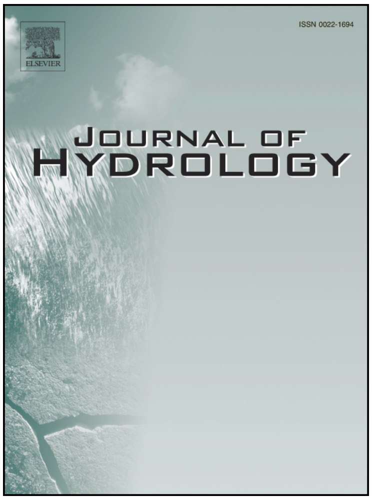

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M. Beyer, P. Koeniger, M. Gaj, J.T. Hamutoko, H. Wanke, T. Himmelsbach

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A Deuterium-based labeling technique for the investigation of rooting depths, water uptake dynamics and unsaturated zone water transport in semiarid environments

M. Beyer[a,b*], **P. Koeniger**[a], **M. Gaj**[a,d], **J. T. Hamutoko**[c], **H. Wanke**[c] **and T. Himmelsbach**[a]

[a] Federal Institute for Geosciences and Natural Resources (BGR), Hannover, Germany

[b] Institute of Water Resources Management, Hydrology and Agricultural Hydraulic Engineering (WAWI), University of Hannover, Germany

[c] Geology Department, University of Namibia (UNAM), Windhoek, Namibia

[d] Chair of Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Germany

[*]Corresponding author. Email: matthias.beyer@bgr.de

Abstract

Non- or minimum-invasive methods for the quantification of rooting depths of plants are rare, in particular in (semi-)arid regions; yet, this information is crucial for the parameterization of SVAT (Soil-Vegetation-Atmosphere Transfer) models and understanding of processes within the hydrological cycle. We present a technique utilizing the stable isotope deuterium (^2H) applied as artificial tracer to investigate the vertical extent of the root zone, characterize water uptake dynamics of trees and shrubs at different depths and monitor transport of water through the unsaturated zone of dry environments.

One liter of 35% deuterated water (^2H_2O) was punctually applied at several depths (0.5 m, 1 m, 2 m, 2.5 m and 4 m) at six different plots at a natural forested site in the Cuvelai-Etosha

Basin (CEB), Namibia/Angola. Subsequently, uptake of the tracer was monitored by collecting plant samples (xylem and transpired water) up to seven days after tracer injection. Soil profiles at the plots were taken after the campaign and again after six months in order to evaluate the transport and distribution of ^{2}H within the unsaturated zone.

Of 162 plant samples taken, 31 samples showed clear signals of artificially introduced ^{2}H, of which all originate from the plots labeled up to 2 m depth. No artificially injected ^{2}H was found in plants when tracer application occurred deeper than 2 m. Results further indicate a sharing of water resources between the investigated shrubs and trees in the upper 1 m whilst tree roots seem to have better access to deeper layers of the unsaturated zone. The soil profiles taken after six months reveal elevated ^{2}H-concentrations from depths as great as 4 m up to 1m below surface indicating upward transport of water vapor. Purely diffuse transport towards the soil surface yielded an estimated 0.4 mm over the dry season.

Results are of particular significance for a more precise parameterization of SVAT models and the formulation of water balances in semiarid areas. The developed methodology is beneficial for site-specific investigations in complex and data scarce environments, where the root zone plays a major role for the overall water balance. For arid and semiarid environments experiencing low recharge rates, water transported in its vapor phase is found to play an important role for the overall soil water balance. The use of ^2H_2O is cost-effective and provides the opportunity to investigate multiple effects along the soil-vegetation interface that have been difficult to deal with previously.

Keywords: rooting depth, deuterium, labeling, stable water isotopes, tracer, semiarid

1. Introduction

In recent history, two scientific articles raised the recognition of the role of plants within the hydrological cycle to a new level. Jasechko et al. (2013) stated that "transpiration is the largest water flux from Earth's continents, representing 80 to 90 percent of terrestrial evapotranspiration" (Jasechko et al., 2013). One year later, McDonnell (2014) published his idea of what he called *"two water worlds"* hypothesis which is based on the findings that in certain watersheds streams and trees return different pools of water to the hydrosphere (McDonnell, 2014). Even though heavily debated (e.g. Brooks et al., 2015; Evaristo et al.; 2015; Good et al., 2015) and partially rectified (e.g. Coenders-Gerrits et al., 2014; Schlaepfer et al., 2014), both studies emphasize the urgent need for an increased number of studies along the soil-plant-atmosphere interface. Despite of advances in applied techniques, measuring root activity is recognized as one of the greatest challenges in plant ecology (Casper et al., 2003; Kulmatiski et al., 2010; Schenk, 2008) and ecohydrology in general. In dry environments, where plant adaptations to the local conditions such as the development of deep tap roots or hydraulic redistribution are common (Canadell et al., 1996; Dawson, 1993; Richards and Caldwell, 1987), it is crucial to obtain information on the lateral and vertical extent of the root zone in order to parameterize SVAT (Soil Vegetation Atmosphere Transfer) and climate models (Schulze et al., 1996). Furthermore, the knowledge of rooting depth and distribution is critical for the calculation of deep percolation and for the identification of thresholds of precipitation duration and intensity that support groundwater recharge (Seyfried et al., 2005). From an ecohydrological perspective, the dominance of certain functional groups (e.g. trees, shrubs, herbaceous plants) or species (Canadell et al., 1996) and effects such as bush encroachment (Hipondoka et al., 2003) or hydraulic redistribution (e.g. Dawson, 1996, 1993; Prieto et al., 2012; Schulze et al., 1998) are of particular interest. Information on rooting depth and distribution is also highly beneficial for any investigation along the soil-vegetation-atmosphere interface because water is the limiting factor in arid and semiarid environments (Canadell et al., 1996; Oliveira et al., 2005; Schenk and Jackson, 2005, 2002a; Schulze et al., 1996; Seyfried et al., 2005).

An overview of studies on maximum rooting depths of plants is presented by Canadell et al. (1996). The authors pointed out that plants from environments experiencing a long dry season on average have deepest roots (15 ± 5.4 m). The deepest recorded rooting depths were found in the Kalahari (*Boscia albitrunca* – 68 m, *Acacia erioloba* – 60 m; Jennings, 1974). Deepest roots can be expected where thick sandy soils are present (Canadell et al., 1996). For such soils, a dependence of maximum rooting depth on rainfall amount and distribution has been found previously (Burke, 2006; Schenk and Jackson, 2002b). On a global scale, results from existing studies have been used in order to map the global distribution of deep roots in relation to climate and soil characteristics (Schenk and Jackson, 2005), to derive parameters for modeling (Zeng, 2001) or to investigate the lateral zone of influence of plants (Casper et al., 2003). Schenk and Jackson (2002b) evaluated existing data and derived relationships for below- and aboveground allometries in water-limited ecosystems.

Different approaches for investigating rooting depths

Traditionally, approaches for investigating root structure and functioning included manual digging, excavation techniques and even the use of dynamite throughout the 20th century (Canadell et al., 1996). Schulze et al. (1996), for example, excavated trenches of 5-10 m length and 3 m depth to investigate rooting depths in Patagonia. Jennings (1974) made a rather coincidental finding during borehole drilling in Botswana: They found roots of *Boscia albitrunca* and *Acacia erioloba* at 68 m and 60 m depth, respectively. In contrast, Ringrose et al. (2000) found no evidence of roots appearing deeper than 6 m in a similar environment.

Since the beginning of the new century, innovative techniques have developed with different approaches.

Ground-penetrating radar (GPR), for instance, has been utilized in several studies (Bassuk et al., 2011; Butnor et al., 2003; Hruska et al., 1999; Raz-Yaseef et al., 2013; Stokes et al., 2002). The technique was shown to be a reliable, non-invasive method for the location of roots and the determination of bulk root densities. However, bulk root density has been found to be not a good indicator for root activity (Kulmatiski et al., 2010). Jackson et al. (1999) used caves and DNA to derive ecosystem rooting depths and were able to assign roots to plant species up to 65 m depth. Regardless of these recent developments, traditional methods are still common (Hipondoka and Versfeld, 2006; Rings et al., 2013) and challenges remain.

Approaches based on stable isotopes of water

Techniques utilizing stable isotopes of water (deuterium, ^{2}H and oxygen-18, ^{18}O) as environmental or artificial tracer (subsequently, we refer to as 'labeling') have been found to be particularly suitable for plant-related studies and are used extensively (Casper et al., 2003). In early studies, Allison et al. (1984) evaluated the effect of climate and vegetation on water stable isotope compositions (Allison, 1984). Other applications of environmental isotopes include the identification of water use strategies of certain species (i.e. Brunel et al., 1995; Chimner and Cooper, 2004; Edwin et al., 2014; Li et al., 2007), changes in water-use strategies (Wu et al., 2013), quantifications of preferential flow (Stumpp and Maloszewski, 2010) and investigations on hydraulic lift (Ceperley et al., 2014). Dawson et al. (2007) used measurements of stable isotopes in woody plants to identify nighttime transpiration.

Artificial applications of the stable isotope deuterium (^{2}H) are only now becoming increasingly popular in hydrology and soil sciences. Labeling with deuterated water ($^{2}H_2O$) is of advantage because it is not radioactive and has no toxicological concerns during both labeling and measurement (Becker and Coplen, 2001), compared to the previously used tritium (^{3}H). Being part of the water molecule, ^{2}H is considered a conservative tracer and therefore ideal for studies in the unsaturated zone (Koeniger et al., 2010). It can be measured in very low concentrations (Becker and Coplen, 2001); therefore only small amounts of tracer are necessary and experiments become economically feasible. It has been shown previously that the use of artificial ^{2}H enables a quantification of transpiration (i.e. Calder et al., 1986, 1992; Calder, 1992; Lambs and Saenger, 2011; Marc and Robinson, 2004). Meinzer et al. (2006) used 2H2O to study the dynamics of water transport in conifers and found maximum sap flow velocities of 2.4 to 5.3 m d-1. Studies directly related to root distribution are, however, rare and limited to shallow depths (e.g. Bishop and Dambrine, 1995; Hawkins et al., 2009; Peñuelas and Filella, 2003; Plamboeck et al., 1999; Sternberg et al., 2005). Only one attempt was made to use artificial ^2H_2O to investigate the spatiotemporal distribution of plant water use of trees and grasses in a tropical savanna (Kulmatiski et al., 2010) to a maximum depth of 120 cm. It is this latter investigation by Kulmatiski et al. (2010) that prompted the idea for the present study. The authors injected ^2H_2O at four different depths (5, 20, 50 and 120 cm) and analyzed plant samples taken from up to five meters distance from the labeled plots in order to test the two-layer hypothesis (Walter, 1971). This theory suggests that woody plant species develop deep roots to escape competition with grasses at shallow depths. The findings by Kulmatiski et al. (2010) do not support this theory and are of high relevance for future research. Based on their studies, one could also investigate water uptake by plants from deeper regions of the unsaturated zone (which is essentially the purpose of this study).

In numerous studies ^2H_2O was applied as artificial tracer to investigate water movement in the unsaturated zone. Applications include studies of preferential flow (Hangen et al., 2005; Schumann and Herrmann, 2001; van der Heijden et al., 2013), transport velocities (Blume et al., 1967; Koeniger et al., 2010; Mali et al., 2007; Saxena, 1984; Zimmermann et al., 1966) and capillary rise (Grünberger et al., 2011). Koeniger et al. (2010) conclude ^2H_2O to be a suitable water tracer extending possibilities for field studies in the field of biogeosciences. Allison et al. (1994) state that in dry areas with sandy soils and high porosity water vapor transport might diffuse a tracer into soil and groundwater systems (Allison et al., 1994). Similarly, several researchers studied the possibility of upward water vapor transport, but clear experimental evidence from the field is lacking (DePaolo et al., 2004; Dincer et al., 1974; Phillips et al., 1988; Scanlon, 1992; Soderberg et al., 2012; Walvoord et al., 2002a, 2002b).

In the past ^2H_2O has primarily been used at shallow depths. Experimental setup is difficult for deeper application of the tracer, which might enable investigating water uptake dynamics not only in lateral direction, but also in the vertical direction. As shown above, a number of processes within the soil-vegetation-atmosphere interface of arid and semiarid environments have been difficult to investigate and describe in the past. In particular, the determination of rooting depth and water uptake dynamics remains challenging, especially with non- and minimally invasive methods. Although it is possible to investigate bulk root mass with currently available methods (i.e. ground-penetrating radar techniques), water transport mechanisms remain challenging to characterize mainly because bulk root mass has been found to not be a good indicator of root activity (Kulmatiski et al., 2010). Rooting depth and depth-specific applications of ^2H_2O, on the other hand, provide a measure for root activity; hence, any advance in quantifying rooting depth and evaluating water uptake from different depths is beneficial.

The scope of our investigation is to combine a comprehensive analysis of depth-dependent water uptake dynamics and monitoring water transport through individuals of multiple plant species and within the unsaturated zone using a minimally invasive experimental procedure employing ^2H_2O as an artificial tracer. In particular, our objectives are:

i) To propose and test a methodology to investigate rooting depths in semiarid environments;

ii) to investigate depth-dependent water uptake dynamics and access of different plants to soil water reservoirs;

iii) to monitor water transport within the unsaturated zone, in particular to assess vapor phase movement during the dry season in a semiarid environment.

The experimental design and sampling strategy further allows as a sub goal to iv) investigate if indicators for processes such as hydraulic redistribution (e.g. displacement of tracer towards shallow depths) can be identified at the studied site. To our knowledge, deuterium labeling has not yet been carried out deeper than 2 meters below ground. Similarly, the tracer has not been used to quantify rooting depths before.

2. Materials and Methods

2.1 Study Area

The Cuvelai-Etosha Basin (CEB) is a transboundary endorheic watershed shared almost equally by Angola and Namibia with a total size of 173,000 km^2. A digital elevation model of the CEB is presented in Figure 1a. The Cuvelai-Etosha region is home to a large number of people both on the Namibian and Angolan side, mainly due to the fact that shallow groundwater and relatively fertile soils are accessible to many areas (Mendelsohn et al., 2013). The basin has vivid hydrogeological history as both the deltas of the Cunene and Okavango Rivers were once situated within the CEB (Lindenmaier et al., 2014; 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), likely a remainder of the former Okavango Delta. At present all of its surface water is either draining towards the Etosha Pan, an enormous salt pan 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 Etosha Pan. No perennial river exists and the basin receives all of its water concentrated over the rainy season from November to April. Mean annual precipitation on the Namibian side varies between 200 and 600 mm y^{-1} along a distinct rainfall gradient from the west to the east of the basin (Mendelsohn et al., 2013). In the Angolan Highlands, mean rainfall can reach up to 1,500 mm y^{-1} (Mendelsohn et al., 2013). Temperature average throughout the basin is greater than 22°C with maximum values reaching up to 40°C in summer (Mendelsohn et al., 2013). In winter temperatures can drop to around zero at night. Evaporation rates can reach up to 3,000 mm y^{-1} and exceed yearly rainfall by a factor of five.

The study site chosen for our experiment (Elundu) is situated in the Eastern Sand Zone (Figure 1a). It is characterized by deep Kalahari sand (locally up to 400 m deep), a remainder of the former Okavango Delta. Around the study site the pure sand can reach depths of several hundred meters and deep infiltration of rain water can be expected. Resulting from visual inspection and preliminary irrigation experiments, no hydrophobic behavior of the soils was observed. At the top layer (first 3 cm) a biological soil crust was identified which likely developed due to extreme temperatures and dryness during the Namibian winter in combination with microbiological activity. Within the first 20 cm below surface fingered like flow was observed during irrigation experiments using dye tracer. The soil is homogeneous and according to its soil hydraulic properties highly permeable (refer to Table 2). In this area rainfall is higher than anywhere else on the Namibian side of the catchment and can reach up to 700 mm y^{-1}. Figure 1b shows the mean monthly precipitation for the closest meteorological station with long-term records, namely Ondangwa. Over time these favorable conditions in terms of rainfall allowed a forest with trees as high as 20 m to develop. Mendelsohn et al. (2013) categorize the vegetation type as north-eastern Kalahari woodland (Mendelsohn et al., 2013). Tree species consist of *Acacia erioloba (Camelthorn), Baikiaea plurijuga (Zambezi teak), Combretum collinum (Red bushwillow)* and, in a lower abundance, *Terminalia sericea (Silver cluster leaf), Ochna pulchra (Mermaid tree)* and *Erythrophleum africanum (Ordeal tree).* Especially the large trees are often almost circular surrounded by a high number of *Salacia luebertii (Wild mango)*, which is the dominating shrub throughout the forest. Even in the rainy season no grass cover can be found, which might be explained by overgrazing and subsequent bush encroachment (Hipondoka et al., 2003). The groundwater table on site (also called ‘perched aquifer’) is situated approximately 30 m below surface (Lindenmeier et al., 2014; Miller et al., 2013). The recently discovered deep, artesian aquifer (namely Ohangwena II) is located at greater depths. The origin of water in this deep freshwater reservoir is not known until now (Lindenmaier et al., 2014); hence, extensive research is conducted currently to determine its recharge areas and mechanisms.

-----*Figure 1a: Study area* ----

-----*Figure 1b Rainfall Ondangwa Monthly* ----

2.2 ^2H_2O tracer experiment: setup, tracer injection, sampling procedure and analysis

Six quadratic plots (1 m²) were chosen within the study area. When establishing the plots, it was desired to have at least one individual of the main species under investigation (which are the most abundant: *Baikiaea plurijuga, Acacia erioloba* and *Salacia luebertii*) around each of the plots. However, this was practically difficult and could not be achieved for all plots (see Appendix 1). We also aimed on choosing trees with similar heights at the plots to allow comparability. For the main species under investigation, preferably adult individuals were chosen. A minimum distance of 100 m between the plots was assigned, so that the tracer injection at the treated plots would not interfere with one another. This assumption neglects the possible occurrence of mycorrhizal fungi, which have been associated to water transport through soils (Allen, 2007; Duddridge et al., 1980; Plamboeck et al., 2007). Considering the time-scale of this experiment, however, transport over distances greater than 100 m seems unlikely. Hence, we treat potentially present fungal species as a major unknown, since micobiological feedbacks are not the scope of this research. The targeted injection depths (0.5, 1, 2, 2.5 and 4 m) were assigned randomly to the plots. An additional plot with a targeted injection depth of 4 m was established close to a *Baikiaea plurijuga* surrounded by numerous individuals of *Salacia luebertii*. This additional plot was established because at the initial 4 m plot, no individual of *Baikiea plurijuga* was present, but this species was one of the targeted. In addition we observed that individuals of *Baikiaea plurijuga* (and *Acacia erioloba*; compare the second plot with 4 m target depth) were often surrounded by a circle of *Salacia luebertii*, a small shrub that is well-supplied with water even at the end of the rainy season, when top soils are already dry. One explanation for this could be some kind of hydraulic redistribution through the roots (tap root or lateral) of *Baikiaea plurijuga*. In order to examine this, a label was placed close to the stem. If hydraulic redistribution occurs within the experimental time-frame, the upper part of the soil would display elevated concentrations of ^{2}H.

At each plot (with exception of the above mentioned additional plot), three holes were drilled until the target depth using a hand auger. The extracted soil was stored separately for each hole. A schematic illustration of the plots and plant distribution around each plot is depicted in Appendix 1.

Subsequently, five small balloons were filled with a 35% ^2H_2O solution using a custom-made syringe. Each balloon had a capacity of ~ 65 ml resulting in a total amount of ~ 325 ml per hole or ~1 liter per plot, respectively. The balloons were attached to a thin cord and then inserted into the holes. A wooden stick with a metal tip was then used to destroy the filled balloons at the target depth. Prior investigations carried out on site to explore the labeled volume showed that per cord or injection hole (five balloons) surrounding soil was wetted 20 cm in horizontal and 30 cm in vertical direction. This corresponds to a total labeled soil volume of 0.14 m³ (144000 cm³) for the plots with three holes and 0.048 m³ (48000 cm³) for the plot with one hole, respectively. After the labeling procedure the soil taken out with the hand auger was carefully re-inserted into the holes. This setup allows investigating for each plot, if the surrounding plants sampled have access to the injected water, i.e. if a plant root would be present in the labeled soil compartment, immediate uptake can be assumed due to the fact that the rest of the soil is dry. We explicitly aim at the investigating the vertical extent of the lateral root zone and exclude a potentially present deep, groundwater-penetrating tap root which has been found by previous researchers (Canadell et al., 1996; Schulze et al., 1996). The importance of such deep roots differs depending on the ecosystem. In the Amazonian forests, for example, the impact of deep roots has been shown to be significant (Nepstad et al., 1994; Oliveira et al., 2005). In a mesic savanna the effects of severing deep taproots were found to be rather small in recent studies (Verweij et al., 2011). The method presented here is not suitable for the investigation of deep tap roots, mainly because of the limitation to which holes can be dug using a hand auger. Figure 2 illustrates the experimental setup and depicts the common understanding of root structure and water transport processes at the soil-vegetation interface.

-----*Figure 2: Concept chart* ----

Starting two days before the injection of ^2H_2O, plant samples were taken at the established plots as well as throughout the study site in order to obtain background isotopic concentrations. During the experiment control samples were collected from all species under investigation at non-labeled locations at different times every day. We used both xylem and transpired water samples for the analysis. Xylem sampling took place only once per day to avoid harm to the trees under investigation, using an increment borer, whereas bags for collecting transpired water (Calder et al., 1986, 1992; Calder, 1992; Edwin et al., 2014; Lambs and Saenger, 2011; Luvall and Murphy, 1982) were used at least twice per day. The xylem samples were taken at chest height and only suberized stems were sampled (Dawson and Ehleringer, 1993). In comparison to xylem, which has to be vacuum-extracted in the laboratory, the transpiration samples can be measured directly. Fractionation, however, becomes relevant and has to be accounted for when using the stable isotopes of water as an environmental tracer. For our study using very high concentrations of ^2H_2O this was not relevant: if a plant takes up deuterium one would be easily able to distinguish between fractionation and artificial ^2H_2O because of the high input concentration of the tracer (35 % ^2H_2O); hence the sampling with transpiration bags is useful. Following the assumption of xylem flow rates of 1-5 m d^{-1} (Fravolini et al., 2005; Meinzer et al., 2006) we assigned a sampling period of 5 days to assure that potential uptake even in the deepest of the drilled holes can be detected. In total, 162 plant samples were collected. Xylem cores were split into three replicates and extracted separately in order to improve the measurement accuracy. Additionally, the upper 50 cm of the soil at each plot was sampled for ^2H concentrations 7 days after the experiment to examine if hydraulic redistribution took place. These soil samples were collected at a vertical resolution of 10 cm with an Eijkelkamp hand auger. For a long-term investigation of upward water transport within the soil, deep soil profiles were taken after the dry season within the carefully marked (using GPS and local markers) plot extent. In total, 109 soil samples were collected throughout the two campaigns.

Due to the small amount of tracer necessary, this method can be regarded being cost-effective. The price of 70 % ^2H_2O is currently 200 € per kg. For the experiment at the six plots approximately 3 kg were used (500 ml 70% ^2H_2O plus 500 ml tap water per hole). Sample analysis at BGR (Federal Institute for Geosciences and Natural Resources, Hanover, Germany) is currently 20 € per sample, but with increasing popularity of using stable isotopes the prices constantly decrease. For 162 plant and 109 soil samples this equals to around 5500 €. This results in an overall cost of such an experiment far below 7000 € (and the sample amount, especially for soil samples, can be minimized in future studies). As alternative tracer, only tritium or oxygen-18 could be used (because they will equally be taken up by plants). Oxygen-18 is about 100 times more expensive than deuterium (Speakman, 1997). Tritium is, due to its toxic character, not allowed anymore in most countries. As stated, at present other methods in the unsaturated zone are not available at present other than manual excavation.

Gravimetric water content of the soil samples was determined after drying the samples for 24 h at 105°C. Grain size distribution was derived by dynamic image analysis. For fine soils the grain size distribution smaller than 63 µm was analyzed by sieving and sedimentation (Altuhafi et al., 2012). Soil hydraulic properties and saturated conductivity were obtained in the laboratory using the evaporation method (Schindler et al., 2010). Saturated conductivity was additionally examined in the field using a double ring infiltrometer.

The transpired water, xylem and soil samples were stored in headspace glass vials and sealed with a crimping device. Transpired water samples were measured maximum two weeks after collection in the laboratory. For the extraction of water from xylem and soil samples cryogenic vacuum distillation was used, which is described in detail by West et al. (2006) and was modified for higher throughput by Koeniger et al. (2011). We slightly modified the method again by using a custom-made aluminum block as heating device in order to achieve higher and more stable temperatures within the vials. Despite recent developments of measuring stable isotopes in-situ (Volkmann and Weiler, 2013; Gaj et al., 2015), cryogenic vacuum extraction remains the most commonly used method and applied for soil, xylem, and plant water (Hangen et al., 2005; Koeniger et al., 2011; West et al., 2006). We used extraction times of 120 min for xylem samples and increased the extraction temperature to 180°C because pre-investigations showed signs of incomplete extraction of water when using lower temperatures.

The extracted water samples were analyzed for ^{2}H concentrations using a Picarro L2120-i cavity – ringdown (CRD) water vapor analyzer after vaporization. Isotope values are expressed in δ-notation in per mill [‰] following the definition of Coplen (2011) given in equation 1:

$$\delta\ {}^{2}H/{}^{18}O = \left[\frac{(R_{Sample})}{(R_{Standard})} - 1\right] \cdot 1000 \qquad \text{(eq. 1)}$$

where R_{Sample} is the ratio (^{2}H/^{1}H or ^{18}O/^{16}O) of the less abundant to the more abundant isotope in the sample and $R_{Standard}$ the ratio (^{2}H/^{1}H or ^{18}O/^{16}O) in a standard solution, respectively. The analytical long-term precision for a quality standard (non-labeled sample) is 0.1 ‰ for ^{18}O and 0.8 ‰ for ^{2}H, respectively. For highly elevated concentrations of ^{2}H the standard deviation can be up to 5 times higher. The samples were corrected for drift and memory using the procedure described in van Geldern and Barth (2012). The software ChemCorrect™ (Picarro Inc., Santa Clara, CA, USA) was used to check whether organic contamination was an issue. Contaminated samples should be ignored for further analysis; however, this was not an issue in any of the taken samples. 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):

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

The lower d, the more evaporation occurred. The deuterium excess 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. if d is unreasonably high, it could indicate incomplete extraction).

In order to identify whether tracer was present in a sample we use an adaptation of the criteria proposed by Kulmatiski et al. (2010). If a sample had a ^{2}H-concentration with at least four standard deviations (*std*) higher than the background value (control samples), tracer was assumed to be present (Kulmatiski et al., 2010 used two *std*). We therefore applied a linear regression to all samples belonging to one type of sampling (T - transpiration bag or X - xylem) containing no artificial ^{2}H (which is the control or background samples) and then defined a four-*std*-line for each. This resulted in a robust criterion for an objective evaluation of the samples and takes into account contingently occurring differences in isotopic ratios in particular for the samples collected in transpiration bags.

3. Results

3.1 Evaluation of isotopic signatures

In Figure 3 the dual-isotope plot for δ^2H and δ^{18}O is presented.

-----*Figure 3: Main Graphic*----

The graphic displays the isotopic compositions of local precipitation, soil water, groundwater (shallow and deep) and plants (xylem and transpired water). Precipitation and groundwater data was obtained during previous campaigns using a recently developed cumulative rain sampler (Gröning et al., 2012) and groundwater pumping at observation wells, respectively. Figure 3 further shows the global (GWML) and local (LWML) meteorologic water lines as well as a transpiration line and the four-*std*-line for the objective decision whether a sample contains artificial ^{2}H or not.

Of the 162 plant samples collected, 31 clearly contained artificial ^{2}H. Separated by sample type, 4 of 48 xylem and 27 of 114 transpiration samples, respectively, displayed highly elevated values for ^{2}H concentrations (displayed in violet colors in Figure 3). The highest measured concentration was slightly above 2,000 ‰ and found in a xylem sample of a tall individual of *Baikiea plurijuga*. The maximum encountered concentration in transpiration samples was 825 ‰, encountered for the same species. Further statistics of the experiment are compiled in Table 1. From the samples not containing artificial deuterium, a clear separation of the xylem and transpiration samples due to fractionation during the latter process can be identified (light and darker green symbols in Figure 3, respectively).

The mean of the deuterium excess over all background samples, -1 for xylem and -64 for transpiration bag samples, respectively, emphasizes the high influence of the latter method of sample collection on fractionation. Non-artificially-enriched samples for both sampling types fall on a distinct line each. The transpiration line has a slope (*sl*) of 2.3 with a coefficient of determination (R^2) of 0.86 and is displaced from the LMWL (sl = 7.3, R^2=0.96). The transpiration line falls on an approximate parallel to the regression line of shallow soil water isotopes (depth < 0.5 m; sl = 2.1, R^2=0.79). Deep soil water (depth > 0.5 m) plots mainly in the range of isotopic values for precipitation and groundwater and shows very little enrichment (sl = 7.0, R^2=0.97). Analysis of the xylem samples yielded in values of 6.8 for *sl* and 0.9 for R^2, indicating no (or negligible) influence of evaporation on the source water. A number of xylem samples plot in the same area on the plot as the analyzed groundwater samples (both shallow and deep). Almost half of the taken non-artificially-enriched xylem samples show isotopic ratios depleted in heavy isotopes which coincide with those measured in precipitation.

3.2 Rooting depth and water uptake dynamics

By evaluating the course of ^{2}H concentrations for each experimental plot over time an approximation of rooting depths for the species of interest and water uptake dynamics can be derived. The temporal evolution of isotope ratios for each plot is presented in Figure 4 a-f. In the graphic, we use indicators for each plant (e.g. 'far', 'young', etc.) in accordance to Appendix 1. For a differentiation of the sampling type the subscripts '(T)' and '(X)' are used for transpiration bag or xylem samples, respectively.

-----*Figure 4: Tracer uptake over the sampling period*----

As can be seen in the figures, the dynamics of the uptake of ^{2}H by different plant species can be tracked to a depth of 2 m. At the deeper labeled plots, no artificial tracer was found besides two samples of the shrub species *Salacia luebertii* (refer to discussion section).

In the experimental plots where labeling occurred in 0.5, 1 and 2 m (Figure 4 a-c), respectively, water becomes available for the plants immediately. This gets reflected in the elevated ^{2}H values for the samples taken one day after the injection characterizing those as clearly containing artificial tracer. At the 0.5 m labeling plot (Figure 4a) the concentration of artificial ^{2}H reaches its maximum for the afternoon sampling one day after the injection for the species close to the experimental plot (*Combretum collinum* and *Salacia luebertii*). In contrast, trees far from the plot show no artificial ^{2}H. During the third day of the experiment, concentrations of ^{2}H for the nearby plants return to background values. At the same time, uptake through one of the farther *Combretum collinum* occurs. In the larger trees (which were not sampled continuously at this plot), no artificial tracer was found. Small trees and shrubs dominate water uptake at this depth. A similar situation can be observed for the plot labeled in 1 m depth. The sampled individuals of *Salacia luebertii* and *Combretum collinum* immediately react to the introduced ^{2}H with highest isotopic ratios one day after injection and then return to background concentration. At this plot, the young *Combretum collinum* under investigation had no access to the labeled water. However, an individual of the same species as far as 6 m from the application area could be associated with tracer uptake. In this *Combretum collinum*, artificial ^{2}H was found again in the afternoon of day four of the experiment. In contrast to the 0.5 m plot, ^{2}H was also encountered in the taller trees that were sampled. The *Baikiea plurijuga* at the 1 m plot contained high concentrations of ^{2}H both in xylem and transpiration samples. The peak values were encountered almost two days later than in the smaller *Combretum collinum* and shrubs. For the 2 m labeling depth (Figure 4c) the pattern of ^{2}H uptake displays similar patterns. One day after labeling, artificial ^{2}H was found in both far and close individuals of *Salacia luebertii* as well as in the tall *Baikiea plurijuga*. Whilst in the subsequent days no further uptake is registered in the shrubs, a clear tracer pulse was found in the *Baikiea plurijuga* on day 4 of the experiment. No uptake of the labeled water was registered in the far *Acacia erioloba* tree throughout the whole sampling campaign.

In Figure 4d to f the temporal evolution of ^{2}H-concentrations for the plots labeled in 2.5 and 4 m are shown. With two exceptions, namely two samples of *Salacia luebertii* at the 4 m plot where the soil was labeled near the stem of *Baikiea plurijuga* (Appendix 1f), no traces of ^{2}H were found. The uptake encountered in the two mentioned samples, however, needs to be examined in detail (refer to discussion section).

3.3 Soil profiles at the end of dry season as indicators for water vapor transport

In order to investigate the possibility of up- or downward water or water vapor transport, the soil at the plots was sampled at the end of the field campaign (upper 50 cm) as well as six months later (up to 4.2 m depth; at the end of the dry season). No artificial deuterium was present in the profiles taken immediately after the campaign (i.e. 7 days after tracer injection; profiles not presented here) indicating no hydraulic redistribution of water to the upper soil layer within the experimental plots during the sampling campaign. The profiles taken after the dry season for four of the experimental plots are shown in Figure 5.

-----*Figure 5: Soil profiles after dry season*----

At all sampled plots, elevated concentrations of ^{2}H can be observed not only at the depth of tracer application but also in both directions vertically. Upward tracer transport from depths as great as 4 m is clearly recognizable by δ^2H-ratios greater than 100 ‰ up to a depth of 1.2 m (Figure 5d). In the 4 m profile, the position of the introduced ^2H_2O prior to the dry season is still distinguishable by the elevated water content around this depth (Figure 5d). At both plots labeled deeper than 1 m the tracer peak coincides approximately with the targeted application depths. Rather than a sharp peak at the target depth, however, a plateau of peak concentration is present (Figure 5c, d). The decrease of tracer concentration towards the surface has an approximate exponential shape at all sampled plots (Figure 5a to d). In contrast, Figure 5c clearly displays a rather steep decrease of ^{2}H concentrations towards the soil compartments deeper than the depth of label injection. In other words, more tracer was transported towards the soil surface (in upward direction) than downwards during the period of observation (i.e. the dry season). Hence; there exist indicators for a dominance of upward transport. The plots labeled at 0.5 and 1 m, respectively, are influenced by rainfall occurring prior to the sampling campaign after the dry season (compare the water contents at all plots; Figure 5). As a result, the tracer at these plots is shifted downwards. It can further be seen that the deeper labeling occurred, the more tracer remained within the soil. This gets reflected by the higher deuterium concentrations found in the 2 and 4 m profiles compared to the surface near plots and clearly points out the influence of evaporation and transpiration to which the latter are exposed. Summarizing our findings, it can be stated that at all plots a pronounced transport of tracer towards the soil surface was observed (either purely diffusive or with an additional advective component). At the plot labeled in 2 m depth (Fig. 5c), a dominance of upward over downward transport could be shown.

4. Discussion

4.1 Isotopic signatures

The dual-isotope plot presented in Figure 3 provides an overview of the interaction of different processes at the soil-vegetation-atmosphere interface. The separation of xylem, transpiration bag samples and samples containing artificial ^{2}H can be clearly distinguished; thus, the chosen methodology proved to be suitable for the main purposes of this study. The use of four standard deviations for the objective decision on whether a sample contains artificial ^{2}H was beneficial, because the natural isotopic composition of transpired water is variable throughout the day and depending on how long the transpiration bag is left on the tree. In addition to the decision if artificial ^{2}H is present (or not), the dual-isotope plot allows an interpretation of plant-water-atmosphere interaction, which is presented in the following.

The non-tracer-containing transpiration bag samples plot on a line that is significantly displaced from the xylem samples. This displacement clearly shows the effect of fractionation at the leaf surface. In the regression lines this gets reflected by a lower slope of the transpiration line compared to the xylem line (compare Table 1). The intercepts, however, are comparable (-12.17 ‰ for transpiration bags and -13.17 ‰ for xylem, respectively), which might be coincidence. Cooper et al. (1991) discuss the role of the slope in the regression equation and state that the lower the slope in this equation, the larger the effect of kinetic fractionation (Cooper et al., 1991). The results we obtain for our regression lines of different types of water support this finding (Table 1). Remarkably, the regression lines for transpired water and shallow soil water are approximately parallel. This indicates that evaporative enrichment is similar in the upper soil and in the leaves.

However, the Figure 3 reveals that the source water for the soil samples from the shallow unsaturated zone, transpired water and also xylem water is not the same. In regard to the different source water for soil and transpired water, the explanation could be that light rains (which or more enriched in heavy isotopes) might be taken up by plants immediately; thus, transpired water coincides with more enriched events at the LMWL. The same might hold true for rain events at the end of the rainy season (which are also more enriched): When rains slowly recede, evapotranspirative demand by plants is still high and water might be taken up immediately. In contrast, the source soil water might result from more intense rainfall events infiltrating slightly deeper into the soil.

In regard to the step-shift between xylem and transpired water, we believe that this is caused by the sampling method using transpiration bags and/or leaf-level effects causing this step-shift. In theory, the intersection of xylem and transpired water, respectively, should be in a similar range of δ-values. It would be worth studying this effect in greater detail (i.e. by sampling individual trees for both xylem and transpired water over a longer period of time) to reveal relations between the results of these two types of sampling. This is out of the scope of the presented study; however, we discuss the two ways of sampling in greater detail in section

4.4. For applications using ^{2}H as artificial tracer, transpiration bags are undoubtedly useful (refer to section 4.4).

The xylem samples (which are not subject to fractionation) plot predominantly in the range of isotopic compositions of precipitation, deep soil water and groundwater, respectively. Hence, the source water could be any of these, or a mixture; hence, a clear conclusion cannot be drawn. It is obvious, however, that none of the xylem samples coincides with shallow soil water (<0.5 m depth). This might be due to the fact that the upper soil was completely dry (< 1 vol.-%) and plants were not able to extract water from here anymore. Or, a small proportion is taken from there and the rest from deep soil water (>0.5 m depth) or groundwater.

4.2 Rooting depth and tracer uptake dynamics

A pronounced uptake of introduced tracer was observed in soil profiles labeled at 2 m or above, however, none of the sampled individuals took up water from profiles labeled at 2.5 m and deeper. Whether 2 m is the maximum depth lateral roots can reach is uncertain for three reasons: i) *Acacia erioloba* was not present at the 2.5 m plot and not all species present on the site were investigated; hence, a generalized conclusion cannot be drawn; ii) there is still a chance that no roots were present within the covered volume at any of the plots labeled deeper than 2 m, but somewhere else; and iii) heterogeneity of rooting depths (e.g. caused by tree age, height or soil heterogeneity) has to be recognized. Nevertheless, our study shows that the presented method is effective for approximating rooting depth on site and capable of capturing water uptake dynamics at different soil layers. In addition, the results are in agreement with the findings of Beyer et al. (2015) who found a step-shift of soil water contents in profiles taken one month after the rainy season at a depth of 2.3 to 2.4 m at the same study site, indicating the end of the main lateral root zone at this depth. The authors further observed a change in the color of the sand towards a clear, almost white color, whereas organic contamination could be visually identified at shallower depths. In regard to ii) and iii) we state that the injection of ^2H_2O was designed in a way that a rather large volume of soil is labeled. With 0.14 m³ labeled soil volume, the area is much bigger than 0.005 m³ in the study of Kulmatiski et al. (2010). It can therefore be assumed that if a certain plant has lateral roots in the investigated depth, the labeled soil volume is sufficient to cover at least a portion of that root. The experimental plots were chosen carefully and at representative locations within the forest where root structure is undisturbed. Since we aimed on marking a bigger soil volume not only the horizontal, but also the vertical distance from the application that was labeled increased. Therefore, we had to sacrifice a more precise vertical labeling in a way that the peak concentration spreads over a vertical depth of ~30 cm, which is confirmed by the soil profiles (Figure 5 c and d). Heterogeneity of rooting depths caused by different sizes of trees, different soil properties or even locally varying precipitation amounts can only be tackled by establishing more (repetitive) plots. The same holds true for i). However, this issue is typical for point investigations; hence, final evidence might only be achievable with tremendous effort. The results imply that there is water available frequently at depths reaching ~2 m; otherwise, there would simply be no roots at these depths (in accordance with Schenk and Jackson, 2002b). Applying a previously proposed conceptual model relating mean annual precipitation to rooting depth (Schenk and Jackson, 2002b), predicted rooting depth would be around 1.5 m (less than the supposed rooting depth in the present study) using a mean precipitation of 500 – 600 mm for the area (Mendelsohn et al., 2013). In November 2015 we further excavated one exceptionally tall individual of *Baikiea plurijuga.* The tree showed strong lateral roots which proceed almost horizontally at 2 – 2.2 m depth, but no tap root. An extensive rooting network of different trees and shrubs (*Salacia luebertii, Burkea africana, Terminalia sericea*) was found up to 2 m. From this depth downwards, root density declined exponentially. At 2.5 m soil was without roots other than occasional fine roots of low density;

the soil color changes to almost whitish sand at this depth. Even though no generalization can be drawn from excavating one tree, these observations coincide with what was found during the labeling experiment.

Water uptake dynamics

When examining the dynamics of plant water uptake, we speculate that there are indications that shrubs and smaller trees might dominate the upper root zone, while trees might have favorable access to water in deeper layers (Figure 4 a to c). For instance, at the 0.5 m and 1 m plots, an immediate reaction to the tracer injection by *Salacia luebertii* (height of shrubs ~1.5 m) and *Combretum collinum* (lower than 3 m in height) was observed. In contrast, no (at the 0.5 m plot) or delayed (1 m plot) uptake by the tall trees of *Baikiea plurijuga* (8 m height), *Terminalia sericea* (6 m height) and *Acacia erioloba* (8 m height) was found. At the 2 m plot the situation seems to turn around. Very high tracer concentrations and pulse-like uptake were measured in the tall individual of *Baikiea plurijuga* (8 m height). However, this interpretation has to be examined in further studies aimed explicitly on evaluating partitioning of the root zone between different species.

The presence of tracer in *Salacia luebertii* at the plot labeled in 4 m depth near the stem of *Baikiea plurijuga* (Figure 4f) was rather unexpected. No clear evidence can be drawn from these results; hence further experiments will be beneficial. From our understanding, there are four possible explanations: i) During the injection, tracer accidently entered areas of the soil above the targeted labeling depth; ii) the roots of the investigated individuals of *Salacia luebertii* are exceptionally deep; or, hydraulic redistribution through iii) the tap root of *Baikiea plurijuga* or iv) mycorrhizal fungi occurred. Option i) can be almost excluded, because if this would have happened, the tracer would have probably been present in other sampled species as well. Option ii) is also unlikely due to the fact that *Salacia luebertii* was also sampled at the 2.5 m and the second 4 m plot, respectively. At these, no uptake was registered. From our perspective, the most likely explanation is either iii) or iv). This would imply that the tap root of *Baikiea plurijuga* was able to access the labeled water and distribute it to the upper soil layers or, a rapid transfer through the mycorrhizal tissue (e.g Allen, 2007; Duddridge et al., 1980; Plamboeck et al., 2007) took place, respectively. As stated in section 3.3, a shallow soil profile was taken at each of the plots indicating no hydraulic redistribution. However, this only holds true for the position where this profile was taken (which is within the area of the experimental plot). Therefore, redistribution to the soil elsewhere cannot be excluded. The latter aspect is far out of scope of our research; thus, it was not investigated if such fungi are present on site. We did not find tracer in this exceptionally tall individual of *Baikiea plurijuga* (height approximately 20 m). An explanation for this might be that timing of xylem sampling was chosen in a way that we missed the tracer throughput. Similarly, it could be possible that the chosen branches for transpiration bag sampling were unfavorable, so that no tracer could be found. However, this hypothesis cannot be completely proven by our results and requires further examination.

The significance of tap roots

In our investigation we excluded the role of tap roots within the soil-vegetation-system. No evidence – both isotopically and based on drilling - was found that tap roots are present on site. As stated previously, the excavated individual of *Baikiea plurijuga* did not have a tap root. Local farmers, elders and people who are responsible for well digging observed that the only local trees in this region capable of developing deep tap roots are *Acacia erioloba* and taller individuals of *Combretum collinum* (*personal communication during several interviews conducted in the last three years*). From a scientific perspective, a number of xylem samples coincide with shallow and deep groundwater. However, precipitation and deep soil water are also plotting in the same range; hence, no clear conclusions can be drawn from our results (Figure 3). Using the presented technique to investigate such roots at great depths is not possible with the current setup (drilling deeper than 10 m with a hand-auger is simply not feasible). The importance (in terms of quantitative estimates of uptake and their role on the overall water balance) of potentially present deep tap roots is yet not fully understood. Canadell (1996) state in their review that ‘we know of the potential of some species to have very deep roots at few sites, yet very little is known about how common the habit of deep rooting is across species and environments’ (Canadell, 1996). The authors further found that deep roots are mainly limited to sandy loose soils where mechanical impedance to root penetration is least’. Up to now it is known that certain trees and shrubs are occasionally – depending on the local climate and soil type – capable of supplying themselves with water during droughts by developing deep tap roots in the unsaturated zone with the ultimate goal of reaching the groundwater table and ‘pump’ water from there (Jennings, 1974; Lemmens, 2012; Moore and Atwell, 1999; Moustakas et al., 2006; Oliveira et al., 2005; Phillips, 1963; Ringrose et al., 1998; Schenk and Jackson, 2005; Seymour and Milton, 2003). Still, the occasional character of deep tap roots needs to be recognized. Ringrose et al. (2000), for example, drilled two individuals of *Acacia erioloba* trees to 60 m in Botswana. The authors found no evidence of roots appearing deeper than 6 m. In their study in the Etosha National Park, Hipondoka et al. (2003) discovered that roots of the species under investigation, *Terminalia sericea*, displayed tap roots more often in the wetter part of the park than the ones in the drier (Hipondoka et al., 2003).

Thus, we state that at the studied site deep tap roots are likely but if this is really the case remains uncertain. Nevertheless, the potential of deep tap roots needs to be recognized because they seem to be very efficient in the acquisition of water (Canadell, 1996; Reicosky et al., 1964).

Future research should focus on the distribution and number of deep rooting trees and species and the quantification of water uptake through the tap root in order to incorporate such effects. For example, the deuterium tracing method proposed by Calder (Calder et al., 1986, 1992; Calder, 1992) could be used for this.

4.3 Upward water vapor transport and importance for estimations of groundwater recharge

The occurrence of ^{2}H in the upper soil layers of all profiles that were sampled after the dry season (i.e. 6 months after injection) was unanticipated. The clear sign of tracer transport from the 4 m plot towards the surface was particularly unexpected. We interpret this in accordance to findings of Phillips et al. (1988), Scanlon (1992) and Walvoord et al. (2002a, 2002b) as follows: During the dry season water movement in regions where the groundwater table is far from the surface is mainly limited to vapor transport resulting from temperature gradients within the soil. These gradients change from day to night, i.e. they are diurnal. In the upper soil layer, temperature gradient is steepest but downward vapor flux during the day is small because of steep water potential gradients introduced by evaporation. In the night, when the upper soil layer cools down rapidly, the thermal gradient changes and upward water vapor transport occurs. For desert soils, Scanlon (1992) found that 'below 1 m soil depth downward vapor flux in summer is balanced by upward vapor flux in winter; net vapor flux on an annual basis should be negligible.' Extending this concept to semiarid environments including the occurrence of a rainy season and plant cover, one could say that the downward water vapor transport during the days in the dry season is very small due to very low water potentials (very dry soils) throughout the whole root zone. In contrast, the overall upward transport during the night will be greater than in deserts due to the availability of water in deeper soil layers (introduced by rainfall in the rainy season), but also due to the very low water potentials in the lower part of the root zone. As a consequence, there would be a net upward water vapor flux during the dry season which results in the tracer profiles presented in Figure 5. This theory is supported by the findings of Seyfried et al. (2005) who state that plants are amongst the main determinants for the control of deep drainage in semiarid regions (Seyfried et al., 2005). The authors found that due to very low water potentials at the end of the root zone, a constant upward water vapor flux can develop even from deep soil layers or groundwater tables as deep as 50 m (Seyfried et al., 2005). At the study site groundwater is approximately 30 m deep (Lindenmaier et al., 2014). The concept of vapor phase diffusion is also supported by the study of DePaolo et al. (2004). On a strictly porous-media-property standpoint, the theoretical depth at which diffuse water vapor transport is initiated (Stage I evaporation), can be calculated by applying the concepts published by Lehmann et al. (2008):

$$L_G = \frac{2\sigma}{\rho g}\left(\frac{1}{r_1} - \frac{1}{r_2}\right) = \frac{1}{\alpha(n-1)}\left(\frac{2n-1}{n}\right)^{(2n-1)/n}\left(\frac{n-1}{n}\right)^{(1-n)/n} \qquad \text{(eq. 3)}$$

where L_G [m] is the gravity characteristic length or length associated with stage I evaporation, σ [Nm^{-1}] surface tension, ρ [kgm^{-3}] the liquid density, g [ms^{-2}] the gravity acceleration, r_1 and r_2 [m] the radii of the small and large capillaries, respectively and α [1/m] and n [-] the shape parameters of the van Genuchten model. The calculation can be performed either with the pore size distribution (first part of the equation) or, if available, the fitted parameters from the van Genuchten model. Shokri et al. (2009) (summarized in Or et al., 2013) presented an equation to estimate diffusive water vapor transport from a vaporization plane at a certain depth (Stage II evaporation):

$$e_{II}(t) = D_S \frac{C_{sat} - C_\infty}{L_{dried}(t)} = \frac{\theta_a}{\phi} D_0 \frac{C_{sat} - C_\infty}{L_{dried}(t)} \qquad \text{(eq. 4)}$$

where $e_{II}(t)$ is the diffusive flux as a function of time, D_S [m²s^{-1}] the vapor diffusion coefficient through (nearly) dry sand which is approximated using the model of Moldrup et al.

(1997) for intact soils, C_{sat} [gm^{-3}] saturated water vapor density, C_{∞} water vapor density at surface [gm^{-3}], $L_{dried}\,(t)$ the time-dependent depth of the vaporization plane [m], θ_a volumetric air content [-],ϕ porosity [-] of the soil and D_0 [m²s^{-1}] the vapor diffusion coefficient in free air.

In order to obtain an estimate of the length associated with stage I evaporation (in isotope hydrology it is also referred to as the zero flux plane), we applied equations 3 and 4 to our data assuming i) a dry soil (θ = 0.01, which has been observed on site; Beyer et al., 2015), ii) a temperature of 25 °C, iii) a water vapor density at surface ranging from 5 to 10 gm^{-3} (taken from estimations by the International Telecommunication Union, http://www.itu.int/) in June/July/August and iv) a vaporization plane at a constant depth. The results, together with soil hydraulic characteristics of the soil on site, are summarized in Table 2. We used data derived from soil cores, pore size distribution and fitted model parameters from a labeling experiment conducted by Beyer et al. (2015) for comparison. Based on the fitted model (which is believed to be the best-estimate), a characteristic depth of ~22 cm (based on the calibrated parameters) is associated with Stage I evaporation. This means that below this depth upward water transport takes place in the vapor phase. The diffuse water loss from a saturated layer of water at 4 m depth (i.e. the depth where the tracer solution was applied) results in a total of 0.4 mm over the whole dry season. Assuming the vaporization plane immediately underneath the root zone (i.e. 2.5 m; this would be the approximate depth based on the results where water surpassing the root zone is located after the rainy season) the diffuse loss of water increases to ~0.7 mm. For very wet rainy seasons (e.g. 2013/14) this is negligible because potential recharge in such is fairly high (Beyer et al., 2015). However, the mean recharge rates in this part of the CEB are estimated as ranging between 2 – 20 mm (Struckmeier and Richts, 2008). Therefore the diffusive loss has to be accounted for, especially for the lower end of this spectrum. In addition, water vapor fluxes caused by thermal gradients (as explained above) can be much higher than purely diffusive (Hendrickx et al., 2005). In order to quantify these, however, further experiments and subsequent numerical modeling is crucial. Practically, this requires injecting exactly predetermined amounts and concentrations of ^{2}H at a certain depth and detailed monitoring of tracer distribution, soil temperatures and meteorological variables. Especially the prior is challenging, but might be possible soon with recent developments of measuring soil water stable isotopes in situ (Gaj et al., 2015; Volkmann and Weiler, 2013). This comprises great potential for future studies. For example, diurnal cycles of stable isotopes and water vapor transport could be investigated contributing to an enhanced understanding of this important process.

In essence, the existence of a net upward water flux affects estimation of groundwater recharge through the unsaturated zone which is commonly referred to as water infiltrating underneath the root zone. In humid environments this might be true since groundwater tables are usually close to the surface. In semiarid and arid climates, however, the unsaturated zone can be very thick and the time from infiltration of water underneath the root zone to the groundwater can be long. If in such cases the effect of upward vapor transport is present, it has to be accounted for in the form of a negative component – or loss term – in the water balance. While there might be a significant amount of water surpassing the root zone on site due to generally favorable conditions for infiltration during the rainy season (e.g. Beyer et al., 2015), this same water (or a fraction of it) might be lost in the subsequent dry season due to water vapor transport. If there was a water table at that depth, loss would be from there. Quantification of such effects requires numerical modeling on which future research should focus. Similarly, if present, uptake of deeper soil water through the tap root of certain species might have to be considered and be accounted for. Having these concepts in mind, it is crucial in dry environments to distinct between potential groundwater recharge (equal to deep drainage) and actual groundwater recharge (water arriving at the groundwater table).Actual groundwater recharge is then to be determined as follows:

$$R_A = D_D - V_u - UP_D \qquad \text{(eq. 5)}$$

where R_A [L] is actual groundwater recharge. D_D [L] represents deep drainage, Vu [L] upward water vapor transport and UP_D [L] uptake of deep soil water through the tap root of certain species (refer to section 4.2). Nevertheless, until now, the quantification of the latter two components remains very difficult (Hendrickx et al., 2005) and has to be investigated further, as stated previously. Quantification of UP_D becomes possible by applying a known amount and concentration of ^2H_2O in the unsaturated zone of a bare soil and subsequently monitoring tracer movement, moisture, matrix potential and soil temperature. Numerical models can then be fitted to the data and water vapor transport might be quantified The role of deeper-penetrating tap roots is labor intensive and appropriate methods are yet not available.

Since previous studies show that year-to-year change of soil water storage within the root zone is zero (Seyfried et al., 2005), precipitation or water input P [L] must exceed the storage capacity C [L] of a soil in a given period of time (i.e. the rainy season) in order to create deep drainage D_D [L] (Seyfried et al., 2005):

$$C = P_S * D * A_W \qquad \text{(eq. 6)}$$

where P_S [-] represents the soil porosity, D [L] the effective rooting depth and A_W the water available to the plants [-]. For the presented study site, C calculates as 73 mm for a rooting depth of 2 m and 91 mm for a rooting depth of 2.5 m. This means, that a period where P is exceeding 73 or 91 mm, respectively, is necessary in order to create groundwater recharge. This fairly low storage capacity indicates favorable conditions for the creation of deep drainage (not taking into account the unknown negative terms defined in equation 5). However, as stated previously, this does not necessarily coincide with high actual groundwater recharge. Nevertheless, such estimations can provide an initial assessment of an investigated site.

Ecohydrological separation?

We conclude this chapter with a brief discussion on how the presented research fits into the presented concepts of recent studies on ecohydrological separation (e.g. Brooks et al., 2010, 2015; Evaristo et al.; 2015; Goldsmith et al., 2012; Good et al., 2015; McDonnell, 2014; Phillips, 2010). In these studies it was found that in certain areas water used by trees is isotopically different than water from nearby streams and groundwater (Brooks, 2010; Goldsmith, 2012) and, thus, ecohydrological processes might be decoupled (McDonnell, 2014). In a global synthesis of studies (Evaristo et al., 2015) supporting, but also opposing evidence was presented. Related to this ecohydrological separation (or, the '*two water worlds – hypothesis*') we interpret our findings as follows: In the dual-isotope plot (Fig. 3), xylem samples clearly plot in the same isotopic range as groundwater, precipitation and deep soil water (> 0.5 m). Shallow soil water (< 0.5 m), on the other hand, does not coincide with any of the xylem samples. In the authors' opinion, this simply is due to the fact that the upper soil dries out very fast after the rainy season or individual rain events and at a certain point plants are not able to extract anymore water from there. Water contents in the upper soil layer during the time of data collection were less than 0.5 vol.-% (data not shown herein). If water in deeper zones of the soil or groundwater is accessible by plants, we suppose that the local vegetation would utilize it. Thus, based on the data presented in Fig. 3, we do not see in our case that 'plants are using soil water that does itself not contribute to groundwater recharge or streamflow' (Evaristo et al., 2015). Rather than that, the mechanism in our case could be explained as follows: In deep unsaturated zones (such as our study site) water needs many years to reach the groundwater table. Eventually, only extreme events or very wet seasons create recharge (Beyer et al., 2014; Evaristo et al., 2015; Taylor et al., 2013). This water, once reaching the groundwater, could be isotopically different from water present in the upper unsaturated zone (refer to Fig. 3). Small rain events will be completely consumed from the upper part of the soil by plants and evaporation. It seems obvious that these events do not contribute to groundwater recharge. Hence; if water supply to plants is from the upper soil - which is mainly in the rainy season - the reservoir used by plants is different than the water recharging the ground, but this is mainly due to the nature of rainfall events (extreme events and long wet spells). In the dry season, on the other hand, plants use deeper soil water (and/or potentially groundwater), because the upper soil simply is too dry and this is what Fig.3 reflects. We believe that this concept mainly applies to arid and semiarid environments, but needs to be considered when investigating ecohydrological separation.

5. Conclusion

A field methodology for the investigation of rooting depths (or, maximum depth of water uptake), water uptake dynamics and water transport through soils using the stable water isotope deuterium as artificial tracer has been developed. The use of ^2H_2O was proven to be appropriate for minimum-invasive investigations in the unsaturated zone of semiarid environments. The technique can be used to investigate root activity, which proposes a major benefit for future application.

We conclude that in order to potentially create groundwater recharge, single (extreme) rain events or wet spells have to reach depths of at least 2.5 m. Even if this is the case, processes such as upward water vapor transport and potential uptake of groundwater through the tap root of certain tree species have to be accounted for. We therefore propose incorporating such processes into groundwater recharge estimations in arid and semiarid environments. The proposed methodology enables studying water uptake dynamics and partitioning of water between different plant species in certain areas of the root zone. The application of the proposed methodology can support the parameterization of SVAT – models, where rooting depth is one of the main parameters related to plant water uptake but also one that is generally treated as unknown. The study also showed that upward water vapor transport through the unsaturated zone during the dry season at the investigated site is present. The diffusive component of this effect was approximated in this study, but the overall contribution of this process (diffusive and thermal) transport of water vapor needs to be investigated further. Using a known concentration of tracer, monitoring in higher spatiotemporal resolution and the application of SVAT models which are able to simulate vapor phase transport processes might enable a quantification of this effect. ^2H_2O has been shown to have a great potential for studies at the soil-vegetation-atmosphere interface. Small amounts of tracer are necessary; hence, field experiments become increasingly economically feasible. Furthermore, the use of ^2H_2O enables quantitative studies of water fluxes. Future research should focus on incorporating the complete range of processes within the unsaturated zone of dry environments important for the water balance. As a first step, the magnitude of effects such as groundwater uptake through trees or, as proven to be of importance in the presented study, upward water vapor transport, should be determined. Tracer methods and, in particular ^2H_2O, are valuable and necessary tools for such applications.

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Table 1. Summary of the experiments related to rooting depth and water uptake dynamics at the six plots.

vegetation type	Kalahari woodland/forest	
Dominant plant species **(main focus of investigation)**	*Baikiea plurijuga (Zambezi Teak); Acacia erioloba (Camelthorn); Salacia luebertii (Wild mango)*	
Other plant species investigated	*Baikiea plurijuga (Zambezi Teak); Acacia erioloba (Camelthorn); Combretum collinum (Kalahari Bushwillow);Salacia luebertii (Wild mango); Terminalia sericea (Silver cluster leaf); Erythrophleum africanum(Ordeal tree); Ochna pulchra (Mermaid tree)*	
mean isotopic composition of background samples	**δ^{18}O / δ^{2}H / d-excess [‰]**	
	xylem samples	**transpiration bag samples**
▪ all samples	-8.9 / -72.1 / -1	8.9 / 7.3 / -64
▪ *Baikiea plurijuga*	-7.4 / -60.4 / -1	12.3 / 16.3 / -82
▪ *Acacia erioloba*	-10.1 / -82.0 / -1	10.9 / 11.5 / -7
▪ *Combretum collinum*	-9.8 / - 73.6 / 5	7.8 / 5.4 / -57
▪ *Salacia luebertii*	-8.7 / -75.3 / -6	10.7 / 14.6 / -71
▪ *Terminalia sericea*	-7.5 / -60.9 / -1	-
▪ *Erythrophleum africanum*	-10.0 / -80.4 / -1	5.0 / -7.8 / -48
samples containing ^{2}H	**no. of samples with $^{2}H_2O$ (total no. of samples)**	
▪ total	31 (162)	
▪ depth of labeling: 0.5 m	10 (29)	
▪ depth of labeling: 1 m	9 (37)	
▪ depth of labeling: 2 m	10 (28)	
▪ depth of labeling: 2.5 m	0 (17)	
▪ depth of labeling: 4 m	0 (29)	
▪ depth of labeling: 4 m near stem	2 (22)	
farthest species with ^{2}H found [species / lateral distance to labeling]	*Combretum collinum* / 6 m	
deepest uptake [species / vertical distance to labeling]	*Salacia luebertii* / 4 m	
highest concentration of ^{2}H encountered for xylem samples [species / concentration]	*Baikiea plurijuga /* 2015 ‰	
highest concentration of ^{2}H encountered for transpiration samples [species / concentration]	*Baikiea plurijuga /* 825 ‰	
regression coefficients & coefficient of determination (R^2)	**$\delta D = sl\ \delta^{18}O + b$**	
▪ xylem samples	sl = 6.80; b = -13.17; R^2 = 0.90	
▪ transpiration samples	sl = 2.29; b = -12.17; R^2 = 0.86	
▪ precipitation samples (LMWL)	sl = 7.31; b = 5.38 ; R^2 = 0.97	
▪ shallow (< 0.5 m) soil water samples	sl = 2.08; b = -30.42; R^2 = 0.79	
▪ deep (> 0.5 m) soil water samples	sl = 6.96; b = -0.50; R^2 = 0.97	

Table 2: Soil hydraulic properties of the soil at the study site and derived parameters from i) soil cores, ii) pore size distribution (parameters estimated using the software Rosetta; Department of Soil, 2002) and iii) calibration (based on data published in Beyer et al., 2015) using the SVAT model DAISY (Abrahamsen and Hansen, 2000). Based on these parameter sets, the depth associated with stage I evaporation and diffusive flux over the dry season (stage II evaporation) were calculated.

Parameter	Soil cores (mean)	Pore size distribution	Calibrated	Sources/Method
bulk density	[1]1.59 g cm^{-3}	-	1.53 g cm^{-3}	-
porosity	0.4			-
van Genuchten parameters **alpha** **n** **residual water content** **saturated water content** **saturated hydraulic conductivity**	[1]0.025 [1]5.819 1/cm [1]0.033 [1]0.35 [1]2491 cm d^{-1}	[2]0.033 [2]3.881 [2]0.05 [2]0.38 [2]1057 cm d^{-1}	[3]0.125 [3]2.200 [3]0.005 [3]0.36 [3]2500 cm d^{-1}	[1]Schindler et al. (2010) [2]Department of Soil (2002) [3]Abrahamsen and Hansen (2000)
saturated hydraulic conductivity (field)	2304 cm d^{-1}			double ring infiltrometer
Stage I evaporation – associated depth (L_G)	32.0 cm	21.2 cm	22.2 cm	Lehmann et al. (2008)
a) Stage II diffusive flux with vaporization plane = 4 m (tracer application depth) **C_∞ = 10 gm^{-3}** **whole dry season (165 d)** **C_∞ = 5 gm^{-3}** **whole dry season (165 d)**	1.79 x 10^{-3} mm d^{-1}0.30 mm 2.44 x 10^{-3} mm d^{-1} 0.40 mm			Shokri et al. (2009) Or et al. (2012)
b) Stage II diffusive flux with vaporization plane = 2.5 m (supposed end of root zone) **C_∞= 10 gm^{-3}** **whole dry season (165 d)** **C_∞= 5 gm^{-3}** **whole dry season (165 d)**	2.87 x 10^{-3} mm d^{-1} 0.47 mm 3.91 x10^{-3} mm d^{-1} 0.65 mm			Shokri et al. (2009) Or et al. (2012)
grain size distribution 0 – 100 cm 100 cm >>	**% sand / % silt+ clay** sand (97.7 / 2.3) sand (97.3 / 2.7)			dynamic image analysis
total rainfall 2013/14 **potential recharge 2013/14** **percent of precipitation**	660 mm y^{-1} 45 mm y^{-1} ~7 %			Beyer et al. (2015)
mean groundwater recharge	2 – 20 mm y^{-1}			Struckmeier & Richts (2008)

Appendix 1: Plant distribution at the labeled plots, height of plants (in brackets) and tracer application area. Indicators used in the scheme (e.g. 'young', 'far', etc.) are in accordance to Figure 4.

Figure 1a: Digital Elevation Model (DEM) and drainage network of the Cuvelai-Etosha Basin (CEB). The study site (Elundu) is located in the Eastern Sand Zone. Marked are climate stations implemented within the framework of SASSCAL (Southern African Science Service Centre for Climate Change and Adaptive Land Management) and major towns.

Figure 1b: Mean monthly precipitation at the closest meteorological station with longer term records (1902 – current). The two field campaigns relevant for the present study took place at the beginning and end of the dry season, respectively.

Figure 2: Schematic visualization of the tracer application via the auger-drilled holes and the typical composition of different functional groups (grasses, shrubs, trees) typically present in semiarid environments. Notes: Deep penetrating tap roots are common in dry environments, but species-, soil- and climate-dependent and not necessarily present on the study site. Grasses were not present at the study site.

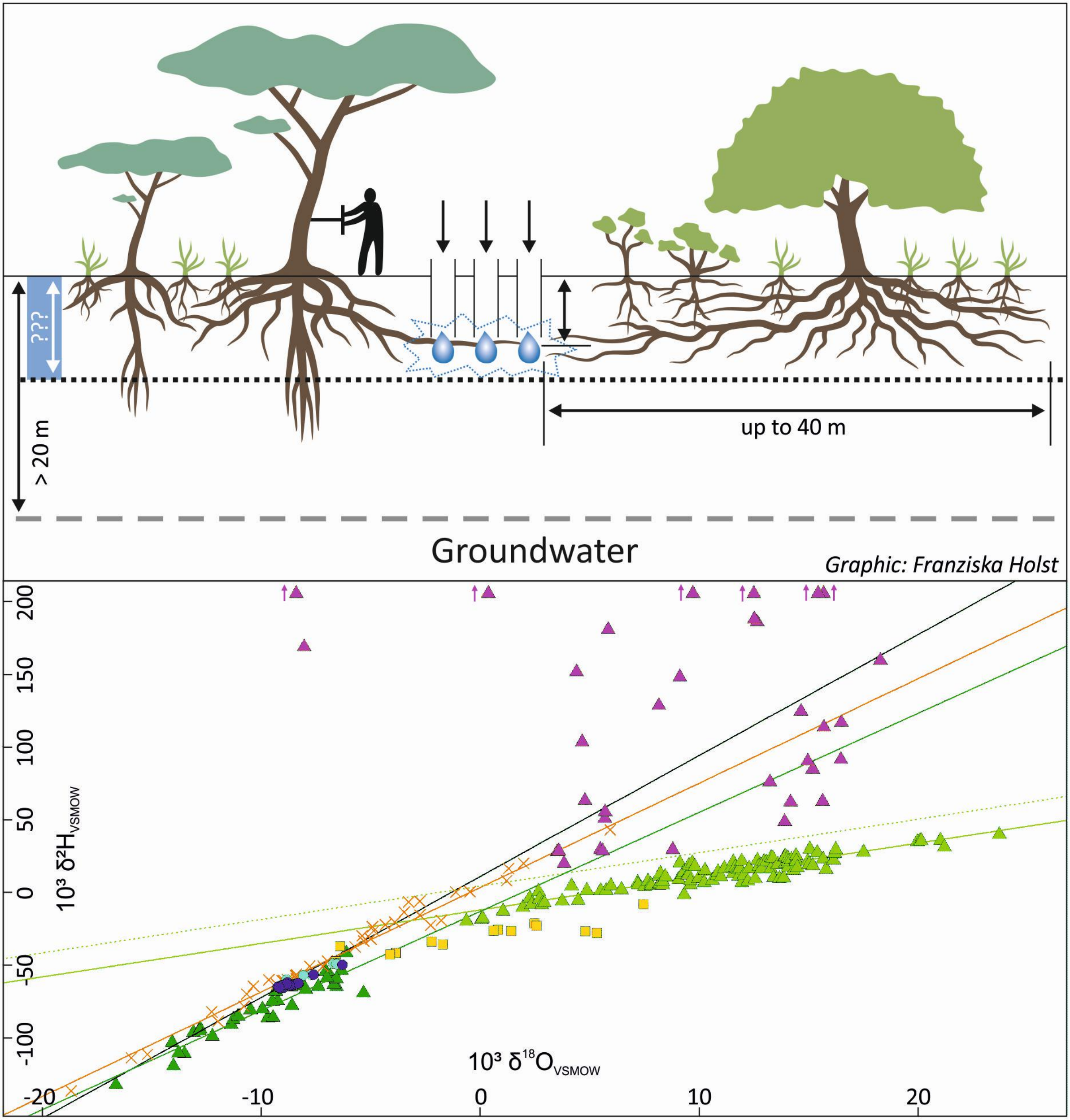
Figure 3: Dual-isotope plot of all samples collected and analyzed throughout the field campaigns. All plant samples taken are indicated by triangles. Violet symbols represent samples clearly containing artificial ^{2}H. The equations of the regression lines are compiled in Table 2.

Figure 4: Dynamics of tracer uptake during the course of the experiment for labeling depths of a) 0.5 m, b) 1 m, c) 2 m, d) 2.5 m, e) 4 m and f) 4 m (near stem of *Baikiea plurijuga*). In the graphic indicators for each plant (e.g. 'far', 'young', etc.) are employed in accordance to Appendix 1. For a differentiation of the sampling type the subscripts '(T)' and '(X)' are used for transpiration bag or xylem samples, respectively. For simplification, a line was inserted

above which samples are clearly artificially enriched in ^{2}H. Symbols not connected by lines represent additional (control) samples taken at the particular plot.

Figure 5: δ^2H and water content profiles after the dry season for labeling depths of a) 0.5 m, b) 1 m, c) 2 m and d) 4 m (near stem of *Baikiea plurijuga*). A distinct signature of artificially injected δ^2H can be identified at all plots. Note that in log scale (inset graphics) negative isotopic ratios cannot be displayed. (i.e. the missing values close to the surface represent background concentration due to recent rain events)

Graphical Abstract_R3



Graphic: Franziska Holst

Fig1a

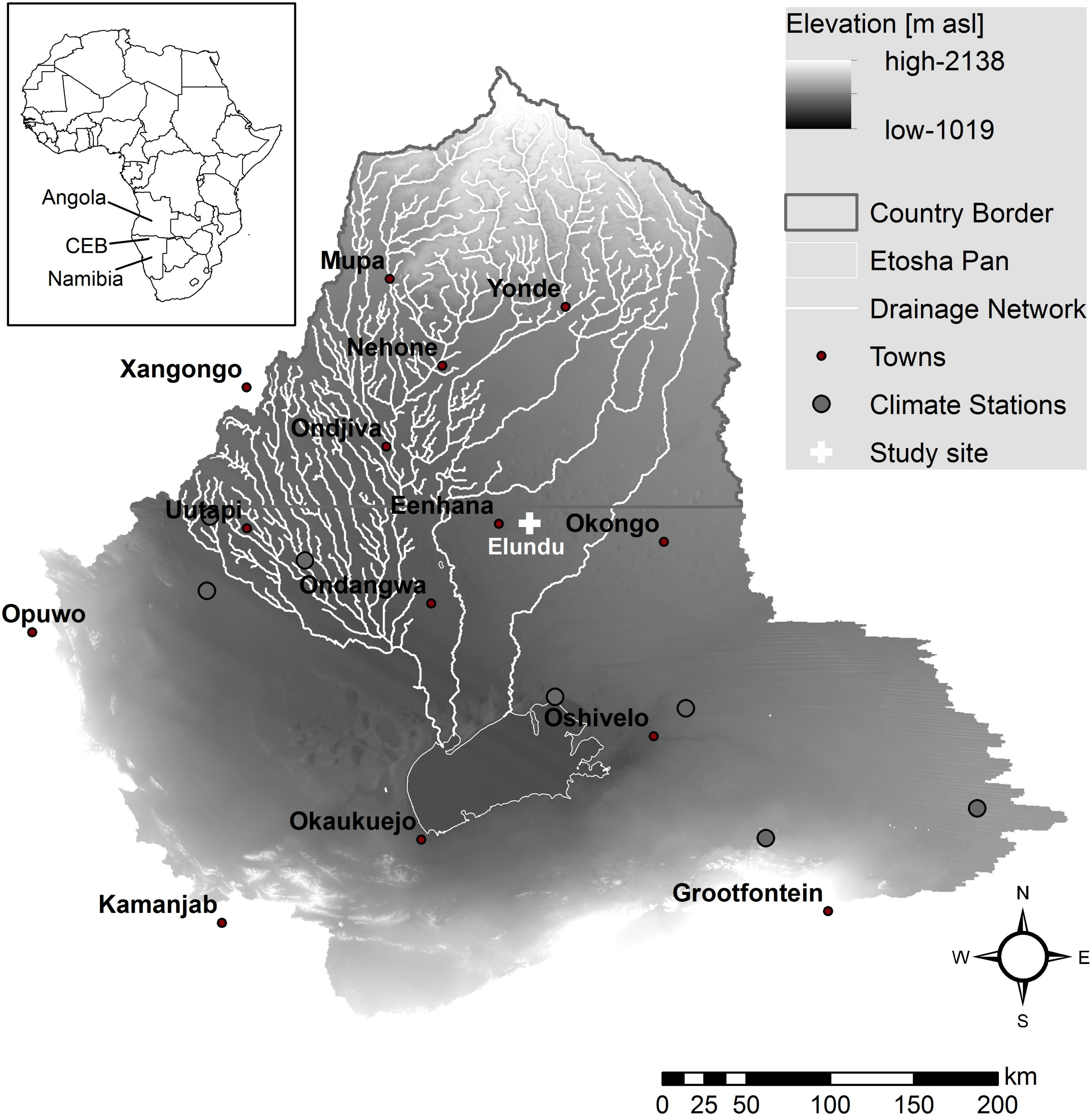

Angola
CEB
Namibia
Elevation [m asl]
high-2138
low-1019
Country Border
Etosha Pan
Drainage Network
Towns
Climate Stations
Study site
Mupa
Yonde
Nehone
Xangongo
Ondjiva
Eenhana
Elundu
Okongo
Uutapi
Ondangwa
Opuwo
Oshivelo
Okaukuejo
Grootfontein
Kamanjab
N
W
E
S
km
0
25
50
100
150
200

Fig1b

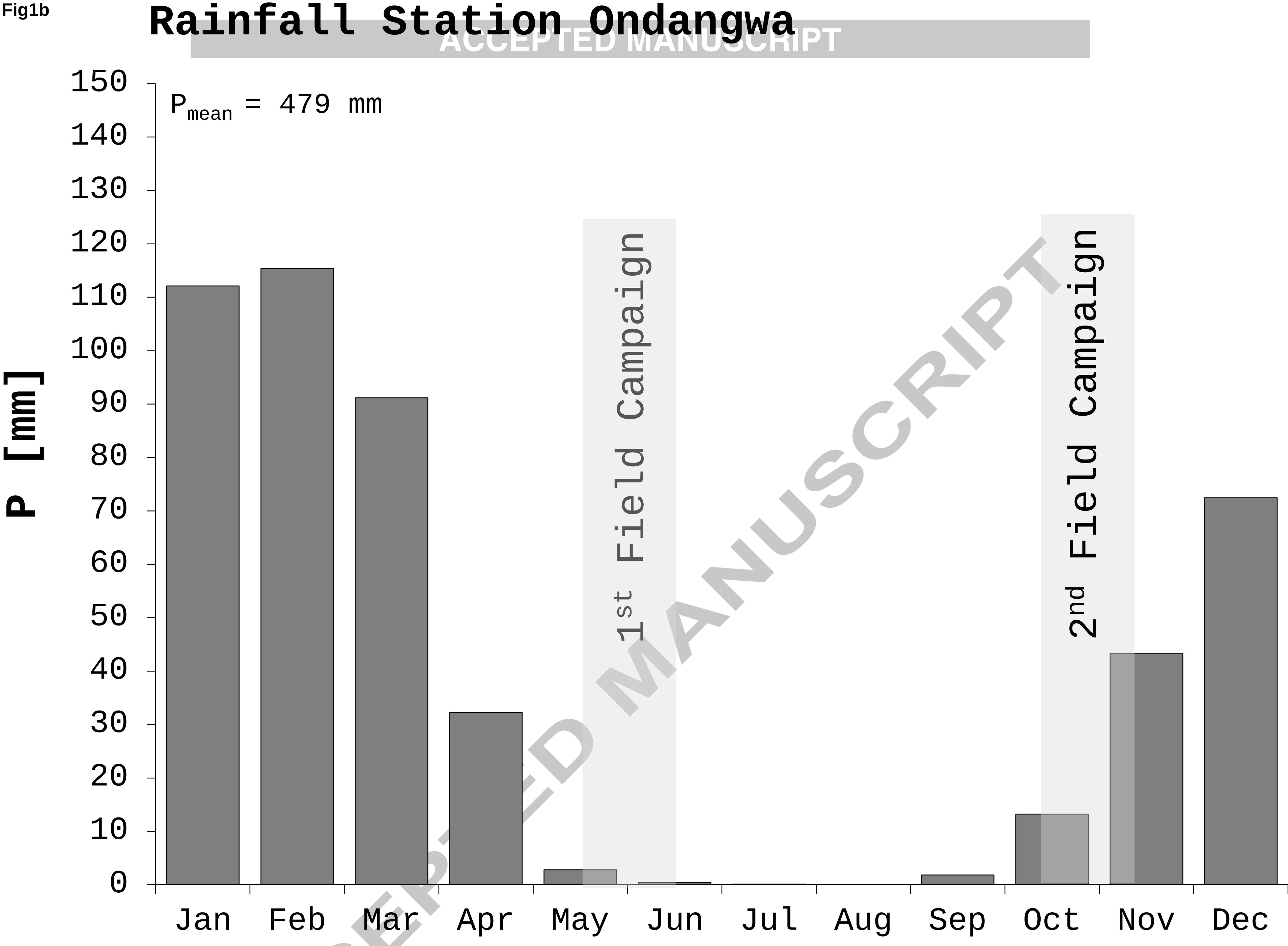
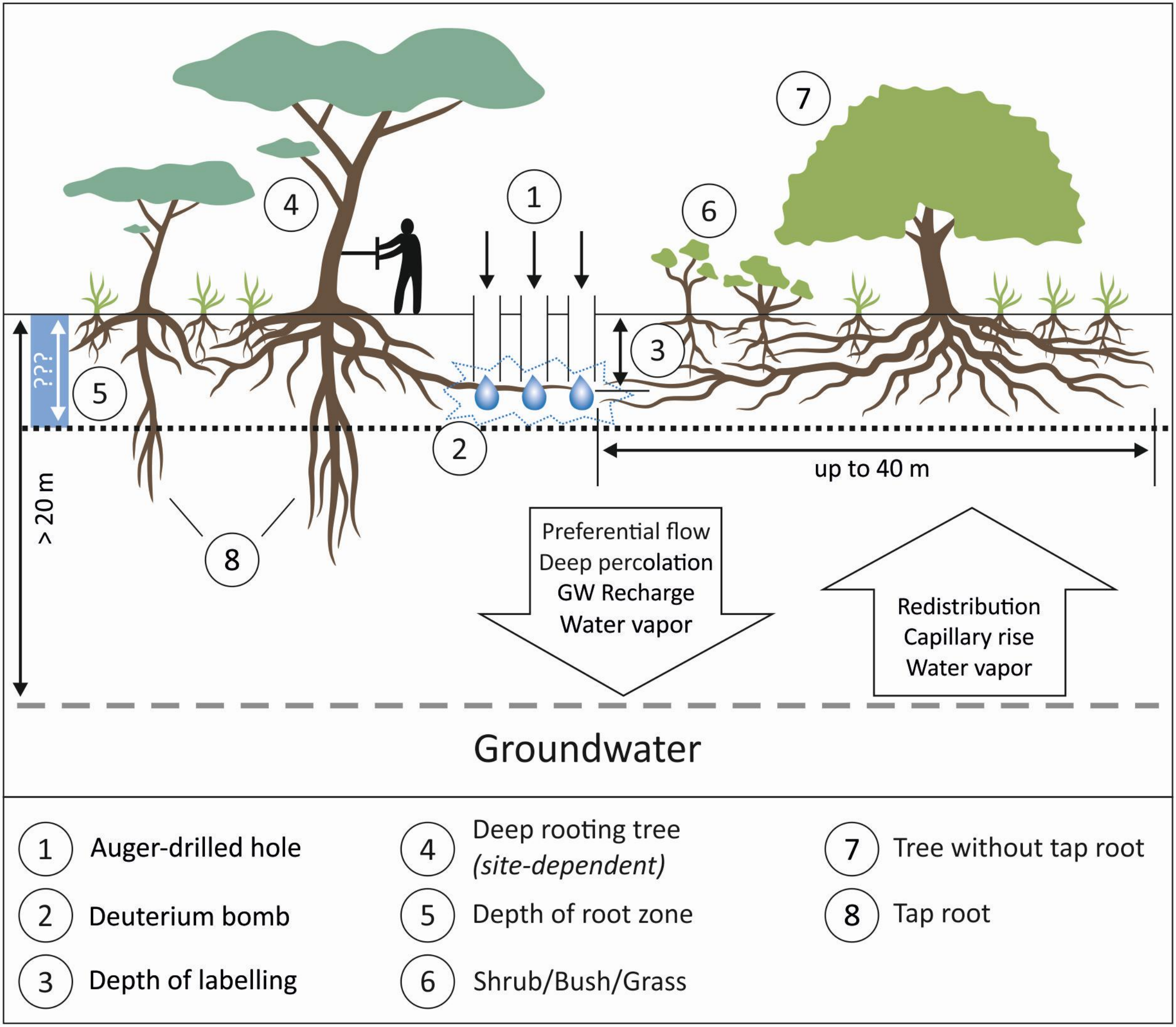

Rainfall Station Ondangwa
P_{mean} = 479 mm
P [mm]
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
1st Field Campaign
2nd Field Campaign

Fig2_R3



4
1
6
7
3
5
???
2
up to 40 m
> 20 m
8
Preferential flow
Deep percolation
GW Recharge
Water vapor
Redistribution
Capillary rise
Water vapor
Groundwater
1 Auger-drilled hole
2 Deuterium bomb
3 Depth of labelling
4 Deep rooting tree (site-dependent)
5 Depth of root zone
6 Shrub/Bush/Grass
7 Tree without tap root
8 Tap root

Fig3_R4

Fig. 3)

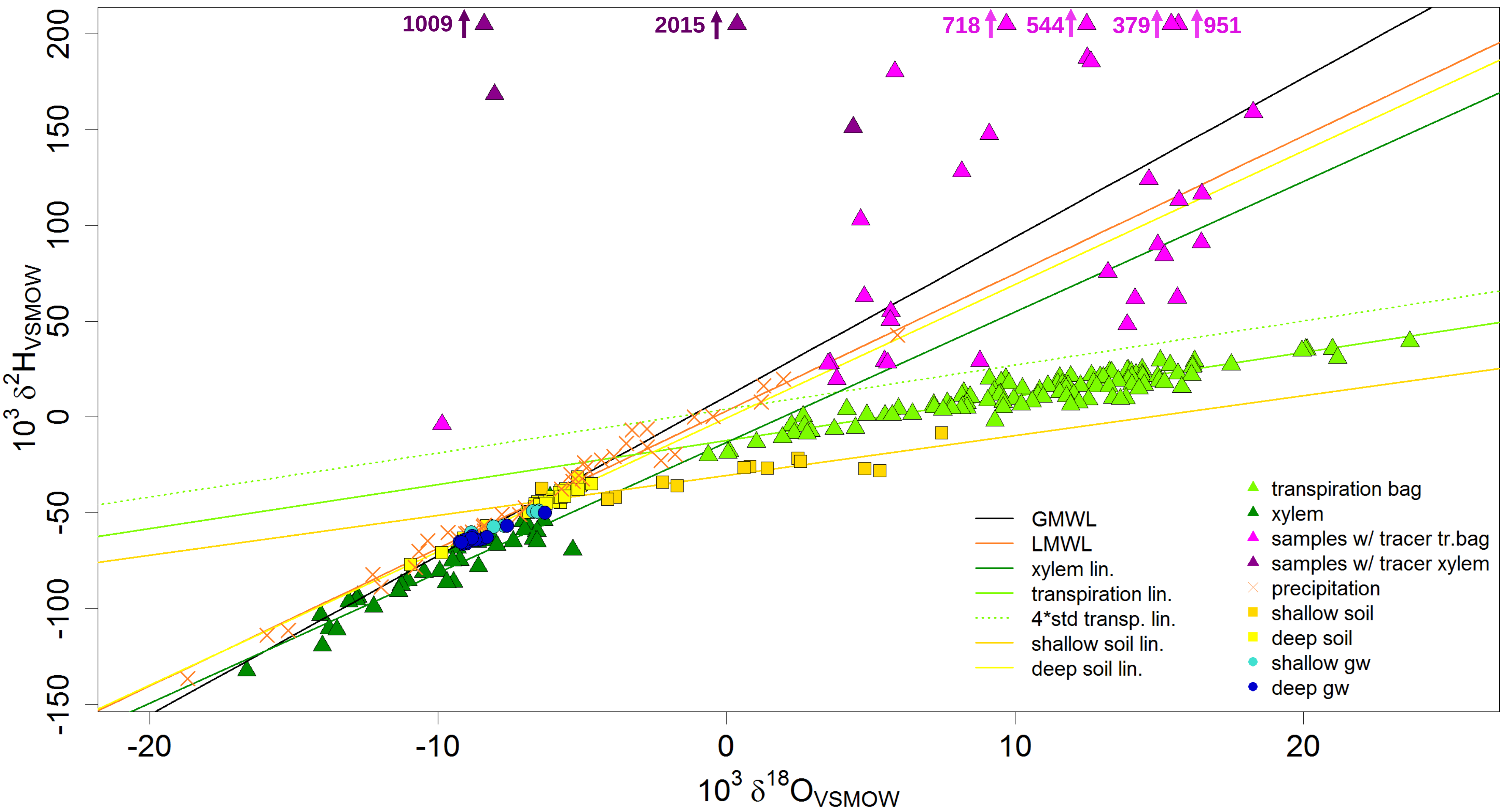
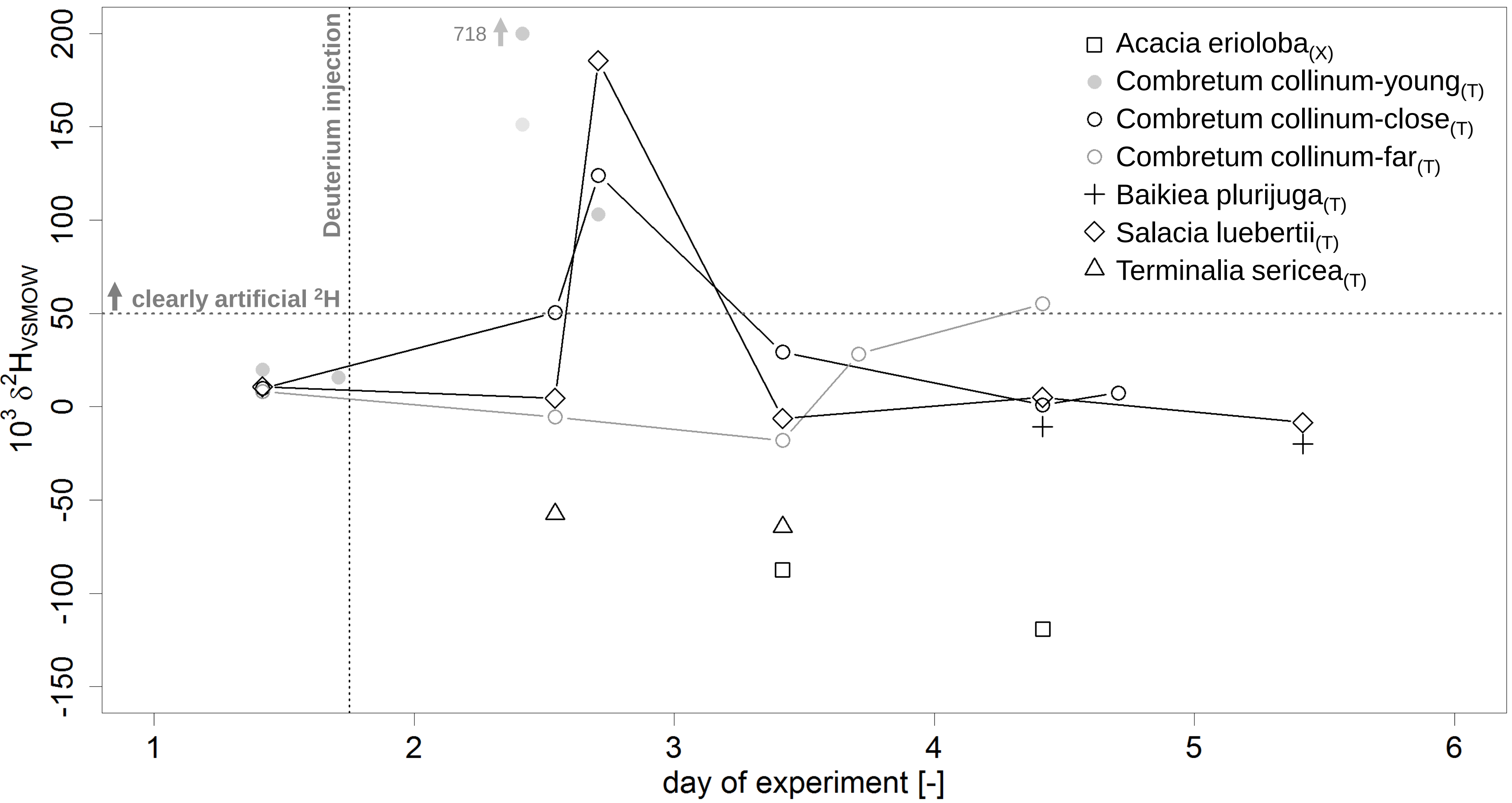
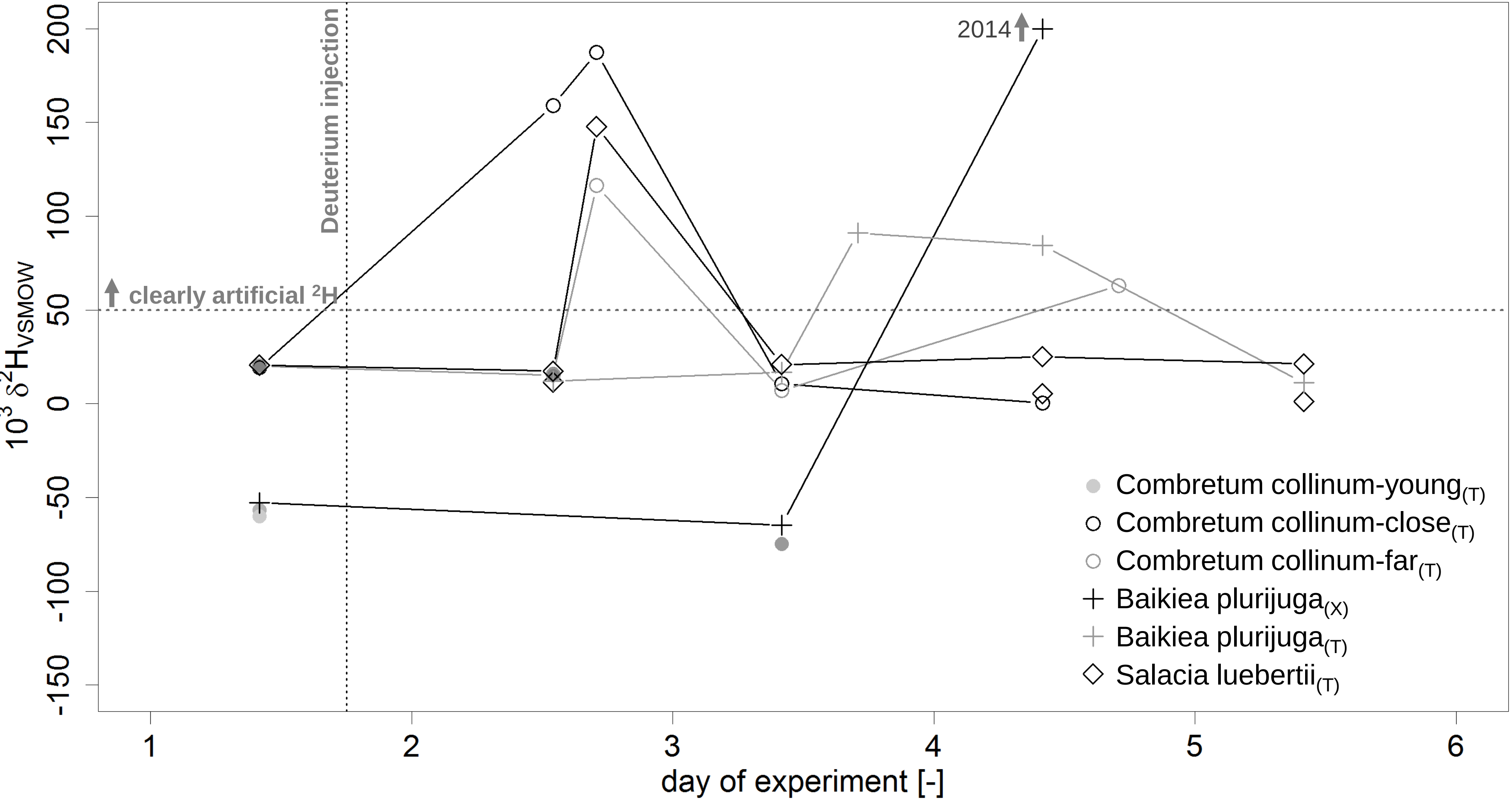
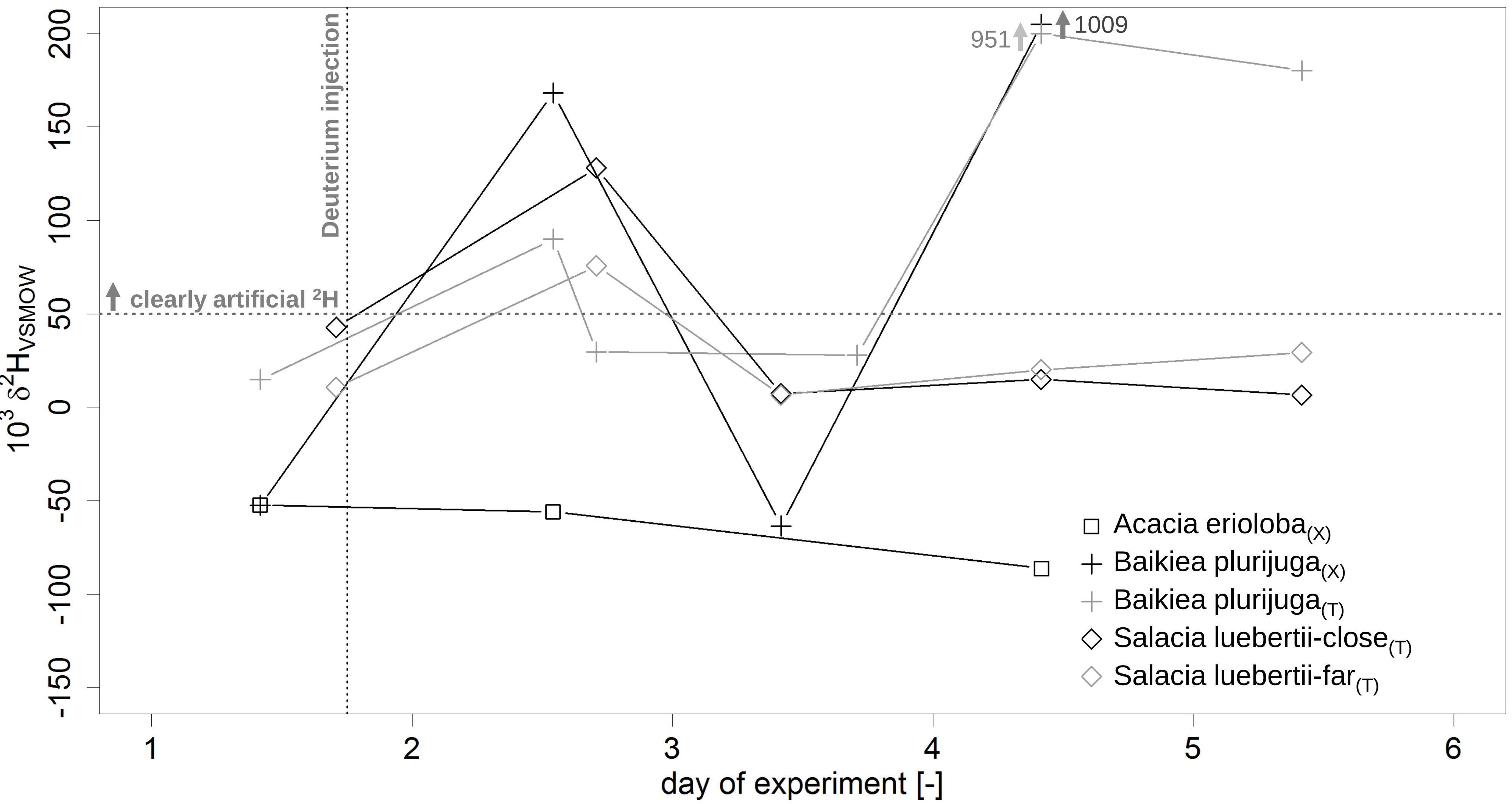

1009
2015
718
544
379
951
200
150
100
50
0
-50
-100
-150
$10^3\ \delta^2H_{VSMOW}$
-20
-10
0
10
20
$10^3\ \delta^{18}O_{VSMOW}$
GMWL
LMWL
xylem lin.
transpiration lin.
4*std transp. lin.
shallow soil lin.
deep soil lin.
transpiration bag
xylem
samples w/ tracer tr.bag
samples w/ tracer xylem
precipitation
shallow soil
deep soil
shallow gw
deep gw

Fig4

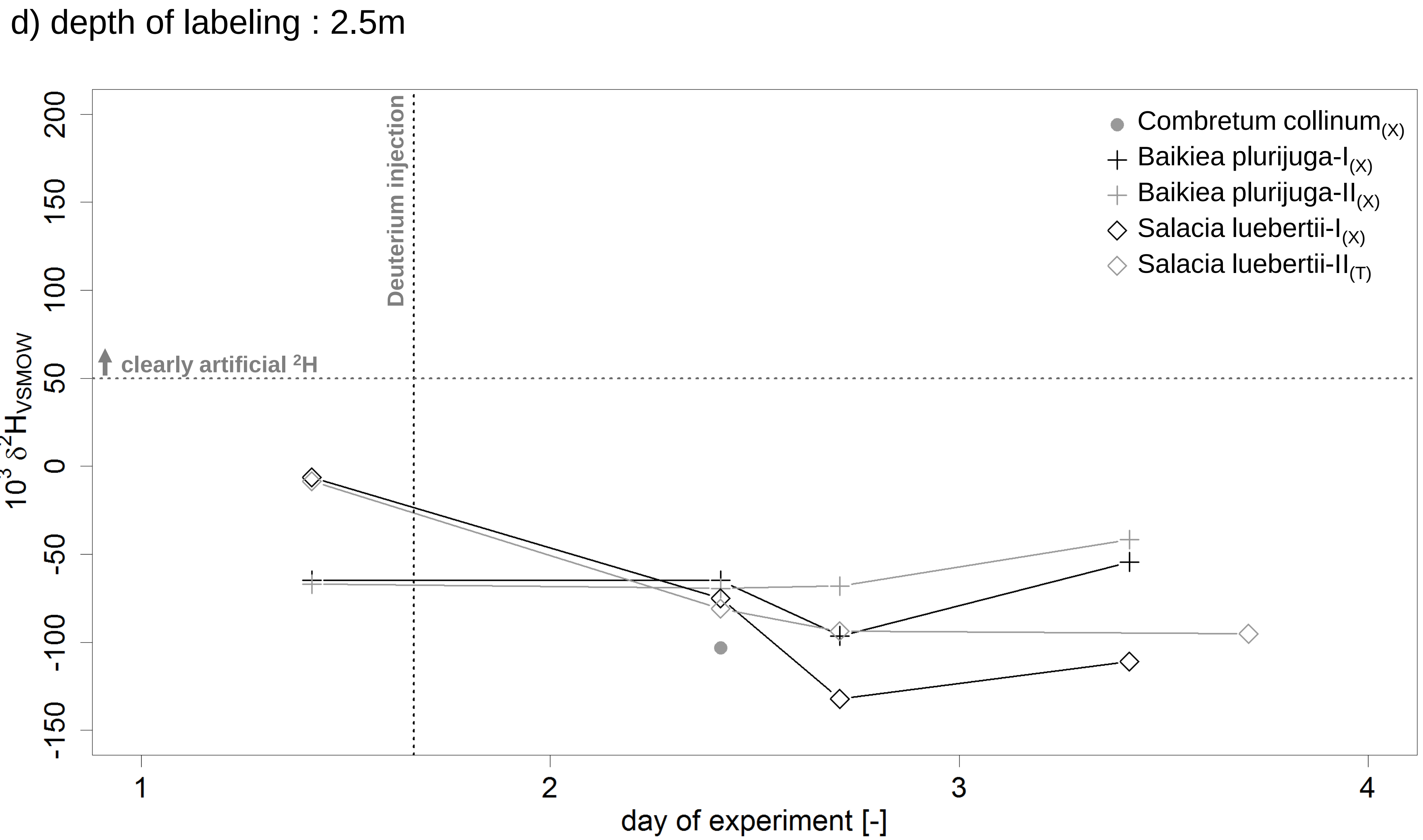


a) depth of labeling : 0.5m

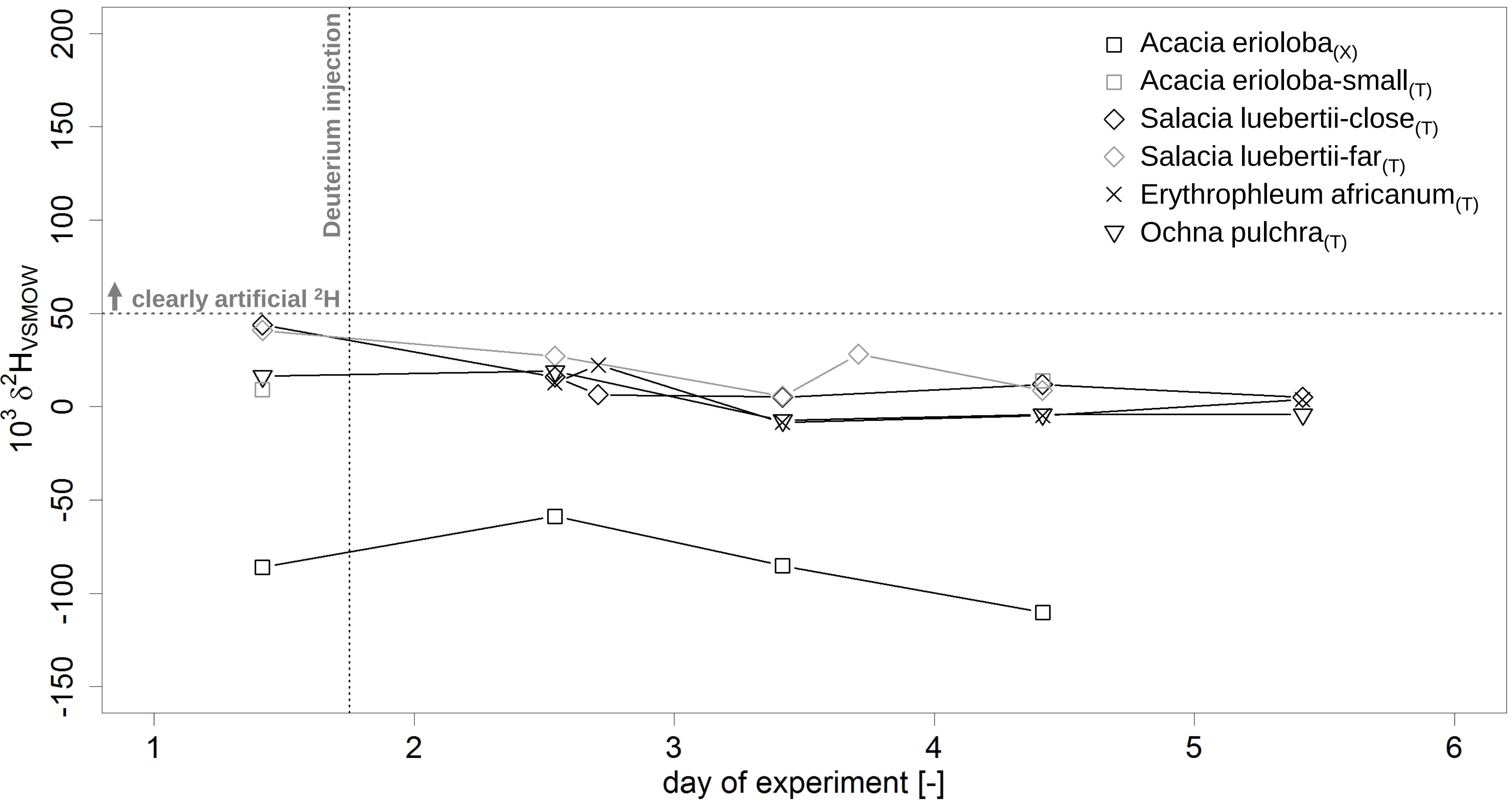

b) depth of labeling : 1m



c) depth of labeling : 2m


d) depth of labeling : 2.5m
Deuterium injection
clearly artificial ²H
Combretum collinum(X)
Baikiea plurijuga-I(X)
Baikiea plurijuga-II(X)
Salacia luebertii-I(X)
Salacia luebertii-II(T)
10³ δ²H VSMOW
200
150
100
50
0
-50
-100
-150
1
2
3
4
day of experiment [-]

e) depth of labeling : 4m



f) depth of labeling : 4m – near stem

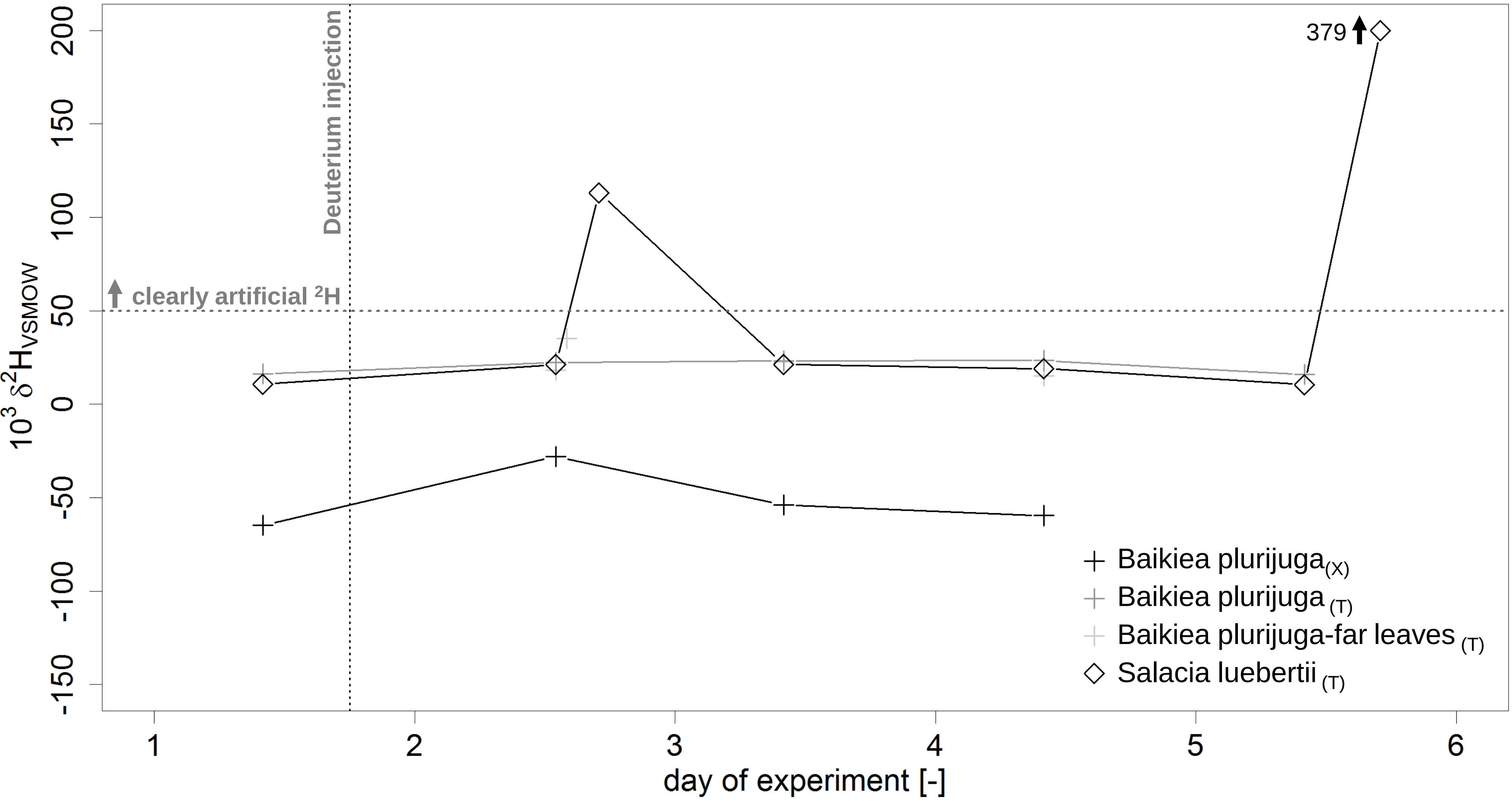
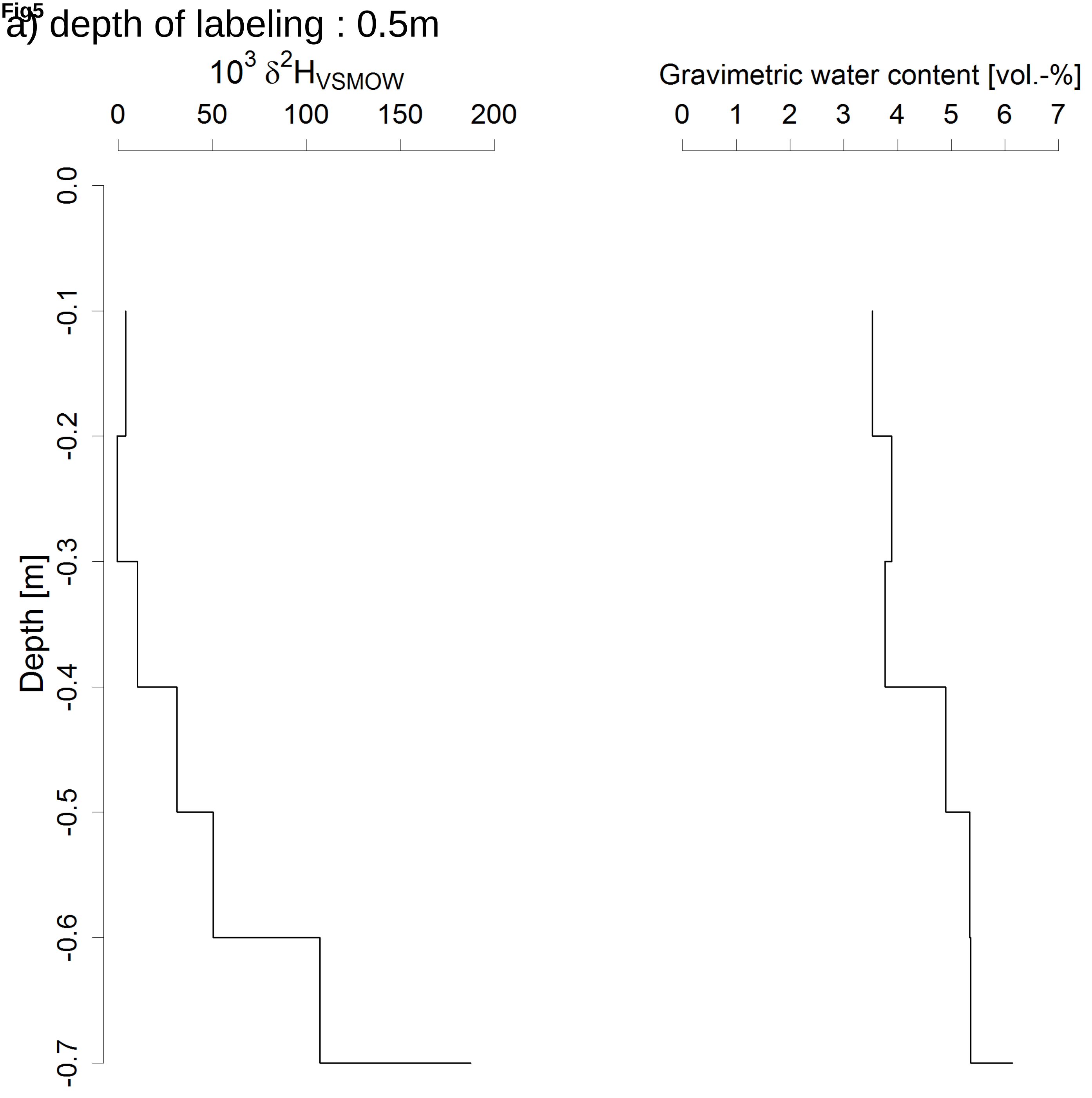
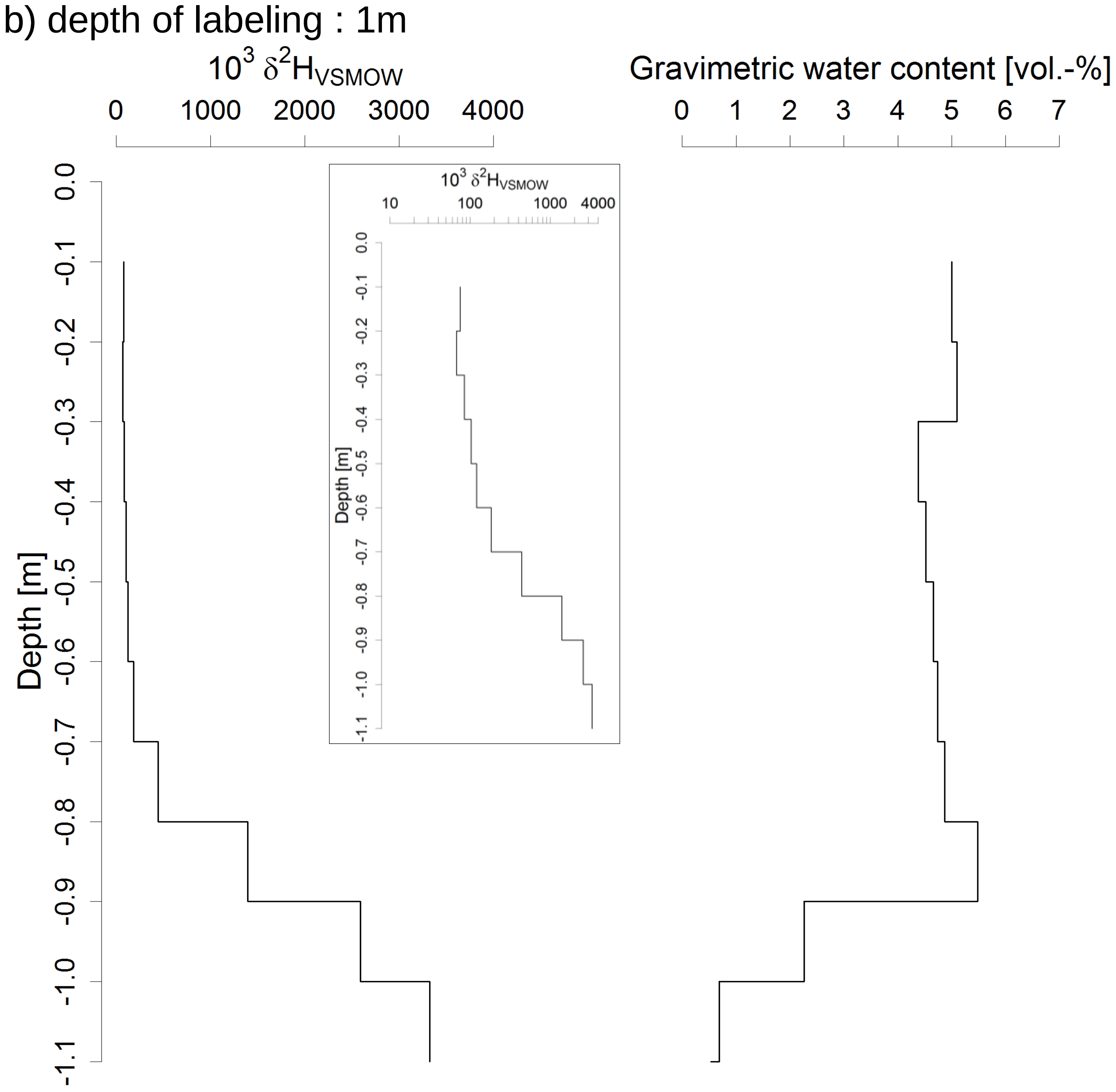
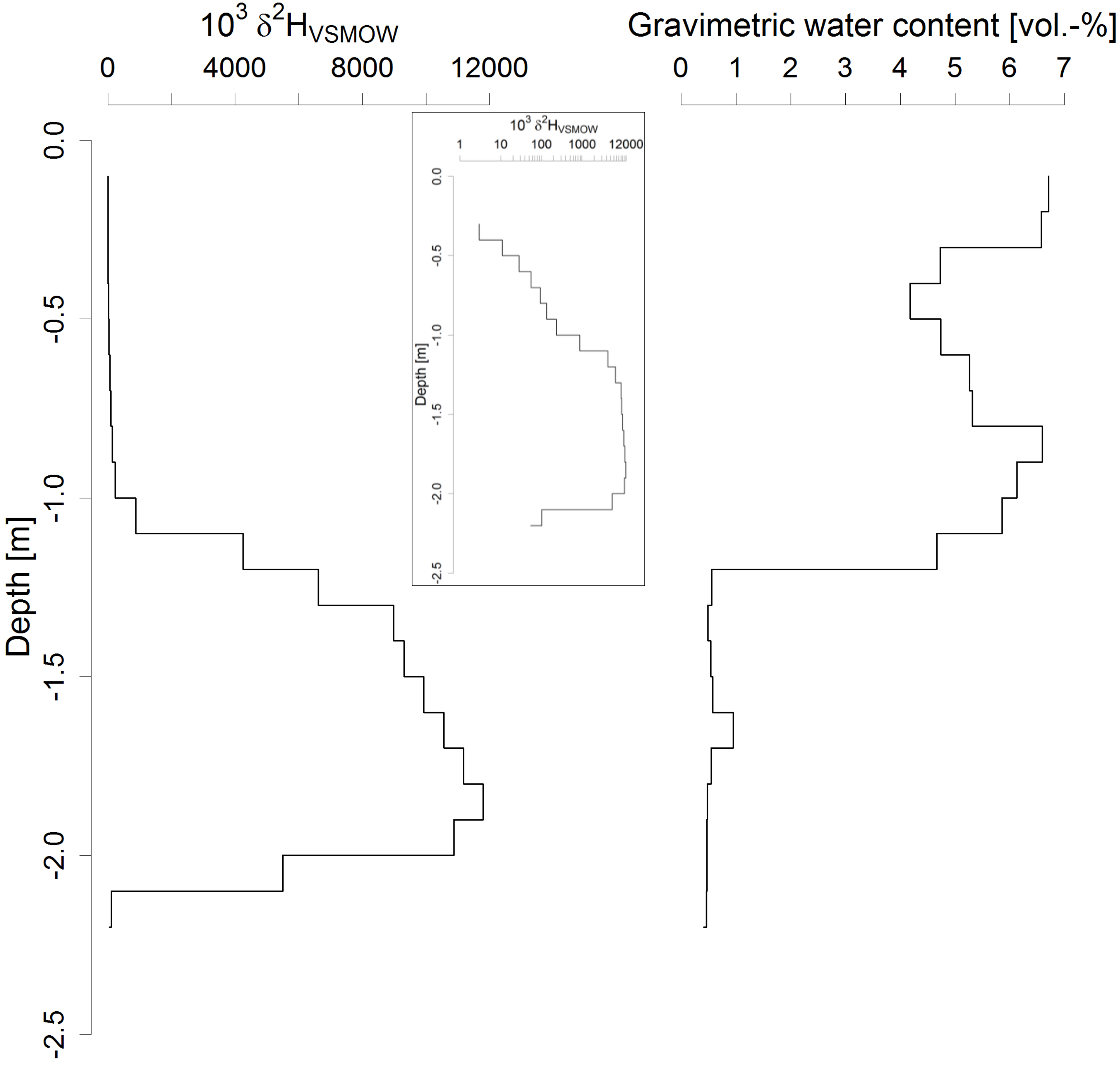


Fig5


a) depth of labeling : 0.5m
$10^3\ \delta^2H_{VSMOW}$
0
50
100
150
200
Gravimetric water content [vol.-%]
0
1
2
3
4
5
6
7
Depth [m]
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7

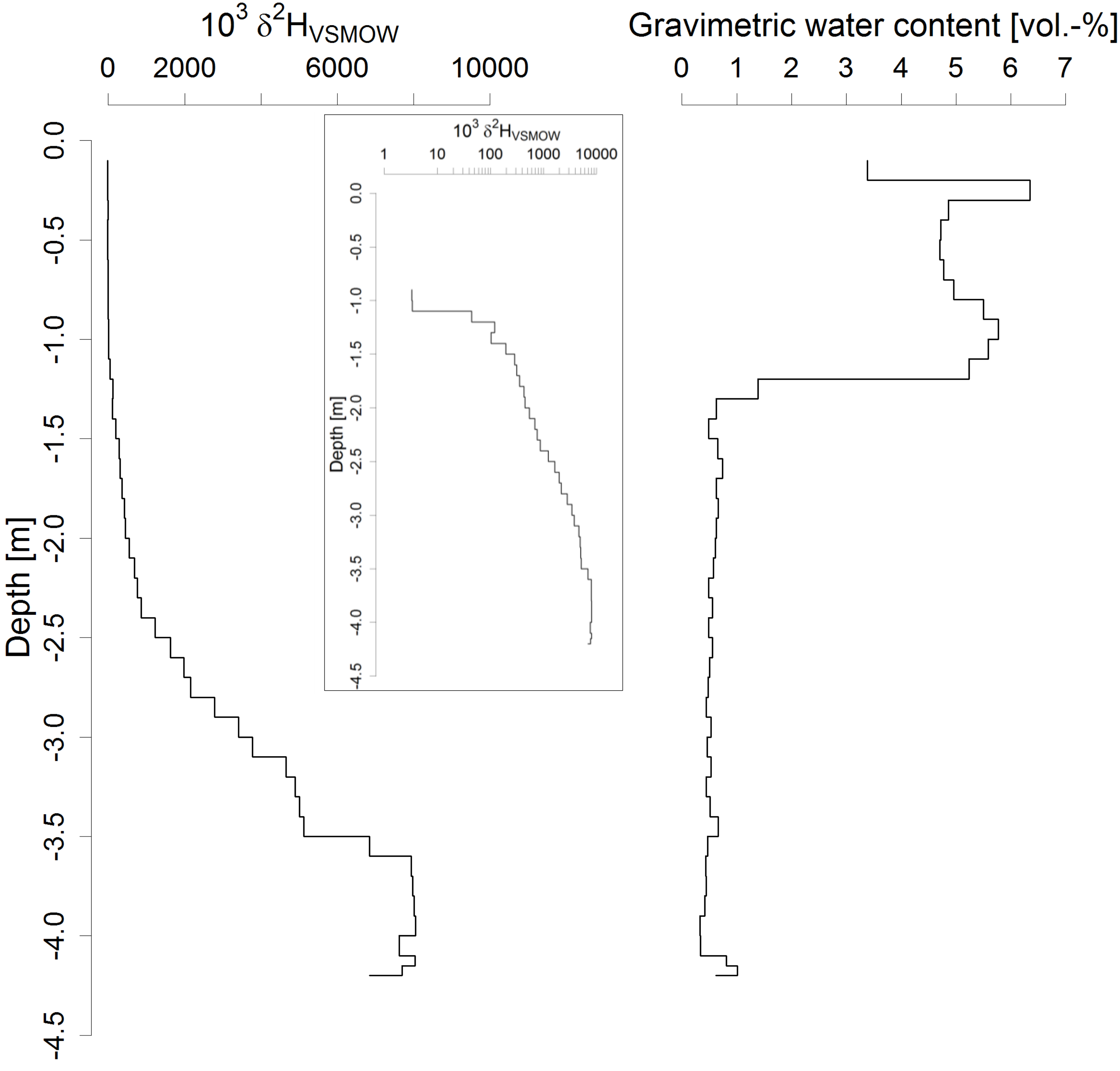


b) depth of labeling : 1m

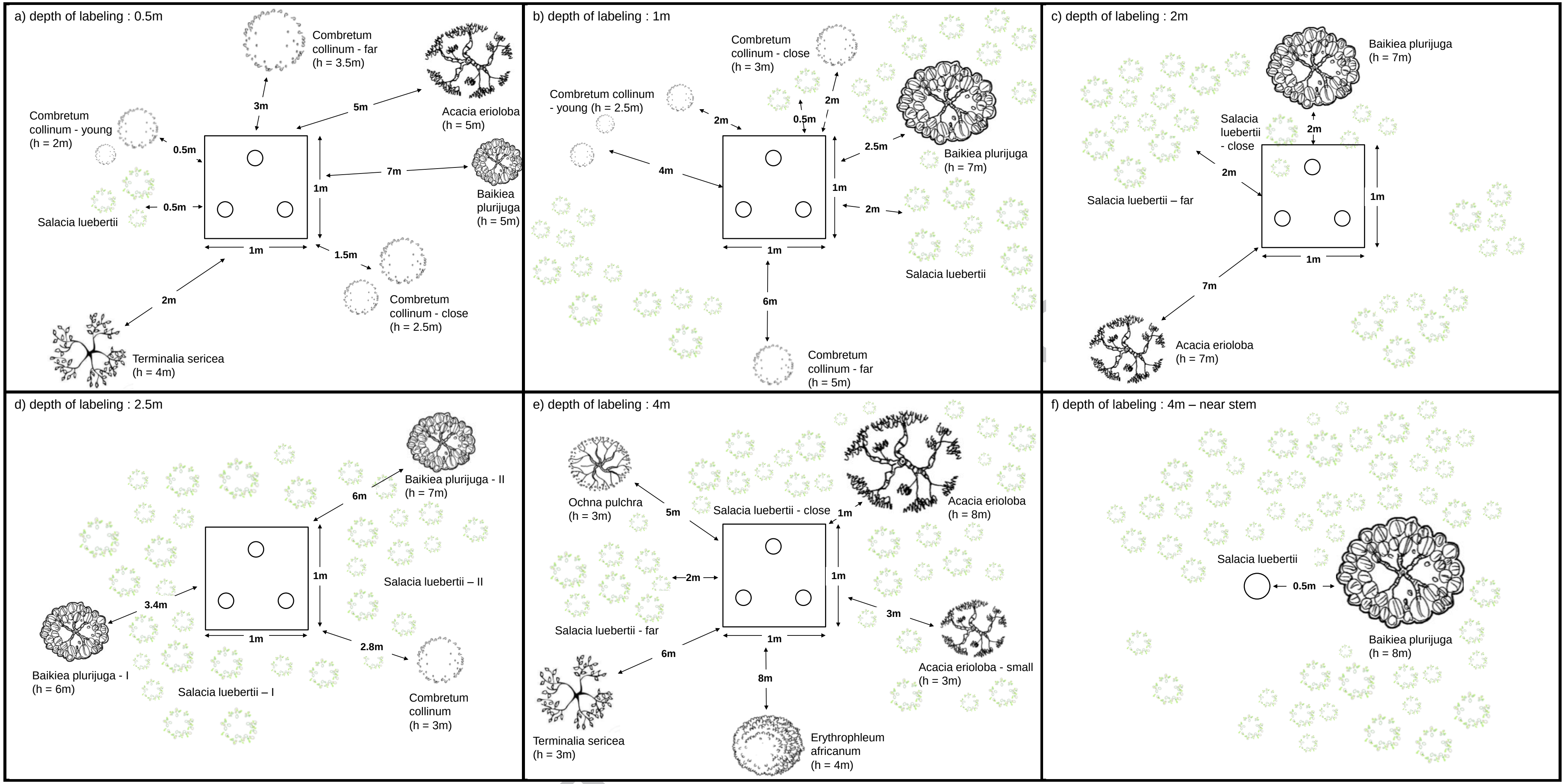


c) depth of labeling : 2m

d) depth of labeling : 4m – near stem



Appendix1


a) depth of labeling : 0.5m
Combretum collinum - far (h = 3.5m)
3m
5m
Acacia erioloba (h = 5m)
Combretum collinum - young (h = 2m)
0.5m
7m
1m
Baikiea plurijuga (h = 5m)
0.5m
Salacia luebertii
1m
1.5m
Combretum collinum - close (h = 2.5m)
2m
Terminalia sericea (h = 4m)
b) depth of labeling : 1m
Combretum collinum - close (h = 3m)
Combretum collinum - young (h = 2.5m)
2m
0.5m
2m
2.5m
Baikiea plurijuga (h = 7m)
4m
1m
2m
1m
Salacia luebertii
6m
Combretum collinum - far (h = 5m)
c) depth of labeling : 2m
Baikiea plurijuga (h = 7m)
Salacia luebertii - close
2m
2m
Salacia luebertii – far
1m
1m
7m
Acacia erioloba (h = 7m)
d) depth of labeling : 2.5m
Baikiea plurijuga - II (h = 7m)
6m
1m
Salacia luebertii – II
3.4m
1m
2.8m
Baikiea plurijuga - I (h = 6m)
Salacia luebertii – I
Combretum collinum (h = 3m)
e) depth of labeling : 4m
Ochna pulchra (h = 3m)
5m
Salacia luebertii - close
1m
Acacia erioloba (h = 8m)
2m
1m
Salacia luebertii - far
3m
Acacia erioloba - small (h = 3m)
6m
1m
8m
Terminalia sericea (h = 3m)
Erythrophleum africanum (h = 4m)
f) depth of labeling : 4m – near stem
Salacia luebertii
0.5m
Baikiea plurijuga (h = 8m)

Highlights

We present a minimum-invasive method for examining rooting depths.

The method allows the simultaneous study of plant water uptake and vapor transport.

Diffusive transport of water vapor over the dry season was quantified.

Deuterium is valuable for studies along the soil-vegetation-atmosphere interface.

The developed method is cost-effective and straightforward.