



Determining the source water and active root depth of woody plants using a deuterium tracer at a Savannah site in northern Stampriet Basin, Namibia

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Abstract

Woody plants play a significant role in the global water cycle through water uptake by roots and evapotranspiration. A deuterium tracer was used to assess the active root depths for *Salvia mellifera* and *Boscia albitrunca* in the Ebenhaezer area (western Namibia). The tracer was inserted at different soil depths in December 2016. Xylem cores were obtained using an increment borer, and transpired water was collected using transpiration bags zipped around the plants' leaves. Groundwater was collected from boreholes. Soil samples were collected after the rainy season using a hand auger. Xylem and soil water were extracted using a cryogenic vacuum extraction method and analysed for stable water isotopes. Only one *S. mellifera* transpiration sample showed a high deuterium content (516‰) where the tracer was inserted at 2.5-m soil depth. Elevated deuterium contents were observed in two *S. mellifera* xylem samples; tracer had been applied at 2.5 and 3 m depth (yielding 35 and 31‰ deuterium, respectively), which constitutes a possible active-root depth range for *S. mellifera*. At the end of the study period (May 2017), the average $\delta^{18}\text{O}$ value for *B. albitrunca* xylem samples was similar to that of groundwater. The $\delta^{18}\text{O}$ value for *S. mellifera* was between that of soil water and groundwater, indicating that this species uses groundwater and soil water available for groundwater recharge. Determination of the active root depth and source water for these species would help improve hydrological modelling by incorporating the influence of woody plants on groundwater recharge.

Keyword Arid regions · Stable water isotopes · Deuterium tracer · Namibia

Introduction

Woody plants play a fundamental role in the global water cycle and as part of the ecosystem, particularly with respect to water uptake by roots, evapotranspiration, and processes involved in groundwater recharge. Plants may use soil water, groundwater, or both, depending on their rooting system. Vertical niche separation model studies have demonstrated

that woody plants take up water from both shallower and deeper depths (Case et al. 2020; Scanlon et al. 2005). The models suggest that the competition for soil moisture among woody plants and grasses would be minimal, as they rely on soil water occurring at different soil depths, which allows niche separation to take place (Walter 1939). The depth from which plants can potentially extract source water is defined by the rooting depth and distribution (Kulmatiski et al. 2020; Zencich et al. 2002). Plants in environments with low moisture often experience water stress. As a result, they adapt by developing root morphologies that include shallow lateral roots and deep groundwater-tapping roots (Lubczynski 2009).

Woody plant encroachment impacts the sustainable management of groundwater in water-limited environments like Namibia (NAU 2010; Stafford et al. 2017). This study focuses on two woody species, namely *Senegalia mellifera* subsp. *detiens* (*S. mellifera*) and *Boscia albitrunca* (*B. albitrunca*). *S. mellifera* was chosen because it is considered one of the main bush encroacher species and is widely distributed in Namibia

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(Bester 1999; de Klerk 2004; Shikangalah and Mapani 2020). One of the impacts of woody plant encroachment is that it decreases groundwater recharge (NAU 2010; Acharya et al. 2018). Many bush encroacher species in arid and semiarid environments have shallow, spreading root systems that capture infiltrating water and extract water by roots, thereby decreasing recharge through the unsaturated zone (Lubczynski 2009; NAU 2010).

S. mellifera appears to have primarily shallow and straight roots and it was observed to have an extensive root system in the upper layers of the soil, thus extending uniformly from its stem. Due to the concentration of roots in the upper soil layers, this species has the advantage of receiving water from even low precipitation events (Geißler et al. 2019; Sala et al. 1992). *S. mellifera* has also been demonstrated to develop a tap root with a depth of more than 30 m for a plant observed at the farm Aiams in the Otavi district, Namibia (NAU 2010). As a result, the species may be adapted to seek out groundwater while exploiting soil water with an extensive lateral root system, either to support growth until groundwater is reached or to absorb water moved to the surface by hydraulic redistribution (Burgess et al. 1998; Scott et al. 2008).

An evergreen species, *B. albitrunca*, was chosen because it is documented to have deep roots and is widely distributed in Namibia (Curtis and Mannheimer 2005). Jennings (1974) encountered *B. albitrunca* roots at about 70 m depth in borehole cores in the Kalahari sands. A deep-rooted *B. albitrunca* can easily access groundwater, and hence affects groundwater resources. *B. albitrunca* has a strategy of extending individual roots at much greater depths than *S. mellifera*; *B. albitrunca* with deep-rooted, sinuous roots shows a different distribution of lateral root biomass with depth, with hardly any roots in the upper soil layers (O'Donnell et al. 2015). Kalahari trees with extremely deep roots reach groundwater and remain green throughout the dry season, which is the case for *B. albitrunca* (Obakeng 2007).

Water stable isotopic composition can distinguish source water by comparing such compositions from plant xylem to those of groundwater, soil water, and precipitation at the same study site (Geißler et al. 2019; von Freyberg et al. 2020). To trace the isotopic composition of precipitation from which plant xylem water originated, intersection points of local plant xylem evaporation lines with local meteoric water lines (LMWLs) are calculated using Eqs. (1) and (2), to derive plant xylem source values (Evaristo et al. 2015).

$$\delta^2\text{H}_{\text{intercept}} = \delta^2\text{H} - m\delta^{18}\text{O} \quad (1)$$

$$\delta^{18}\text{O}_{\text{intercept}} = (\delta^2\text{H}_{\text{intercept}} - b)/a \quad (2)$$

whereby m is the slope of the evaporation line, a is the LMWL slope, and b is the LMWL intercept. However, Eq. (1) was revised by Javaux et al. (2016) to Eq. (3):

$$\delta^2\text{H}_{\text{intercept}} = \delta^2\text{H} - m(\delta^{18}\text{O} - \delta^{18}\text{O}_{\text{intercept}}) \quad (3)$$

Alternatively, the point on the LMWL where the plant xylem water evaporation line intersects can provide a good approximation of the mean isotopic value of plant xylem source precipitation (Evaristo et al. 2015). Plant xylem water is preferred over transpired water for determining the water source because fractionation at a leaf level causes a more enriched isotope value (Beyer et al. 2016; Evaristo et al. 2015; Flanagan et al. 2019; Kulmatiski and Forero 2021). Nevertheless, new approaches account for fractionation processes using the Craig and Gordon model to map leaf water back to its respective precipitation event water sources (Benettin et al. 2021).

Lubis et al. (2014) determined plant water sources using stable water isotopes in Riau, Indonesia. Their study found that oil palms absorb water from 0–50 cm depths, corresponding to the most active root of oil palm that absorbs nutrients, water, and oxygen. Zencich et al. (2002) studied seasonal water sources for species growing on a coastal dune system that overlies a shallow sandy aquifer in southwestern Australia. The authors found that plants use more water from the upper layers of the soil profile during the wet winter. A study by Beyer et al. (2016) using deuterium as an artificial tracer to investigate rooting depths in a semiarid environment in northern Namibia suggests the primary root zone ended between 2 and 2.5 m below the ground surface.

Knowledge of the influence of vegetation, especially bush encroacher species, on groundwater recharge in semiarid regions such as Namibia still needs to be improved, with only one preliminary report in the grey literature for Namibia (NAU 2010). The objective of this study is thus to determine the influence of woody plants on groundwater recharge at the farm site Ebenhaezer, in the Stampriet Basin region of southeast Namibia, using two species, namely *S. mellifera* and *B. albitrunca*, by determining their effective root depths and source water.

Materials and methods

Study site

The study site is in the Ebenhaezer area in Namibia's northern part of the Stampriet Basin (Fig. 1). The mean annual precipitation within the basin ranges between 175 and 240 mm, and the mean potential evaporation varies between 3,000 and 3,500 mm/year (DWA 1988). The study area is generally flat with an elevation of ~1,200 m above mean sea

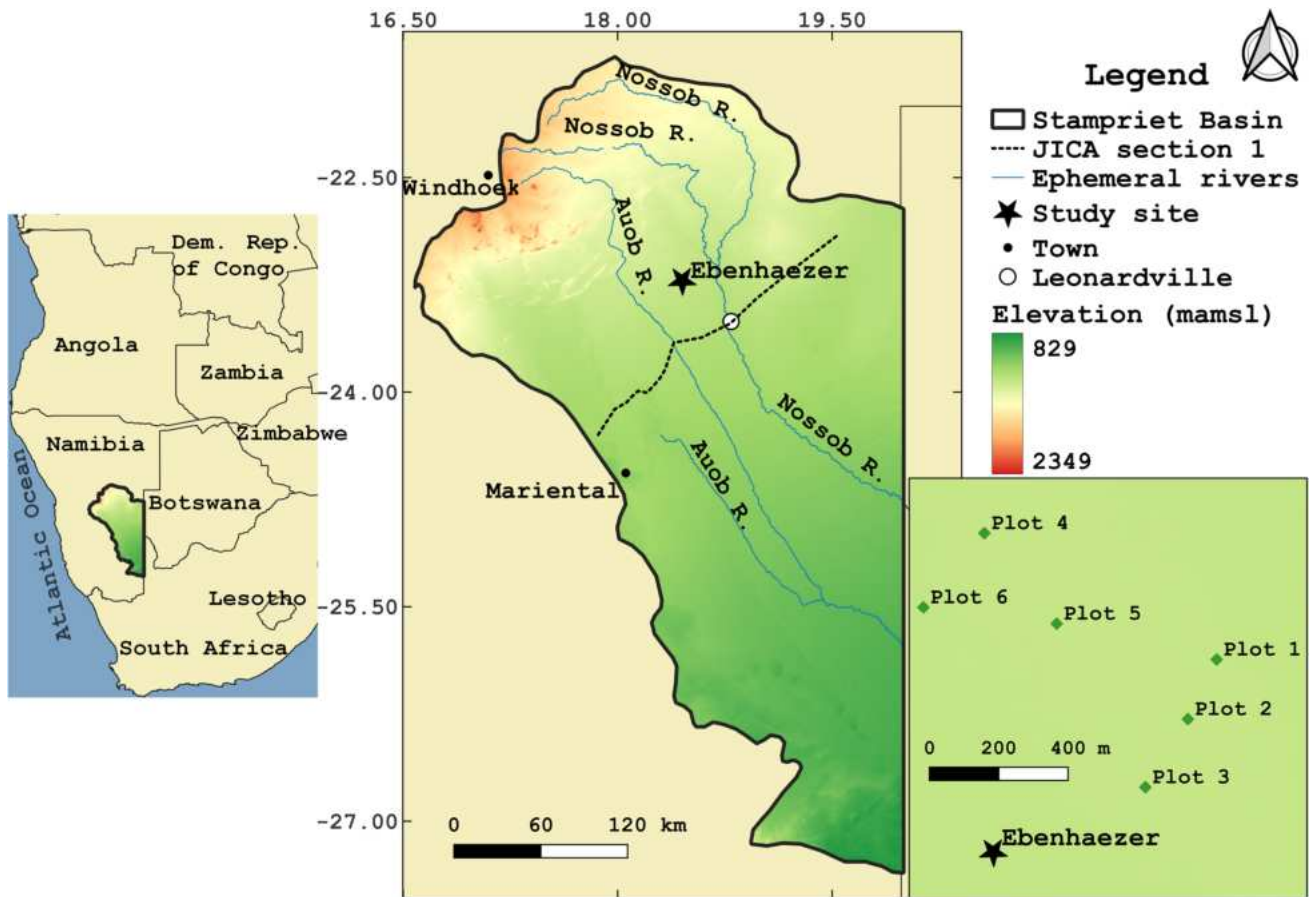


Fig. 1 Digital elevation model (DEM) and drainage network of the Stampriet basin, Namibia. The study site (Ebenhaezer) is located in the northern part of the basin. Diamond shapes represent the spa-

tial distribution of plots used in this study. Precipitation and xylem samples collected at Tsumeb and Waterberg are considered for water sources in this study

level (amsl) and flanked by ephemeral rivers, the Nossob River to the east and the Olifants River to the west (Fig. 1). The area is covered by Kalahari dune sands partially underlain by calcrete (Mendelsohn et al. 2002). Biogeographically, the study area is characterised as Kalahari shrubland within the Central Kalahari Camelthorn Savannah (Geißler et al. 2019; Mendelsohn et al. 2002).

Geology and hydrogeology of the area

Groundwater in the Stampriet Basin occurs in three main aquifers: the Kalahari beds, the Auob sandstone and the Nossob sandstone (Fig. 2a; Christelis and Struckmeier 2011; JICA 2002; Stone and Edmunds 2012). The Auob and Nossob aquifers lie in the Ecca Group of the Lower Karoo Sequence. These aquifers are confined and may be free-flowing (artesian) in some parts of the basin, such as in the Auob Valley and downstream of the Stampriet settlement and in the Nossob Valley around Leonardville (JICA 2002). Elsewhere in the basin, groundwater is subartesian. Figure 2

shows a simplified cross-sectional view through the basin as well as a description of the production of each unit.

Groundwater at Ebenhaezer is hosted in sedimentary rocks as well as in porous alluvium layers (Fig. 2). The groundwater potential in these units ranges between low to moderate potential; the Auob aquifer has the highest potential, followed by the Kalahari Aquifer, and the Nossob Aquifer has the lowest potential (Fig. 2b; JICA 2002). This study focused on the unconfined Kalahari aquifer, with a groundwater depth ~34 m below ground level (bgl). The study site at Ebenhaezer is located on Kalahari beds made up of consistent dune sand at all six plots. The maximum thickness of these beds is estimated to be ~250 m (JICA 2002). The entire basin, therefore, relies on water from the subsurface. Groundwater in the basin is used for stock watering and irrigation purposes.

Demarcation of plots, tracer injection, and sampling

Field campaigns were carried out in December 2016, March and May 2017. A total of 15 woody plants were selected,

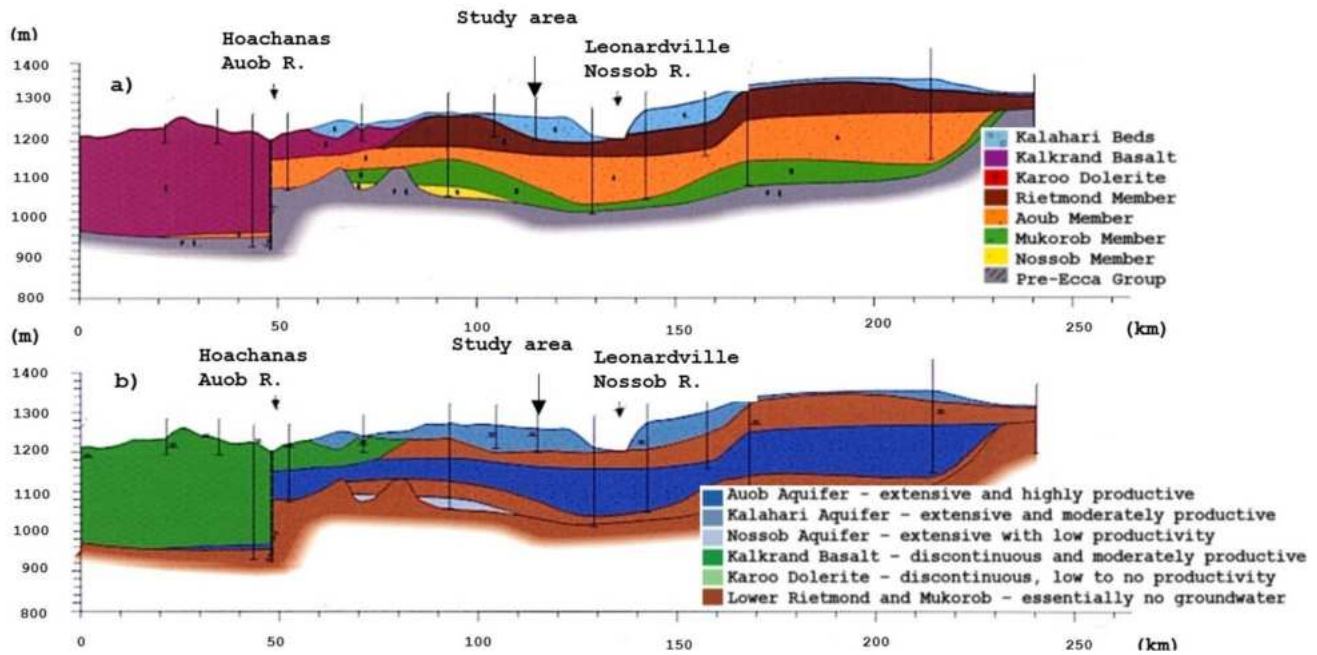


Fig. 2 a Geological and b hydrogeological cross-sections, modified from JICA (2002)

with at least one *S. mellifera* and one *B. albitrunca* per plot. In December 2016, the deuterium tracer (input concentration of the tracer is 30% $^2\text{H}_2\text{O}$) was injected at different target depths at six plots (see Fig. 1 for the spatial distribution of the plots) using similar procedures as outlined by Beyer et al. (2016). The deuterium tracer was inserted at 0.5 m for plot 5; 1 m for plot 4; 2.5 m for plot 2; 3 m for plot 3; 3.5 m for plot 6, and 4 m for plot 1. Three holes 1 m apart were drilled at each plot until the target depth using a hand auger. Each hole was inserted with five small balloons filled with a 30% deuterium tracer with a capacity of about 65 ml each. The balloons were attached to a thin cord and inserted into the holes. The balloons were busted at the target depth using a sharp object. The distance from the injected holes to the traced woody plants was within 6 m.

S. mellifera and *B. albitrunca* were sampled for both xylem cores and transpired water to get the background isotopic values before injection of a deuterium tracer. Xylem core samples were obtained using an increment borer. Transpired water samples were obtained using transpiration bags that were zip-locked around the leaves in the mornings, with the transpired water collected in the evenings. Both transpired water samples and xylem cores were collected once per day for 4 days following the injection of the tracer.

Soil samples were collected during the second field campaign in May 2017. A new hole was drilled at the centre of the earlier three holes at plots 2, 3, 4, and 5 to collect soil samples at different depths (Table 1).

Groundwater samples were collected from 15 boreholes at Ebenhaezer farm. The closest borehole to the plots is

Table 1 Summary of the soil sample depths in May 2017

Plot No.	Tracer injection depth (m)	Soil sample depths (m)
1	4	–
2	2.5	0.5, 1.0, 1.5, 2.0, 2.5, 3.0
3	3	0.5, 1.0, 1.5, 2.0, 2.5, 3.0
4	1	0.35, 0.7, 1.0, 1.5, 2.0
5	0.5	0.25, 0.5, 0.75, 1.0, 1.5
6	3.5	–

within a distance of ~400 m. The average measured depth to groundwater level is 34 m. Samples were drawn from the boreholes using a bailer where there was no solar pump installed, and immediately transferred to a 30-ml clear glass bottle which was then tightly closed. Groundwater samples were collected in December 2016 as well as May 2017.

Xylem and soil water extraction and analysis

Water was extracted from both xylem cores and soil samples using a cryogenic vacuum distillation method, following (Gaj et al. 2016; Koeniger et al. 2011). A sample was inserted into a capped exetainer vial and connected to an empty vial using a capillary tube. The soil sample within was frozen with liquid nitrogen to prevent water vapour loss during evacuation. The connected and frozen vials were subsequently evacuated using a syringe needle attached to an evacuation system. A frozen sample

was placed in an aluminium vial holder over a hot plate (180 °C), while the tip of the empty vial was inserted in a Dewar flask containing liquid nitrogen, which ensured that the flask was filled with liquid nitrogen during the entire extraction process.

After 30 min of the extraction process, the water sample was removed, recapped to prevent evaporation, and stored in a fridge until measurement took place for stable water isotopes using a Picarro L2120-i cavity-ring down spectrometer at the isotope hydrology laboratory at BGR in Hanover, Germany. The results obtained from the analysis were checked with ChemCorrect®, a software package that identifies and flags contamination from a broad range of organics, providing confidence in the accuracy of isotope ratios reported.

Groundwater and transpired water were analysed at the University of Namibia using a Los Gatos Research Inc., LGR DLT 100 laser spectrometer at the hydro-lab. All isotope ratios were reported in δ notation given in ‰ relative to the international Vienna Standard Mean Ocean Water (VSMOW) standard as shown in Eq. (4):

$$\delta = \left(\frac{R_{\text{sample}} - R_{\text{VSMOW}}}{R_{\text{VSMOW}}} \right) \times 1000 \quad (4)$$

where δ -value is the deviation of the isotope ratio of a sample relative to that of VSMOW, R_{sample} is the isotope ratio of $^2\text{H}/\text{H}$ or $^{18}\text{O}/^{16}\text{O}$ in the sample, and R_{vsmow} is the isotope ratio of $^2\text{H}/\text{H}$ or $^{18}\text{O}/^{16}\text{O}$ of VSMOW standard.

Analytical errors involved with stable isotope analyses are better than 0.1 and 1‰ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of pure water samples, respectively. For xylem and soil water extractions, analytical errors of up to 5× higher should be considered, depending mainly on the clay contents of the soils. For measurements of highly enriched deuterium samples, analytical errors can be higher than 10‰ due to memory effects, depending on the degree of enrichment (Koeniger et al. 2011).

Estimation of source water

Estimating source water for *S. mellifera* and *B. albitrunca* was conducted by determining source water from xylem water rather than transpired water. To trace the isotopic composition of precipitation from which xylem water originated, intersection points of local xylem evaporation lines with LMWLs were determined on $\delta^{18}\text{O}$ vs $\delta^2\text{H}$ plots. LMWLs determined by Uugulu and Wanke (2021) for Ebenhaezer ($\delta^2\text{H} = 7.16 \delta^{18}\text{O} + 9.88$, $R^2 = 0.96$), Waterberg ($\delta^2\text{H} = 7.37 \delta^{18}\text{O} + 5.77$, $R^2 = 0.97$) and Tsumeb ($\delta^2\text{H} = 7.78 \delta^{18}\text{O} + 6.74$, $R^2 = 0.95$) were used. Xylem water sources for *S. mellifera* and *B. albitrunca* were determined at Ebenhaezer and compared to those at the Tsumeb and Waterberg sites.

Results

Isotopic description of groundwater and xylem water and estimation of their source water

Figure 3 shows stable isotope values for groundwater, *S. mellifera* and *B. albitrunca* xylem water for Ebenhaezer (Fig. 3a) in comparison to Waterberg (Fig. 3b) and Tsumeb (Fig. 3c). The groundwater samples plot close to the LMWL of Waterberg and Tsumeb but far from Ebenhaezer LMWL. Regression lines for all stable isotope data are compiled in Table 2. The slope of the regression lines for both groundwater and *B. albitrunca* range between 4.0 and 4.8 for the three sites. *S. mellifera* slope variations are more significant (2.1–8.7) and show lower R^2 values; hence, the data do not fit the linear regression model as compared to *B. albitrunca*. At Waterberg, groundwater samples plot directly on the LMWL with a regression line of $\delta^2\text{H} = 7.5 \delta^{18}\text{O} + 7.1$, $R^2 = 0.89$. As for *S. mellifera* in Tsumeb, a regression line equation of $\delta^2\text{H} = 8.7 \delta^{18}\text{O} + 0.7$, $R^2 = 0.99$ is derived, almost parallel to the LMWL.

Intersects between species-specific isotope regression lines and groundwater with LMWLs for all three sites are shown in Table 3. Intersects for *B. albitrunca* are relatively similar between all three sites. *B. albitrunca* intersects are similar to the groundwater intersects at Waterberg and Ebenhaezer, but *B. albitrunca* intersects lower than groundwater at Tsumeb. Intersects for *S. mellifera* are above those for groundwater and *B. albitrunca* at Ebenhaezer and Waterberg but could not be determined for Tsumeb as the regression line is parallel to the LMWL. Moreover, there is a more significant divergence in the intersection values (22‰ different for $\delta^2\text{H}$) for *S. mellifera* for the two sites compared to *B. albitrunca*.

Deuterium tracer experiment at Ebenhaezer

Active root depth

The results of the deuterium tracer experiment conducted at Ebenhaezer are presented in “Deuterium tracer experiment at Ebenhaezer” section. Figure 4a shows stable isotope values for groundwater, *S. mellifera* xylem and transpiration water from Ebenhaezer. Regression line equations and the range of stable isotope values for xylem and transpiration water are compiled in Table 4. Groundwater plots close to the LMWL. *S. mellifera* xylem sampled after the deuterium tracer was inserted showed more negative isotope values (represented as squares in Fig. 4a). These values have become more negative during the sampling campaign than on the first sampling day,

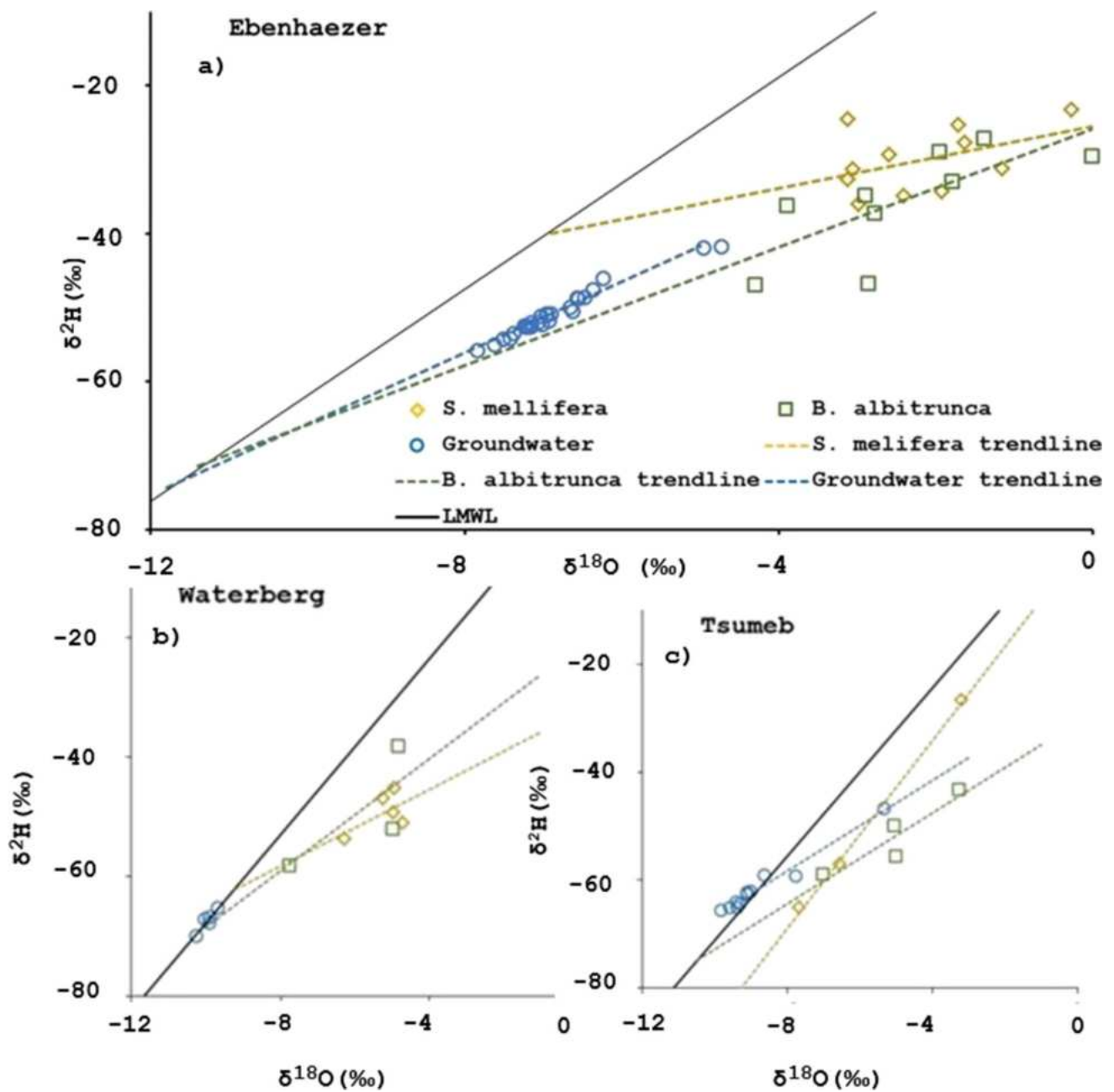


Fig. 3 Estimation of source water at **a** Ebenhaezer, compared to **b** Waterberg and **c** Tsumeb. Data were collected during November/December 2016

Table 2 Summary of regression parameters for groundwater and xylem water for Ebenhaezer compared to Waterberg and Tsumeb

Site name	Equations for the regression lines; R^2 values		
	Groundwater	<i>S. mellifera</i>	<i>B. albitrunca</i>
Ebenhaezer	$\delta^2\text{H} = 4.8\delta^{18}\text{O} - 17.7$ $R^2 = 0.97$	$\delta^2\text{H} = 2.1\delta^{18}\text{O} - 25.5$ $R^2 = 0.20$	$\delta^2\text{H} = 4.0\delta^{18}\text{O} - 25.8$ $R^2 = 0.54$
Waterberg	$\delta^2\text{H} = 7.5\delta^{18}\text{O} + 7.1$ $R^2 = 0.89$	$\delta^2\text{H} = 3.2\delta^{18}\text{O} - 32.7$ $R^2 = 0.34$	$\delta^2\text{H} = 4.7\delta^{18}\text{O} - 21.5$ $R^2 = 0.59$
Tsumeb	$\delta^2\text{H} = 4.2\delta^{18}\text{O} - 24.7$ $R^2 = 0.97$	$\delta^2\text{H} = 8.7\delta^{18}\text{O} + 0.7$ $R^2 = 0.99$	$\delta^2\text{H} = 4.2\delta^{18}\text{O} - 30.7$ $R^2 = 0.85$

Table 3 Source water intersects of the LMWL and the regression line for groundwater and xylem water at Ebenhaezer compared to Waterberg and Tsumeb

Site name	Intersect of regression lines with the LMWL					
	<i>S. mellifera</i> (xylem)		<i>B. albitrunca</i> (xylem)		Groundwater	
	$\delta^{18}\text{O}$ (‰)	$\delta^2\text{H}$ (‰)	$\delta^{18}\text{O}$ (‰)	$\delta^2\text{H}$ (‰)	$\delta^{18}\text{O}$ (‰)	$\delta^2\text{H}$ (‰)
Ebenhaezer	-6.9	-40	-11.4	-70	-11.7	-74
Waterberg	-9.2	-62	-10.2	-71	-10	-67
Tsumeb	-	-	-10.4	-75	-8.8	-63

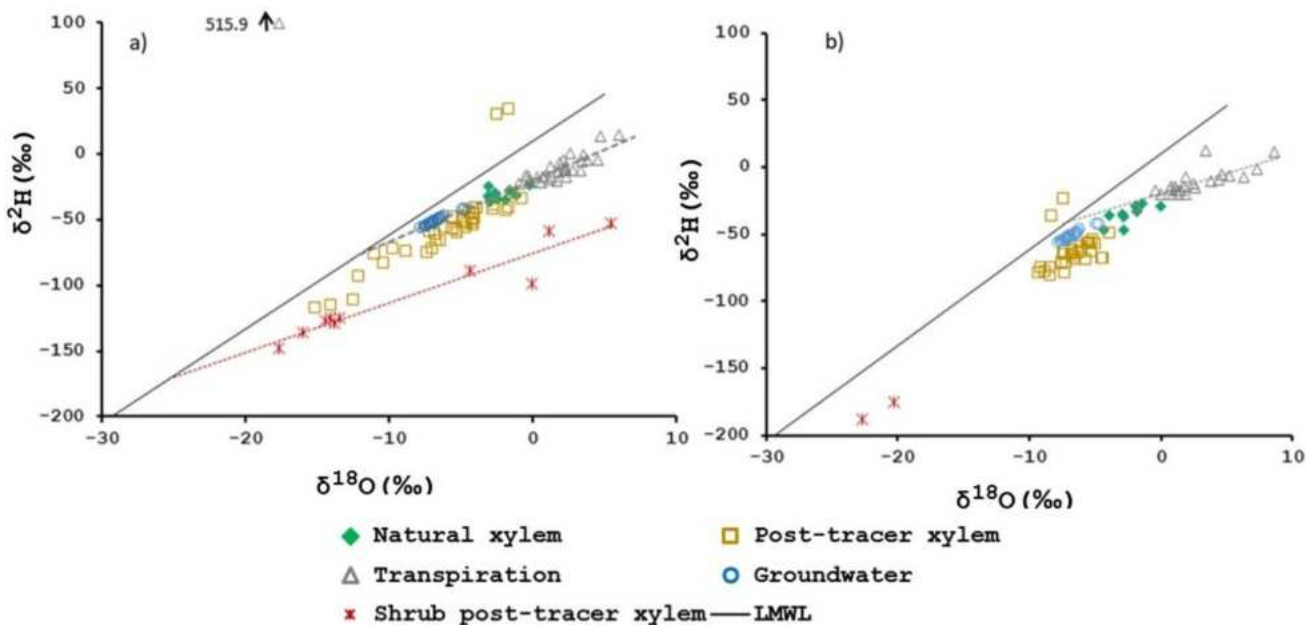


Fig. 4 Dual isotope plot for **a** *S. mellifera* and **b** *B. albitrunca* transpired and xylem water, sampled in December 2016 at Ebenhaezer. Natural xylem samples were taken before the deuterium tracer was inserted; post-tracer xylem samples were taken after introducing the tracer

Table 4 Regression lines and isotopic value ranges at Ebenhaezer

Site name	<i>S. mellifera</i>	<i>S. mellifera</i>	<i>S. mellifera</i>	<i>B. albitrunca</i>	<i>B. albitrunca</i>
Ebenhaezer	Transpiration	Post-tracer (xylem)	Shrubs (xylem)	Transpiration	Post-tracer (xylem)
Regression lines	$\delta^2\text{H} = 4.7\delta^{18}\text{O} - 20.0$ $R^2 = 0.70$	$\delta^2\text{H} = 6.9\delta^{18}\text{O} - 14.0$ $R^2 = 0.71$	$\delta^2\text{H} = 3.8\delta^{18}\text{O} - 75.7$ $R^2 = 0.92$	$\delta^2\text{H} = 3.0\delta^{18}\text{O} - 19.6$ $R^2 = 0.63$	$\delta^2\text{H} = 2.8\delta^{18}\text{O} - 44.4$ $R^2 = 0.11$
Ranges	$\delta^{18}\text{O}$ (‰) -0.55 to -5.96 $\delta^2\text{H}$ (‰) -16.30 to 14.60	-15.17 to -1.69 -116.40 to 34.90	-17.64 to 5.50 -147.60 to -54.90	-1.86 to 8.64 -28.6 to 12.80	-9.80 to -3.78 -89.23 to -18.46

representing natural background values. Ranges for all isotope values are presented in Table 4. Only two xylem samples plot clearly above the LMWL, showing elevated deuterium contents (35 and 31‰); these two samples are from plots where the deuterium tracer was applied at 2.5 and 3 m, respectively. *S. mellifera* shrubs' isotopic composition varies from enriched to depleted values in heavy isotopes and demonstrates a regression line of $\delta^2\text{H} = 3.8 \delta^{18}\text{O} - 75.7$, $R^2 = 0.92$.

Figure 4b shows isotopic data for groundwater, *B. albitrunca* xylem and transpiration water from Ebenhaezer. *B. albitrunca* xylem water plots near groundwater with less variation than *S. mellifera* in Fig. 4a. There is no evidence of a tracer being picked up by *B. albitrunca*, as all the xylem isotopic values are below 0‰ $\delta^2\text{H}$. The only *B. albitrunca* shrub xylem sampled during the last two days of the campaign indicates more negative isotope values than groundwater and *B. albitrunca* xylem (Fig. 4b). Not much variation

is visible in *B. albitrunca* xylem water isotope composition compared to *S. mellifera*.

Transpiration

Groundwater, *S. mellifera* transpiration water and *S. mellifera* xylem samples were taken before the deuterium tracer was inserted, and they plot along one regression line (Fig. 4a). Out of 49 transpired samples, only one *S. mellifera* sample showed an enriched deuterium signal of 516‰. This sample was taken from plot 2, where the deuterium tracer was inserted at 2.5 m depth (Fig. 4a). Transpiration water for *S. mellifera* is enriched in heavy isotopes as compared to xylem water with a regression line of $\delta^2\text{H} = 4.7 \delta^{18}\text{O} - 20$ ($R^2 = 0.70$) (Table 4).

Transpiration water isotope values for *B. albitrunca* are also enriched in heavy isotopes as compared to xylem water. *B. albitrunca* transpiration samples show a regression line of $\delta^2\text{H} = 3.0 \delta^{18}\text{O} - 19.6$ ($R^2 = 0.63$; Table 4). A similar trend to that of *S. mellifera* is observed for *B. albitrunca* whereby groundwater, *B. albitrunca* transpiration water and *B. albitrunca* xylem taken before the deuterium tracer was inserted appeared on one regression line (Fig. 4b). Two transpiration water samples show slightly enriched deuterium values of 11‰ $\delta^2\text{H}$ (plot 2) and 13‰ $\delta^2\text{H}$ (plot 3; Fig. 4b). The only *B. albitrunca* shrub sampled during the last 2 days of the campaign indicates more negative isotope values than groundwater, xylem and transpiration water isotope compositions (Fig. 4b).

Seasonal variation in source water

Figure 5 shows xylem water composition (and transpiration water in one sampling interval) compared to groundwater. This indicates a seasonal variation in source water and/or isotopic fractionation processes within these species.

In December 2016, the xylem samples from both species overlapped with groundwater samples and each other, and both have a wider range of values (or wider distribution) than groundwater (Fig. 5a). In comparison, the transpiration samples for both species have more positive (isotopically enriched) values, with their interquartile ranges above 0‰.

In March 2017, the pattern was different, with xylem values from both species being more negative (isotopically depleted) compared to groundwater, although with some overlap with groundwater for *B. albitrunca* (Fig. 5b). The range of values is larger, and the mean values are more negative for *S. mellifera* than *B. albitrunca*.

In May 2017, shortly after the rainy season, *S. mellifera* xylem samples had more positive values (enriched) than groundwater, whilst *B. albitrunca* overlaps that of groundwater (with a broader range; Fig. 5c). Soil water samples also sampled during this interval contain more enriched (positive) values, overlapping with *S. mellifera* xylem samples (Fig. 5c). Overall, it can be seen that *S. mellifera* xylem $\delta^{18}\text{O}$ composition is more variable through time than *B. albitrunca*.

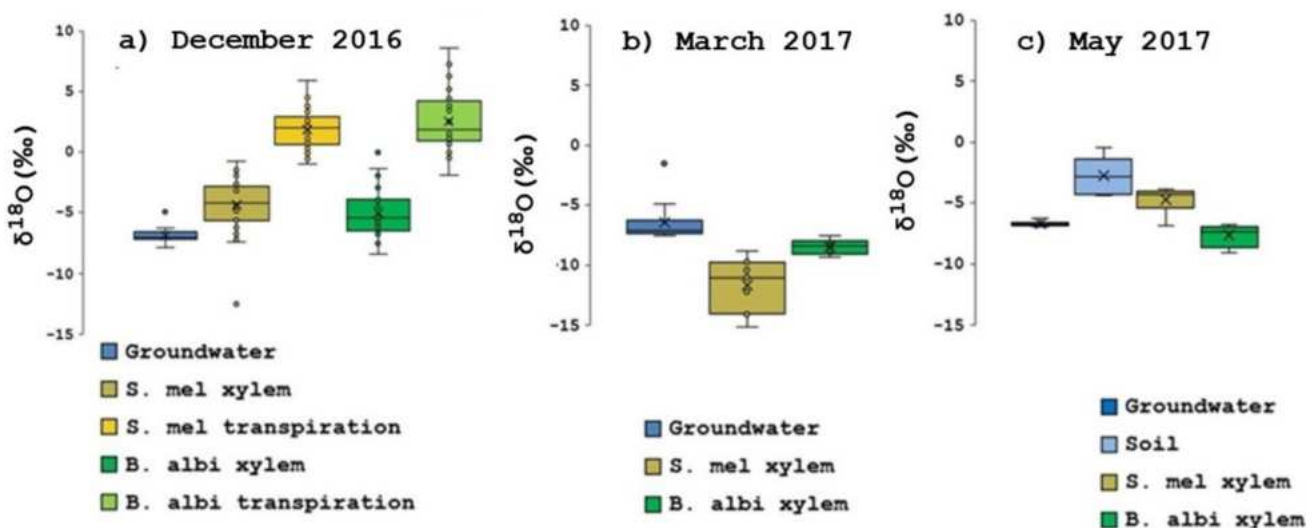


Fig. 5 $\delta^{18}\text{O}$ values for *S. mellifera* and *B. albitrunca*, soil water and groundwater, taken in **a** December 2016, **b** March 2017 and **c** May 2017

Interpretation and discussion

Groundwater and xylem water isotopic compositions, sources, and processes

The groundwater isotopic regression line at Ebenhaezer shows an evaporative enrichment of groundwater compared to its LMWL (Figs. 3a and 4a); this is examined with respect to the Tsumeb and Waterberg sites where groundwater isotopic compositions are comparable to their LMWLs (Fig. 3b,c). A similar trend is seen for xylem water at Ebenhaezer (Fig. 3a), and together this indicates a strong kinetic isotope effect due to evaporation (Craig 1961; Evaristo et al. 2015; Uugulu and Wanke 2020). Such kinetic effects produce a systematic deviation of isotopic compositions of a pool of water from the LMWL that evolves along an evaporation line (Bowen et al. 2018). The regression line equations' slopes for groundwater and *B. albitrunca* are typical evaporation slope lines. Slopes ~5 indicate that evaporation is the dominant factor governing the isotopic relationship (Craig 1961). Therefore, this indicates that the deep-rooted *B. albitrunca* is tapping evaporated source water. Subsequently, it has been demonstrated that an isotopic enrichment of water in woody plants can also occur; hence, this has an implication on the interpretation of plant source water (Dawson and Ehleringer 1993). However, this enrichment was mainly observed in younger stems that are not yet suberised to prevent gaseous exchange (carbon dioxide and water vapour) with the atmosphere, while mature stems showed little or no isotopic enrichment (Dawson and Ehleringer 1993).

S. mellifera showing a wide range of slopes (2.1–8.7) suggests it is tapping from evaporated to nonevaporated source water. The ability to switch among different source waters puts a plant at an advantage if competition for water occurs within the ecosystem (Ehleringer and Dawson 1992). The isotopic composition of the source water of *S. mellifera* and *B. albitrunca* being different indicates that these woody plants exploit different source water at different active rooting zones and hence a large variation in isotopic composition. For Waterberg, *S. mellifera* seems to be using source water which is slightly enriched in heavier isotopes, whereas *B. albitrunca* traces back to the same source water as groundwater. As for Ebenhaezer, *S. mellifera* uses source water enriched in heavier isotopes. A study by Geißler et al. (2019) indicated that *S. mellifera* uses soil water traced back to smaller precipitation events. *B. albitrunca* traces back to the same source as groundwater.

The source water for *S. mellifera* and *B. albitrunca* in this study was inferred based on the assumption that stable water isotopes are largely conservative tracers (with the primary exception of evapotranspiration); thus, the

isotopic composition of xylem water remains constant despite other physical and chemical transformations undergone by the water as it moves into roots and up through the plant (Bowen et al. 2018; Dawson and Ehleringer 1993). Furthermore, some studies have demonstrated that there is little or no isotopic fractionation occurring between the soil water pool and the plant during root uptake (Chen et al. 2020; Dawson and Ehleringer 1993; Lubis et al. 2014; White et al. 1985). On the contrary, some studies have demonstrated that fractionation occurs during root water uptake (Ellsworth and Williams 2007; von Freyberg et al. 2020). Hence the source water identified in this study should be used as an approximation because of the fractionation effect that creates uncertainty.

Deuterium experiment at Ebenhaezer

Active root depth

The more negative isotope values observed (Fig. 4), especially for shrubs and some *S. mellifera* xylem samples at Ebenhaezer, reflect the isotope composition of heavy precipitation events. Such negative isotope values could be explained by higher monthly rainfall, heavier precipitation events, the contribution of recycled moisture to precipitation or a combination of those (Callow et al. 2014; Wanke et al. 2018). It has to be noted that it rained during the second day of sampling (8 December 2016), and the closest SASSCAL weather station to Ebenhaezer (SASSCAL station Sandveld; ID 31198) recorded a precipitation amount of 9.2 mm. *S. mellifera* develop a dense network of roots extending uniformly from the tree stem in the upper soil layers at dry sites (O'Donnell et al. 2015). It could be that *S. mellifera* and shrubs used their extensive lateral root system in the upper layers of the soil to scavenge water from that precipitation event that could have been available for groundwater recharge.

Elevated deuterium contents observed in two *S. mellifera* xylem samples where the tracer was applied at 2.5 and 3 m are a possible indication of the active root depth for *S. mellifera*. The possible active root depth determined in this study is similar to that of Beyer et al. (2016), who determined the end of the primary root zone to be 2.0–2.5 m using a deuterium tracer in northern Namibia, although different species were used. Also, *S. mellifera* is known for its extensive lateral root system (NAU 2010; O'Donnell et al. 2015), which makes it ideal for accessing soil water at such depths. The active root depth for *B. albitrunca* could not be determined due to the absence of the deuterium tracer in *B. albitrunca* xylem water. However, a study by Obakeng (2007) using LiCl tracer concluded that *B. albitrunca* in Kalahari Basin, Botswana, is one of the species that are extracting water at

a depth of more than 3 m, thus below the main root zone of shrubs and grasses.

S. mellifera xylem water having a high isotopic composition variation (see Fig. 4a) indicates that this species uses a mixture of groundwater and soil water. An extensive root system gives *S. mellifera* an advantage in accessing soil water and reduces infiltration to groundwater levels (NAU 2010). Ebenhaezer having a general depth to groundwater of ~34 m, makes it likely for *S. mellifera* to access groundwater using its tap roots since it is demonstrated to develop a tap root of more than 30 m. Kanyama (2017) indicated that woody plants at Ebenhaezer use groundwater and soil water which correlates well with the findings of this study for *S. mellifera*. Moreover, a study on *S. mellifera* tree ring growth formation by Shikangalah et al. (2020) indicated a variation in water supply throughout the growth period, whereby *S. mellifera* uses less water during drier seasons. As a result, *S. mellifera* may be adapted to seek out groundwater, while exploiting surface water with an extensive lateral root system, either to support growth until groundwater is reached or to absorb water moved to the surface by hydraulic redistribution (Lubczynski 2009; O'Donnell et al. 2015; Scott et al. 2008).

B. albitrunca xylem isotopic composition is similar to that of groundwater, although it is slightly depleted in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ (Table 2 and Figs. 3 and 4b). This suggests that the *B. albitrunca* active root depth is closer to the groundwater depth. *B. albitrunca* having a similar isotopic composition to groundwater could be explained by the fact that there are barely any roots in the upper soil layer, thus it extends its roots to much greater depths (O'Donnell et al. 2015). The deep roots of this species were encountered at ~70 m depth in borehole cores in the Kalahari sands (Jennings 1974). Such deep roots make it easier for *B. albitrunca* to access groundwater easily, hence the isotopic composition of the source water is similar to that of groundwater. Slightly depleted isotopic values of the source water can be attributed to very few roots in the upper soil layers tapping soil water (Fig. 4b).

As noted, groundwater and *B. albitrunca* xylem water have similar regression line slope values, which are higher than for *S. mellifera*. A lower slope value indicates a higher evaporation effect (Craig 1961; Gat et al. 2000). The evaporation effect is usually more pronounced at shallower depths than at deeper depths. As a result, soil profiles typically have heavier isotopic ratios at shallow depths and lighter isotope ratios at greater depths (Barnes and Allison 1984; von Freyberg et al. 2020). A lower slope value for *S. mellifera* could be attributed to a portion of soil water being tapped by its dense extensive shallow root system. Kalahari profiles indicated an isotopic enrichment of soil moisture in the upper unsaturated zone of approximately less than 5 m due to direct evaporation (Lubczynski 2009). Furthermore, it

was demonstrated that bulk soil water showed greater levels of evaporative enrichment near the surface and gradually declined with depth (Gokool et al. 2021).

A slight variation of the intersects of regression lines with LMWLs across a precipitation gradient is observed, whereby the isotopic composition of source water decreases along a precipitation gradient—Tsumeb has the highest values, and Ebenhaezer has the lowest values (Table 3). This contrasts with the evaporation factor because a site with a higher evaporation rate is expected to be enriched, with heavier isotopic values. Furthermore, Kanyama (2017) found a lack of correspondence between plant source water and precipitation amount, and thus along a precipitation gradient, in Namibia.

Transpiration

A high deuterium content of 515‰ in transpired water of an *S. mellifera* sample where the tracer was inserted at 2.5-m soil depth indicates an active root depth for *S. mellifera* or possible contamination because such high deuterium content is only observed in one sample. Slightly elevated deuterium contents in *B. albitrunca* transpired water at plots 2 and 3 could be attributed to either fractionation at a leaf level or to the tapping of soil water by its rare roots that are found in the upper soil layer.

Transpired water for both species being enriched in heavy isotope composition could be attributed to the fractionation at the leaf level. When a plant is transpiring, water vapour molecules containing the lighter isotopic composition escape from the leaf more readily than heavier ones, resulting in transpired water being enriched with heavy isotope composition (Dongmann and Nürnberg 1974; Flanagan and Ehleringer 1991). As a result, all linear regression lines fitted to transpired water samples for *B. albitrunca* have a lower slope (slope = 3.03) than those fitted from xylem water samples (slope = 4) in Fig. 4. However, the same cannot be said for *S. mellifera*, as the slope of the transpiration line (slope = 4.7) is higher than that derived from xylem water (slope = 2.1). This is an indication that *S. mellifera* is using different source waters. Water extracted from the upper layers of soil has been found to produce slopes in the range of 2–5, with the lowest slopes generally produced by drier soils (Barnes and Allison 1984).

Seasonal variation in source water

Both *S. mellifera* and *B. albitrunca* xylem having similar boxplots of $\delta^{18}\text{O}$ indicates that these two species used more or less the same source water in December 2016, at the beginning of the rainy season (Fig. 5a). *S. mellifera* and *B. albitrunca* xylem $\delta^{18}\text{O}$ values overlapping with those of groundwater indicate that groundwater is one of the main source waters for these woody plants. Both *S. mellifera* and

B. albitrunca transpired water samples are enriched in $\delta^{18}\text{O}$ values as compared to xylem water due to fractionation, as an evaporative enrichment of stable isotopes occurs in the leaves (Allison et al. 1985; Dongmann and Nürnberg 1974; Sheshshayee et al. 2005).

In March 2017 (Fig. 5b), during the rainy season, *S. mellifera* made a substantial shift in source water, probably using soil water from heavy precipitation events, indicated by negative $\delta^{18}\text{O}$ values usually associated with such precipitation events, which eventually also contribute to groundwater recharge (Geißler et al. 2019). Generally, it is observed that there is a positive relationship between the amount of precipitation and the negative isotopic composition of that precipitation (Dansgaard 1964; Flanagan and Ehleringer 1991). In May 2017 (Fig. 5c), *S. mellifera* used both groundwater and soil water, and positive $\delta^{18}\text{O}$ values are attributed to evaporated soil water at Ebenhaezer. Evaporative processes in the soil water usually make the surface layers become isotopically enriched (Zencich et al. 2002); *B. albitrunca* xylem water, on the other hand, has not shown a significant variation.

Conclusions

An investigation to assess the influence of woody plants on groundwater recharge was carried out at Ebenhaezer farm, Namibia. A deuterium tracer was inserted in December 2016 at different depths in each of the six plots to determine the source water and active root depth of both *S. mellifera* and *B. albitrunca*. This was done by measuring $\delta^2\text{H}$ and $\delta^{18}\text{O}$ compositions for groundwater, soil water, xylem water, and transpired water. The source water for the woody plants was compared to those obtained at Tsumeb and Waterberg. This work indicates that *S. mellifera* does not exclusively use groundwater but also water from the unsaturated zone via its extensive root system. *S. mellifera* roots capturing water from the unsaturated zone could influence groundwater recharge. The study allowed the estimation of the active root depth for *S. mellifera* to be between 2.5 and 3 m. The absence of the tracer in the *B. albitrunca* xylem water indicates no evidence of shallow root activity in the soil zone. Additionally, the *B. albitrunca* xylem water isotope composition matches the groundwater signature year-round, indicating that the active root depth for *B. albitrunca* is closer to the groundwater depth. Estimating the active root depth helps to better identify and quantify groundwater use by vegetation and improve groundwater recharge models. This knowledge helps improve hydrological modelling by incorporating the influence of woody plants on groundwater recharge into such models. Further experimental studies focusing on woody plants and groundwater recharge are necessary, especially along a precipitation gradient, as the

impact of woody plants on groundwater resources is essential for long-term planning of water resources in arid and semiarid countries.

The use of statistical methods for a comparison of stable isotopes of the xylem water and source water would benefit future studies. It would be good to interrogate variability within the dataset and across stable isotopes within different woody plant species. Moreover, it is recommended to undertake observations of time series of change in plant uptake through time and its variability. Moreover, bush encroachers such as *S. mellifera* should be controlled and monitored as they have an effect on groundwater recharge. Such encroachers can be harvested for economic purposes, e.g. for charcoal production. Harvesting of these woody plants will reduce their uptake of water that would be otherwise available for groundwater recharge and, as a result, groundwater levels would rise.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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