the median value of the x_{IsoS} probability range (e.g., for the experiment with the shallow groundwater table and wet upper layer at low transpiration rate – ShWe_lT / layer I, Fig. 5g) or matched exactly the x_{IsoS} unique value

5   (i.e., for the experiment with the deep groundwater table and dry upper layer at low transpiration rate – DeDr_lT / layer I, Fig. 5m). In contrast, the statistical methods succeeded best in providing x estimates similar to those of the model of Couvreur et al. (2012) in case of a shallow groundwater table and at low T only (Fig. 5a-c and g-i), thus when water availability was high and root compensation was low. In these cases, x_{I_Couv} was included in the estimated x_{I_IsoS} range and the mean absolute difference (MD) between x_{I_Couv} and x_{SIAR} MFV was equal to 8.6 %. This difference was the greatest

10   (129.2 %) for experiment DeDr_lT when hydraulic redistribution by the roots was simulated by the analytical model (Fig. 5m-o).

Considering eight soil layers instead of three added uncertainty in the assessment of their relative contribution to T as determined by IsoSource: the estimated probability ranges increased in most of the cases (results not shown). However it considerably improved the results of the bayesian model: the mean absolute difference between x_{I_Couv} and the most frequent

15   x_{SIAR} value was equal to 4.7 % for the scenarios with a shallow groundwater table and low transpiration rate and equal to 52.1 % in case of hydraulic redistribution by the roots (Table 3).

Independent of the number of defined soil layers, lowering the value of increment to 5 % in IsoSource refined the analysis where the probability distribution was already narrow (i.e., in the case of a well identified x_{IsoS} value, e.g., Fig. 5m) while it produced distributions that were flatter and contained less gaps when no clear solutions had emerged before (results not

20   shown). Artificially increasing the value of tolerance had the consequence that more solutions to Eq. (10) were found for each experiment / transpiration value / layer combinations and *vice versa* (results not shown). An increase or decrease of a factor 2 of the number of runs as well as the number of runs to be discarded from the analysis had only a marginal impact on the density distribution curves obtained with the SIAR model in the case of three or four soil layers.

The modelling exercise illustrated the disparities of outcome between the graphical method on the one hand and the

25   statistical and mechanistic methods on the other: there simply cannot be a single or multiple "root water uptake depths" but rather a continuous RWU profile or statistical solutions of contribution to transpiration (provided by IsoSource and SIAR). Significant changes of δ_{Ti} do not necessarily mean important changes in the depth of RWU but rather slight (nevertheless significant) modification of the RWU profile. The authors believe that the relatively novel statistical tools presented in this review should be therefore preferred over the graphical inference method, especially since the two former are available as

30   user-friendly programs and packages and do not require significant computing time, therefore can be run locally on a personal computer. As highlighted in this series of virtual experiments, the Bayesian method showed much more convincing results than the method of Phillips and Gregg (2003), especially in the case of eight soil layers, illustrating the interest of reaching the best vertical resolution and maximizing the number of identified potential sources.

One can also show from this inter-comparison of methods that labelling of soil water in either ^{18}O or ^{2}H has potentials for improving the different methods presented here theoretically if water is taken up by the roots from the labeled region predominantly. However this was never the case looking at the results of the analytical model. A dual isotope (^{18}O or ^{2}H) labelling pulse experiment that would artificially disconnect the strong link between δ^{18}O and δ^{2}H would on the other hand much more constrain the inverse problem and provide accurate estimate of contribution of S to transpiration flux.

5 CHALLENGES AND PROGRESSES

5.1 Soil and plant water sampling strategies

For determination of δ_S, soil profiles are usually destructively sampled, typically with an auger down to a depth of a few centimeters (Rothfuss et al., 2010) to a few meters (Moreira et al., 2000) (see Table 1), depending on the depths of the root system and of the water table. The sampling depth interval should, when possible, match the exponential decrease of isotopic composition (Wang et al., 2010) due to fractionating evaporation and it should capture sudden variations with time at the soil surface due to precipitation, i.e., be minimal at the surface and maximal deeper in the soil profile where isotopic dynamics are less pronounced. A minimal sampling interval at the surface is also crucial as it provides the isotopic composition of the layer contributing the most to transpiration in the case of a low T flux (e.g., morning transpiration) under non-limiting water availability. Not measuring this maximum soil isotopic composition (between precipitation event) can lead to a situation where source partitioning is not feasible from isotopic measurements. Under field conditions (i.e., ~95 % of the studies reviewed in this work, summarized in Table 1) soil material is generally not a limiting factor, thus can be sampled twice or thrice to average out or characterize lateral heterogeneity without significant disturbance of the soil (Leroux et al., 1995).

For grasses and nonwoody plants the root crown (e.g., Leroux et al., 1995), the aerial nodal roots (e.g., Asbjornsen et al., 2007), the meristematic petiole, or else the collars (e.g., tillers) at the base of the plant (e.g., Dawson and Pate, 1996; Sánchez-Perez et al., 2008) can be sampled for determination of δ_{Ti}. In the case of ligneous plants the fully suberized stem (Asbjornsen et al., 2007) or sapwood (e.g., White et al., 1985) is sampled. Special attention is to be given to remove any organ potentially transpiring that would have a water isotopic composition not representative of δ_{Ti}, e.g., dead material or the sheath of the oldest living leaf around tillers (Barnard et al., 2006; Durand et al., 2007). Non-conducting heartwood should discarded from conducting sapwood (White et al., 1985).

5.2 Off-line destructive versus on-line nondestructive isotopic measurements in plant and soil waters

Water from plant and soil materials is predominantly extracted by cryogenic vacuum distillation (Araguás-Araguás et al., 1995; Ingraham and Shadel, 1992; Koeniger et al., 2011; Orlowski et al., 2013; West et al., 2006). This consists in (i) introducing the plant or soil sample into an extraction flask attached to one end of the extraction line, while at the other end a collection tube is connected, (ii) freezing the sample by immersing the collection flask into liquid nitrogen (temperature ~ −

200°C), (iii) pumping the extraction line down to a pressure of $\sim 10^{-3}$ mbar, (iv) heating the sample to a certain temperature ($\sim 60 < T < \sim 100$°C) depending on its nature while immersing the trap into liquid nitrogen. The water vapor produced condenses in the trap following a stepwise procedure (~lasting one to a few hours), in order to avoid condensation elsewhere on the water vapor path between sample and collection trap. Accuracy of this extraction method was shown to be maximal at

5   higher water content and for sandy soils and lower for soils with high clay content (e.g., Koeniger et al., 2011; West et al., 2006). In the latter case, extraction times should be longer and temperatures higher to mobilize water strongly bound to clay particles, which has a distinct isotopic composition from that of pore "bulk" water (Araguás-Araguás et al., 1995; Ingraham and Shadel, 1992; Oerter et al., 2014; Sofer and Gat, 1972). In other studies, plant and soil waters are extracted following azeotropic distillation with kerosene as solvent (e.g., Brunel et al., 1995; Thorburn and Ehleringer, 1995), or direct

10   equilibration with CO_2 (Asbjornsen et al., 2007) following the method of Scrimgeour (1995), or liquid water – water vapor equilibration (Wassenaar et al., 2008), or else the mild vacuum method (Dawson and Pate, 1996; Jeschke and Pate, 1995). Certainly one of the main limitations of all isotopic approaches for quantifying RWU is the destructive character of isotopic sampling (see section 3.1) and associated offline analyses (sections 2.2 and 2.3). This usually leads to poor spatial (maximum a few cm^2) as well as temporal (minimum hourly) resolution of the inferred results, when comparing with

15   measuring frequency of other soil and plant state variables, e.g., soil water content and potential, and leaf water potential (section 3.2.2). In addition, one may question the representativeness of plant samples, in which tissues (and thus water) with very different water residence time is mixed. Similarly, given the expected high lateral and temporal variability of the hydraulic redistribution process, the representativeness of δ_S should be questioned for soils, in particular when combined with 1D models.

20   Recently developed methods take advantage of laser-based spectroscopy which allows on-line and continuous isotopic measurements in the gas phase. These methods rely on coupling a laser spectrometer (e.g., Wavelength-Scanned Cavity Ring-Down Spectroscopy – WS-CRDS, Picarro Inc., Santa Clara, CA, USA; Cavity-Ringdown Laser Absorption Spectroscopy – CRLAS and Off-Axis Integrated Cavity Output Spectroscopy - ICOS, Los Gatos Research, Los Gatos, USA) with specific soil gas sampling probes consisting of gas-permeable microporous polypropylene membranes or tubing. These

25   membranes or tubing exhibit strong hydrophobic properties while their microporous structures allow the intrusion and collection of soil water vapor. Several authors (Gaj et al., 2015; Gangi et al., 2015; Herbstritt et al., 2012; Oerter et al., 2016; Rothfuss et al., 2013; Sprenger et al., 2015; Volkmann and Weiler, 2014) could determine the soil liquid water isotopic composition in a nondestructive (yet invasive) manner from that measured in the collected soil water vapor considering thermodynamic equilibrium between vapor and liquid phase in the soil. In contrast to "traditional" isotopic methods, these

30   novel isotopic monitoring methods have also the distinct advantage of determining soil liquid water isotopic composition at very low water content, since water vapor, in contrast to soil liquid water, is not limiting for analysis. These novel methods allow a vertical resolution down to 1 cm and an approximately hourly time-resolution. However, they do not allow horizontal resolution along the tube and the laser spectrometers could be, as pointed out by Gralher et al. (2016) for the

specific case of a Picarro WS-CRDS greatly sensitive to the carrier gas used. In their opinion papers, McDonnell (2014) and Orlowski et al. (2016) also urged for a comparison between methods, which was addressed by Pratt et al. (2016).

Leaf and plant gas chamber systems provide indirect means for a nondestructive determination of δ_{Ti} when assuming full steady-state conditions at the evaporative sites of the leaves ($\delta_{Ti} = \delta_T$) (e.g., Volkmann et al., 2016b). In the coming years, effort should be made towards developing novel methods for a direct and nondestructive determination of δ_{Ti} based on the use of gas-permeable membranes, which was recently initiated for trees (Volkmann et al., 2016a). This should be further investigated to test applicability to other (non-woody) plant species. This will imply the major challenge of not disrupting the water columns in the active xylem vessels when installing such a membrane-based system. Another potential issue to be investigated is the species-specific extent of water exchange between xylem and phloem conductive tissues which might lead to isotopic "contamination" of the xylem sap water (Farquhar et al., 2007).

5.3 Isotopic assessment of hydraulic redistribution

Hydraulic redistribution has been observed using isotopic measurements in a number of studies (e.g., Caldwell and Richards, 1989; Dawson, 1993; Kurz-Besson et al., 2006). However, in contrast to nondestructive "traditional" methods allowing for direct monitoring of redistribution dynamics (i.e., psychrometry, time domain reflectometry, and frequency domain capacitance, Brooks et al., 2002; Dawson, 1996; Richards and Caldwell, 1987; Wan et al., 2000), isotopic methods provide a destructive and indirect assessment. These methods are based on (i) labelling of soil or roots of deep-rooted plants at a given depth in the soil or at a certain location in the experimental field and (ii) measuring the δ_{Ti} of plants not having access to labeled water (i.e., of which the roots do not reach the isotopic labeled depth or location). When hydraulic redistribution occurs, the xylem sap water (of measured isotopic composition $\delta_{Ti,m}$) of these plants can be conceptualized as a mixture of antecedent soil water (at natural isotopic abundance) and isotopically enriched water released to the soil by the deep-rooted vegetation. From simple mass balance at the release location, δ_S at a given depth z in the soil and at time ($t+\Delta t$) deviates from that at time t as a function of the (negative) S (i.e., hydraulic redistribution) at time t and change of soil volumetric water content (θ):

$$\delta_S(z, t+\Delta t) \cdot \theta(z, t+\Delta t) - \delta_S(z,t) \cdot \theta(z,t) = \delta_{Ti,m} \cdot V_{HR}(t)/V(z) = \delta_{Ti,m} \cdot |S(z,t)| \cdot \Delta t \qquad (11)$$

If θ and δ_S at times t and $t+\Delta t$, and δ_{Ti} are measured, the water volume lifted by the roots (V_{HR}, L^3) can be calculated knowing the volume of soil representative of the hydric and isotopic measurements (V, L^3). Note that hydraulic redistribution is observable at a certain soil depth if and only if uptake and release locations in the soil have distinct water isotopic compositions. Finally, the obtained volume can be compared with the water volume transpired by the vegetation on the following day.

To the authors' knowledge, no precise observation (other than the study of Zegada-Lizarazu and Iijima, 2004) of change of soil water isotopic composition has been attributed with certainty to hydraulic redistribution and simultaneously provided amount of water involved in the process. Such observations however should be feasible under controlled experimental

conditions where (i) the initial soil water isotopic profile before labelling is known and (ii) natural isotopic changes (due to, e.g., soil redistribution and moisture input from a precipitation event) can be avoided, and (iii) the lateral heterogeneity of soil water and isotopic composition profiles can be minimized (see for instance the setups of Armas et al., 2012; Querejeta et al., 2012). As highlighted in section 2.3, hydraulic redistribution can be conceptualized as a negative S (Eq. (4b)) and should

5 therefore be exempt of isotopic fractionation. However, to the authors' knowledge this point has not yet been proven experimentally.

5.4 Call for a coupled experiments-modelling approach for determination of plant water sources on the basis of isotopic data

In order to fully benefit from the potential of water stable isotopic analysis as tools for partitioning transpiration flux, the
10 authors call for the development of approaches making use of physically based models for RWU and isotopic fractionation to analyze experimental data, especially since several soil-vegetation-atmosphere transfer (SVAT) models are available that can simulate flow of isotopologues in the soil and the plant (i.e., SiSPAT-Isotope, Braud et al., 2005; Soil-litter-iso, Haverd and Cuntz, 2010; TOUGHREACT, Singleton et al., 2004; HYDRUS, Sutanto et al., 2012).

To the authors' knowledge, there are only a few studies which attempted to do so. An example is the work of Rothfuss et al.
15 (2012) who ran an experiment under controlled laboratory conditions where they measured on four dates (corresponding to four different stages of vegetation and therefore root development) soil water potential and isotopic composition profiles, and root length density distribution profiles. In their experiment, the isotopic composition of transpiration was also known. The authors used a global optimization algorithm to obtain the set of parameter of SiSPAT-Isotope that best reflected the experimental dataset. Distributions of RWU could be determined on these four dates. Also, in the study of Mazzacavallo and
20 Kulmatiski (2015), the RWU model of HYDRUS could also be parameterized during a labelling (heavy water ^2H_2O) pulse experiment on the basis of measurements of xylem water hydrogen and oxygen isotopic compositions. This provided insights into the existence of niche complementarity between tree (mopane) and grasses species. Note however that this HYDRUS version did not incorporate isotopic transport through the soil and the roots.

Another example is the work of Ogle et al. (2004) who could reconstruct active root area and RWU profiles from isotopic
25 measurements using the 1D analytical macroscopic model of Campbell (1991) in a Bayesian framework (root area profile and deconvolution algorithm – RAPID). By assuming normal a priori distributions for the xylem water oxygen and hydrogen isotopic compositions and considering prior knowledge on RWU distribution (i.e., synthetic information based on measurements of other studies), Ogle et al. (2004) obtained a posteriori distributions of x of a desert shrub (Larrea tridentate).

30 Simple analytical models, such as the formulation of Couvreur et al. (2012), can be applied and confronted with isotopic data. In comparison with statistical tools, such physical models provide profiles with high spatial resolution and lower uncertainty, on the condition that all required (isotopic) data is available. We recognize that in comparison with the statistical

and conceptual methodologies presented in this review, using a physical (analytical or numerical) model implies the measurements of additional state variables to be fed as input to the model, and of one parameter (K_{plant}) (when considering the assumption $K_{plant} = K_{comp}$ valid, see Appendix B). Some of these variables are laborious to obtain (e.g., *RLD*) or not straightforward to measure (H_S, H_L, and *T*) – especially in the field – but are mandatory to be able to determine contributions

5   to *T* across a set of identified water sources. In addition, they are necessary to gain insights into soil-plant interactions, e.g., dynamics of root function (active versus non-active roots in the soil profile) in water uptake and thus quantify the disconnection between measured *RLD* and the prognostic variable *SSF* (see Appendix B1). For doing this, controlled conditions in state-of-the-art climatic chambers are ideal, as they allow reducing the inherent spatial heterogeneity present under natural conditions and, thus, the deconvolution of environmental effects on RWU. Experimental facilities that not only

10   control atmospheric forcing (soil upper boundary conditions for latent and heat flow), but impose lower boundaries for the soil compartment (e.g., drainage and capillary rise dynamics) and provide means to close the hydrological balance are required. Moreover, macrocosm experiments (~m^3 scale) should be favored over mesocosm (~dm^3 scale) experiments to avoid or reduce inherent side effects that would ultimately hamper mimicking natural conditions.

6 CONCLUSION

Root water uptake is a key process in the global water cycle. More than 50% of total terrestrial evapotranspiration crosses plant roots to go back to the atmosphere (Jasechko et al., 2013). Despite its importance, quantification of root water uptake remains difficult due to the opaque nature of the soil and the spatial and temporal variability of the uptake process.

5 Water stable isotopic analysis is powerful and valuable tool for the assessment of plant water sources. In an inverse modelling framework, isotopic analysis of plant tissues and soil also allow for obtaining species-specific parametrization of physically based analytical and numerical RWU models. They provide at the plant scale a unique way to tackle the difficulty of disentangling actual RWU profiles with root traits and characteristics.

In this review we tried to highlight the importance of systematically reporting uncertainties along with estimates of
10 contribution to T of given plant water sources. The inter-comparison exercise could quantify the impact of the definition of the plant water sources (i.e., whether they are spatially disjoint or not and whether their isotopic compositions values are significantly different or not) on the outcome of the two end-member mixing model. The inter-comparison also illustrated the limitations of the graphical inference method and the multi-source mixing model of Phillips and Gregg (2003), whereas it underlined the performance of the Bayesian approach of (Parnell et al., 2010), which uses a more rigorous statistical
15 framework, if the number of considered water sources matches the number of isotopic measurements in the soil profile. However, contrary to the analytical model none of the graphical and statistical methods could locate and quantify hydraulic redistribution of water.

Finally, the authors call for (i) the further development of nondestructive and on-line isotopic measurement methodologies to circumvent the necessity of sampling soil material and plant organs destructively, and (ii) a generalization of coupled
20 approaches relying on the confrontation between labelling experiments under controlled conditions and three dimensional RWU numerical modelling.

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AUTHOR CONTRIBUTION

Y. Rothfuss reviewed the published literature. Y. Rothfuss and M. Javaux designed the virtual experiments, analysed, and discussed the obtained results.

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Table 1

Table 1: Summary of the reviewed studies that use one of either the three methods (graphical inference), two-end members mixing model, and "multi-source mixing models) for plant water sources partitioning.

Authors	Experimental conditions (field: F / laboratory: L)
Graphical inference	
Leroux et al., 1995	F (tropical)
Thorburn and Ehleringer, 1995	F (semi-arid; cold desert, subhumid)
Weltzin and McPherson, 1997	F (temperate semi-arid savanna)
Jackson et al., 2009	F (tropical forest / savanna)
Moreira et al., 2000	F (eastern Amazon)
Chimner and Cooper, 2004	F (desert)
Kulmatiski et al., 2006	F (shrub-steppe)
Li et al., 2007	F (cold continental-semiarid)
Wang et al., 2010	F (warm temperate / monsoon climate)
Stahl et al., 2013	F (tropical)
Two-end members mixing model	
White et al., 1985	F (temperate)
Dawson and Ehleringer, 1991	F (riparian zone)
Brunel et al., 1995	F (aeolian sand dune)
Dawson and Pate, 1996	F (mediterranean)
McCole and Stern, 2007	Field conditions (subtropical)
Goebel et al., 2015	F (semi-arid)
Multi-source mixing model	
Asbjornsen et al., 2007	F (cornfield, prairie, oak savanna, and woodland)
Wang et al., 2010	F (warm temperate / monsoon climate)
Huang and Zhang, 2015	F (desert)
Prechsl et al., 2015	F (temperate)
Volkmann et al., 2016	F (temperate)

Plant species

Hyparrhenia diplandra, Andropogon schirensis, Imperata cylindrica (grasses), Cussonia barteri, Crossopteryx febrifuga, Bridelia ferruginea (shrubs)

Eucalyptus largiflorens, camaldulensis, Acer negundo and grandidentatum, Atriplex canescens, Chrysothamnus, nauseosus, Vanclevea stylosa

Quercus emoryi Torr., Trachypogon montufari (H.B.K.) Nees. (deciduous) Qualea grandiflora Mart., Q. parviflora Mart., Kielmeyera coriacea (Spr.) Mart., Pterodon pubescens Benth., Dalbergia myscolobium Benth. / (evergreen) Didymopanax macrocarpum, Sclerolobium paniculatum, Miconia ferruginata, Roupala montana

(invasive) Solanum crinitum Lamb. (native) Panicum maximum Jacq.

Sarcobatus vermiculatus, Chrysothamnus nauseosus, and Chrysothamnus greenei

(invasive) Centaurea diffusa, (native) Pseudoroegneria spicata; Bromus tectorum L.

Larix sibirica

Summer corn and cotton (species not specified)

tropical rainforest trees

Taxodium distichum / Pinus strobus

Acer grandidentatum Nutt., A negundo L., Quercus gambelii Nutt.

"Mallee Tree" (Eucalyptus sp.)

Banksia prionotes, Dryandra sessilis, Grevillea (species unknown)

Juniperus ashei

Gossypium hirsutum L.

Quercus macrocarpa, Umus americana L. (trees), Zea mays L. (crop), and Andropogon gerardii (grass)

Summer corn and cotton (species not specified)

Caragana korshinskii and Artemisia ordosica

Phleum pratense, Lolium multiflorum, Poa pratensis, Taraxacum officinale, Trifolium repens, Rumex obtusifolius, Trisetum flavescens, Phleum rhaeticum, Carum carvi, and Achillea millefolium, Rumex alpestris, Taraxacum officinale and Trifolium, pratense. Quercus petraea and Fagus sylvatica

SAMPLES FOR ISOTOPIC MEASUREMENTS

Soil profile (soil depth: SD [m] / increment: l [m] / number of profiles: Nps / Replicates)	Roots profile (soil depth: SD [m] / increment: l [m] / number of profiles: Npr)
$0.10 < SD < 2.00 / 0.01 < l < 0.10 / R = 3$	$SD = 1.80 / l = 0.10 / R = 16$
No profiles, but soil directly surrounding roots are sampled	No profiles, but single roots sampled after excavation
$SD = 1.50 / l = 0.05 / 3 < R < 4$	none
$SD = 5.00$ (depending on the site) $R = 2 / 0.05 < l < 0.20$	none
$4 < SD < 6 / 0.05 < l < 2.00$	$SD = 4 / 0.25 < l < 0.50 / R = 3$
(1st campaign) $SD = 0.6 / l = 0.10 / R = 2$ at each site / $4 < Nps$ (per site) $< 6 /$ (2nd campaign) $SD = 2.1 / 0.2 < l < 0.30 / Nps = 10$	none (reference made to Cooper and Chimner, unpublished data)
$1.05 < SD < 2.20 / 0.10 < l < 0.60$	$1.20 < SD < 2.20 / l = 0.15$
$SD = 1.00 / 0.05 < l < 0.30$	none
$SD = 1.50 / 0.05 < l < 0.30 / Nps = 7$	none
$SD = 2.00 / 0.20 < l < 0.30 / R = 6$	none (reference made to other literature)
none	none
$SD = 0.50$	none
$50 < SD < 4.2 / 0.10 < l < 0.25$	none (qualitative observation)
none (sand around the root system)	none (reference made to other literature)
$SD = 0.30 / 0.05 < l < 0.10 / 4 < Nps$ (per site) < 5	none
$SD = 0.3 / l \sim 0.02 / Nps = 4$ per irrigation treatment	
$1.40 < SD < 2.00 / 0.05 < l < 0.20 / R=2$ per site	none
$SD = 1.50 / 0.05 < l < 0.30 / Nps = 7$	none
$SD = 2.00 / 0.05 < l < 0.50$	none
$0.30 < SD < 0.40 / 0.04 < l < 0.10 / R = 3$	$SD = 0.30 / 0.075 < l < 0.125 / 6 < Npr < 7$
$SD = 0.60 / 0.05 < l < 0.10 / Nps > 17$	$SD = 0.60 / 0.05 < l < 0.10 / R = 4$

Natural Abundance: NAb / labelling Experiment: LE	Water extraction method (cryogenic vacuum distillation: Cr / azeotropic distillation Az / direct equilibration: GI / mild vacuum distilation: Mi)	Plant (organ: O / number of samples: Ns / replicates: R / temporal resolution: TR [hrs])
Nab	Cr	O: sapwood trunks (shrub), Crown (grasses) / 8 < Ns < 24 / 0.5 < TR < 1
Nab	Cr / Az	O: non-green, suberised stems / Ns = 3
Nab	Cr	O: stem with phloem tissue / R = 4 (tree and sapling) / O: stem without green tissue / R = 4 (seedling) / O: culm base with sheaths removed / 3 < R < 4
Nab	Cr	O: wood or suberized, mature, stem segments (outer bark and phloem are removed) / R = 2
LE	Cr	O: well suberized stems (trees) / thick fleshy culms covered with dry leaves (grass) / 3 < R < 5
Nab	Cr	O: fully suberized stem sections from the base of plants / (1st campaign, S. Vermiculatus) 4 < Ns (per site) < 8 / R = 3 / (1st campaign, C. nauseosus) 5 < Ns (per site) < 6 / R = 3 / (2nd campaign) 3 < Ns < 5 (depending on the species)
Nab	Cr	O: stem
Nab	Cr	O: stem / Ns (per sampling date) = 5
Nab	Cr	O: stems (epidermis contacted with air was removed) / Ns = 7 / TR = 1 per vegetation stage
LE	Cr	O: branch (length = 0.07 m; diameter = 0.1 – 0.3 m). Bark tissue is immediately removed
Nab	none (Sap flow water)	O: wood samples taken at breast height
Nab	Cr	O: mature suberized stems
Nab	Az	O: twigs (bark is removed) / R = 2
Nab	Mi	O: roots, trunks, stem base
Nab	Cr	O: stem
Nab	Cr	O: meristematic petiole reduced in size to 5mm. / 14 < Ns < 17 (depending on the irragtion treatment) / R = 2 / TR = 1
Nab	Di	O: stem (trees); aerial nodal roots just above the soil surface (Zea mays L.); stem (grass) / Ns = 2 per specie
Nab	Cr	O: stems (epidermis contacted with air was removed) / Ns = 7 / TR = 1 per vegetation stage
Nab	Cr	O: twigs (1–2 cm of stem with bark immediately removed) / R = 3
NAb	Cr	O: root crown / 2 < Ns < 10
LE	none	none (measurement of transpiration isotopic composition)

Single: S / Dual: D / "Double Single": DS isotope approach
S (δ^{18}O)
S (δ^{2}H)
D
S (δ^{2}H)
S (δ^{2}H)
S (δ^{18}O)
DS
DS
DS
D
S (δ^{2}H)
S (δ^{2}H)
D
S (δ^{2}H)
S (δ^{18}O)
S (δ^{18}O)
S (δ^{18}O)
DS
DS
DS
S (δ^{2}H)

Main results (RWU depth: z_{RWU} [m] / soil depth z [m] / fraction of *transpiration*: x / Source: So)

$0.00 < z_{RWU} < 0.05$ (grasses, early morning) / $0.05 < z_{RWU} < 0.10$ (grasses, midday) / $z_{RWU} = 0.30$ or $z_{RWU} > 1.50$ (shrubs, no unique solution)

Dominant source: groundwater (Mountain and floodplain) / $0.3 < z_{RWU} < 0.4$ (cold desert)

$z_{RWU} > 0.50$ (trees and sapling) / $z_{RWU} < 0.15$ (2-months-old seedling) / $0.20 < z_{RWU} < 0.35$ (1- and 2-year-old seedlings and grasses)

$z_{RWU} < 2.00$ (four evergreen and one deciduous species) / $z_{RWU} > 2.00$ (three deciduous and one evergreen species)

Fraction of root water uptake from the labeled region $x = 0.20$ (Solanum crinitum Lamb.) $z_{RWU} < 1.00$ (P. maximum Jacq.)

$0.00 < z_{RWU} < 0.50$ (C. nauseosus, pre-monsoon and monsoon periods) / (S. vermiculatus and C. nauseosus) dominant source: groundwater (pre-monsoon period) and switched to precipitation recharged water ($0.30 < z_{RWU} < 0.40$, during mansoon).

$z_{RWU} = 0.15$ (Bromus tectorum L., early season) / $z_{RWU} = 1.20$ (Centaurea diffusa Lam, late season)

$0.10 < z_{RWU} < 0.40$ from $\delta^{18}O$ meas / $0 < z_{RWU} < 0.80$ from δ^2H meas $z_{RWU} = 0.10$ (Corn, jointing stage) / $z_{RWU} = 0.50$ (Corn, flowering stage) /

$z_{RWU} = 0.10$ (Corn, full ripe stage) / $z_{RWU} = 0.40$ (Cotton, seedling stage) / $z_{RWU} = 0.50$ (Cotton, bud stage) / $z_{RWU} = 1.10$ (Cotton, bolls open stage) / More diffuse z_{RWU} for shorter trees

$z_{RWU} > 1.00$ (dry periods, tall trees) /

Groundwater: $0.46 < x < 0.64$ wet site) / $0.16 < x < 0.25$ (intermediate site)

So: Groundwater (streamside mature trees) / So: stream water (younger streamside trees) / So: Precipitation (younger non-streamside trees)

$0 < z_{RWU} < 0.4$ with $0.7 < x < 0.9$ (1st site) / $0 < z_{RWU} < 1.5$ with $x = 0.07$ (2nd site)

So: deeper soil layers (dry season) / So: shallow soil layers (wet season)

$z_{RWU} > 0.30$ m (hot, dry summer) / $0.10 < z_{RWU} < 0.30$

Evidence for shifting to rainwater predominantly

$0.0 < z_{RWU} < 0.20$ m with $x < 45\%$ and $x < 36\%$ (crop and grass, resp.) / $0.00 < z_{RWU} < 0.20$ m with $x < 40\%$ and $x < 20\%$ and $z_{RWU} > 0.60$ m with $x < 60\%$ and $x < 80\%$ (Q. macrocarpa and U. americana L., resp.).

$0 < z_{RWU} < 0.20$ with $96 < x < 99$ (Corn, jointing stage) / $0.20 < z_{RWU} < 0.50$ cm with $58 < x < 85$ (Corn, flowering stage), $0.00 < z_{RWU} < 0.20$ with $69 < x < 76$ (Corn, full ripe stage) // $0.00 < z_{RWU} < 0.20$ with $27 < x < 49$ (Cotton, seedling stage), $0.20 < z_{RWU} < 0.50$ cm with $79 < x < 84$ (Cotton, bud stage) / $0.50 < z_{RWU} < 0.90$ with $30 < x < 92$ (Cotton, blooming stage) / $z_{RWU} > 90$ cm with $69 < x < 92$ (Cotton, boll open stage)

$0.1 < z_{RWU} < 1.0$ (wet seasons) / z_{RWU} not affected by small rainfall events / $z_{RWU} > 1.00$ with x observed to increase from $2 (\pm 0.7)$ to $10 (\pm 1.4)$ % for both plants after large rainfall event.

$0 < z_{RWU} < 0.10$ with $0.43 < x < 0.68$ (Drought treatment) / $0 < z_{RWU} < 0.10$ with $0.04 < x < 0.37$ and $0.20 < z_{RWU} < 0.35$ with $0.29 < x < 0.48$ (control treatment)

constant RWU depth profile with $0.15 < x < 0.18$ (beech) / $x < 0.15$ for $z < 0.20$ and $0.15 < x < 0.25$ for $z > 0.30$ (beech/oak mixture and oak monoculture)

Table 2

SOIL DATA	Shallow groundwater table (Sh)						Deep groundwater table (De)						RLD (cm cm^{-3})
z (m)	Dry surface conditions (ShDr)			Wet surface conditions (ShWe)			Dry surface conditions (DeDr)			Wet surface conditions (DeWe)			
	θ (cm^3 cm^{-3})	H_S (cm)	δ_S (‰)	θ (cm^3 cm^{-3})	H_S (cm)	δ_S (‰)	θ (cm^3 cm^{-3})	H_S (cm)	δ_S (‰)	θ (cm^3 cm^{-3})	H_S (cm)	δ_S (‰)	
-0.01	0.235	-454	5	0.372	-2	-7	0.044	-9875	11	0.372	-51	-5	6.0
-0.03	0.325	-267	3	0.372	-8	-6	0.055	-3581	7	0.371	-77	-5,5	3.0
-0.07	0.347	-215	1	0.372	-11	-5	0.081	-1661	1	0.372	-14	-7	2.0
-0.15	0.360	-179	-4	0.372	-70	-6	0.105	-1165	-3,5	0.135	-869	-3,5	0.8
-0.30	0.367	-155	-6	0.370	-125	-6,5	0.122	-989	-4	0.134	-889	-4	0.5
-0.50	0.371	-135	-7	0.371	-135	-7	0.165	-730	-5	0.165	-730	-5	0.4
-1.00	0.372	-125	-7	0.372	-125	-7	0.210	-620	-6	0.210	-620	-6	0.3
-2.00	0.372	-125	-7	0.372	-15	-7	0.259	-600	-7	0.259	-600	-7	0.2
PLANT DATA	T (mm h^{-1})	H_L (cm)		T (mm h^{-1})	H_L (cm)		T (mm h^{-1})	H_L (cm)		T (mm h^{-1})	H_L (cm)		
IT	0.01	-587		0.01	-491		0.01	-2347		0.01	-918		
hT	0.30	-12330		0.30	-12234		0.30	-14090		0.30	-12661		

Table 2: Soil, plant, and isotopic synthetic input data for the different modelling approaches (depth (z) profiles of soil water content θ, total soil water potential H_S, soil water oxygen isotopic composition δ_S, root length density RLD, transpiration rate T, and leaf water potential H_L) "collected" during eight virtual experiments differing in the depth of the groundwater table (Shallow –Sh / Deep – De) and the water status at the soil surface (Dry – Dr / Wet – We).

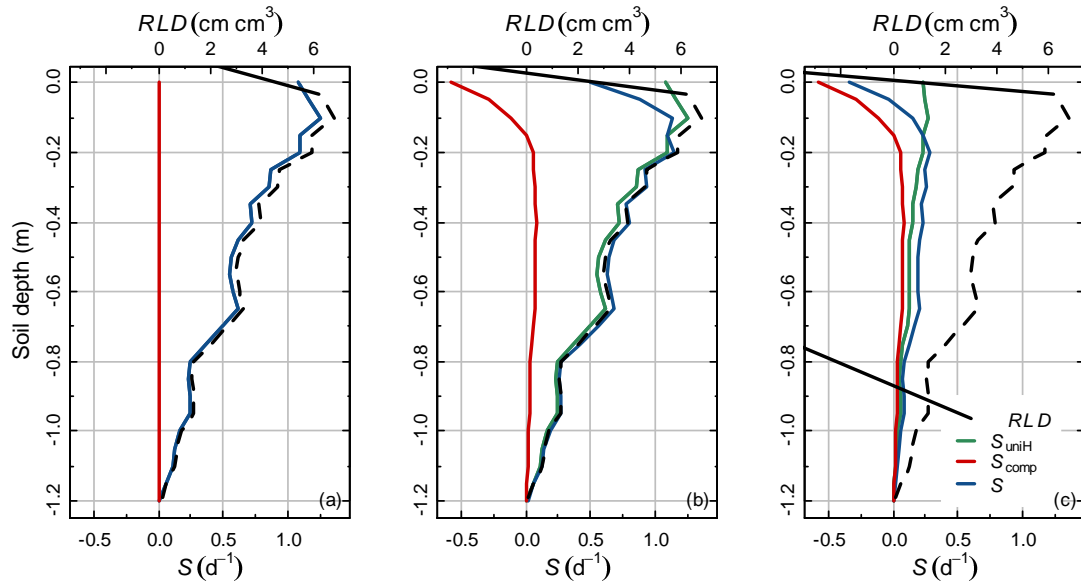
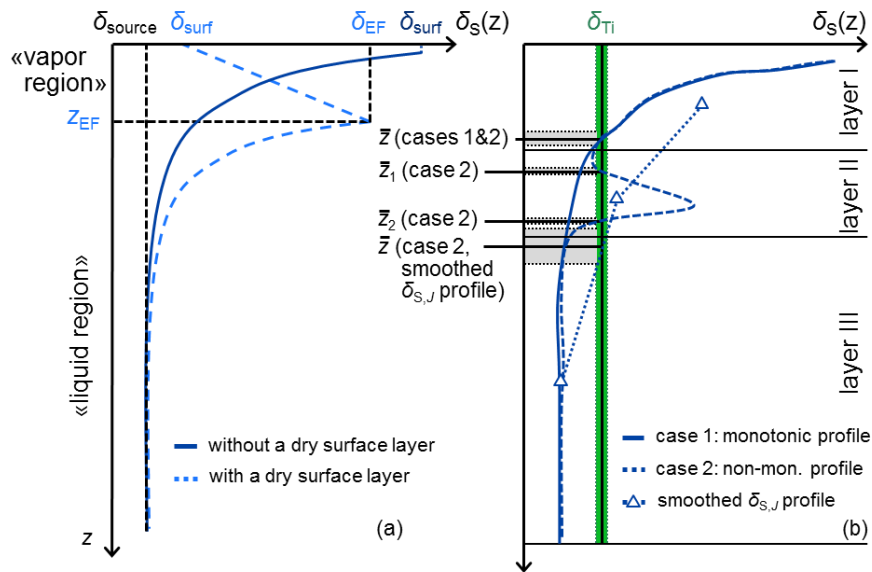
1   **Table 3**

Soil layer (m)	Shallow groundwater table (Sh)							
	Dry surface conditions (ShDr)				Wet surface conditions (ShWe)			
	Low T (ShDr_lT)		High T (ShDr_hT)		Low T (ShWe_lT)		High T (ShWe_hT)	
	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)
0-0.02	1(0-35)	5(1)	6(0-37)	11(1)	18(0-48)	13(1)	16(0-53)	11(1)
0.02-0.05	1(0-35)	7(1)	5(0-38)	9(1)	13 (0-42)	10 (1)	7 (0-43)	9(1)
0.05-0.11	3(0-41)	11(1)	10(0-48)	11(1)	11(0-41)	13(1)	7 (0-41)	11 (1)
0.11-0.225	15(0-57)	10(1)	14(0-47)	10(1)	11 (0-46)	10(1)	3 (0-43)	10(1)
0.225-0.4	19(0-57)	11(0)	16(0-55)	10(0)	16(0-53)	9(0)	16 (0-49)	10(0)
0.4-0.75	16(0-55)	16(0)	17(0-48)	14 (0)	18(0-44)	13(1)	15 (0-48)	14 (0)
0.75-1.5	17(0-52)	27(2)	18(0-46)	23 (2)	16(0-48)	21(2)	16 (0-53)	23(2)
1.5-2	17(0-59)	14(2)	17(0-47)	12(2)	15 (0-52)	11(2)	16(0-51)	12(2)

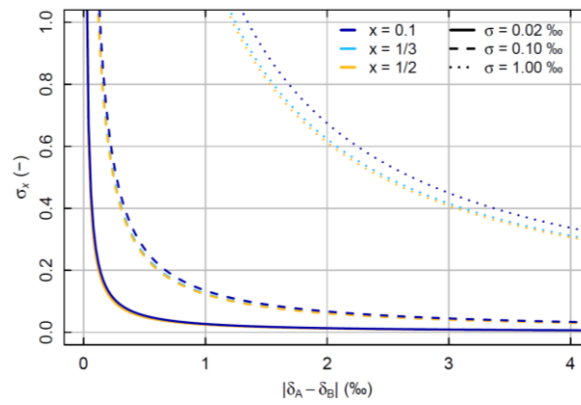
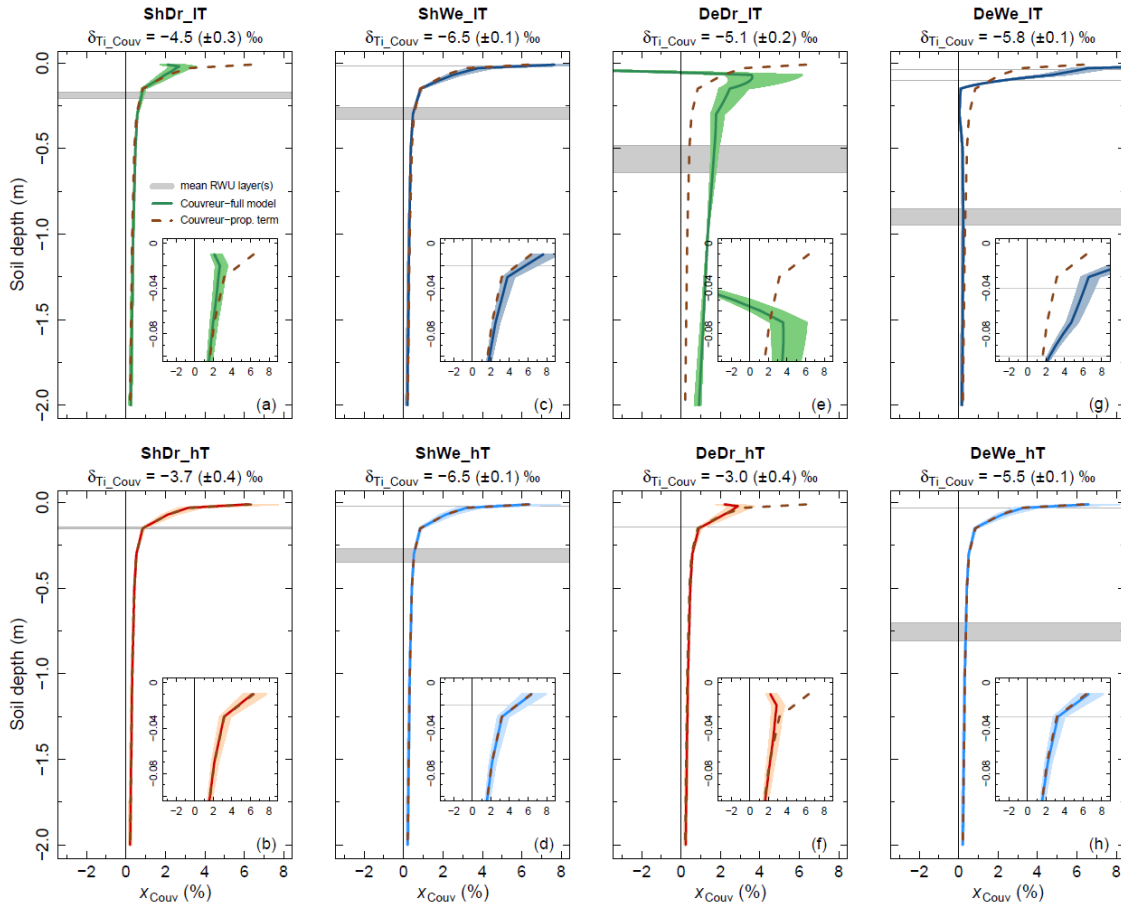
Soil layer (m)	Deep groundwater table (De)							
	Dry surface conditions (DeDr)				Wet surface conditions (DeWe)			
	Low T (DeDr_lT)		High T (DeDr_hT)		Low T (DeWe_lT)		High T (DeWe_hT)	
	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)	x_{SIAR} mfv(range) (%)	x_{Couv} (1sd) (%)
0-0.02	1(0-42)	-170(16)	1(0-41)	5(1)	2(0-49)	24(2)	10(0-52)	11(1)
0.02-0.05	1(0-42)	-17(1)	2(0-45)	8(1)	17(0-55)	18 (2)	13(0-54)	9(1)
0.05-0.11	1(0-44)	19(6)	5(0-47)	12(1)	16 (0-58)	21(2)	16(0-51)	12(1)
0.11-0.225	3(0-55)	28(5)	11(0-51)	10 (1)	1(0-39)	3(0)	12(0-43)	12(1)
0.225-0.4	7(0-75)	33 (4)	17(0-51)	11(0)	0,8(0-38)	1 (0)	9(0-38)	10(0)
0.4-0.75	15(0-68)	57 (3)	17(0-56)	16(0)	5(0-46)	7(1)	15(0-53)	14 (0)
0.75-1.5	16(0-74)	98(1)	16(0-54)	26(2)	16(0-51)	17(2)	16(0-45)	23(2)
1.5-2	17(0-76)	51 (4)	18(0-53)	13 (2)	18(0-53)	9 (2)	16(0-46)	12(2)

Table 3: Most frequent value (mfv) and range of the density distribution curve of the relative contribution to transpiration across eight defined soil layers as determined by the Bayesian method of Parnell et al. (2010) (x_{SIAR}, %) and mean relative contribution (with standard deviation) provided by the analytical model of Couvreur et al. (2012) (x_{Couv}, %). Profiles of relative contribution were computed for eight soil-plant virtual experiments differing in the depth of the groundwater table (shallow – Sh / deep –De), the soil surface water status (dry – Dr / wet – We), and the plant transpiration rate (low – lT / high – hT).

1 **FIGURES**

2 **Figure 1**

4 **Figure 1: Some examples of root water uptake sink term (S, in d^{-1}) profiles (blue lines) conceptualized as the sum of**
5 **S_{uniH} (green lines), the root water uptake term proportional to root length density *RLD* (dotted black line) and the**
6 **compensatory root water uptake (S_{comp}, red lines). (a) $S_{comp} = 0$ (no root compensation) leading to $S = S_{uniH}$. (b) $S_{comp} \neq$**
7 **0 and becomes negative towards the surface but remains smaller (in absolute term) than S_{uniH}. (c) $S_{comp} \neq 0$ and is**
8 **negative at the surface while is greater than S_{uniH} for z > −0.08 m. In the last case, S is negative at the surface, meaning**
9 **hydraulic lift is observed.**

1   **Figure 2**

3   Figure 2: (a) Simulated soil water isotopic composition (δ_S) profiles for a water saturated (dark blue line) and
4   unsaturated (light blue dotted line) soil following Barnes and Allison (1983). Indices "surf" and "EF" refer to soil
5   surface and Evaporation Front. "vapor" and "liquid" regions refer to soil regions where water flow occurs
6   predominantly in the liquid and vapor phase, respectively. (b) Illustration of the "graphical inference" method for
7   determining the "mean root water uptake depth" ($\bar{z}$). "Ti" stands for the sap xylem water at the plant tiller. Case 1:
8   one unique solution is found; case 2: more than one solution is found. Smoothed profile is designated by the symbols).
9    $\bar{z}$ range is indicated by the gray horizontal stripes.

1 **Figure 3**

3 Figure 3: Standard error (σ_x) associated with the estimation of the relative contribution (x) of source A water to root
4 water uptake in case of two distinct sources (A and B of isotopic compositions δ_A and δ_B). Three x values (0.1, dark
5 blue color; 1/3, light blue; ½, orange) and three values of standard errors associated with sampling and measurement
6 of δ_A, δ_B, and of the isotopic composition of the tiller sap water (δ_{Ti}) (0.02, solid line; 0.10 and 1.00, dashed lines) are
7 tested.

1 **Figure 4**

3 **Figure 4: Simulated depth (z, in m) profiles of x_{Couv} (%) (solid colored lines), the simulated relative contributions to**
4 **transpiration provided by the model of Couvreur et al. (2012) for experiments "ShDr" (soil with shallow groundwater**
5 **table and relatively dry soil surface), "ShWe" (soil with shallow groundwater table after a rainfall event)", "DeDr"**
6 **(soil with deep groundwater table with relatively dry soil surface), "DeWe" (soil with deep groundwater table and wet**
7 **soil surface). Suffices "lT" and "hT" refer to "low" and "high" transpiration rate simulations. The color-shaded areas**
8 **depict the uncertainty of the model estimates on the account of the precision of the measurements. The horizontal**
9 **gray-shaded areas delimit the mean root water uptake layer(s) obtained by the "graphical inference" method. At the**
10 **bottom right corner of each plot is a detail presented for $z \geq -0.10$ m. Finally, results from the first term of the model**
11 **of Couvreur et al. (2012) which considers uptake proportional to root length density is plotted as a dashed brown line**
12 **for comparison. Note that negative x_{Couv} means hydraulic redistribution by the roots.**

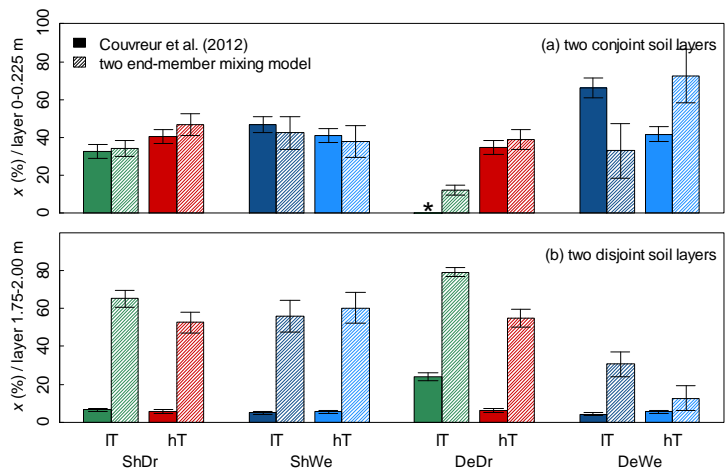



Figure 5

Figure 5: Comparison between relative contributions to transpiration (x, in %) simulated by the analytical RWU model of Couvreur et al. (2012) and the two end-member mixing model in case of two defined soil layers. Figure 5a displays x from the topmost soil layer (0 – 0.225 m) in case of a two conjoint soil layers (0 – 0.225 m and 0.225 – 2.00 m) whereas Figure 5b displays x from the lowest soil layer (1.75 – 2.00 m) in case of a two disjoint soil layers (0 – 0.225 m and 0.225 – 2.00 m), i.e., information on soil water isotopic composition is lacking between 0.225 and 1.75 m. "Sh" ("De") stands for the virtual experiments where the soil has a shallow (deep) groundwater table while "Dr" and "We" stand for when the soil is dry or wet at the surface. Suffices "lT" and "hT" refer to "low" and "high" transpiration rate simulations. "*" refers to when hydraulic redistribution is simulated by the analytical model, leading to a negative x. Error bars are either one standard deviation (for the RWU analytical model) or one standard error (for the TM approach).

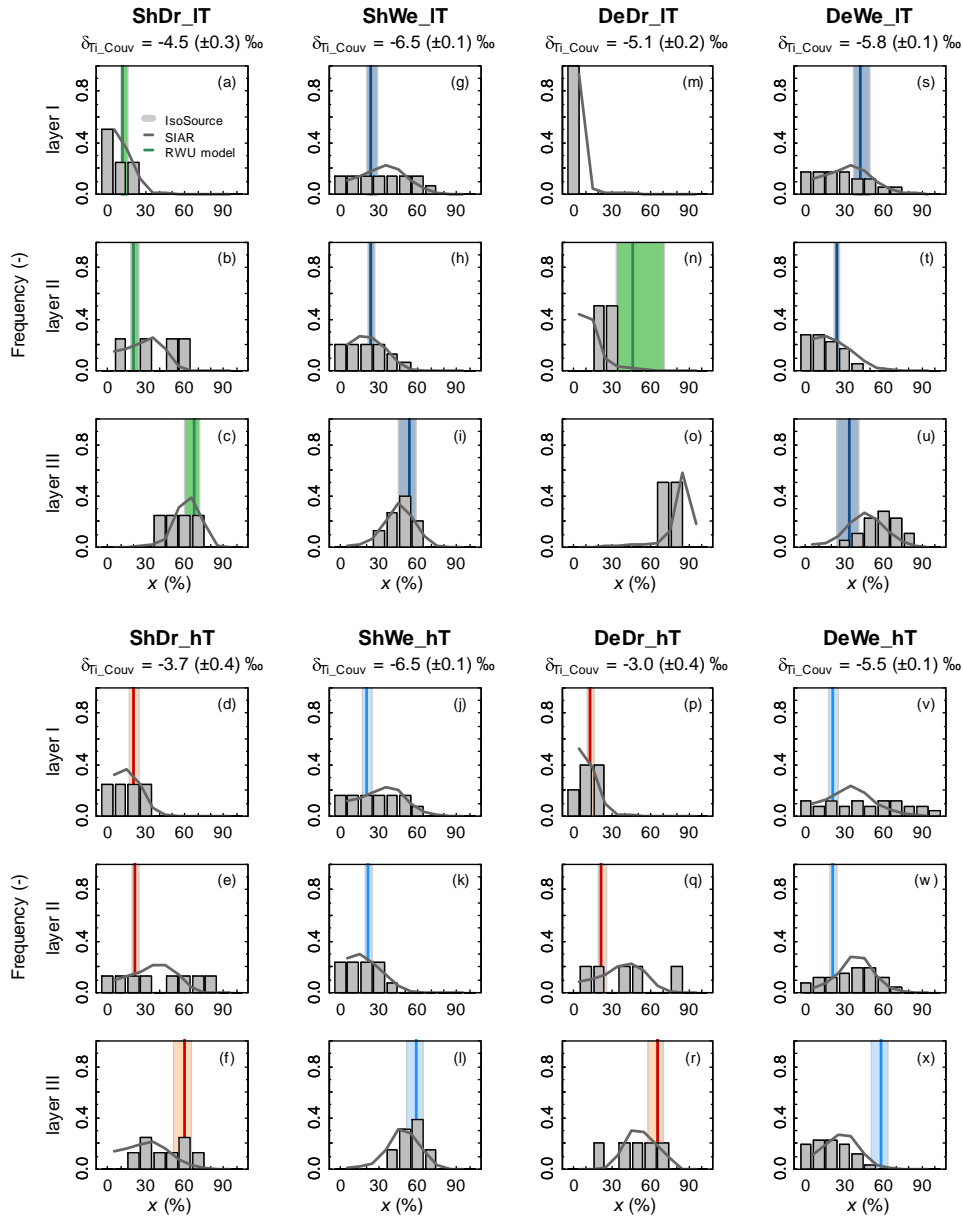



Figure 6

Figure 6: Simulated ranges of possible relative contributions to transpiration from three defined soil layers (I: 0.00 – 0.05 m, II: 0.050 – 0.225 m, and III: 0.225 – 2.000) provided by the multi-source mixing model IsoSource (Phillips and Gregg, 2003) (displayed
5 **in the form of gray histograms). Density distribution functions following the Bayesian model SIAR (Parnell et al., 2010) (gray lines). "Sh" ("De") stands for the virtual experiments where the soil has a shallow (deep) groundwater table while "Dr" and "We" stand for when the soil is dry or wet at the surface. Suffices "lT" and "hT" refer to "low" and "high" transpiration rate simulations. The colored vertical lines give x_{I_Couv}, x_{II_Couv}, and x_{III_Couv}, the relative contributions to transpiration from layers I, II, and III simulated by the analytical model of Couvreur et al. (2012). The color-shaded areas associated with x_{I_Couv}, x_{II_Couv}, and**
10 **x_{III_Couv} refer to their uncertainty associated with input data uncertainty.**

APPENDIX

Appendix A: List of symbols

Symbol	Description	Dimension	Equation number	Measured (m) / simulated (s) / prescribed (p)
C, C_S, C_A, C_B, C_{Ti}	Water stable isotopic concentration, soil water stable isotopic concentration, sources A and B water stable isotopic concentrations, xylem sap water isotopic concentration, root water uptake isotopic concentration	$M\,L^{-3}$	2, 3, 6a, 6b	m s
E, E_i	Evaporation rate for $^1H_2^{16}O$ isotopologue, Evaporation rate for $^1H^2H^{16}O$ or $^1H_2^{18}O$ isotopologue	$L^3\,T^{-1}$	B1-B4	m/s
h	Matric head	L		m
H_{eq}, H_L, H_S	Soil water equivalent and leaf water potentials, total soil water potential	P		m
J_A, J_B, J_{Ti}	Fluxes of water originating from water sources A and B, and at the plan tiller	$L^3\,T^{-1}$	6b	m
J_i^A, J_i^B, and J_{Ti}^i	Fluxes of isotopologues originating from water sources A and B, and at the plan tiller	$M\,T^{-1}$	6a	m
K_{plant}, K_{comp}	Plant and compensatory conductances to water flow	$L^3\,P^{-1}\,T^{-1}$	B1-B4	m/p
M_w, M_i	Molar masses of water and isotopologue ($^1H^2H^{16}O$ or $^1H_2^{18}O$)	$M\,L^{-3}$	3	m
RLD RLD_{1D}	Root length density Root length density per unit of surface area	$L\,L^{-3}$ $L\,L^{-1}$	B3	m/p m/p
R_{ref}	Vienna-Standard Mean Ocean Water (V-SMOW) hydrogen or oxygen stable isotopic ratio	-	3	m
S, S_{uniH}, S_{comp},	Total root water uptake sink term as simulated by the analytical model of Couvreur et al. (2012), Root water uptake sink term under uniform soil water potential distribution, compensatory root water uptake sink term.	$L^3\,L^{-3}\,T^{-1}$	1-4, 6b, B4,B5, 6b	s
SSF	Standard sink fraction	-	B2, B4, B4'	m/p
t, Δt	Time, time step	T	11	m
T	Transpiration flux	$L^3\,T^{-1}$	2, 4a, 4b, B1, B3, B4	m
x, x_j x_{Couv}, x_{J_Couv}, x_{J_IsoS}, x_{J_SIAR}	Contributive proportion to transpiration, source j contributive proportion to transpiration, continuous and integrated (layer J) contributive proportions to transpiration as simulated by the analytical model of Couvreur et al. (2012), integrated (layer J) contributive proportions to transpiration as determined by the IsoSource and SIAR models of Phillips and Gregg (2003) and Parnell et al. (2010). Contributive proportion to transpiration under conditions of uniform soil water potential	-	7, 8b, 9, 9'	s
z, z_j, z_{j+1}, Δz_j, z_{max}, $\bar{z}_{RWU}$	Soil depth, soil depth of layers j and $j+1$, thickness of soil layer j, depth of the root system, "mean root water uptake depth"	L	4b, 5, B2-B4'	m/p

Symbol	Description	Dimension	Equation	
δ, δ^2H, $\delta^{18}O$, δ_{source}, δ_{surf}, δ_S, $\delta_{S,j}$, $\delta_{S,J}$, δ_A, δ_B, δ_{Ti}, $\delta_{Ti,m}$, δ_{Ti_Couv}, δ_E	Water stable isotopic composition, water hydrogen and oxygen stable isotopic compositions, source, soil surface, soil water, soil layer j and J water isotopic composition, sources A and B water stable isotopic compositions, isotopic composition of xylem sap water at the plant tiller, isotopic composition of xylem sap water measured at the plant tiller, isotopic composition of xylem sap water at the plant tiller as simulated by the model of Couvreur et al. (2012)	- (expressed in ‰)	3-5, 7-9, 11	m/s
ε_j	Residual error term	- (expressed in ‰)	9'	s
θ	Soil volumetric water content	$L^3 L^{-3}$	5, 11	m
ρ	Volumetric mass of water	$M L^{-3}$	3	m
σ_x, σ_{δ_A}, σ_{δ_B}, $\sigma_{\delta_{Ti}}$, $\sigma_{\delta_{Ti\_AM}}$, σ_x	Sandard errors associated with the measurements of x, δ_A, δ_B, δ_{Ti} and estimated uncertainty of δ_{Ti_Couv} as simulated by the analytical model of Couvreur et al. (2012), error associated with the estimation of the contributive proportion to T of water source A in the case of two distinct sources	- (expressed in ‰)	8a, 8b	s
τ	Isotopic tolerance	- (expressed in ‰)	10	p

Appendix B: The macroscopic RWU model of Couvreur et al. (2012)

B1: Presentation of the model

In the approach of Couvreur et al. (2012), RWU is based on physical equations describing the water flow processes but without the need of the full knowledge of the root system architecture and local hydraulic parameters. Instead, three macroscopic parameters are needed. The first equation defines plant transpiration:

$$J_{Ti} = K_{plant} \cdot \left(H_{eq} - H_L \right) \tag{B1}$$

where J_{Ti} [L³ T⁻¹] is the sap flow rate in the root tiller and considered to be equal to the transpiration rate, K_{plant} [L³ P⁻¹ T⁻¹] is the plant conductance to water flow (the first macroscopic parameter of Couvreur et al., 2012's model). H_L[P] is the leaf water potential and H_{eq} [P] the "plant averaged soil water potential" defined as the mean soil water potential "sensed" by the plant root system in the one dimensional (vertical) space:

$$H_{eq} = \int_z SSF(z) \cdot H_S(z) \tag{B2}$$

where z is the soil depth, H_S [P] is the total soil water potential, and SSF [-] the standard sink term fraction (the second macroscopic parameter of the model of Couvreur et al., 2012). SSF is defined as the RWU fraction under the condition of totally uniform soil water potential (i.e., when $H_S(x,y,z) = H_S = $ cst). Under such conditions, if all the root segments had the same radial conductivity (and the xylem conductance would not be limiting), the RWU distribution in a uniform soil water potential profile would be exactly the same as the root length density per unit of surface area (RLD_{1D} of dimension [L L⁻¹]) profile. SSF could be defined as:

$$SSF(z) = \frac{S_{uniH}(z)dz}{q_{Ti}} \approx \frac{RLD_{1D}(z) \cdot dz}{\int_z RLD_{1D}(z) \cdot dz} \tag{B3}$$

where $q_{Ti} = J_{Ti}/(\Delta x \Delta y)$ represents the sap flow rate in the root tiller per unit surface area [L T⁻¹], S_{uniH} [T⁻¹] is the RWU sink term under uniform soil water potential profile. The RWU under conditions of heterogeneous soil water potential is described with the following equation:

$$S(z) = S_{uniH}(z) + S_{comp}(z) = q_{Ti} \cdot SSF(z) + K_{comp} \cdot \frac{\left(H_s(z) - H_{eq} \right) \cdot SSF(z)}{V(z)} \tag{B4}$$

where K_{comp} [L³ P⁻¹ T⁻¹] is the compensatory conductance and S_{comp} [L³ T⁻¹] the compensatory RWU accounting for the non-uniform distribution of the soil water potential and $V(z)$ is the volume of soil considered. If the soil water potential is uniform, this term vanishes from the equation, as $H_S = H_{eq}$ for any z, and water is extracted from the soil proportionally to RLD. When the water potential at a certain location is smaller (more negative, which means drier) than H_{eq}, less water is extracted from this location. On the other hand, when the soil is wetter (H_S less negative), a larger amount of water can be taken up from the same location as compared. Note that if $H_S < H_{eq}$ and if the compensatory term is higher than the first one,

S can become positive, and water is released to the soil (i.e., hydraulic redistribution). From Eq. (B4), it can be concluded that hydraulic redistribution will preferably occur when q_{Ti} is small and when large soil water potential gradients exist. Plant root hydraulic characteristics will control compensation through the K_{comp} term. The importance of the compensatory RWU term has been discussed in the literature for a long time (e.g., Jarvis, 1989). Except if plants activate specific mechanisms to avoid it, compensation always takes place under natural conditions due to the spatially heterogeneous distribution of soil water potential (Javaux et al., 2013).

A simplifying hypothesis that can be made (Couvreur et al., 2014; Couvreur et al., 2012) is to consider that K_{plant} and K_{comp} are equal, which substituted in Eq. (B4) leads to:

$$S(z) = SSF(z) \cdot K_{plant} \cdot \left(H_s(z) - H_L\right)/V(z) \tag{B4'}$$

Finally, the uptake of water stable isotopologues, i.e., the "isotopic sink term" (S_i [M T^{-1}]) is defined as:

$$S_i(z) = S(z) \cdot C(z) \tag{B5}$$

where C [M L^{-3}] is the water isotopic concentration.

B2: Running the model for the inter-comparison

The root water uptake (S) depth profiles and corresponding δ_{Ti_Couv} were simulated using the model of Couvreur et al. (2012) (Eq. (B4')) for all eight scenarios. For this, H_S, δ_S, and RLD input data were interpolated at a 0.01 m vertical resolution and the resistance of the xylem vessels was assumed to be neglible so that $H_{Ti} = H_L$. A K_{plant} value of 2.47 10^{-6} h^{-1} was taken and was determined based on concomitant T, H_{eq} and H_L data measured for *Festuca arundinacea*. δ_{Ti_Couv} was then calculated from Eq. (4b) (section §2.3). From these simulations, the depth profiles of x_{Couv} (%), the ratio $\dfrac{S(z)dz}{T/(\Delta x \cdot \Delta y)}$ at each interpolated depth z was determined, and x_{J_Couv}, the ratio $\dfrac{S(z)dz}{T/(\Delta x \cdot \Delta y)}$ from each of the integrated soil layers J ($J \leq$ III or $J \leq$ VIII) were calculated. In order to account for uncertainty of the input data (i.e., total soil water potential and oxygen isotopic composition H_S and δ_S, and root length density RLD), the model was run a 1000 times where a single offset randomly selected between −5 and +5 cm, −0.2 and +0.2 ‰, and −0.1 and +0.1 cm cm^{-3} was added to the initial values (reported Table 2) of H_S, δ_S, and RLD, respectively. By doing this we obtained *a posteriori* distributions of S and corresponding δ_{Ti_Couv} standard deviations ($\sigma_{\delta_{Ti\_Couv}}$);

Appendix C: Inter-comparison methodology

The graphical inference method (GI), the two end-member mixing model (TM), and the multi-source mixing models IsoSource (Phillips and Gregg, 2003) and SIAR (Parnell et al., 2010) were compared to each-other in the following manner for each of the eight virtual experiments:

5 (i) Single (or multiple) mean RWU depth(s) ($\bar{z}$) were graphically identified following the GI method as the depth(s) of the intersection between a vertical line of value $\delta_{\text{Ti\_Couv}}$ with the δ_S profile. The uncertainty of method GI was determined on the basis of the $\delta_{\text{Ti\_Couv}}$ *a posteriori* distribution: by taking into account $\sigma_{\delta_{\text{Ti\_Couv}}}$, $\bar{z}$ results were translated into "RWU layers";

(ii) relative contribution of RWU to transpiration (x_{TM}, %) to two defined soil layers (either conjoint: 0 – 0.225 m and 10 0.225 – 2.00 m or disjoint: 0 – 0.225 m and 1.75 – 2.00 m) were determined using the TM approach. For this, representative values for the water oxygen isotopic compositions of these soil layers were computed using Eq. (5) which uses soil volumetric water content (θ, in m^3 m^{-3}) as input data. θ distribution was obtained from H_S distribution and the van Genuchten (1980) closed-form equation. Values for its different parameters, i.e., the soil residual and saturated water contents (θ_{res} and θ_{sat}), and the shape parameters related to air entry potential and pore-size distribution 15 (α and n) were equal to 0.040 and 0.372 m^3 m^{-3}, 0.003 cm^{-1}, and 3.3, respectively;

(iii) Possible range of x_{J_IsoS}, the relative contribution of RWU to transpiration for each of the integrated soil layers following the IsoSource model was computed based on smoothed $\delta_{S,J}$ profile and $\delta_{\text{Ti\_Couv}}$ by solving the following equation:

$$\sum_J x_{J\_\text{IsoS}} \cdot \delta_{S,J} \leq \left| \delta_{\text{Ti\_Couv}} \pm t \right| \tag{C1}$$

20 with $\tau = \sigma_{\delta_{\text{Ti\_Couv}}}$.

$\delta_{S,J}$ was computed similarly to for the TM method;

(iv) Density distribution of x_{J_SIAR}, the relative contribution of RWU to transpiration for each of the three (or eight) soil layers following the IsoSource model was determined based on smoothed $\delta_{S,J}$ profile and $\delta_{\text{Ti\_Couv}}$ data as well. To compare with the IsoSource model (i) the number of δ_{Ti} replicates was fixed to three and equal to $\delta_{\text{Ti\_Couv}} - \sigma_{\delta_{\text{Ti\_Couv}}}$, 25 $\delta_{\text{Ti\_Couv}}$, and $\delta_{\text{Ti\_Couv}} + \sigma_{\delta_{\text{RWU\_Couv}}}$, and (ii) x_{J_SIAR} was computed at a 10 % increment (i);

(v) Results obtained at steps (i)-(iv) were compared to each other;

(vi) Sensitivity of IsoSource to the values of i and τ, and of SIAR to values of arguments *iterations* and *burnin* were finally briefly tested.