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Examination of deep root water uptake using anomalies of soil water stable isotopes, depth-controlled isotopic labeling and mixing models

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ABSTRACT

Knowledge on the water uptake depths of vegetation is crucial for understanding water transport processes of the soil-vegetation atmosphere continuum and relevant for many applications (e.g. the estimation of groundwater recharge, irrigation planning and the parameterization of (eco-) hydrological models). The identification and quantification of water uptake from deep soil layers and groundwater remain challenging. This study uses a combined framework based on natural abundances of stable water isotopes and isotopic labeling experiments with deuterium oxide (^2H_2O) to study root water uptake and identify uptake from deep soil in a semi-arid environment.

Between 2013 and 2016, more than 1000 soil (isotope depth profiles); plant (xylem and transpiration) and water (precipitation and groundwater) samples for the analysis of isotope ratios were collected. Two experiments using isotopic labeling were carried out in order to assess root water uptake depths. Herein, we i) present series of deep soil water isotope depth profiles, interpret water transport dynamics and ecohydrological feedbacks; ii) examine the suitability of natural isotope depth profiles for identifying deep root water uptake; iii) apply the Bayesian mixing model MixSIAR to quantify deep root water uptake and iv) constrain water uptake depths using isotopic labeling experiments and derive an active root water uptake distribution.

Our results show that the form of isotope depth profiles of soil in water-limited environments follows characteristic shapes for the end of the rainy and dry seasons, respectively. Isotope ratios in the upper 4 m of the soil are heavily dependent on the character of the respective rainy season. Under certain conditions – e.g. droughts or weak rainy seasons – the isotope depth profile displays an enrichment in heavy isotopes up to 4 m depth. Such pronounced anomalies provide an opportunity for studies on source water partitioning. In the present experiment, the studied individuals of *Acacia erioloba* were found to obtain 37% [24–52%] of their water from deep soil (> 4 m) and groundwater at the end of the dry season of 2015. All other investigated trees (individuals of *B. plurijuga, S. luebertii, T. sericea* and *C. collinum*) mainly utilize water originating from 1 m to 2.5 m depth. Under "average" rainy season conditions, the similarity of isotope ratios of potential plant water sources hinders a conclusive identification of water uptake depths.

Deep natural isotope depth profiles in dry environments can – under certain conditions – be used to identify and quantify access of vegetation to deep water resources. Isotopic labeling enables to determine active root distributions for the lateral root zone. Combined frameworks contribute to a better understanding of deep water uptake. A differentiated consideration of water uptake from the lateral root zone and deep, potentially groundwater-tapping roots is required in order to fully investigate ecohydrological feedbacks and for a proper parameterization of models.

1. Introduction

Transpiration through plants' leaves is the largest of all terrestrial water fluxes, despite the ongoing debate on the exact magnitude and uncertainty (Coenders-Gerrits et al., 2014; Jasechko et al., 2013; Schlaepfer et al., 2014). Water being transported through the stem and transpired by vegetation ultimately is a mixture of all water reservoirs a particular plant has access to and is actively using via its roots, i.e.

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water originating from precipitation, different soil layers and, if accessible, groundwater. Under certain conditions, plants might even "prefer" water originating from reservoirs that are hydraulically more difficult to access for them (Dawson and Ehleringer, 1991; McDonnell, 2014). Hence, quantifying the magnitude of each contributor remains challenging and is subject of current research efforts (Barbeta and Peñuelas, 2017; Berry and Evaristo, 2017; Evaristo et al., 2015, 2017; Zhang et al., 2017). Qualitative and quantitative approaches for the investigation of water uptake originating from deep soil layers and/or groundwater require increased scientific efforts (Maeght et al., 2013; Pierret et al., 2016).

Deep roots exist in different forms, of which tap, sinker and descending lateral roots are the most common (Canadell et al., 1996). Their main ecological functions are to provide stability for the plant and supply them with water and nutrients. For some species (e.g. phreatophytes), survival can completely depend on the capability of roots to access the water table (Canadell et al., 1996; Stone and Kalisz, 1991). Especially when the supply from shallow soil water and precipitation are insufficient, the water uptake function of deep roots is crucial (e.g. in dry and rocky environments). In such cases – even if the fraction of roots reaching the water table is small – the efficiency of tap roots can be hundreds of times larger than roots in drier soil and large amounts of water can be transported (Canadell et al., 1996; Reicosky et al., 1964)). Deep roots also play a role in physical and chemical weathering, can influence the biogeochemistry of carbon and have an impact on the soil fauna and microbial communities (Maeght et al., 2013). Deep roots are not only able to take up water but are also capable to redistribute water between different soil layers. Hydraulic lift (HL) or hydraulic redistribution (HR) is the nocturnal transport of water from moist to dry regions of the soil profile and has been studied since decades (Burgess, 2011, Burgess et al., 1998, 2000, Dawson, 1993, Ludwig et al., 2003, Richards and Caldwell, 1987). The morphology of root systems affects the plant's ability to take up nutrients. If there is availability of essential nutrients at depth, but not at the surface (e.g. trace elements such as strontium, potassium or iodine) plants might prefer the deeper source even if water availability is lower. This mechanism can also work the other way around. Similar to transporting of water, the before mentioned mechanism of hydraulic redistribution can also be used by the plant to redistribute nutrients (da Silva et al., 2011, Lambers et al., 2006, but refer to Maeght et al., 2013 for more examples).

The methodological aspect of identifying deep water uptake through plants' roots requires further attention (Maeght et al., 2013). Methods based on water isotopes have been found particularly suitable to study water uptake of plants (refer to and Pierret et al., 2016 for an overview of methods for studying deep roots).

1.1. Natural abundances of water isotopes for quantifying deep soil/groundwater uptake

Under well-defined conditions, techniques based on stable water isotopes can be used to identify the contribution of each water source (e.g. soil and groundwater) to a mixture (the water transported through the plant) by applying isotopic mixing models (for a recent review on mixing models, refer to Rothfuss and Javaux, 2016). Prerequisites for this are that the isotopic composition of all potential sources is captured, and the isotopic compositions of these sources differ from each other (i.e. isotopic ratios from groundwater are different from soil water isotopes and the isotopic ratios of the soil water profile are not constant). Many studies applying these principles have been carried out in the past (e.g., Cook and O'Grady, 2006; Dawson and Ehleringer, 1991; Dawson and Pate, 1996; Grossiord et al., 2016; Rings et al., 2013; Zhao et al., 2016). Chimner and Cooper (2004), for instance, used stable isotopes to examine the water sources of native shrubs in the desert environment of San Luis Valley, Colorado (USA). The authors found that different desert shrubs can tap groundwater deeper than 2 m depth, even though their isotopic analysis is based on an incomplete soil profile. In addition, their deep soil and groundwater isotopic compositions are almost identical, which makes an interpretation only based on water isotopes non-conclusive. The latter aspect is crucial limiting the applicability of natural abundances and/or mixing models for inferring deep water uptake because the before mentioned prerequisites are not fulfilled (i.e. the isotopic signatures of the potential water sources for plants do not differ substantially). Thus, using natural abundances and mixing models for inferring deep water uptake in such cases will lead to ambiguous results. This is particularly true for thick unsaturated zones of many water-limited environments as the characteristic profile of soil water isotopes at depth approaches a constant value due to mixing and diffusion (Barnes and Allison, 1984; Clark and Fritz, 1997; Cook et al., 1994). In their investigation of water uptake in an Amazonian Tropical Forest, Moreira et al. (2000) and Sternberg et al. (2002) further state that if the isotopic composition of water in the soil profile is redundant (i.e. different depths show the same isotopic composition) natural stable isotope ratios cannot be used with confidence.

Zhang et al. (2017) used tritium in order to overcome this issue. The authors used profiles of a thick unsaturated zone site in China where the tritium bomb peak from the mid-1960 s was well preserved. Applying a mixing model, they found that 40 ± 30% of the water used by Fuji apple trees (*Malus pumila* Mil) originates from depths between 4 m and 9 m. The average age of water used by these trees was found to be 29 years old. Though innovative and highly useful for understanding ecohydrological dynamics in thick unsaturated zones, this method is not generally applicable because tritium analysis requires a large amount of water for it to be analyzed, which is often not feasible from soil samples. Secondly, the tritium bomb peak is long gone in many places (refer to Koeniger et al., 2016 for a recent compilation of studies).

1.2. Isotopic labeling

In addition to investigate active water uptake depths (e.g. Beyer et al., 2016; Kulmatiski et al., 2010; Priyadarshini et al., 2016), labeling methods allow to allocate unknown roots to a particular tree or for deriving *active* root distributions as any tracer will only be taken up by living roots (Mazzacavallo and Kulmatiski, 2015; Piayda et al., 2017). Many investigations used labeling techniques to study water uptake and rooting depths (e.g., Beyer et al., 2016; Burgess et al., 2000; Haase et al., 1996; Kulmatiski et al., 2010; Milchunas et al., 1992; Priyadarshini et al., 2016). For identifying groundwater use by plants, however, labeling techniques are not the method-of-choice because of the large amounts of tracer required.

In summary, investigating the occurrence of deep roots and their impact on the hydrological cycle – both qualitatively and quantitatively remains challenging (Beyer et al., 2016; Lubczynski, 2011; Maeght et al., 2013; Pierret et al., 2016; Verweij et al., 2011). Especially minimum- and non-invasive approaches for studying deep roots and suitable methods for deriving root distributions are rare. Despite the fact that water uptake through deep roots can be an important component of the hydrological cycle (compare Evaristo et al., 2017) none of

the presented methods in isolation has the capability to provide a holistic description of the ecohydrological implications. Isotope based approaches using natural abundances do not allow for determining an active root distribution but offer the potential to infer groundwater uptake – if the water sources are isotopically distinct. Labeling methods, though being a direct approach to infer deep uptake (yes/no – answer) and deriving active root distributions (e.g. Mazzacavallo and Kulmatiski, 2015; Piayda et al., 2017), are not suitable for inferring groundwater uptake. A combination of depth-controlled labeling and a thorough ecohydrological analysis using natural abundances of stable isotopes might provide a more comprehensive description of soil–vegetationatmosphere interactions.

The intention of this paper is to examine how drought-induced variations in the soil water isotope depth profile and labeling experiments using $^{2}H_2O$ as applied tracer can be effectively combined in order to understand root water uptake depths in water-limited environments. In particular, our main goals are are to

i) Present a series of soil water isotope depth-profiles over a period of three years at a semi-arid site, interpret these in terms of root water uptake depths and improve the understanding of water transport processes in thick unsaturated zones
ii) Identify deep-soil/groundwater uptake of the local vegetation (*Acacia erioloba, Baikiea plurijuga, Combretum collinum, Salacia luebertii, and Terminalia sericea*) using natural abundances of stable water isotopes in thick unsaturated zones by leveraging on drought-induced anomalies of the soil water isotope depth profiles; and
iii) Utilize isotopic labeling experiments to constrain the water uptake depths and derive an active root water uptake distribution.

We provide a comprehensive combination of methods to improve the understanding of ecohydrological dynamics. A three-year dataset of deep (4 m) isotope depth profiles allowis for a systematic analysis of water transport processes in water-limited environments. Drought-induced shifts of the soil water isotope depth profile observed in northern central Namibia are further used to infer deep water uptake. Such pronounced shifts – to the authors' knowledge – have not been documented previously and represent an opportunity for studies on water source partitioning and the application of any sort of mixing models.

2. Study area

The Cuvelai-Etosha Basin (CEB) is a transboundary endorheic surface watershed shared almost equally by Angola and Namibia with a total size of 173,000 km^2. The Cuvelai-Etosha region is home to a large number of people both on the Namibian and Angolan sides, mainly because of the presence of shallow groundwater and relatively fertile soils in many areas (Lindenmaier et al., 2014). The basin has a vivid hydrogeological history as both the deltas of the Cunene and Okavango Rivers were once situated within the CEB (Lindenmaier et al., 2014;

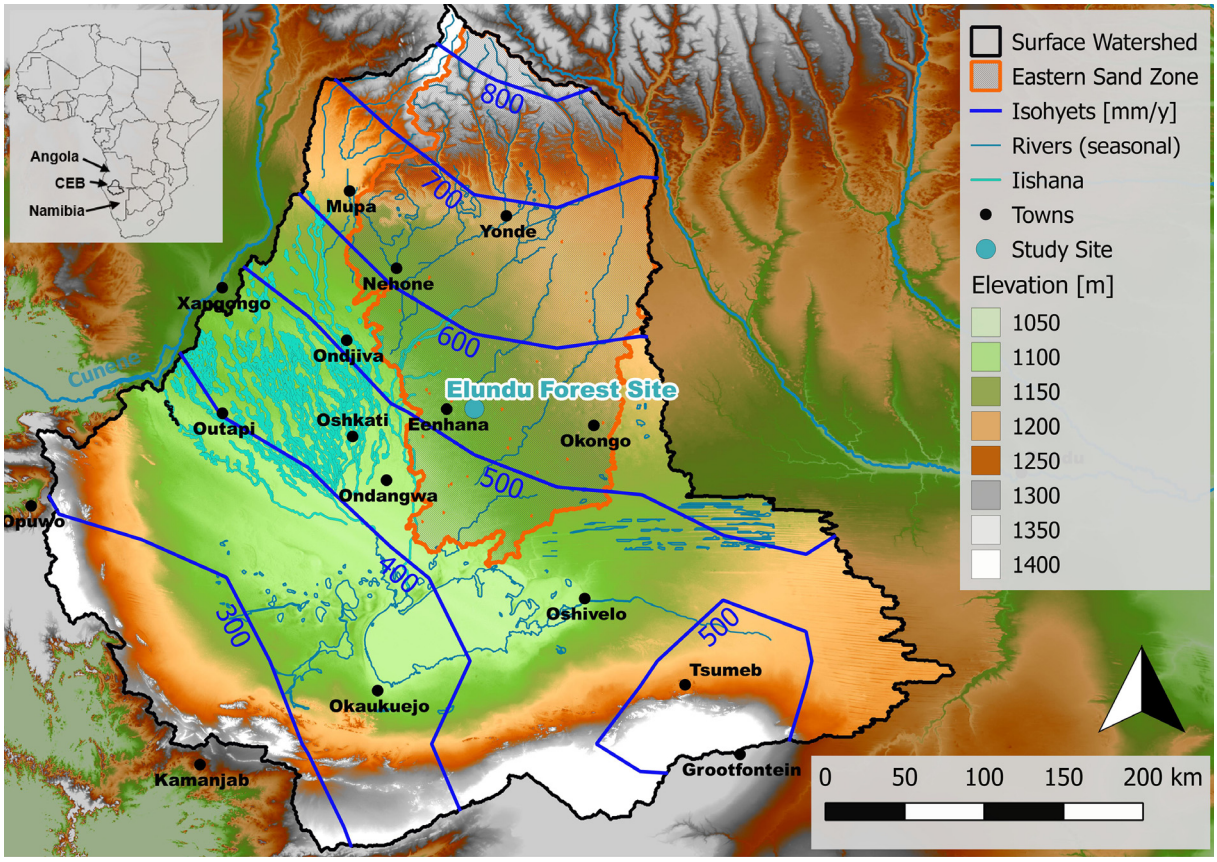

Fig. 1. DEM of the Cuvelai-Etosha Basin, river network and isohyets (dark blue). "*Elundu Forest Site*" is located in the Eastern Sand Zone (shaded area). The site is characterized by deep Kalahari sand and medium-dense forest cover with numerous large individuals (> 10 m height) of *B. plurijuga*, *A. erioloba* and *C. collinum*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Miller et al., 2010). In recent history, a deep aquifer containing fresh water was discovered in the northeastern part of the basin (Lindenmaier et al., 2014). At present all of the surface water is either draining towards the Etosha Pan, a salt pan located in the southern part of the CEB or remains in surface depressions (locally called *iishana*) that are forming a vast, partly inter-connected channel-like system north-west of the Etosha Pan (Mendelsohn et al., 2013). No perennial river exists, and the basin receives all of its water concentrated over the rainy season from November to April. Mean annual precipitation varies between 200 and 600 mm y^{-1} along a distinct rainfall gradient from the south-west to the north-east of the basin (Fig. 1). In the years 2014 and 2015 a major drought due to exceptionally low rainfall occurred throughout southern Africa causing many surface water reservoirs to dry out and water consumption to be limited in many places. Temperature average throughout the basin is 22 °C with maximum values reaching up to 40 °C in summer (Mendelsohn et al., 2013). In winter, temperatures can drop below zero at night. Potential evaporation ratesreach up to 3000 mm y^{-1} and exceed yearly rainfall by a factor of five.

Between 2013 and 2016 several field campaigns were carried out with the goal to improve the understanding of groundwater recharge related processes in the Eastern Sand Zone (shaded are in Fig. 1). Extensive sampling (soil water isotope depth profiles, bulk isotope precipitation, bulk soil, xylem) took place and continuous monitoring of key variables (soil water content, matric potential, precipitation, meteorological information) was established at sites with differing soil types, geology, vegetative cover and geomorphology. *Elundu Forest Site* is situated approximately 20 km east of the township Eenhana (Fig. 1). A number of studies on groundwater recharge estimation (Beyer et al., 2015), hydrogeochemical characterization (Hamutoko et al., 2017), root water uptake (Beyer et al., 2016) and water balance estimations using isotope data (Gaj et al., 2016) have been carried out here previously. Wanke et al. (2018) established a LMWL and analysed water stable isotopes in rainfall for the region. The site is classified as a medium-dense forest (Mendelsohn et al., 2013) underlain by thick Kalahari sheet sand which is characteristic for the Eastern Sand Zone (Fig. 1). The Kalahari sands in this area are classified as fine-grained medium sand (Ad-Hoc-AG Boden, 2005) which are highly permeable (saturated hydraulic conductivity ~2400 cm d^{-1}; Beyer et al., 2016) and homogeneous with depth. Groundwater at this site is located deeper than 20 m below ground and contains brackish water (Hamutoko et al., 2017). In this part of the Eastern Sand Zone, the perched, hanging aquifers commonly found in the region are not present (Hamutoko et al., 2017). The local vegetation consists of numerous large (heights greater 10 m) individuals of *B. plurijuga, A. erioloba, C. collinum*) as well as smaller trees (*T. sericea, Burkea Africana, Ochna pulchra, Erythrophleum africanum*) and shrubs (mainly *S. luebertii*). Grasses are only present in the rainy season and generally have a low density due to livestock grazing.

3. Materials and methods

3.1. Field and laboratory methods

3.1.1. Natural soil profiles, xylem samples & labeling experiments

Four extensive sampling campaigns were carried out between 2013 and 2016 (Fig. 2). Rain collectors (Gröning et al., 2012) for isotope analysis of precipitation were installed at several sites in the CEB and in Windhoek, respectively. For the analysis of groundwater (water stable isotopes and hydrochemistry), extensive well- and borehole-sampling campaigns were carried out (Hamutoko et al., 2017). At each campaign at least one natural soil depth profile with a vertical resolution of 10 cm was taken using a hand auger at the site-of-interest. The samples were stored in crimp-sealed headspace vials (IVA-Analysentechnik, Meerbusch, Germany) to avoid evaporation. Additional soil samples were collected for soil hydraulic analyses (see Beyer et al., 2016).

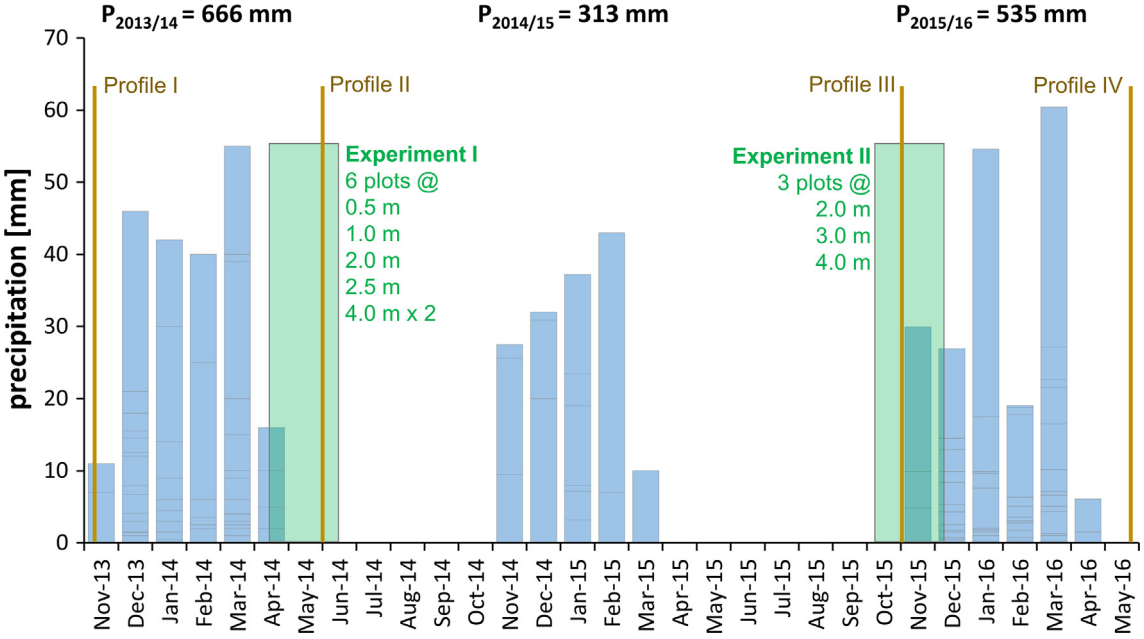

Fig. 2. Monthly rainfall time-series from November 2013 to May 2016. The separation of the blue rainfall bars indicates the amounts of rain per event in the month. Indicated are further the time, when isotope-depth profiles were collected and the period, when the isotopic labelling experiments were conducted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In June 2014 (immediately after the rainy season) and October 2015 (at the end of the dry season), sampling of the most abundant plant species, namely *Acacia erioloba* (Camelthorn), *Baikiea plurijuga* (Zambezi Teak), *Combretum collinum* (Red bushwillow), *Terminalia sericea* (Silver cluster leaf) and *Salacia luebertii* (Wild mango) took place. During both campaigns, all sampled individuals had leaves, i.e. they were transpiring water. A hollow-stem auger (Haglöf, Västernorrland, Sweden) was used to collected xylem samples of numerous individuals of each species and stored in crimp-sealed headspace vials (IVA-Analysentechnik, Meerbusch, Germany). All xylem samples were taken at chest-height and only suberized stems were collected (Dawson and Ehleringer, 1993). After establishing a background isotope inventory (see laboratory methods), a labeling experiment using a 35 vol-% ^2H_2O solution was carried out to examine root water uptake depths. A thorough description of the experimental design can be found in Beyer et al. (2016); hence, only a summary is provided here: multiple quadratic plots with an area 1 m^2 were established at the study site with each of the plots surrounded by at least one mature individual of the most abundant plant species (see above). We attempted to establish the plots in a way that none of the trees was farther than 5 m from the tracer injection area. A specific labeling depth (between 0.5 m and 4 m) was assigned randomly to each plot and four auger-drilled holes to this depth established using a hand auger. The ^2H_2O (35 vol-%) solution was inserted into common water balloons with a volume of approximately 50 ml each. Five of such balloons were attached to a string, inserted to the auger hole and destroyed at the bottom. This enabled a depth-specific labeling of each plot. The total volume of injected tracer was 250 ml per auger hole, i.e. approximately 1 L per plot (four holes per plot). In the following seven days (we assumed xylem flux rates of 1–5 m d^{-1}; refer to Fravolini et al., 2005; Meinzer et al., 2006), tracer uptake of all surrounded individuals was monitored twofold: Increment borer samples (xylem) of the surrounding vegetation were taken once a day (in the late morning)and transpired water using the bag method was collected twice per day (Calder et al., 1986, 1992; Lambs and Saenger, 2011). It should be noted that the latter way of sampling involves fractionation and therefore can only be used for the identification of tracer uptake (yes/no answer). If the deuterium concentration of any sample was at least four standard deviations (*sd*) higher compared to the regression line through the background samples, tracer was assumed to be present (refer to Beyer et al., 2016; Kulmatiski et al., 2010). The initial experiment described in Beyer et al. (2016) was conducted in June 2014 and the results are published therein. A repetition of this experiment with a focus on the deeper unsaturated zone (2–4 m, respectively) was carried out at the end of the dry season 2015 after an exceptionally weak rainy season with only 313 mm of rain (2014/15, Fig. 2). Throughout the experiment control samples were collected from additional individuals of all species under investigation at non-labeled locations.

3.1.2. Laboratory methods

Three replicate samples of soil (approximately 10 g for each soil depth) and plant material were prepared in exetainer vials and the water cryogenically extracted using the methods proposed by Koeniger et al. (2011). Minor modifications were introduced: instead of a water bath, a custom-made aluminum block was used for heating. This allows for higher and more stable extraction temperatures (see current discussions on tightly bound water, e.g., Gaj et al., 2017). In the present case a temperature of 180 °C was used both for soil and xylem samples to ensure complete extraction.

The extracted soil water samples were analyzed for isotope ratios using a Picarro L2120-i cavity – ringdown (CRD) water vapor analyzer after vaporization. For plant samples (transpired leaf water and extracted xylem), a Thermo Fisher Scientific Delta V Advantage infrared mass spectrometer (IRMS) coupled with a high temperature conversion elemental analyzer (TC/EA) was used (see current discussions on organic contamination, e.g., Martín-Gómez et al., 2015; Schultz et al., 2011; West et al., 2011, 2010). We express isotope values as -values in per-mill following recommendations for notation given by Coplen (2011) as shown below for ^2H:

$$10^3\delta^2H = \left(\frac{R_{Sample}}{R_{Standard}} - 1\right) \quad (1)$$

with R_{Sample} being the ratio ($^2H/^1H$) of the less abundant to the more abundant isotope in the sample and $R_{Standard}$ the ratio ($^2H/^1H$) of a standard, respectively. The analytical long-term precision for a quality standard (non-labeled sample) is better than 0.2‰ for ^{18}O and 0.8‰ for ^2H, for the CRD measurements and better than 0.5‰ for ^{18}O and 2‰ for ^2H for TC/EA-IRMS measurements, respectively. For highly elevated concentrations of ^2H the standard deviation can be up to five times higher. The samples were corrected for drift and memory using the procedure described in van Geldern and Barth (2012). The deuterium excess (*d*) is a measure of fractionation (or, isotopic enrichment) of individual samples and can was calculated using the relationship proposed by Dansgaard (1964):

$$d = \delta^2H - 8\delta^{18}O \quad (2)$$

The lower *d*, the more a particular sample was subjected to evaporation. The deuterium excess *d* therefore is an important measure for the degree of fractionation a sample was exposed to. It can also be used as a quality check for the cryogenic vacuum extraction (i.e., an unreasonably high value of *d* indicates incomplete extraction).

Table 1
Isotope values and variation of isotope values (expressed as standard deviation) for a) the water sources derived from the isotope depth profiles from November 2015 and b) consumers (i.e. plants). The means and standard deviations for the sources were calculated from the three analyzed soil depth profiles and groundwater sampling efforts between 2013 and 2017. Means and standard deviations for the consumers were calculated from all background and control samples taken in November 2015. For all samples, n represents the number of samples used for averaging.

	Mean δ^2H	sd* δ^2H	Mean $\delta^{18}O$	sd* $\delta^{18}O$	n
a) Sources					
Soil surface (0.0–0.2 m)	34.6	13.0	14.3	8.7	6
Soil 0.2–0.5 m	11.7	7.3	3.5	1.8	12
Soil 0.6–1.0 m	1.9	7.1	4.2	2.6	9
Soil 1.0–4.0 m	−12.6	5.2	−0.8	1.5	17
Groundwater/deep soil	−58.5	4.3	−8.1	0.8	30
b) Consumers					
Acacia erioloba	−22.2	14.7	−3.0	1.9	29
Baikiea plurijuga	−10.3	11.6	−0.5	1.7	32
*Burkea Africana***	−6.3	1.9	−0.2	1.0	3
Combretum collinum	−8.0	12.0	−0.2	1.5	26
Salacia luebertii	−8.4	12.7	0.3	1.5	7
Terminalia sericea	−3.0	3.6	2.0	0.7	6

* Standard deviation in this case represents the variation of the isotope values for the averaged depths and multiple soil profiles, groundwater samples and plant samples. Hence, it should not be confused with analytical precision.

** This data was excluded from the MixSIAR analysis because of an insufficient number of samples.

3.2. Bayesian mixing model

The software package MixSIAR (Stock and Semmens, 2013) was used for the analysis of source water contributions to the plant isotopic composition. MixSIAR is a flexible framework to create mixing models based on the Bayesian theory (Erhardt and Bedrick, 2013; Moore and Semmens, 2008; Parnell et al., 2013). Bayesian techniques use Markov Chain Monte Carlo (MCMC) methods to determine proportions and probability distributions of sources (in our case potential water sources) to a mixture (the consumer, i.e. the isotopic composition of the sampled plant). The purpose of the model was to examine deep soil and groundwater uptake and to derive the water uptake proportions of the investigated plant species (not individual plants) as we want to generate a mean characterization of the study site. Hence, the analysis was performed on a species level. The isotopic ratios of three soil depth profiles (all taken on the same day up to 4 m depth) were averaged for each depth and acted as source data. The isotopic composition of groundwater was also used as potential source. For all sources, the input included mean and standard deviation for both ^{18}O and ^{2}H to account for uncertainty. Sources were then grouped based on soil depth and statistical similarity of isotopic ratios. This resulted in the following discretization: (1) soil surface – 0.2 m (maximum enrichment), (2) soil 0.2–0.5 m, (3) soil 0.6–1 m, (4) soil 1–4 m and (5) deep unsaturated zone/groundwater. The consumer data was composed of the background isotope inventory of vegetation established immediately before the labeling experiment. As no rainfall occurred during the experiment, the isotopic ratios of vegetation can be expected to remain constant. In total, 101 consumer samples were used (29 of *A. erioloba*, 32 of *B. plurijuga*, 26 of *C. collinum*, 7 of *S. luebertii* and 7 of *T. sericea*). In Table 1 the source and consumer data for the established model are summarized.

We ran the model with three chains, 1,000,000 iterations and a burn-in (no. of discarded first iterations) of 500,000. Gelman-Rubin and Geweke tests (implemented into the MixSIAR package in R, Stock and Semmens, 2013) were used to evaluate if the MCMC converged on the posterior distributions for all variables. We used a generalist prior ($\alpha = 1$) assuming water uptake from all potential sources is equally likely.

The result of a Bayesian mixing model are probability distributions of source contributions to a mixture that are accounting for uncertainty of the sources. In order to illustrate statistical measures (i.e. median and Bayesian credibility intervals) together with the probability distribution calculated by the model, violin plots were created using the R package *Vioplot* (https://cran.r-project.org/web/packages/vioplot/index.html).

Only the isotopic data from the experiment in November 2015 are used for the investigation of water uptake depths using the Bayesian

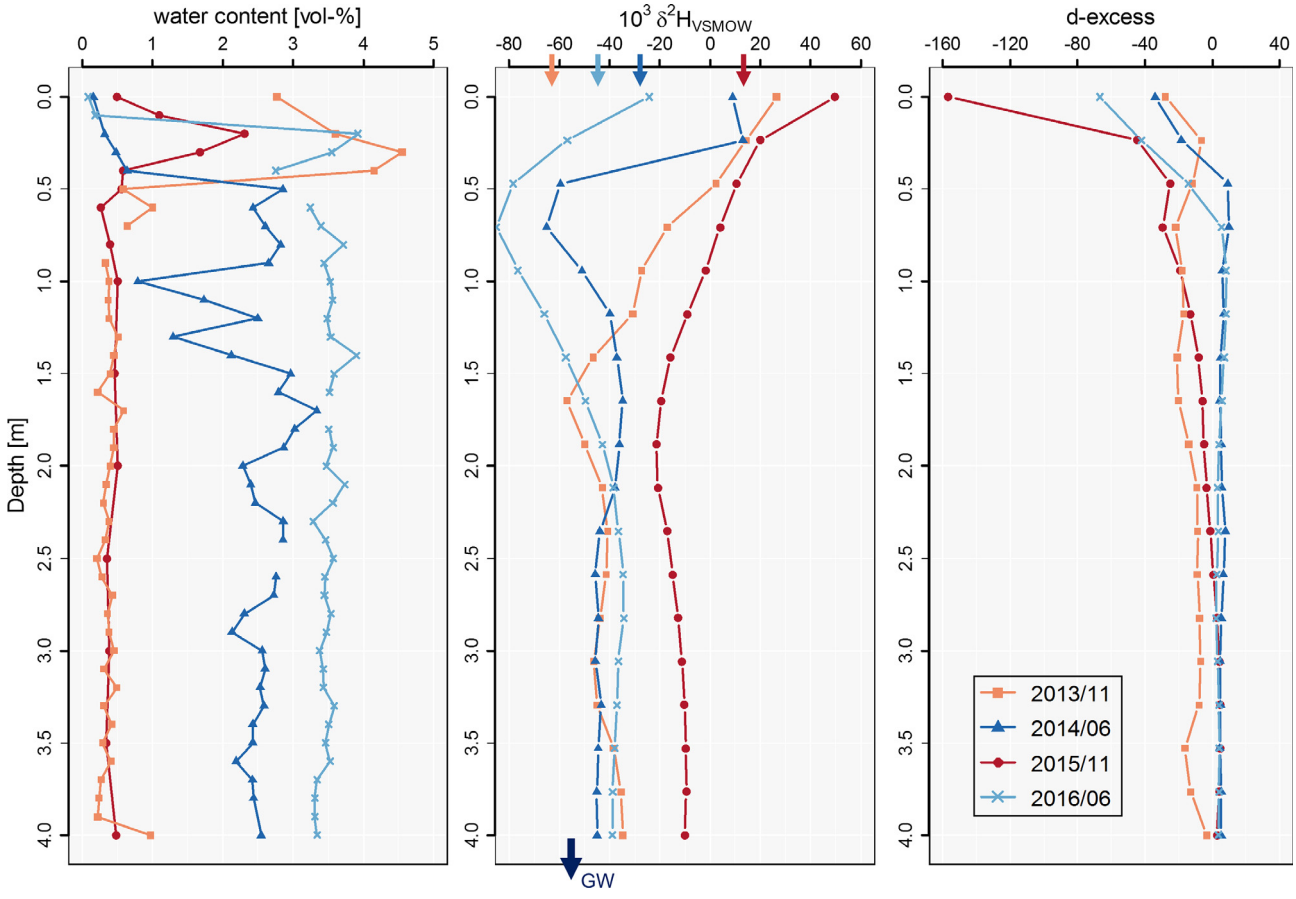

Fig. 3. Soil water ^{2}H-values, water content and deuterium excess depth profiles for four sampling campaigns. The arrows in the ^{2}H plot indicate weight-averaged isotopic composition of rainfall (top arrows) for the particular rainy season (orange: 2012/13; dark blue: 2013/14; red: 2014/15 and light blue: 2015/16). The blue arrow at the bottom indicates the isotopic composition of groundwater. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mixing model for the following reasons (refer to discussion for further elaborations): i) the pronounced shift of soil water isotopes for the profile taken in November 2015 allows a differentiation of sources (i.e. the constant isotope value of soil water at depth (> 1 m) for this profile and groundwater are clearly distinctive); ii) the upper soil is not carrying an isotope ratio similar to the one of groundwater (compare the two profiles from after the rainy season, i.e. 2014/6 and 2016/6); and iii) isotopic ranges from rainfall, deep soil and groundwater for all other profiles similar or very close to each other and lead to ambiguous results (compare Rothfuss and Javaux, 2016).

Recent research suggests that analyzing xylem water with isotope ratio infrared spectroscopy (IRIS) might lead to erroneous results (e.g. West et al., 2011, 2010). Even though the post-processing software ChemCorrectTM (Picarro Inc., Santa Clara, CA, USA) which is used to identify whether organic contamination was an issue indicated no contamination of the samples, the xylem samples measured with IRIS (i.e. those from the experiment in June 2014) were not considered to infer uptake depths from natural abundances of water stable isotopes.

3.3. Root water uptake distribution for the lateral root zone

Using the results of the labeling experiments, uptake in the lateral root zone can be further constrained. Mazzacavallo and Kulmatiski (2015) proposed a method of approximating an active root distribution based on their labeling experiments using ^2H_2O as tracer:

$$d_z = \frac{S_n - C}{\sum_{n=1}^{j} (S_n - C)} \quad (3)$$

with d_z – active root proportion at depth z, Sn – mean d-excess of experimental samples from treatment level n (e.g., labeling at 0.5 m), C – mean d-excess of control samples. In the denominator the values (i.e., Sn-C) are summed across all labeling depths with j representing the deepest application depth (i.e., 4 m). The approach is basically comparing d-excess of the background plant samples with the d-excess after labeling and, thus, constitutes a soft criterion of tracer uptake. Data from both labeling experiments (total of 389 samples) was used in order to derive a mean active root water uptake distribution for the study site.

4. Results

4.1. Drought-induced shift of soil water stable isotopes

In Fig. 3 the soil water isotope and water content profiles from the four campaigns are presented.

Fig. 3 reveals significant differences between the end of the rainy and dry seasons, respectively, but also between the two isotope profiles taken at the end of the dry season (2013/11 and 2015/11). The two profiles taken after the rainy season (2014/06 and 2016/06, respectively) have a similar shape and can be summarized as follows: i) strong isotopic enrichment near the soil surface representing the effect of evaporation; ii) isotopic depletion due to infiltrated end-of-rainy-season rainfall in the upper two meters and iii) a constant isotopic composition because of mixing and diffusion in the deeper unsaturated zone. The end-of-dry-season profiles (2013/11 and 2015/11, respectively) have a similar shape but one that is different from the profiles taken after the rainy season. The soil is isotopically highly enriched at the surface as a result of evaporation and early season rainfall. Subsequently, soil water stable isotope values are depleted with a maximum at approximately 1.7 m depth. Compared to the after-rainy-season profiles, the magnitude of this depletion is significantly lower. At greater depths (> 2 m), the after-dry-season-profiles approach a constant isotopic composition (mixing and diffusion). The most remarkable fact is that the after-dry-season profile taken in 2015 displays an isotopic shift of approximately 20–25‰ in ^2H (and 2–3‰ in ^{18}O, not shown herein) at depths greater two meters compared to all other depth-profiles.

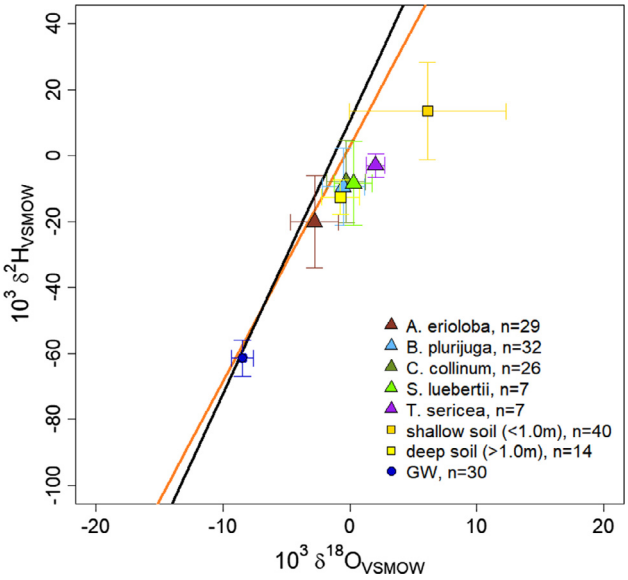

Fig. 4. Mean values and ranges of isotopic compositions of δ^2H and $\delta^{18}O$ for the five investigated plant species (xylem), shallow and deep soil water and groundwater, respectively. All data other than groundwater originates from the campaign conducted in Nov 2015. The groundwater isotopic composition was derived from samples taken from shallow boreholes.

The observed water contents clearly reveal generally higher moisture in depth profiles taken after the rainy season than after the dry season. The d-excess suggests the after-dry-season profiles were exposed to increased evaporation compared to the after-rainy-season ones. Whereas the former shows a negative d-excess up to depths of approximately two meters, the latter approach zero at approximately 0.7 m soil depth. For all profiles, deuterium excess is the lowest (i.e. highest impact of evaporation) near the soil surface and approaches a constant value of around zero (i.e., negligible to no fractionation due to evaporation) at greater depths.

For the subsequent analysis of water uptake depths using MixSIAR only the profile from November 2015 was used because only this one allows for a clear isotopic distinction of water sources available for the local vegetation (refer to methods).

4.2. Root water uptake depths determined from drought-affected soil water isotope profiles

4.2.1. Dual-isotope analysis

In Fig. 4 mean values and ranges of ^2H and ^{18}O for the five investigated plant species, shallow and deep soil water and groundwater for the sampling campaign conducted in November 2015 are presented (detailed information on the investigated trees, i.e. height, stem diameter and mean isotope values are provided in Appendix 1).

Fig. 4 reveals distinct differences in the isotopic compositions of plant water. The results can be summarized as follows:

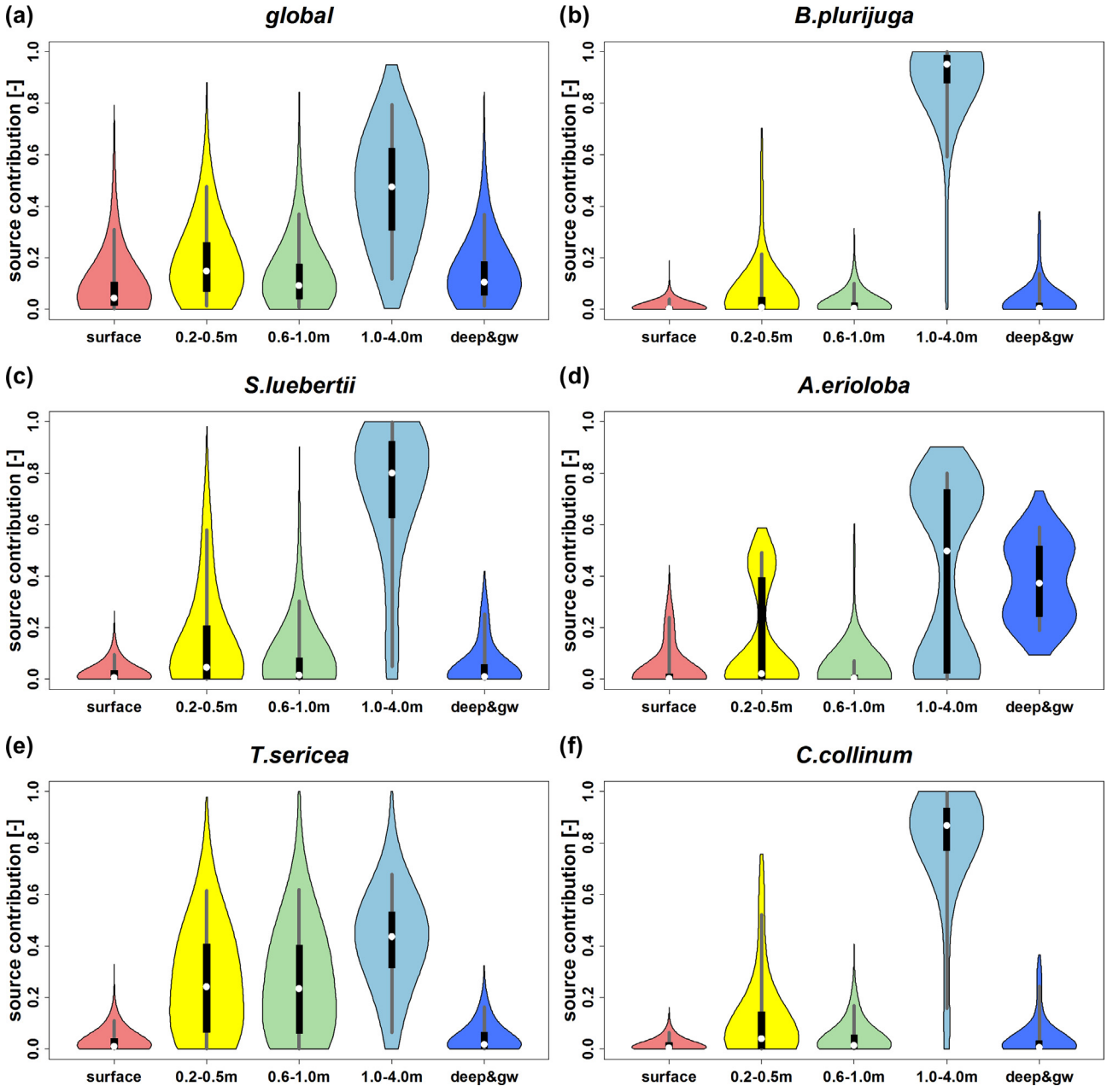

Fig. 5. Violin plot of source water contributions to the mean isotopic composition of xylem of different tree and shrub species. The shape of the violins represent the probability distributions determined from the MixSIAR realizations. White points indicate the median of the probability distribution. The black boxes represent 25% and 75% Bayesian credibility intervals and the grey whiskers the 5% and 95% Bayesian credibility intervals.

i) Both shallow and deep soil water are isotopically enriched. When analyzing all soil samples (the data of which Fig. 4 is composed of) both deep and shallow soil samples fall on an evaporation line with a slope of approximately two at the end of the dry season 2015. In comparison, the slopes of shallow and deep soil from profiles taken shortly after the rainy season were two and seven, respectively (refer to Beyer et al., 2016). For the dry season profile taken in November 2013, slopes for shallow and deep soil are four and five, respectively. In other words, deep soil immediately after the rainy season is not enriched by evaporation. It is slightly enriched after the dry season of 2013 and highly enriched after the dry season 2015.

ii) Xylem values for all investigated species plot along an evaporation line indicating either uptake of enriched water (slope of five when considering all xylem samples) or fractionation along the stem. The mean isotopic composition of four out of five species (*B. plurijuga, C. collinum, S. luebertii and T. sericea*, respectively) under consideration ranges in between deep and shallow soil water. Only one species, namely *A. erioloba*, carries an isotopic signature plotting in between deep soil water and shallow groundwater. Further, a high intra-

Table 2
Median source water contributions and Bayesian credibility intervals as determined by MixSIAR.

	Median source contribution [25% and 75% Bayesian credibility intervals]				
	Soil surface	0.2–0.5 m	0.6–1 m	1–4 m	Deep soil & Groundwater
global	**0.04 [0.02/0.11]**	**0.15 [0.07/0.26]**	**0.09 [0.04/0.18]**	**0.47 [0.31/0.63]**	**0.11 [0.06/0.19]**
B. plurijuga	0 [0/0.04]	0.01 [0/0.05]	0.01 [0/0.30]	0.95 [0.88/0.99]	0 [0/0.02]
S. luebertii	0 [0/0.03]	0.05 [0/ 0.21]	0.02 [0/0.08]	0.80 [0.63/0.92]	0.01 [0/0.06]
A. erioloba	0 [0/0.02]	0.02 [0/ 0.40]	0 [0/0.02]	0.50 [0.02/0.74]	0.37 [0.24/0.52]
T. sericea	0.01 [0/0.04]	0.24 [0.06/ 0.41]	0.23 [0.06/0.40]	0.44 [0.31/0.53]	0.02 [0/0.07]
C. collinum	0.01 [0/0.02]	0.04 [0/ 0.15]	0.01 [0/0.06]	0.87 [0.77/0.94]	0 [0/0.03]

species variation of isotope values is evident from Fig. 4. This can be attributed to the fact that different individuals with different ages and development stage of the root system were selected for this study.

4.2.2. Mixing model MixSIAR

The advantage of the profile taken in November 2015 compared to all others is that the potential sources of water taken up by the plants and transpired by them differ substantially. This is one main requirement for robust results when using isotopic mixing models. We leveraged on this fact and used all data from this extended sampling campaign in order to conduct an analysis of source contributions to the mean plant isotopic compositions using MixSIAR. The results of this analysis are presented in Fig. 5.

The global analysis of source water contributions to the xylem composition of different plant species suggests that most water is taken up from soil water between 1 and 4 m depth (median of 0.47) at the end of the dry season. Water originating from the all other depths are minor contributors (medians: soil surface 0.04, 0.2–0.5 m 0.15, 0.6–1.0 m 0.09 and deep soil and groundwater 0.10). There is a high uncertainty associated with the global analysis (as indicated by the shape of the violin as well as the box and grey whiskers) which can simply be explained by the fact that all individuals are lumped into one "bucket" (Fig. 5a). Performing an analysis for each species yields a more differentiated picture (Fig. 5b–f). The investigated individuals of the tree species *B. plurijuga* and *C. collinum* and the shrub *S. luebertii* (Fig. 5 b, c and f) are supplied almost completely by water originating from 1 to 4 m depth (median source contributions of 0.95, 0.87 and 0.80, respectively). The uncertainty in the water uptake proportions is lower compared to the global analysis. The violin plot of *A. erioloba* (Fig. 5d) shows significantly different uptake depths compared to all other species analyzed. The median source compositions suggest a contribution of 0.50 for water from 1 to 4 m depth, but also 0.37 for deep/groundwater. The shape of the violin reveals that the investigated individuals might originate from two groups which might be explained by different tree ages (or heights). We refer to the discussion section for a thorough analysis. The fifth species under investigation, *T. sericea* obtains its water from soil depths between 0.2 m and 4 m (median source contributions: soil surface 0.01, 0.2–0.5 m 0.24, 0.6–1.0 m 0.23, 1–4 m 0.44 and deep soil and groundwater 0.02). Table 2 summarized the results of the mixing model.

4.3. Constraining uptake depths: labeling experiments

The results of the initial experiment (Beyer et al., 2016) can be summarized as follows: i) frequent uptake of the injected ^2H_2O by all investigated trees and shrubs up to a labeling depth of 2 m; ii) no uptake of the tracer by trees when injecting ^2H_2O deeper than 2 m and iii) two positive samples from the shrub species *S. luebertii* at one of the plots labeled in 4 m depth, which however was not interpreted as conclusive evidence.

Table 3
Results of the second experiment and summary of both experiments carried out in May 2014 and November 2015. In total, nine plots were labeled with one liter 35 vol.-% ^2H_2O per plot.

vegetation type dominant plant species (main focus of investigation)	Kalahari woodland/forest *Baikiea plurijuga (Zambezi Teak); Acacia erioloba (Camelthorn); Salacia luebertii (Wild mango); Combretum collinum (Kalahari Bushwillow); Terminalia sericea (Silver cluster leaf)*	
samples containing ^2H_2O	no. of samples with ^2H_2O (total no. of samples)	
	2nd experiment	Both experiments
▪ total	9 (227)	40 (389)
▪ depth of labeling: 0.5 m	–	10 (29)
▪ depth of labeling: 1 m	–	9 (37)
▪ depth of labeling: 2 m	3 (92)	13 (120)
▪ depth of labeling: 2.5 m	–	0 (17)
▪ depth of labeling: 3 m	2 (65)	2 (65)
▪ depth of labeling: 4 m	4 (70)	6 (121)
δ^2H concentrations encountered for positive samples	2nd experiment [δ^2H]	
▪ depth of labeling: 2 m	86 (*T. sericea*); 140 (*B. plurijuga*); 261 (*S. luebertii*)	
▪ depth of labeling: 3 m	264 (*B. africana*); 58 (*A. erioloba*)	
▪ depth of labeling: 4 m	119; 94; 347; 80 (all *S. luebertii*)	

The results of this repetition experiment are summarized in Table 3. In Fig. 6, the dual-isotope plots for the initial experiment from 2014 (Fig. 6a) and the repetition experiment from 2015 (Fig. 6b) are shown.

Out of 227 samples taken up to seven days after the labeling with ^2H_2O, nine showed a clear tracer signal. Similar to the initial experiment (Beyer et al., 2016), more positive samples were found when collecting the samples via "transpiration bags" compared to xylem samples (eight vs. one positive samples, respectively). Three positive samples originated from the plot labeled at 2 m depth: one from *T. sericea* (transp. bag), one from *S. luebertii* (transp. bag) and one from *B. plurijuga* (xylem). Three tracer-containing samples were found at the 3 m plot, namely *B. africana* and *A. erioloba* (both transp. bags). Four

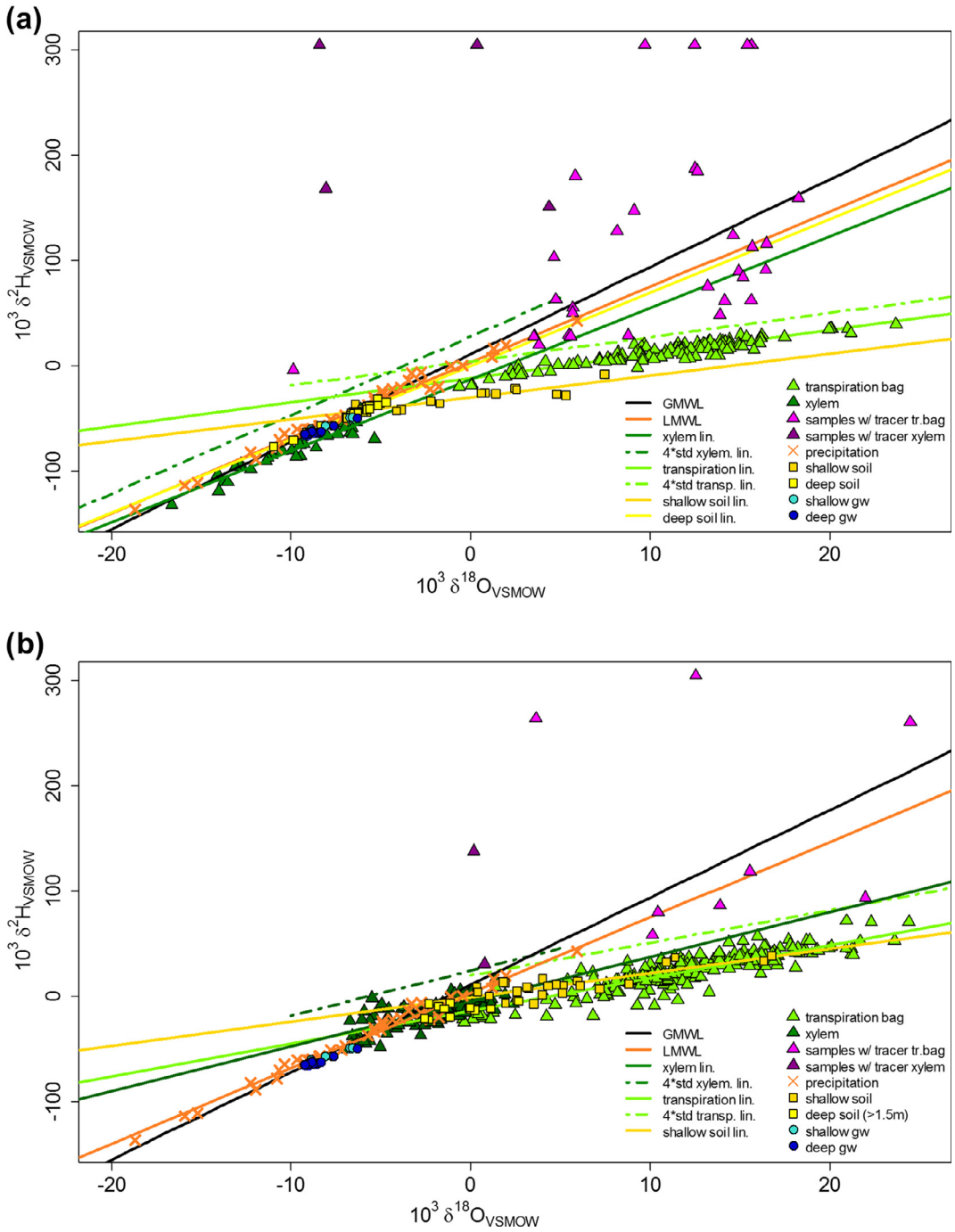

Fig. 6. Dual-isotope plot of all samples (precipitation, plant water, soil water, groundwater) collected and analyzed throughout the field campaigns. All plant samples taken are indicated by triangles (light green: via bag method; dark green: via increment borer sampling). Violet symbols represent samples clearly containing artificial ^2H_2O. The dotted lines indicate the four *sd* – line used for the identification if a plant sample contained tracer or not. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

positive samples originating from *S. luebertii* (all transp. bags) were found during the last three days of the experiment at the plot labeled in 4 m depth.

4.3.1. Root water uptake distribution for the lateral root zone

The resulting mean (i.e. averaged over all investigated species) active root water uptake distribution for "*Elundu Forest Site*" based on the labeling experiments is presented in Fig. 7.

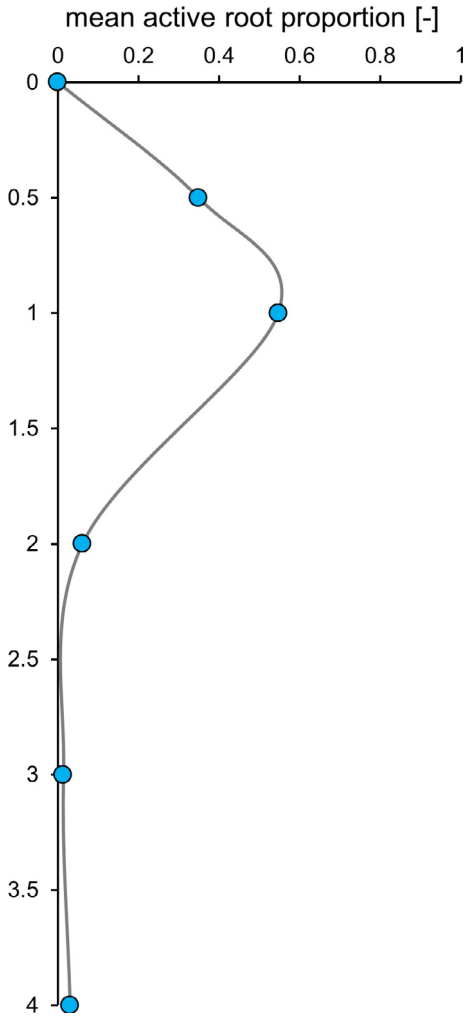

Fig. 7. Mean active root proportion for the study site as derived from the labeling experiments.

The mean active root distribution shows that most of the water uptake occurs in the upper two meters. The largest water uptake originates from 1 m soil depth. Underneath this depth, there are almost no active roots present other than the slight increase at 4 m (due to the few positive samples encountered at this depth).

5. Discussion

5.1. Drought-induced differences of soil water stable isotopes

The soil water stable isotope profiles taken at different times over a three-year period (Fig. 3) provide valuable insights into unsaturated zone water transport dynamics and water uptake. Clearly outstanding is the fact that three of the analyzed profiles (2013/11, 2014/6 and 2016/6, respectively) approach a constant isotopic ratio of −25‰ to −35‰ for δ^2H. In contrast, the after-dry-season profile taken in Nov 2015 approaches −12‰ for δ^2H at depth. Measurement and handling errors were thoroughly checked in three ways: i) three independent profiles were taken at the study site; ii) three replicates were prepared, extracted and measured in the laboratory for each depth and iii) complete extraction was verified via gravimetric and d-excess control. All profiles show the pronounced isotopic shift at depth. The interpretation we offer for the isotopic shift discovered in the depth-profiles of Nov 2015 is described in the following (see Fig. 3). With 313 mm measured on site, the anteceding rainy season received significantly less rainfall than the average (mean for the region is 530 mm y^{-1}). Consequently, no (or very little) deep infiltration occurred (groundwater recharge for this season was estimated 0 mm, refer to Beyer et al., 2015). Thus, the observed pattern could mean that either the deeper soil layers did not receive isotopically depleted rainfall which is typically the case during a "normal" rainy season (refer to Fig. 3); or, some deep infiltration of enriched rainfall occurred explaining the parallel shift shown in Fig. 3 (for further explanation on the effect of different rainfall and cloud types refer to Aggarwal, 2016; Clark and Fritz, 1997; Dansgaard, 1964). Both interpretations would explain the observed isotopic shift.

The little rainfall occurring during this rainy season was enriched in heavy isotopes compared to the rainfall from all succeeding rainy season (Fig. 3). From the authors perspective, diffusion and mixing caused the observed isotopic parallel-shift of the depth-profile taken in Nov 2015 (red lines in Fig. 3). We explain this as a result of what can be called "breathing of the soil", an effect of diurnal and seasonal changes of soil temperatures (recorded matric potential expressed in pF and temperature time series and diurnal cycles from the dry season 2015 are provided in Appendix 2, we further refer to Hendrickx et al., 2005; Scanlon et al., 2003; Walvoord and Scanlon, 2004): if no rainfall occurs, water vapor movement is dependent on temperature differences (i.e. from warm to cold). During the day (isotopically enriched) water vapor would thus move downwards. During the night, this system turns around as the soil surface and upper soil layers cool down and deeper soil are warmer. Though the enrichment effect might be small for one day it accumulates over a complete dry season. The d-excess profile presented in Fig. 4 supports this finding (negative d-excess until 2.5 m depth). The situation documented here is therefore an extreme one induced by drought. One can leverage on this fact in order to analyze root water uptake depths, because the isotope depth-profile for soil water allows for a clear distinction in the source water composition (refer to the succeeding section) of water available for the vegetation.

Another aspect is that both after-rainy-season profiles and both after-dry-season profiles have a similar shape; the shape of the two "*system states*" (wet and dry), however, is completely different. Both after-rainy-season profiles have most depleted δ-values at 0.7 m depth which is much likely the isotopic signature of recent rainfall. In contrast, the most depleted δ-values in both end-of-dry-season profiles are found at a depth of approx. 1.7 m. Around this depth, also the d-excess approaches zero. Whereas the drying out of the soil can be explained by root water uptake, the isotopic fractionation certainly cannot. Rather, the water vapor dynamics (see explanation above) are likely to have caused this (refer to the seasonal variation of soil temperature up to 2.5 m; Appendix 2). Thus, for the situation during the *dry season* not the surface-near zero flux plain might be crucial for evaluating water vapor transport, but rather the position of the highest depletion, i.e. the soil layer located between 1.5 m and 2 m. Underneath this depth, temperature-driven water vapor transport effects might vanish.

Finally, the four analyzed profiles reveal another finding: even though all isotope-depth profiles approach a constant ratio at depth, this value is not similar to the isotopic signature of groundwater (Fig. 4) – neither for the rainy season profiles nor for the dry season profiles. The likely explanation is that the presented depth-profiles represent the mean weighted average of rain events infiltrating deeper (i.e., out of the soil layers that are effect by immediate evaporation) in the associated rainy season (a detailed explanation of the process is provided in Blavoux et al., 2013). It needs to be verified further, at which depth the soil water isotope ratios and groundwater approach each other; however, it appears based on our data that it is not within the first four meters of the unsaturated zone. A practical implication of this would be that even for the after-rainy-season profiles, a distinction between groundwater and deep soil water might be possible; however, the difference between the two is much smaller than for the drought-affected profile which would result in a greater uncertainty. Further, Fig. 3 reveals that isotope signatures similar to the deep soil are found in the upper soil (due to recent rain events) which means that still two similar sources are present.

5.2. Root water uptake depths determined from drought-affected soil water isotope profiles

Given the explanation above (i.e., sources – precipitation, shallow soil water and deep soil water – for plant water are isotopically similar), a differentiation of plant water sources with mixing models for the after-rainy-season profiles is basically inapplicable. In order to obtain robust results, isotopically distinct source waters are required (Rothfuss and Javaux, 2016). The end-of-dry-season profile taken after the drought in 2014/15 allowed for this. Our explicit goal with the mixing model was to derive a mean image of the water uptake zones for different species on site. Thus, the mixing model was not run for each tree and depth individually. Rather, potential water sources were lumped into the groups shallow soil, deep soil and groundwater. For the visualization of the results from MixSIAR we chose violin plots because they illustrate the complete probability distribution of the different model realizations. The advantage is that in this way different groups within each species might be identified. To demonstrate that, provide two examples:

i) The mixing model results for *A. erioloba* are subject to large uncertainty (Fig. 5d and Table 2) if only looking at the median and Bayesian credibility intervals. The shape of the violin, however, suggests that two groups exist: one with a high and one with a lower source contribution of deep/groundwater. Perhaps, the latter group uses additionally soil water originating from 1 to 4 m and 0.2 to 0.5 m depth, respectively. *Acacia* species have been long known for their capability of developing deep penetrating tap roots (Canadell et al., 1996; Dawson, 1993; Lubczynski, 2009; Ludwig et al., 2003; Seymour and Milton, 2003). They are also known for an extensive surface-near lateral root network (Leistner, 1967; Wang et al., 2007). Combining this, our interpretation is that the group with the high proportion of groundwater is the older individuals of *A. erioloba,* i.e., those that were able to reach groundwater via their tap root. They no longer rely exclusively on their lateral root system to satisfy their transpirative demand. The other group is (most likely) younger individuals which are still developing their tap root and additionally rely on the extensive lateral root system. The results from *A. erioloba* might provide indicators for the root architecture and their capability to take up water from very dry soils: an extended shallow lateral root system and a large tap root in order to survive droughts. Dawson and Pate (1996) found such a *dimorphic root system* in Australian phraeatophytic plants enabling them to cope with droughts. Based on the mixing model, *T. sericea* might also have an extended shallow lateral root system as this species shows the highest contribution of shallow soil water. However, this species seems to not have access to deeper water sources (compare Hipondoka and Versfeld, 2006).

ii) The statistics for *B. plurijuga* show a lower uncertainty which is revealed in the violin plot (Fig. 5b and Table 2): 90% of the probability distribution (i.e. the range covered by the grey whiskers) for the investigated population of *B. plurijuga* suggests a source contribution of deep unsaturated zone water ranging from 0.60 to 1.00. In contrast, only 5% of the model realizations indicate the potential of deep soil water and/or groundwater uptake. Therefore, the confidence that the investigated individuals of *B. plurijuga* are almost completely supplied by 1–4 m soil water is high. Statistically, all sampled individuals of *B. plurijuga* behave similar and the same is true for the analysis of *C. collinum* and *S. luebertii*, where comparable results were obtained.

The results of the mixing model provide an overall image of the root water uptake zones of the dominant species on site. This, in turn, enabled to gain valuable insights into species-specific strategies to cope with drought stress. It is impossible, however, to infer rooting depth (or maximum root water uptake depth, respectively) with the results from the mixing model alone. This is for the following reason: generally, the constant isotopic composition in the deep soil water (see Fig. 3) is assumed to persist until (or until close proximity to) the groundwater. Due to the fact that the sampling depth was limited to 4 m, it is not known at which depth the isotopic signal of soil water approaches the groundwater signal. Hence, it is not precisely known from which depths the fraction of 0.47 of 1–4 m source contribution indicated by the mixing model is originating (it might also originate from deeper soil layers). Further, it cannot clearly by distinguished between groundwater use and water uptake deeper than 4 m for the same reason. Furthermore, the uncertainty of the results from the mixing model is high and we acknowledge this explicitly. Nevertheless, the presented evidence allows to state with confidence that:

i) Certain (but not all) individuals of *A. erioloba* have access to deep (> 4 m)/groundwater at the studied site;
ii) in semi-arid environments the end of the dry season is more suitable for identifying water uptake depths using natural abundances of water stable isotopes. Because there is no precipitation, soil water is enriched and hence; isotopic differences can be large; and
iii) drought-induced shifts of soil water isotope depth profiles provide the opportunity to identify deep water uptake using natural abundances of stable water isotopes.

5.3. Labeling experiments to constrain uptake depth estimates

The results of the two labeling experiments imply the following: i) several plant species on site are capable of accessing water deeper than two meters depth; ii) uptake deeper than two meters depth is rather an exception (8 out of 203 samples contained tracer); and iii) *S. luebertii* is the one species where tracer uptake up to four meters depth was registered.

The outcomes of the repeated tracer experiment fortify our initial finding (Beyer et al., 2016) that the main lateral root zone at the study site ends in between 2 and 2.5 m. This is clearly indicated by the results of the labeling experiments (refer to Table 3). We validated this via traditional excavation, water content monitoring (immediate dry-out of the upper 2 m after infiltration; refer to Beyer et al., 2016) and interviews with local farmers. We further analyzed depth profiles for soil organic carbon (SOC) and found the highest proportions at the soil surface (old leaves) and in between 0.3 and 1.5 m soil depth (data not shown herein), though SOC was generally very low. This is not an incontrovertible validation, but it increases the confidence that main root activity occurs in this zone of the soil as it matches with the tracer-derived active root distribution (Fig. 7). Our combined interpretations from utilizing natural abundances and labeling experiments can be summarized as follows:

1. If water is available, water uptake mainly occurs in the upper two meters.
2. The root distribution approximated by the labeling experiments is suitable for describing the lateral root zone; however, occasionally, local and plant-specific deep water uptake needs to be accounted for.
3. Water uptake at the end of the dry season mainly occurs from the deeper unsaturated zone. When combining the mixing model results (main uptake between 1 and 4 m) with the labeling results (end of main lateral root zone between 2 and 2.5 m), this water must mainly originate from soil depths of 1–2.5 m.
4. Individuals of *A. erioloba* (as indicated by the mixing model) and *S. luebertii* (as indicated by the tracer experiments) are capable of accessing deeper soil water and/or groundwater. However, this information should not be included into the mean root distribution as this is a local, species-specific phenomenon. On the other hand, any model for simulating thick unsaturated zone water transport not accounting for this will overestimate deep drainage and groundwater recharge. In order to incorporate this meaningful into hydrological models, different root distributions should be modeled (i.e. one with and one without the deep roots) and some sort of upscaling needs to be applied, perhaps based on vegetation counts.

Using one root distribution only accounting for the deep uptake by *A. erioloba,* for instance by extending the root distribution derived from the labeling experiments until the groundwater, would produce meaningless results, especially when modeling compensated water uptake: the complete transpirative demand (not only the one of *A. erioloba*) on site would be satisfied by taking water from deeper layers affecting drainage rates and groundwater recharge. This issue can only be solved by models capable of incorporating multiple root distributions, e.g., one for the lateral root zone and one for deep roots.

5. The fact that the sampled *A. erioloba* trees did not take up tracer but indicated deep water uptake suggests the presence of tap roots. *Acacia* species have long been associated with the development of deep roots (Canadell et al., 1996; Jennings, 1974; Lubczynski, 2009) and hydraulic lift (Dawson, 1993; Ludwig et al., 2003, 2004).

Admittedly, the presented combination of methods is no complete chain of evidence for describing root water uptake at this thick unsaturated zone site. The ultimate magnitude and seasonal variation in deep water uptake as well as potential water vapor uptake by certain plants, other adaptations like sinker roots and hydraulic redistribution remain to be studied further.

Occasional uptake from deeper zones up to 4 m was registered (Table 3). As no isotopic label was placed deeper than 4 m, it is likely that *S. luebertii* might have roots reaching deeper than this. When excavating individuals of this species, strong deep roots were discovered. In the purely tracer-derived active root distribution shown in Fig. 7, the potential of deeper uptake is incorporated (slightly increased root proportion at 4 m depth), but we explicitly state that this distribution is incomplete, mainly because of the positive samples at 4 m depth for *S. luebertii.* What is also not incorporated in the root distribution derived by labeling is the component deep uptake (> 4 m) by *A. erioloba* which is reflected in the results of the mixing model.

A variety of methods is needed in order to understand deep unsaturated zone water uptake by plants and for source partition: i) different "*system states*" (drought-induced anomalies) provide opportunities for source partitioning using natural isotopic abundances; e.g., for identifying deep water use by the local vegetation. Applying Bayesian mixing models for situations when the potential water sources (i.e., shallow soil, deep soil and groundwater) differ substantially allows for a better understanding of the unsaturated zone and its access by plants' roots. ii) Labeling experiments allow for a direct approach of deriving active root distributions that can be used to improve the parameterization of models for simulating unsaturated zone water transport. However, the maximum vertical extent of such experiments is limited. By leveraging on drought-induced effects on soil water isotope depth profiles and labeling experiments, our understanding of ecohydrological implications of plants for water transport in thick unsaturated zones can be improved.

We further reason that it is crucial to differentiate between the lateral root zone and a deep (tap) root component in semi-arid environments with thick unsaturated zones. In order to incorporate this into (eco-)hydrological models, multiple root distributions need to be established. Lubczynski (2011, 2009) provide an excellent example of how to incorporate "groundwater transpiration" into modeling approaches. Future research efforts should be directed to further develop and parameterize such models, quantify the magnitude of water uptake through (groundwater-) tapping roots and investigate ecohydrological feedbacks such as hydraulic redistribution and the role of nutrient availability on water uptake. The methods proposed herein can help to address and design experiments aiming on answering these questions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhydrol.2018.08.060.

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