Squandering Water in Drylands: The Water Use Strategy of the Phreatophyte *Ziziphus lotus* in a Groundwater Dependent Ecosystem

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- **PREMISE:** Water is the most limiting factor in dryland ecosystems and plants are adapted to cope with this constraint. Particularly vulnerable are phreatophytic plants from Groundwater Dependent Ecosystems (GDEs) in regions that have to face water regime alterations due to climate change and land-use change impacts.
- **METHODS:** We investigated two aspects related to the water use strategy of a keystone species that dominates one of the few terrestrial GDEs in European drylands (*Ziziphus lotus*): where to obtain water from and how to regulate its use. We: 1) evaluated plants' water sources and use patterns, applying a multiple-isotope approach ($\delta^2\text{H}$, $\delta^{18}\text{O}$, and $\Delta^{13}\text{C}$); 2) assessed plant water potential regulation characterizing the species in the iso/anisohydric continuum; and 3) evaluated plants' response to increasing water stress along a depth-to-groundwater (DTGW) gradient, through foliar gas exchange and nutrient concentration measurements.

24 ● **RESULTS:** *Z. lotus* behaves as a facultative or partial phreatophyte with extreme
25 anisohydric stomatal regulation. However, as DTGW increases, *Z. lotus* 1) reduces
26 the use of groundwater; 2) reduces total water uptake; and 3) limits transpiration
27 water loss while increasing water use efficiency. We also found a physiological
28 threshold at 14 meters depth to groundwater, which could indicate maximum rooting
29 length beyond which optimal plant function could not be sustained.

30 ● **CONCLUSIONS:** Species such as *Z. lotus* survive by squandering water in drylands
31 because of a substantial groundwater uptake. However, the identification of DTGW
32 thresholds indicates that drawdowns in groundwater level would jeopardize the
33 functioning of the GDE.

34 **KEY WORDS:** Anisohydry, Arid regions, Deep-rooted shrub, Foliar nutrients concentration,
35 Gas exchange rates, Rhamnaceae, Stable isotopes, Water Potential, WUE.

INTRODUCTION

Plant productivity in terrestrial ecosystems of arid regions, covering 41% of Earth's land surface (Reynolds et al., 2007), is limited by water availability (Newman et al., 2006; Nolan et al., 2018). These ecosystems suffer from prolonged drought periods since they are characterized by less annual precipitation than potential evapotranspiration (Newman et al., 2006). Several species can mitigate these constraints by the access to stable groundwater resources (O'Grady et al., 2006). The current and projected global change scenario for the Mediterranean Region, which involves both precipitation decrease and temperature increase (Giorgi and Lionello, 2008), along with the increasing demand of groundwater for human use that alters the hydrological regime (Eamus et al., 2016), poses a major risk for such species. This is the case of phreatophytes that support groundwater dependent ecosystems (GDEs) (Kløve et al., 2014; Eamus et al., 2015, 2016), whose decoupled productivity from climate conditions represents a critical ecosystem function for drylands. Exploring the physiological response of such phreatophytes along groundwater depth gradients is key to understand their response to reductions in water availability (Garrido et al., 2018), both as consequence of climate change and the increase in human groundwater demand.

Plants have developed coherent physiological and morphological strategies to cope with water limitations (Lambers and Oliveira, 2019). Two main strategies related to the water movement in the soil-plant system have been identified in terrestrial plants that face such constraints: drought avoidance and drought tolerance (Nardini et al., 2014). Drought avoiding species can minimize water loss (water savers) or maximize water uptake (water spenders), thereby maintaining high tissue water status; whereas drought tolerant plants go through large water potential fluctuations while maintaining their metabolic activity (Arndt et al., 2001; Reigosa et al., 2004; Nguyen et al., 2017). Within the drought avoidance strategy, some plants from arid regions exert strict stomatal control (closing their stomata) to reduce

stomatal conductance, and thus limiting water loss in order to keep water potentials within adequate bounds (isohydric species) (Glenn et al., 2013; Martínez-Vilalta and García-Forner, 2016). Other plants reduce their leaf area through leaf shedding during drought to limit transpiration (Newman et al., 2006), or even display a genetically pre-programmed drought-deciduous leaf phenology to avoid water stress during summer when water is scarcer (Sperry and Hacke, 2002). In contrast, some species invest in deep roots to access more stable water sources and maximize water uptake (Jackson et al., 2000). These species are called phreatophytes (well plants; Meinzer, 1927) and can freely access groundwater (Glenn et al., 2013), although some of them have a limited capacity to maintain stable water potentials (anisohydric species). Anisohydric behavior is not restricted to phreatophytes and is observed in desert shrubs like *Juniperus monosperma* (Engelm.) Sarg. and the shallow-rooted *Larrea tridentata* DC., which also shows plasticity in its hydraulic behavior related to environmental conditions (Guo et al., 2020).

Groundwater dependent ecosystems are often characterized by the presence of phreatophytes that can show varying degrees of dependence on groundwater (Hultine et al., 2020). A dual-isotope approach offers the potential to gain insight into temporal and spatial variability of plant water sources and the interrelation of carbon and water fluxes (Werner et al., 2012; Eamus et al., 2016). Indeed, hydrogen ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) stable isotopes are used to find out which is the dominant water source for plants, assuming differences in isotopic composition between water sources (Lin and Sternberg, 1993; Dawson et al., 2002). This approach compares the stable isotope composition of plants' xylem water to that of precipitation, soil water, intermediate water of the vadose zone, or groundwater (Moreno-Gutiérrez et al., 2012; Eamus et al., 2015; Rumman et al., 2018; Nolan et al., 2018). Although this approach is based on the assumption that isotopic fractionation does not occur during root water uptake in terrestrial plants (Dawson and Ehleringer, 1991; Dawson et al., 2002),

several studies have reported fractionated isotopic signatures in xylem water of xerophytic and halophytic species (Lin and Sternberg 1993, Ellsworth and Williams, 2007). Particularly, hydrogen isotope fractionation may occur during water uptake in suberized and lignified roots with less porous membranes, which are associated with salt exclusion functions (Lin and Sternberg 1993). Compared to oxygen, discrimination against deuterium is more common in this type of membranes (Ellsworth and Williams, 2007). Additionally, the carbon stable isotope composition of plant tissues ($\delta^{13}\text{C}$) is a widely used ecological indicator of plants function (Dawson et al., 2002). Particularly, leaf carbon isotope discrimination ($\Delta^{13}\text{C}$) has been used as a time-integrated measure of intrinsic Water Use Efficiency (WUEi) that depends on the ratio between intercellular and ambient CO_2 concentration (c_i/c_a) (Flanagan et al., 1992; Moreno-Gutiérrez et al., 2012; Prieto et al., 2018). Recent studies have shown that leaf (or sapwood) $\Delta^{13}\text{C}$ can respond to changes in rooting depth and groundwater use in phreatophytes; thus, carbon isotopes in leaves can serve as a reliable indicator of depth-to-groundwater (DTGW) in dryland ecosystems (Zolfaghar et al., 2014; Garrido et al., 2016; Rumman et al., 2018).

Depth-to-groundwater is a key parameter that defines groundwater availability for phreatophytes (Eamus et al., 2016; Nolan et al., 2017). Differences in rooting patterns among coexisting species promote water source partitioning and enable them to exploit different ecohydrological niches, which is key for the persistence of functional diverse plant communities in drylands (Zencich et al., 2002; Querejeta et al., 2007; Skelton et al., 2015; Nolan et al., 2017, Guo et al., 2018). Regions with a long dry season, such as Mediterranean climate regions, host species with the deepest root systems (Jackson et al., 1996), but also small shrubs and herbs with other strategies to survive such as sclerophylly or drought-deciduousness (Reigosa et al., 2004). In phreatophytes, hydraulic redistribution is a widely reported strategy that also benefits understory species (Scott et al., 2008). Consisting in lifting

water from deeper moist soil layers to shallower dry ones, it contributes to enhance nutrient availability and to maintain the water and carbon balance of the ecosystem at multiple temporal scales (Cardon et al., 2013; Hultine et al., 2020). Likewise, plants nutrients uptake and their accumulation in leaves are mainly driven by water availability (Salazar-Tortosa et al., 2018). Thus, phreatophytes' ability to access permanent water from depth enables plants not only to survive for prolonged rainless periods while keeping stomata open (Zencich et al., 2002), but may also enhance nutrient acquisition and ensure growth (Cardon et al., 2013).

Anisohydric plants show no discernible water potential thresholds for stomatal closure and can maintain high gas exchange rates during the dry season, although they run the risk of xylem cavitation and hydraulic failure (McDowell et al., 2008; Glenn et al., 2013; Nardini et al., 2014; Roman et al., 2015). Thus, anisohydric species generally show higher water usage and primary productivity in drylands than isohydric species, which reduce their stomatal conductance (g_s) under drought stress imposed by conditions such as high atmospheric demand and low soil water availability (McDowell et al., 2008). However, several studies have challenged the concept of fixed hydraulic behavior by showing that individual plants can vary along the iso/anisohydry continuum, according to differences in spatio-temporal environmental conditions (Feng et al., 2019; Guo et al., 2020). Whether phreatophytes are more or less iso- or anisohydric has important implications for the productivity of arid regions (Nolan et al., 2017) and for the ecohydrology of the GDEs (van der Molen et al., 2011; Roman et al., 2015).

Understanding water sources and transport regulation in phreatophytes is key for disentangling GDE functioning and forecasting response patterns to hydrological and climatic changes. Here we incorporate both questions simultaneously into the study of the deep-rooted shrub *Ziziphus lotus* (L.) Lam (*Rhamnaceae*), an example of keystone species in a GDE of Mediterranean arid region (Le Hourou, 2006). In contrast to drought-deciduous plants that

avoid water loss, *Z. lotus* is a winter deciduous species that is photosynthetically active during the dry Mediterranean summer. Previous studies have established the tight spatial relationship between *Z. lotus* distribution and groundwater availability through satellite images (Guirado et al., 2018) although to date no field analyses have proved this dependence conclusively. The main aims of this study were to reveal the degree of groundwater dependency and use of *Z. lotus*, as well as the mechanism that regulates water transport through the plant along a DTGW gradient. We hypothesized that 1) *Z. lotus* depends on groundwater to sustain its high physiological activity during the dry summer and consequently, to support the ecosystem functioning. If so, we predicted that 2) greater DTGW will foster more negative plant water potentials, and lower stomatal conductance and transpiration (i.e. tighter stomatal regulation and more isohydric behavior), as well as 3) higher water use efficiency because of lower groundwater availability and use.

MATERIALS AND METHODS

Study site

The study was conducted in the coastal plain of Cabo de Gata-Níjar Natural Park, southeast of Spain (36°49'20"N, 2°16'50"W), during 2018 and 2019. Climate is typical semiarid Mediterranean characterized by hot and dry summers and mild winters. The mean annual temperature is 18°C, and the precipitation, 220 mm/year on average, which is unevenly distributed during the year and mainly occurring in autumn and winter (Machado et al., 2011). The plain is located over a small and shallow aquifer that belongs to the western part of the larger aquifer Hornillo-Cabo de Gata. Its geological structure corresponds to the foothills of the sedimentary basin of Sierra Alhamilla mountains (1000 m.a.s.l), ~20 km to the north, as well as from superimposed Quaternary marine deposits (Vallejos et al., 2018).

The arborescent shrub *Ziziphus lotus* (L.) Lam (*Rhamnaceae*) is the dominant species in the study area (Tirado 2009). Due to its large size and hemispherical canopy, *Z. lotus* accumulates substantial amounts of organic matter underneath and fosters favorable microclimatic conditions for plants to thrive in drylands (Tirado and Pugnaire, 2003). Thus, this phreatophyte behaves as the key engineer species of the GDE that it constitutes (Guirado et al., 2018). *Z. lotus* is mainly distributed along the Middle East area and the southern Mediterranean coast, being also sparsely present in the Southeast of the Iberian Peninsula and Sicily (Sánchez-Gómez et al., 2003). In our study area, other Mediterranean shrubs can be found such as the summer deciduous *Lycium intricatum* Boiss., *Withania frutescens* (L.) Pauquy, and the evergreen *Thymelaea hirsuta* (L.) Endl. (Tirado and Pugnaire, 2003; Tirado, 2009). For the study, we selected 16 individuals of *Z. lotus* located along a DTGW gradient from 2 to 26 m depth, and associated to a network of boreholes used for long-term monitoring of the aquifer (two plants located less than 50 m far from each borehole) (Appendix S1; see Supplemental Data with this article). The electrical conductivity of the groundwater at each borehole also shows a wide variability (from 3500 to 11000 $\mu\text{S}/\text{cm}$). We used these 16 individuals for the stable isotope and nutrient composition analyses that we performed to explore the strategies of *Z. lotus* in water acquisition and use. We also selected eight additional plants, randomly distributed along the coastal plain, to increase samples number (24 in total) for the general characterization of the species along the iso/anishohydric spectrum. Water potential and leaf gas exchange measurements were conducted several times during the *Z. lotus*' growing season in May, July, and September of 2018 and 2019 (corresponding to late spring, mid-summer, and late summer respectively), whereas sampling for stable isotope analyses was undertaken in May of 2019 when the species shows its maximum physiological activity (Guirado et al., 2018).

Xylem water extraction and hydrogen and oxygen stable isotopes analysis

We analyzed the isotopic composition ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) of plant stem water, precipitation, and groundwater, aiming to assess the water acquisition strategies of *Z. lotus*. For the stems, we cut and debarked three suberized branch sections from the 16 plants associated to the boreholes. Single stem samples were stored at -20°C in glass vials tightly sealed by lids and wrapped with Parafilm[®] (Fisher Scientific, US) to minimize potential evaporation prior to xylem water extraction in the laboratory. The same glass vials were introduced in the cryogenic vacuum distillation system which achieves yields higher than 99% and low organic contamination (Millar et al., 2018). Defrosted samples (1.8 ± 0.5 g) were heated *in vacuo* (10^{-3} mBar) to a final temperature of 100°C for 2 hours approximately and the extracted water was cryogenically trapped using liquid nitrogen. Once the process was completed, extracted water vapor was defrosted at room temperature, in sealed conditions, and transferred to capped glass vials for isotopic analysis. Additionally, we collected groundwater samples from the boreholes ($n = 24$), 4 m below the water table, in May, July, and September of 2019. However, as we observed signs of evaporated water in some samples, we finally displayed 18 out of the 24 samples. We also collected rainwater samples on a rain event-basis ($n = 20$) from March 2019 to February 2020 from a pluviometer at a weather station 8 km far from the study area.

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in xylem water, groundwater, and precipitation were measured simultaneously by cavity ringdown spectroscopy (CRDS). This technique is increasingly used for isotopic analyses since it allows the simultaneous measurement of oxygen and hydrogen isotopes with high accuracy (Martín-Gómez et al., 2015). A L2140-*i* Picarro water isotope analyzer was used (Steig et al., 2014), interfaced with an A0211 high-precision vaporizer (Picarro Inc., Santa Clara, California, US). Contamination of plant-extracted water by organic compounds can produce spectroscopic interferences in the measurements, affecting the accuracy and precision of the isotopic data (Martín-Gómez et al., 2015; Millar et

al., 2018; Barbeta et al., 2020). To avoid such potential organic contamination, combustible organic compounds were removed from water samples by using a Picarro micro-combustion module (MCM[®], Picarro Inc., Santa Clara, California, US) (Band et al., 2009; Gázquez et al., 2015). The MCM[®] comprises an 8-cm long cylindrical cartridge filled with a pyrolytic catalyst heated at 400°C, which was placed in-line between the vaporizer and the water isotope analyzer. The instrument used dry air (containing 21% O₂) as the carrier gas. Previous studies suggested that the efficiency of this approach for the removal of organics from the water steam can be limited if the concentrations of alcohols and other volatiles are high (Martín-Gomez et al., 2015; Millar et al., 2018; Barbeta et al., 2020); thus, additional data post-processing may be needed to obtain accurate isotopic results. To check whether the pyrolytic catalyst was efficient in removal of organics, we used the ChemCorrect[™] software by Picarro (Santa Clara, California, US). This piece of software uses a suit of spectral indicators, including the spectral baseline and the residual noise, amongst others, to flag the presence of organic compounds in the water steam (Picarro, 2010). ChemCorrect[™] did not find spectroscopic issues in any of the samples analyzed in this study, so additional data processing was not applied.

Each sample (three per plant) was injected ten times into the vaporizer, which was heated to 110°C. Memory effects from previous samples were avoided by rejecting the first three analyses. The results were normalized against V-SMOW by analyzing internal standards before and after each set of twenty samples and are given as per mil (‰). Values for the final 7 injections were averaged with a typical in-sample precision ($\pm 1SD$) of $\pm 0.04\text{‰}$ for $\delta^{18}O$ and $\pm 0.18\text{‰}$ for δ^2H .

Water potential and leaf gas exchange rates

We measured stem water potential in four branches of the 24 selected plants using a Scholander pressure chamber (SKPM1405, Skye instruments Ltd. Powys, UK). Predawn water potential (Ψ_{pd}) was measured between 5:30 and 6:30 (1 hour before sunrise) and midday water potential (Ψ_{md}), between 13:30 and 14:30 in four stems per individual plant. On the same days, we measured leaf gas exchange rates in 8 leaves per individual between 10:00 and 13:00 with a portable infrared gas analyzer (Li-6400XT; LI-COR Inc., Lincoln, Nebraska, US). The following conditions in the chamber were maintained during measurements: ambient temperature at 25-30 °C, flow rate of 400 $\mu\text{mol s}^{-1}$, CO_2 concentration of 400 $\mu\text{mol mol}^{-1}$, and light intensity of 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. We measured vapor pressure deficit (VPD) and leaf transpiration rate (E) to detect the response of E to the diel increment of VPD. We also obtained the photosynthetic rate (A) and the stomatal conductance (g_s). All measures were repeated in the three sampling periods of 2018 and 2019 (late spring, mid-summer, and late summer).

Iso/anisohydric continuum metrics

Several metrics have been proposed to quantify drought response strategies and to place species along the iso/anisohydric continuum (Klein, 2014; Martínez-Vilalta et al., 2014; Li et al., 2019). Here we developed two main metrics that fall into two different categories related to stomatal regulation (Li et al., 2019). First, we performed a linear regression analysis to obtain the slope (σ) of the regression between midday (Ψ_{md}) and predawn water potential (Ψ_{pd}). Following Martínez-Vilalta et al. (2014), the slope of this regression (σ) indicates the degree of iso/anisohydry; $\sigma = 0$ refers to extreme isohydric plants, $\sigma > 1$ refers to extreme anisohydric, and $0 < \sigma < 1$ are intermediate between the two extremes. This analysis highlights the stomatal control to avoid/allow water loss (Li et al., 2019). For this, we used average values of individuals during the whole growing season, but also compartmentalized the analysis monthly, differentiating between periods during the growing season (late spring,

mid-summer, and late summer), and sites related to DTGW. Second, we estimated the maximum range of daily leaf water potential variation (hereafter maximum daily range, $\Delta\Psi_{\max}$), which is obtained from the maximum daily difference between Ψ_{md} and Ψ_{pd} (Klein, 2014; Li et al., 2019). This metric, related to the stringency of stomatal regulation within safety margins to prevent hydraulic failure (Skelton et al., 2015; Li et al., 2019), was analyzed based on DTGW and period during the growing season. Additionally, to complete the characterization of the *Z. lotus* along the iso/anisohydric spectrum, we used gas exchange data (A , g_s , and E) and assessed the relationship between VPD and E (Urban et al., 2017; Drake et al., 2018).

Leaf nutrients and carbon stable isotopes composition

To explore the variability in plant resource-use and establish the link between leaf carbon isotope composition and rooting depth in response of the DGTW gradient (Prieto et al., 2018; Rumman et al., 2018), we also analyzed foliar nutrient concentrations and carbon isotope discrimination in the *Z. lotus* population. We collected 30 leaves per individual from the same 16 plants associated to the boreholes, stored them in paper bags, and ultimately oven-dried them at 60°C for 48 hours to finely ground to powder. A subsample of foliar material was used for stable carbon isotopes analysis and another subsample for nutrients analysis. Leaf Na, P, K, Ca, Mg, S, Fe, Cu and Zn concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP- OES, Thermo Elemental Iris Intrepid II XDL, Franklin, Massachusetts, US) after a microwave-assisted digestion with HNO₂:H₂O₂ (4:1, v:v). Foliar N concentration was also analyzed by a CHNOS Elemental Analyzer (vario ISOTOPE cube, Elementar, Hanau, Germany) interfaced to an IsoPrime100 mass spectrometer (Isoprime Ltd, Cheadle, UK) in the CSIB at the University of California, Berkeley (US).

For $\delta^{13}\text{C}$ analyses, we used a combustion module (CM) coupled to a CRDS System (G2201-i Analyzer, Picarro Inc., California, US). Powdered samples of leaves in tin capsules (~1.25 mg) were loaded into the CM by an autosampler (Costech Analytical Technologies Inc., California, US), achieving a complete combustion at 1200 °C. The CO_2 released passed through a water filter and a GC-column and was transferred to a Picarro Liaison™ A0301 interface and finally inputted into CRDS for analysis. The system assembly uses ultra-high purity (UHP) N_2 as the carrier gas and pure O_2 for combustion. Four replicates per sample were analyzed and $\delta^{13}\text{C}$ -isotope values were referenced to the Vienna PeeDee Belemnite (V-PDB) scale. Two L-glutamic acid standards: USGS40 ($\delta^{13}\text{C}$ VPDB = -26.4 ‰) and USGS41a ($\delta^{13}\text{C}$ VPDB = +37.6 ‰), supplied by USGS/Reston Stable Isotope Laboratory, were used to calibrate the CM-CRDS system. Sugarcane ($\delta^{13}\text{C}$ VPDB = -11.7 ‰), urea ($\delta^{13}\text{C}$ VPDB = -49.2 ‰) and NaHCO_3 ($\delta^{13}\text{C}$ VPDB = -10.9 ‰) were used as working reference standards for consecutive rounds of $\delta^{13}\text{C}$ analyses. We also analyzed four replicates for each standard. Based on these internal (secondary) standards, the precision ranged between 0.06-0.35 ‰ (1 SD, $n = 16$) and the accuracy from -0.25 to 0.28 ‰. We calculated foliar carbon isotope discrimination ($\Delta^{13}\text{C}$) from $\delta^{13}\text{C}$, using the following equation (Werner et al., 2012):

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p) / (1 + \delta^{13}\text{C}_p)$$

where $\delta^{13}\text{C}_a$ and $\delta^{13}\text{C}_p$ were the $\delta^{13}\text{C}$ values of the CO_2 in air and the plant, respectively. The value used for $\delta^{13}\text{C}_a$ was -9.7‰, on average, which was obtained from air samples collected in the study area during the field surveys and through using a portable diaphragm pump and 1-L Ritter bags for air sample storage. We immediately measured $\delta^{13}\text{C}$ of ambient CO_2 in the lab by using the same CRDS analyzer (Picarro G2201-i).

Data analysis

The relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in xylem water, precipitation, and groundwater, was examined through regression analyses. To identify groundwater use patterns, we assessed the response of each stable isotope ($\delta^2\text{H}$ and $\delta^{18}\text{O}$ in xylem water and groundwater, and $\Delta^{13}\text{C}$ in leaves) to a DTGW gradient. In the case of hydrogen isotopes, further analysis was developed to establish the relationship of $\delta^2\text{H}$ with the electrical conductivity of groundwater (i.e. groundwater salinity). Regarding stem water potential, we performed a general linear regression between Ψ_{md} and Ψ_{pd} with monthly average values to obtain the slope of the regression line (σ). We also assessed this relationship by differentiating between sites with different water table depths. Additionally, we evaluated the response of Ψ_{pd} to the DTGW gradient, differentiating between periods. Temporal and spatial differences in $\Delta\Psi_{\text{max}}$ were also tested using a two-way analysis of variance (ANOVA) with a Tukey's honestly significant difference (HSD) post-hoc test. As regards leaf gas exchange rates, the general relationship between E and VPD during the whole growing season was assessed through a regression analysis, as well as the relationship between DTGW with mean leaf gas exchange fluxes (A, g_s , and E). Finally, leaf nutrients concentrations (Na, P, K, Ca, Mg, S, Fe, Cu and Zn) were also regressed against DTGW. To determine thresholds related to groundwater availability and access by roots, we looked for breakpoints in functions with DTGW by applying segmented regression analyses (Toms and Lesperance, 2003). The best fitting function was selected by maximizing the statistical coefficient of explanation. The least squares method was applied to the resulting regression lines while minimizing the sum of squares of the differences between observed and calculated values of the dependent variables. When no significant breakpoint was found, we selected simple linear regression to explain the responses to DTGW. All analyses were undertaken in R 1.1.456 (R Development Core Team) with the packages "stats" and "segmented".

RESULTS

Water Isotope composition

The stable isotope analyses of groundwater revealed $\delta^2\text{H}$ values that ranged from -38.2 to -44.9‰, with an average of $-41.3 \pm 0.4\text{‰}$, and $\delta^{18}\text{O}$ values from -5.47 to -6.52‰, with an average of $-6.10 \pm 0.06\text{‰}$ (Fig. 1). Groundwater $\delta^{18}\text{O}$ showed rather homogeneous values along the DTGW gradient (light blue circles) (Fig. 2). Compared with groundwater, xylem water showed lower $\delta^2\text{H}$ values and higher $\delta^{18}\text{O}$ ones ($-45.4 \pm 0.4\text{‰}$ and $-4.71 \pm 0.09\text{‰}$ on average, respectively), although a significant linear regression between them was found ($R^2 = 0.54$, $P = 0.001$) (Fig. 1). Thus, we observed a mean isotopic offset between groundwater and xylem water of -4.1‰ for $\delta^2\text{H}$ and 1.4‰ for $\delta^{18}\text{O}$. Regarding oxygen isotopes, we found a significant increase in xylem water $\delta^{18}\text{O}$ values (green triangles) with increasing DTGW up to 14 m ($R^2 = 0.71$, $P < 0.001$) (Fig. 2). Beyond this threshold, the segmented regression analysis showed that $\delta^{18}\text{O}$ became independent of DTGW (slope = -0.012‰). We also observed a significant increase in xylem water $\delta^2\text{H}$ values with both DTGW ($R^2 = 0.26$, $P = 0.016$) and electrical conductivity ($R^2 = 0.41$, $P = 0.007$) although xylem water $\delta^2\text{H}$ values were consistently lower than those of groundwater (Appendix S2). Differences between groundwater and xylem water $\delta^2\text{H}$ values decreased with DTGW and conductivity, thus larger offsets of $\delta^2\text{H}$ occurred at sites with the shallowest and less salty groundwater.

Leaf water potential and gas exchange rates

Average predawn water potential (Ψ_{pd}) ranged between -0.3 and -1.4 MPa across time (Fig. 3), being generally lower (more negative) at the end of the dry season (September, red squares). As Ψ_{pd} declined, midday water potential (Ψ_{md}) also decreased linearly with a slope of $1.43 \text{ MPa MPa}^{-1}$ ($F_{1,72} = 76.52$, $R^2 = 0.51$, $P < 0.001$), far from the 1:1 line. In May and July, Ψ_{md} was lower than Ψ_{pd} (Appendix S3) as the linear regression fell significantly below

352 the 1:1 line by 0.92 and 1.62 MPa respectively ($P < 0.001$), with a slope that also differed
 353 from 1 ($F_{1,24} = 13.06$, $P = 0.001$ and $F_{1,22} = 12.5$, $P = 0.002$ respectively). In September, a
 354 negative tendency similar to July could be observed, although it was not significant.
 355 Therefore, the slopes of the Ψ_{md} : Ψ_{pd} regressions (σ) revealed the extreme anisohydric
 356 behavior of the species ($\sigma = 1.43$), which was more pronounced in spring ($\sigma = 1.37$) than in
 357 summer ($\sigma = 0.85$).

358 When comparing between sites, plants with the shallowest water tables (Fig. 4A, B, C and D)
 359 consistently showed Ψ_{pd} values higher (less negative) than -1.1 MPa, whereas the plant with
 360 the deepest DTGW (25.5 m at site B9) had Ψ_{pd} values lower than -1.5 MPa (Fig. 4I). Indeed,
 361 the significant relationships between Ψ_{pd} and DTGW in summer confirmed lower Ψ_{pd} values
 362 at sites with the deepest water tables, both in July and September (Fig. 5B and C). In all
 363 cases, the regression analysis between Ψ_{pd} and Ψ_{md} revealed significant relationships with
 364 slopes higher than 1 MPa MPa⁻¹. Site B2 is noteworthy for its steep slope (3.19 MPa MPa⁻¹)
 365 (Fig. 4B). Additionally, plants from sites B1 and B2, with the lowest DTGW (2 m and 7 m
 366 respectively), showed higher values of maximum daily range in water potentials ($\Delta\Psi_{max}$
 367 values of 1.6 and 1.7 MPa respectively, Fig. 6). The two-way ANOVA revealed that plants at
 368 site B2 had significantly higher $\Delta\Psi_{max}$ values than the rest of the plants ($F_8 = 10.88$, $P <$
 369 0.001). $\Delta\Psi_{max}$ varied during the growing season ($F_2 = 148.66$, $P < 0.001$), being much lower
 370 in spring (May) than in summer (July and September) (inset in Fig. 6). This temporal trend is
 371 also observed in the regression of VPD against transpiration rate (E), where E significantly
 372 increased with VPD ($F_{1,70} = 24.95$, $R^2 = 0.26$, $P < 0.001$) from spring to summer (Fig. 7).
 373 Thus, the response of E did not reveal stomatal limitation with increasing VPD, at least up to
 374 4.5 KPa. However, we observed a significant reduction of both mean E and g_s with increasing
 375 DTGW up to 14 m (Fig. 8).

Leaf nutrients and carbon isotope composition

Leaf nutrient analysis revealed that DTGW did not significantly influence the foliar concentrations of most essential plant macro- and micronutrients (i.e. N, P, Ca, Mg, S, Fe, Cu and Zn; Appendix S4). However, a significant relationship was found for K and Na ($F_{1,7} = 9.91$, $R^2 = 0.58$, $P < 0.05$; and $F_{1,7} = 10.07$, $R^2 = 0.59$, $P < 0.05$ respectively), as higher foliar concentrations of these nutrients were found at sites where DTGW was relatively shallow (Fig. 9). Furthermore, foliar carbon isotope discrimination ($\Delta^{13}\text{C}$) was significantly correlated with DTGW (Fig. 10). The segmented regression ($R^2 = 0.59$, $P = 0.036$) showed a pronounced decline of $\Delta^{13}\text{C}$ with increasing DTGW at the sites where DTGW was relatively shallow (DTGW < 13.3 m, slope = -0.15). From that point, the relationship became more stable (slope = - 0.013, $P < 0.036$).

DISCUSSION

This study assessed two main aspects related to the water use strategy of a deep-rooted shrub of drylands (*Ziziphus lotus* (L.) Lam): the water source and transport regulation. Our findings support partial dependence of this species on groundwater, which points to the partial decoupling of its productivity from current precipitation. Such dependency might be facultative and compatible with the simultaneous utilization of other sources of water (e.g. soil water or intermediate water of the vadose zone), as there was not a perfect match between the stable isotope values of xylem water and groundwater at any site. However, we found a shift in the stable isotope composition of the source of water used along a DTGW gradient, even though there are still some uncertainties about the precise identity of the water sources used along the growing season. Nonetheless, the dependency of predawn water potential, stomatal conductance, transpiration rate, leaf $\Delta^{13}\text{C}$, and xylem water $\delta^{18}\text{O}$ values on DTGW suggest that groundwater is an important source of water for *Z. lotus*, particularly at

sites with shallow water tables and during the harsh dry summer. To mobilize abundant groundwater, the species showed an extreme anisohydric stomatal regulation characterized by the absence of any discernible water potential thresholds for stomatal closure, and by strong increases in transpiration flux during the warmest and driest period when soil water availability is lowest and VPD is highest (mid and late summer). Nevertheless, we also observed spatio-temporal variability in the stomatal regulation in response to groundwater depth, which suggests this can be a response mechanism of the species to contrasting environmental conditions. Overall, these attributes support the general hypothesis that *Z. lotus* squanders water, a profligate resource use strategy that becomes more pronounced as DTGW decreases and groundwater becomes more easily accessible to roots.

Groundwater dependency

Our findings suggest a facultative groundwater dependency in *Z. lotus* that indicates that the productivity of the ecosystem is partially decoupled from current precipitation (Appendix S5) (Hultine et al., 2020). Neither $\delta^{18}\text{O}$ nor $\delta^2\text{H}$ values in xylem water completely match those of the groundwater isotopic signature, which indicates that such dependence is not exclusive. The $\delta^{18}\text{O}$ offset of 1.4‰ on average between xylem water and groundwater might indicate that *Z. lotus* is a partial or facultative phreatophyte that also obtains some water from an additional unknown source (e.g. the soil or the intermediate vadose zone) aside from groundwater.

Fractionation of hydrogen (but not oxygen) isotopes can occur during root water uptake (Dawson et al., 2002; Ellsworth and Williams, 2007; Barbeta et al., 2020). Hydrogen fractionation may occur in most xerophytic and halophytic plants, which possess suberized roots that promote the symplastic pathway of water uptake at the expense of the apoplastic pathway (Ellsworth and Williams, 2007). This mechanism avoids root water leakage to

surrounding dry or saline soils (Reinoso et al., 2004) and has been measured to be up to 9‰ discrimination against deuterium for the phreatophyte *Prosopis velutina* (Ellsworth and Williams, 2007). More recently, isotopic offsets of -11‰ and -15‰ between soil and stem water have been found in the temperate species *Fagus sylvatica* L. (Barbeta et al., 2020) and the xerophytic species *Acacia caven* Mol. (Poca et al., 2019) respectively. Here, our results showed a $\delta^2\text{H}$ mean offset of -4.1‰ between groundwater and xylem water, reaching -7.5‰ at 11.5 m depth, that cannot be attributed to methodological issues or organic contamination. Firstly, the micro-combustion module (MCM[®]) successfully eliminated the organic compounds that could have been co-extracted during cryogenic vacuum distillation of xylem water, which is reported to be one of the techniques that extracts less amount of organic compounds (Millar et al., 2018). Secondly, we found no evidence of potentially contaminated samples with organics (i.e. alcohols, methanol, etc.), as confirmed by the ChemCorrect[™] software. Finally, considering that organic compounds concentration is sample-dependent and that it affects more one isotope than the other (Martín-Gómez et al., 2015), we would expect no correlation between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values if organics are present. Nonetheless, we show a significant relationship between them ($R^2 = 0.54$, $P = 0.001$), which cannot be explained by a systematic bias. The isotopic offset between xylem water and groundwater is more pronounced when groundwater availability and transpiration are greater, which might be due to hydrogen fractionation during water uptake and other plant and soil processes discussed in Barbeta et al. (2020). Larger amounts of water entering the roots through the symplastic pathway at sites with shallow and less saline groundwater tables might lead to greater deuterium depletion of xylem water compared with groundwater. However, more specific studies should be conducted to provide further insights into hydrogen isotope fractionation in *Z. lotus* roots.

Groundwater appears to be the key water source for *Z. lotus*, particularly at sites with shallow water tables where its availability and accessibility to roots would be greater. The increase in xylem water $\delta^{18}\text{O}$ values with increasing DTGW suggests a change in the pattern of water use, which might indicate a progressive reduction in groundwater uptake up to a DTGW threshold of 14 m. Roots might find difficulties to obtain groundwater beyond this depth, switching to greater utilization of soil water or intermediate water of the vadose zone to compensate for this difficulty. Such pattern is supported by more negative values of predawn water potential in summer as DTGW increases. Because of free access to groundwater, deep-rooted species can maintain more stable predawn water potentials (Jackson et al., 2000; Martínez-Vilalta and García-Forner, 2016). However, since *Z. lotus* might behave as a facultative or partial phreatophyte, it is reasonable to expect a negative trend in predawn water potential with DTGW. Besides rooting depth, predawn water potential is largely affected by soil water availability (West et al., 2012; Martínez-Vilalta and García-Forner, 2016; Nardini et al., 2016), which is reduced throughout the dry Mediterranean summer. The sampling of stems for stable isotope analyses of xylem water was conducted in spring during a period of moderate water stress (May), as supported by relatively high predawn water potential values. Therefore, groundwater use might be lower at this point of the growing season, which would explain the lack of perfect overlap between xylem and groundwater isotopic values. Nonetheless, the species might increase the uptake of groundwater during the drier later part of the growing season as consequence of the progressive reduction in soil water availability. Likewise, when DTGW is beyond 14 m, *Z. lotus* plants might become more dependent on water from the unsaturated vadose zone, which will increase drought vulnerability (Zencich et al., 2002). In any case, *Z. lotus* not only shows spatial variations in groundwater uptake, but also a seasonal groundwater disconnection because of leaf

deciduousness during winter, which additionally define it as a facultative phreatophyte in this dryland ecosystem (Hultine et al., 2020).

We acknowledge that characterizing the variability of soil water isotopic composition with depth along the edaphic profile would have contributed to identify the additional water sources and to quantify their proportional contribution to total plant water uptake. Moreover, extending the sampling period to the summer would clarify the main water source of the species throughout its growing season. Unfortunately, this has not been investigated yet. Our findings indicate that *Z. lotus* does not behave like a strict phreatophyte, but rather like a facultative or partial phreatophyte that primarily takes up groundwater for the most part but also uses water from another unknown sources.

Anisohydric behavior

The iso/anisohydric classification is strongly related to the plant water and carbon economy, since stomatal closure is the main mechanism to limit both water loss and carbon exchange at short time scales (Martínez-Vilalta and García-Forner, 2016). The slope of the linear regression between predawn and midday water potential reveals that *Z. lotus* plants show declining daytime water potentials (Ψ_{md}) in response to reductions in water availability (Ψ_{pd}). Plants undergo large fluctuations of their water potentials on a daily basis due to a loose stomatal control, as revealed by the large diel variation of water potentials ($\Delta\Psi_{max}$; Klein, 2014; Nardini et al., 2014). Our results suggest that *Z. lotus* plants do not constrain water loss strongly, indicating little stomatal limitation on photosynthesis and carbon assimilation; quite the contrary, this winter deciduous species shows maximum transpiration rates during the dry summer when VPD is higher. Together with high productivity, anisohydry also promotes leaf evaporative cooling to cope with high thermal stress, particularly in arid regions (Hultine et al., 2020). Both plant water potentials and leaf gas exchange rates confirm the extreme

496 anisohydric strategy of *Z. lotus*, which is supported by groundwater access by roots and high
497 atmospheric water demand. Nonetheless, plants hydraulic behavior emerges from the
498 interplay between plants physiology and environmental conditions, which impede an
499 exclusive and stable interpretation of such results (Feng et al., 2019).

500 The observed temporal variations in predawn and midday water potentials may respond to
501 both the species water use strategy and the environmental condition. Our results showed a
502 decrease in Ψ_{md} from May to September as well as a transition from extreme anisohydric ($\sigma =$
503 1.37) to partial isohydric behavior ($\sigma = 0.85$). This response agrees with the increase in VPD,
504 but also with a reduction in water availability (lower Ψ_{pd}) during summer. Several studies
505 have already shown temporal variations in the hydraulic strategy of desert shrubs across
506 seasons (Feng et al., 2019, Guo et al., 2020). Guo et al. (2020) attributed such dynamic
507 behavior in *Larrea tridentata* to temporal variations in air temperature, VPD, soil water
508 availability, and their interactions. Here, *Z. lotus* showed extreme anisohydry during the wet
509 and productive spring, whereas partial isohydry was most common during the dry and hot
510 summer. Therefore, although *Z. lotus* exhibits a dominant anisohydric behavior, variable
511 environmental conditions may lead to plasticity in water potential regulation. Such plasticity
512 might be critical to the success of this species in seasonally dry areas (Guo et al., 2020).

513 Our findings also point to high spatial variability in stomatal regulation among *Z. lotus*
514 individuals that might result from differences in water availability and rooting depth (Hultine
515 et al., 2020). Feng et al., (2019) reported spatial variations in the hydraulic response of
516 *Quercus douglasii* to drought across different conditions of soil moisture availability. Here,
517 differences among individuals related to spatial differences in DTGW can induce different
518 hydraulic responses. Plants at sites with shallower depths of the groundwater table (lower
519 DTGW) showed an extreme anisohydric behavior. Consistent with our hypothesis, these
520 results point to high transpiration rates and physiological activity because of higher water

availability to roots at these depths (Gries et al., 2003). On the contrary, plants at sites with the greatest DTGW showed the lowest Ψ_{pd} values, what suggests some degree of water stress due to increased hydraulic limitation or physiological restriction (groundwater availability and/or rooting depth) (West et al., 2012; Nardini et al., 2016). In fact, other authors (Rais et al., 2017) have recorded leaf accumulation of osmotically active compounds (proline, water soluble carbohydrates, and soluble N compounds) in water stressed individuals of *Z. lotus*. Conversely, the non-significant relationship between photosynthetic rate (A) and DTGW (Appendix S6) highlights that *Z. lotus* plants keep assimilating carbon despite increasing stomatal limitation (low g_s and E) even at sites with deep water tables. Although sparse measurement of leaf gas exchange rates were obtained, these results further confirm the anisohydric behavior of the species and suggest a great physiological plasticity to adapt to different water table depths. This plasticity in terms of rooting depth and stomatal regulation may partially buffer phreatophytes from climate change effects (Hultine et al., 2020).

Besides spatio-temporal hydraulic regulation shifts and winter deciduousness, *Z. lotus* coexist with both perennial and summer deciduous species at the ecosystem level (Tirado and Pugnaire, 2003). Such contrasting phenologies would promote hydrological niche separation and a dynamic carbon balance in this dryland ecosystem (Dawson et al., 2002; Werner et al., 2012). It has been observed that dryland productivity significantly enhances the terrestrial carbon sink, particularly during wet periods (Poulter et al., 2014). However, under water limitation, photosynthetic activity and plant productivity in these ecosystems are severely restricted (Nolan et al., 2018). This can also be applied to ecosystems relying on groundwater to function (i.e. GDEs) and the species that have the ability of making use of it to survive (i.e. phreatophytes). Shallow water tables and access to plentiful water in GDEs dominated by anisohydric strategies likely promote vegetation productivity and carbon sequestration in drylands, which may have a significant impact on the global terrestrial carbon sink.

However, maintaining relatively high leaf gas exchange rates in *Z. lotus* is a risky strategy that would jeopardize the GDE if aquifer overexploitation, groundwater level drawdown, and climate change trends forecasted for the Mediterranean Region (Giorgi and Lionello, 2008) are fulfilled. These findings are thus highly relevant to the ecohydrology of the GDEs (van der Molen et al., 2011; Roman et al., 2015), and to the primary productivity and carbon balance of drylands over time (Nolan et al., 2017).

Nutrients acquisition and depth threshold for groundwater use

Nutrient cycling and acquisition by plants are strongly driven by water availability (Salazar-Tortosa et al., 2018). Our results showed lower concentration of K and Na in *Z. lotus* leaves when water availability to roots was reduced (at higher DTGW). This poorer nutritional status likely has negative implications for plant osmoregulation and osmoprotection during water stress periods (Sardans and Peñuelas, 2015). Particularly important is K, given that reduced concentrations in leaves may constrain leaf osmoregulation and stomatal regulation and opening (Arquero et al., 2006). This agrees with lower g_s and E with greater DTGW. However, the decoupling of most leaf nutrients (N, P, Cu, Fe, etc) from DTGW indicates a remarkable capacity to take up nutrients even under water stress. We suggest that *Z. lotus* could be carrying out hydraulic redistribution by its deep roots to facilitate nutrient uptake from dry upper soil layers (Caldwell et al., 1998; Cardon et al., 2013). The highest concentrations of soil organic matter and nutrients are present in the dry upper soil layers, which under drought conditions will reduce their microbial activity. Hydraulic redistribution is a key process in drylands that provides moisture to such generally dry soil, thereby facilitating nutrient acquisition by plants (Cardon et al., 2013). Widely reported in facultative phreatophytes (Scott et al., 2008; Hultine et al., 2020), this process might explain the few significant relationships between leaf nutrients concentrations in *Z. lotus* and water availability along a DTGW gradient.

Finally, the negative relationship between groundwater use and DTGW is also supported by leaf carbon stable isotopes. The response of leaf $\Delta^{13}\text{C}$ to increasing DTGW in *Z. lotus* follows the same pattern observed for predawn water potentials and leaf water fluxes (E and g_s), i.e. a decrease up to 13-14m. Above this DTGW threshold, *Z. lotus* plants are less efficient in their water use (higher $\Delta^{13}\text{C}$) and show less water stress and more anisohydric behavior because they have greater groundwater availability and thus, they use more water (higher g_s and E). Conversely, below that DTGW threshold, they show markedly increased stomatal regulation and water use efficiency, and decreased transpiration in response to lower groundwater availability or accessibility by roots. A similar DTGW threshold for groundwater use was also reported for the deep-rooted phreatophyte *Corymbia opaca* in arid regions of Australia (Rumman et al., 2018). The consistency of our results with those of previous studies supports the use of leaf $\Delta^{13}\text{C}$, being potentially indicative of rooting depth and groundwater access in *Z. lotus* and other species of GDEs in arid regions (Zolfaghar et al., 2014; Garrido et al., 2018; Rumman et al., 2018). Furthermore, these findings allow for establishing a threshold in groundwater use that could reflect the maximum rooting depth of the species for optimal functioning in dryland GDEs. Despite the ability of these phreatophytes to develop deep root systems, the resilience of the GDEs will depend on the capacity to overcome drawdowns in water table levels and rises in atmospheric water demand linked to ongoing climate warming and drying.

CONCLUSIONS

We used stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$, and $\Delta^{13}\text{C}$), plant water potentials (Ψ_{pd} , Ψ_{md} , $\Delta\Psi_{max}$), leaf gas exchange measurements (A , g_s , and E), and leaf nutrient concentrations to reveal how the winter deciduous *Z. lotus* thrives in drylands. Here, we uncover the ability of the species to obtain groundwater through deep roots and its extreme anisohydric behavior, which becomes

more pronounced with higher water availability (in spring and at sites with shallower groundwater tables). However, we found that most measured plant traits were strongly affected by DTGW. With this multi-pronged approach we can establish a DTGW threshold at 13-14 m, which differentiates plants according to their water relations (i.e. where they obtain water from and how they use it). As DTGW increases, *Z. lotus* 1) decreases its dependence on groundwater; 2) reduces transpiration water loss while increasing water use efficiency; and 3) fractionates less hydrogen through roots, possibly due to reduced water uptake and transpiration rates. Therefore, this groundwater use threshold may reveal the maximum rooting depth of this phreatophyte for optimal functioning and suggests the existence of breakpoints in phreatophytes of arid regions that affect plant ecophysiological functioning. Our findings provide insight into the response of GDEs to the expected water table drawdowns as a consequence of both progressive climate aridification that would reduce aquifer recharge and water content of the vadose zone, and the increase in groundwater overexploitation by humans. Therefore, we suggest that anisohydric species like *Z. lotus* with facultative dependence on groundwater in drylands, despite having mechanisms to cope with water stress, would be threatened if global change predictions are fulfilled.

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AUTHOR CONTRIBUTIONS

M.T.T., M.J.S., and J.C. conceived the study; M.T.T. and M.J.S. conducted the field work; F.G. and A.F. conducted isotopes analyses and interpretation; M.T.T. analyzed data; J.I.Q. substantially contributed to results interpretation. M.T.T. wrote the manuscript with inputs and revisions of all authors.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article.

APPENDIX S1. Location of the study area and distribution of *Ziziphus lotus*’ plants and boreholes. Big green triangles represent the 16 individual plants for stable isotope and nutrient composition analyses; whereas the small ones are eight additional plants for water potential and gas exchange measurements. Numbers indicate depth-to-groundwater (meters) at each borehole. Note that at 21.5 m to the water table we do not have isotopic data neither from groundwater nor from xylem water.

APPENDIX S2. Relationship between xylem water $\delta^2\text{H}$ values in *Z. lotus* shrubs and depth-to-groundwater (DTGW) (A) and groundwater electrical conductivity (B). Light-blue circles represent groundwater and green triangles, xylem water. The goodness of the fit (R^2), F-

statistics, and degrees of freedom are shown for the regression analysis. Significance values of the xylem: **: $P < 0.01$, *: $P < 0.05$; Error bars represent $\pm$ standard error.

APPENDIX S3. Relationship between predawn (Ψ_{pd}) and midday (Ψ_{md}) water potentials in *Z. lotus* shrubs during the growing season, disaggregated in May (A, green triangles), July (B, yellow circles), and September (C, red squares). Data from 2018 and 2019 pooled together are shown. The solid lines show the significant $\Psi_{pd} : \Psi_{md}$ regressions whereas the dashed ones represent the 1:1 line. The equation of the regressions and the goodness of the fit (R^2) are also shown. Significance value: **: $P < 0.01$, no data: no significant. Error bars represent $\pm$ standard error.

APPENDIX S4. Relationship between depth-to-groundwater (DTGW) and concentration of macro- and micronutrients in leaves.

APPENDIX S5. Monthly precipitation during the studied period (A) and its mean stable isotope composition from March 2019 to January 2020 (B). Black circles in panel B represent $\delta^{18}\text{O}$ values whereas gray circles show $\delta^2\text{H}$ ones. Dashed line at 0‰ differentiates between positive and negative isotopic values.

APPENDIX S6. Relationship (non significant) between depth-to-groundwater (DTGW) and photosynthetic rate, A. Mean values of the 2018 and 2019 growing seasons are shown. Error bars represent $\pm$ standard error.

DATA AVAILABILITY

The data are available at <https://doi.org/10.5281/zenodo.4106409> [Data <https://doi.org/10.5281/zenodo.4106409>. Dataset name: Torres-García et al. 2020_AJB. M.T.T. is the data owner.]

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877 **FIGURE LEGENDS**

878 **Figure 1.** Relationship between mean $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in groundwater (light-blue circles)
 879 and xylem water in *Ziziphus lotus* (green triangles). Averaged values are displayed for each
 880 water source (black circle and light-green triangle, respectively). The local meteoric water
 881 line (LMWL, blue dotted line, $y = 7.2x + 2.36$, $F_{1,18} = 452.4$, $R^2 = 0.96$, $P < 0.001$), the
 882 groundwater line (black solid line, $y = 5.97x - 4.81$, $F_{1,17} = 118.2$, $R^2 = 0.87$, $P < 0.001$), and
 883 the xylem water line (black dotted line, $y = 3.1x - 31.14$, $F_{1,14} = 16.6$, $R^2 = 0.54$, $P = 0.001$)
 884 are also shown. Error bars represent $\pm$ standard error.

885 **Figure 2.** Relationship between xylem water $\delta^{18}\text{O}$ values found in *Z. lotus* shrubs and depth-
 886 to-groundwater (DTGW). Light-blue circles represent groundwater and green triangles,
 887 xylem water. Dashed line indicates the breakpoint of the segmented regression (14 m).
 888 Significance values ***: $P < 0.001$; $F_{1,14} = 11.07$; Error bars represent $\pm$ standard error.

889 **Figure 3.** Relationship between predawn (Ψ_{pd}) and midday (Ψ_{md}) plant water potentials
 890 during the 2018 and 2019 growing seasons. Green triangles refer to May (late spring), yellow
 891 circles to July (mid-summer), and red squares to September (late summer). The solid line
 892 shows the significant $\Psi_{\text{pd}}:\Psi_{\text{md}}$ regression line, whereas the dashed one indicates the 1:1 line.
 893 Significance value: ***: $P < 0.001$. Error bars represents $\pm$ standard error.

894 **Figure 4.** Relationship between predawn (Ψ_{pd}) and midday (Ψ_{md}) plant water potentials at
 895 different sites, ranging from shallow water tables (A), to deeper ones (I). Groundwater depth
 896 at each site is indicated in brackets. Green triangles represent data from May, yellow circles
 897 from July, and red squares from September. Solid lines show the $\Psi_{\text{pd}}:\Psi_{\text{md}}$ regression line
 898 whereas dashed lines represent the 1:1 line. Significance values: ***: $P < 0.001$, **: $P < 0.01$,
 899 *: $P < 0.05$. Error bars represents $\pm$ standard error.

900 **Figure 5.** Relationship between predawn water potentials (Ψ_{pd}) in *Z. lotus* shrubs and depth-
 901 to-groundwater (DTGW) in May (A), July (B), and September (C) of 2019. Lines show
 902 significant linear regressions. The equation of the regression and the goodness of the fit (R^2)
 903 are shown for significant analyses (July: $F_{1,16} = 11.88$, September: $F_{1,16} = 11.53$). Significance
 904 value **: $P < 0.01$. Error bars represents $\pm$ standard error.

905 **Figure 6.** Mean and $\pm$ standard error of plant water potential maximum daily range ($\Delta\Psi_{\max}$)
906 by sites and months (inset) in *Z. lotus* shrubs. Letters represent significant differences
907 between groups ($P < 0.05$).

908 **Figure 7.** Relationship between Vapor Pressure Deficit (VPD) and transpiration rate (E) in *Z.*
909 *lotus*. Data from 2018 and 2019 is shown. Green triangles represent data from May, yellow
910 circles from July, and red squares from September. Solid line shows the significant regression
911 (Significance values: ***: $P < 0.001$). The equation of the regression and the goodness of the
912 fit (R^2) as well as the standard errors (error bars) are also shown.

913 **Figure 8.** Relationship between depth-to-groundwater (DTGW) and transpiration rate, E (A)
914 and stomatal conductance, g_s (B) in *Z. lotus* shrubs. Data represent average values of the 2018
915 and 2019 growing seasons. Solid lines show the significant segmented regressions and R^2 , the
916 goodness of the fit. Dashed lines indicate the breakpoint at 14m (E: $F_{1,14} = 8.74$; g_s : $F_{1,14} =$
917 5.09). Significance values: **: $P < 0.01$, *: $P < 0.05$. Error bars represent $\pm$ standard error.

918 **Figure 9.** Relationship between depth-to-groundwater (DTGW) and foliar concentrations of
919 potassium, K (A) and sodium, Na (B) in *Z. lotus* shrubs. The equation of the regression and
920 the goodness of the fit (R^2) are shown. Significance value: *: $P < 0.05$. Error bars represents
921 $\pm$ standard error.

922 **Figure 10.** Relationship between carbon isotope discrimination ($\Delta^{13}\text{C}$) values in leaves and
923 depth-to-groundwater (DTGW) in *Z. lotus* shrubs. Lines represent significant segmented
924 regression ($F_{1,14} = 11.82$, $R^2 = 0.59$, *: $P = 0.036$). Dashed line indicates the breakpoint of the
925 regression (13.3 m). Error bars represent $\pm$ standard error.

Figure 1

Figure 2

[Click here to
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Figure 3

Ψ_{pd} (MPa)

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Figure 4

[Click here to access/download;Figure;Figure4.pdf](#)

Figure 5

[Click here to access/download;Figure;Figure5.pdf](#)

A) May

B) July

C) September

Figure 6

[Click here to access/download;Figur](#)

Figure 7

[Click here to access/download;Figur](#)

Figure 8

[Click here to access/download;Figure;Figure8.pdf](#)

Figure 198

Figure 10

[Click here to access/download;Figur](#)

APPENDIX S1. Location of the study area and distribution of *Ziziphus lotus*’ plants and boreholes. Big green triangles represent the 16 individual plants for stable isotope and nutrient composition analyses; whereas the small ones are eight additional plants for water potential and gas exchange measurements. Numbers indicate depth-to-groundwater (meters) at each borehole. Note that at 21.5 m to the water table we do not have isotopic data neither from groundwater nor from xylem water.

APPENDIX S2. Relationship between xylem water $\delta^2\text{H}$ values in *Z. lotus* shrubs and depth-to-groundwater (DTGW) (A) and groundwater electrical conductivity (B). Light-blue circles represent groundwater and green triangles, xylem water. The goodness of the fit (R^2), F-statistics, and degrees of freedom are shown for the regression analysis. Significance values of the xylem: **: $P < 0.01$, *: $P < 0.05$; Error bars represent $\pm$ standard error.

APPENDIX S3. Relationship between predawn (Ψ_{pd}) and midday (Ψ_{md}) water potentials in *Z. lotus* shrubs during the growing season, disaggregated in May (A, green triangles), July (B, yellow circles), and September (C, red squares). Data from 2018 and 2019 pooled together are shown. The solid lines show the significant $\Psi_{pd}:\Psi_{md}$ regressions whereas the dashed ones represent the 1:1 line. The equation of the regressions and the goodness of the fit (R^2) are also shown. Significance value: **: $P < 0.01$, no data: no significant. Error bars represent $\pm$ standard error.

APPENDIX S4. Relationship between depth-to-groundwater (DTGW) and concentration of macro- and micronutrients in leaves.

APPENDIX S5. Monthly precipitation during the studied period (A) and its mean stable isotope composition from March 2019 to January 2020 (B). Black circles in panel B represent $\delta^{18}\text{O}$ values whereas gray circles show $\delta^2\text{H}$ ones. Dashed line at 0‰ differentiates between positive and negative isotopic values.

APPENDIX S6. Relationship (non significant) between depth-to-groundwater (DTGW) and photosynthetic rate, A. Mean values of the 2018 and 2019 growing seasons are shown. Error bars represent $\pm$ standard error.