

RESEARCH ARTICLE

Coordination between water uptake depth and the leaf economic spectrum in a Mediterranean shrubland

Angela Illuminati¹  | José Ignacio Querejeta²  | Beatriz Pías³  | Adrián Escudero¹  |
Silvia Matesanz¹ 

¹Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Madrid, Spain

²Departamento de Conservación de Suelos y Aguas, Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Murcia, Spain

³Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense de Madrid, Madrid, Spain

Correspondence

Angela Illuminati

Email: angela.illuminati@gmail.com

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Abstract

1. Water is the most limiting resource for plant survival and growth in arid environments, but the diversity of water-use strategies among coexisting species in dryland communities is not well understood. There is also growing interest in assessing whether a whole-plant coordination exists between traits related to water-use and the leaf economic spectrum (LES).
2. We used water stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$) to quantify water uptake proportions from different soil depths by 24 species in a Mediterranean shrubland. Leaf traits associated with water-use efficiency, stomatal regulation ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) and the LES (SLA, N, P, K concentrations) were also measured. We assessed potential trade-offs between the above-mentioned leaf traits, water uptake depth and their relationship with species abundance.
3. We found distinct ecohydrological niche segregation among coexisting species. Bayesian models showed that our shrubland species used a median of 37% of shallow soil water (0–30 cm) and 63% of deep water (30–100 cm). Still, water source proportions varied considerably among species, as shallow soil water-use ranged from a minimum of 6.4% to a maximum of 68%. Interspecific variability in foliar carbon investment (SLA) and nutrient concentrations was remarkably high, indicating diverse nutrient-use strategies along the LES. Leaf $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values also differed widely among species, revealing differences in stomatal regulation, water-use efficiency and nitrogen acquisition mechanisms. After accounting for evolutionary history effects, water uptake depth was coordinated with the LES: species using shallower soil water from fertile topsoil layers exhibited a more acquisitive carbon- and nutrient-use strategy, whereas water uptake from deeper but less fertile soil layers was linked to a more conservative nutrient-use strategy. Leaf-level water-use traits significantly influenced species abundance, as water-savers with tight stomatal regulation and high water-use efficiency were dominant.
4. *Synthesis.* Greater utilisation of water stored in nutrient-rich topsoil layers favoured a more acquisitive nutrient-use strategy, whereas a deeper water uptake

pattern appeared to constrain access to nutrients. Our findings thus suggest a largely inescapable trade-off and coordination between soil water uptake depth and carbon- and nutrient-use strategies in low-fertility drylands.

KEYWORDS

coexistence, drylands, ecohydrological niche segregation, leaf economic spectrum, Mediterranean shrublands, plant–soil (below-ground) interactions, stable isotopes, water-use strategy, water uptake depth

1 | INTRODUCTION

One of the main questions in plant ecology is understanding whole-plant phenotypic integration driving plant form and function (Freschet et al., 2010). As water is the most limiting resource for plant survival and growth in arid environments and is thus the main driver of competition between coexisting plant species (e.g. Kulmatiski et al., 2010; Kulmatiski & Beard, 2013), increasing attention is paid to traits related to plant water-use strategy, especially in drylands such as Mediterranean shrublands. These ecosystems harbour diverse plant communities highly susceptible to species extinctions or composition shifts under climate change and aridification (León-Sánchez et al., 2020; West et al., 2012). Plant water-use strategy is defined by traits related to leaf-level stomatal regulation, water-use efficiency and root water acquisition (Moreno-Gutiérrez et al., 2012). In dryland ecosystems, leaf $\delta^{13}\text{C}$ and leaf $\delta^{18}\text{O}$ can serve as time-integrated proxies of leaf-level intrinsic water-use efficiency (WUEi) and stomatal conductance (g_s), respectively (e.g. Barbour, 2007; Farquhar et al., 1989; Moreno-Gutiérrez et al., 2012). Prieto et al. (2018) suggested that these leaf isotopic traits could be an overlooked component of the leaf economic spectrum (LES, thereafter; Wright et al., 2004) in water-limited plant communities, based on the tight coupling and coordination observed between LES traits and water-use isotopic traits across Mediterranean shrubland species.

Water uptake depth in the soil profile is another key aspect of plant water-use strategy related to water acquisition (Schulze et al., 1996; Williams & Ehleringer, 2000). Thus far, contrasting patterns have been observed regarding the relationship between water uptake depth and the leaf-level water-use strategy. Some works (e.g. Brum et al., 2017; Ding et al., 2021; Jiang, Meinzer, et al., 2020; Moreno-Gutiérrez et al., 2012) have found that species with a profligate water-use strategy (water-spenders), that is, with high g_s and low WUEi, generally use water stored in shallower soil layers, while coexisting species with a water-saver strategy (water-savers), that is, with low g_s and high WUEi, tend to use comparatively deeper soil water sources. In contrast, other studies (e.g. Beyer et al., 2018; del Castillo et al., 2016; Rodríguez-Robles et al., 2020) have found variable or even opposite patterns that are highly dependent on the species and the sampling season. To obtain an integrative perspective of the whole-plant water-use strategy and niche segregation among species in drylands, it is necessary to further investigate

the relationship between soil water uptake depth and aboveground leaf traits related to stomatal regulation and water-use efficiency (e.g. Moreno-Gutiérrez et al., 2012; Sánchez-Martín et al., 2021). Especially in drylands, ecohydrological niche segregation, that is, the use of water from different soil depths or the spatial partitioning of species along soil moisture gradients in realised assemblages (Araya et al., 2011; Silvertown et al., 2015) among coexisting plant species is expected. Silvertown et al. (2015) reported that ecohydrological niche segregation is commonly found in multiple habitats. Yet, only a few studies to date (e.g. Moreno-Gutiérrez et al., 2012; Palacio et al., 2017) have explored ecohydrological niche separation in drylands at the plant community level, that is, considering many species coexisting at fine spatial scales. In this context, maximum rooting depth has been often considered a proxy of the water source used by plants (Bucci et al., 2009; Zhou et al., 2020). However, rooting depth is difficult to measure in field conditions, and often fails to robustly predict water sources actively accessed by plants (e.g. Ehleringer & Dawson, 1992; Holdo, 2013). As a powerful alternative, the isotopic composition of stem water can provide an estimate of the relative proportion of water sources used by plants, especially when steep vertical gradients of oxygen and hydrogen stable isotopes ratios ($\delta^{18}\text{O}/\delta^{16}\text{O}$ and $\delta^2\text{H}/\delta\text{H}$, respectively) of water along soil depth are formed due to evaporative isotopic fractionation (Allison et al., 1983; Dawson et al., 2002). As a result, plants using water stored in shallow soil layers tend to present stem water that is more enriched in the heavier isotopes (^{18}O and ^2H) because of intense evaporative isotopic fractionation of their main water source, compared with plants using deeper and less evaporated water sources (Ding et al., 2021; Moreno-Gutiérrez et al., 2012; Querejeta et al., 2021).

If species coexisting in diverse dryland communities segregate their water niches by adopting different whole-plant water-use strategies, an additional remaining question is whether such differentiation affects species performance, that is, abundance, at the community level. Contrasting, but equally successful, strategies to cope with water stress possibly coexist in drylands (Jacobsen et al., 2008; Moreno-Gutiérrez et al., 2012; West et al., 2012). Therefore, contrasting water-use strategies could lead to niche complementarity to minimise competition and favour coexistence (McDowell et al., 2008; West et al., 2012). However, eco-physiological constraints determining inevitable trade-offs among plant traits could limit the number of feasible trait combinations and water-use strategies (Moreno-Gutiérrez et al., 2012; Wang

et al., 2021). Several studies have highlighted the advantage of tapping deep water sources in drought-prone ecosystems (McCormick et al., 2021; Rempe & Dietrich, 2018; West et al., 2012), which also concurs with the greater biomass allocation to roots typical of these environments in comparison with more mesic vegetation types (Schenk & Jackson, 2002). It is also known that the most dominant and abundant species in a plant community tend to exhibit trait values closer to the 'optimal' strategy in any given ecosystem (Jiang, Wang, et al., 2020). However, our current understanding of the relationship between depth of water uptake in the soil profile, leaf-level water- and nutrient-use strategy, and species abundances in plant communities at local scales is still poor.

In this study, we aimed to gain insight into the potential role played by plant water-use strategy in the assembly of plant communities in drylands. We measured the soil water isotopic profile with depth along with stem water isotopic composition ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) to identify the water sources used by the different coexisting species during the phenological peak of the community (late spring). We also measured leaf $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ as proxies of time-integrated g_s and WUE_i , respectively, as well as SLA and leaf nutrients (N, P, K) known to be key functional traits in the LES. We first assessed the presence of water niche partitioning between 24 species coexisting in a Mediterranean shrubland. Then, we assessed the potential coordination of water uptake depth with leaf traits related to stomatal regulation ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) and carbon- and nutrient-use strategy (i.e. LES traits). As evolutionary history can importantly affect the level of integration between plant traits (e.g. Long & Medeiros, 2021; Zhou et al., 2018), we accounted for the phylogenetic relatedness among species in our analyses (Caruso et al., 2020). Based on plant functional diversity theory (Reich, 2014) and the findings of recent studies conducted in other water-limited ecosystems (Ding et al., 2021; Prieto et al., 2018), we expected a tight coordination between water uptake depth, leaf-level water-use strategy and LES traits across species. In last instance, we aimed to assess whether any particular plant water- and nutrient-use strategy was advantageous and linked to greater species abundance in this dryland community.

2 | MATERIALS AND METHODS

2.1 | Study area, plant and soil sampling

We sampled a species-rich Mediterranean shrubland located in the south of Madrid (Spain; 40°17'17.5"N 3°12'19.4"W, 760m a.s.l.) characterised by a semi-arid continental climate, with cold winters and intense summer droughts, a mean annual temperature of 12.8°C and a mean annual precipitation of 452mm. The area presents low-fertility calcareous soils with a variable content of gypsum, which creates a fine-scale patchy environment. The perennial plant community is very diverse and dominated mainly by dwarf shrubs and scrubs, hemicryptophytes and grasses, being the most abundant species *Bupleurum frutescens* L., *Thymus vulgaris* L., *Linum suffruticosum* L., *Helianthemum cinereum* Pers. and *Stipa pennata* L.

We established three 30×30m plots with similar slope (≈10%), orientation (north-west) and species composition, located 0.5 km away from each other. We sampled three individuals per species (one per plot) for a total of 24 species and 72 individuals. These species encompass more than the 90% of the plant community in terms of cover and abundance. All samples were collected on the same day in the first week of June. The sampling date was carefully chosen to (a) match the flowering peak of the community; (b) collect samples at the onset of the summer drought period (Spring–Summer transition period), and (c) ensure a preceding 2-week period with no rainfall. The latter was necessary to obtain a steep isotopic soil water gradient, which is formed in response to soil water evaporation and isotopic fractionation during rainless periods (Allison et al., 1983). We collected one segment of the basal stem from each individual to measure stem water isotopic composition, to avoid any potential bias due to the proximity of leaves or photosynthetic stems where transpiration takes place (Schwinning, 2008). In a few cases in which the aerial part of the plant was completely herbaceous (e.g. *Stipa pennata*), we sampled the root crown instead. We immediately stored these plant samples in 5 ml glass vials, capped and wrapped with parafilm, before placing them in a cooler. We also sampled well-developed and healthy leaves of the same individuals for SLA measurement and elemental and isotopic analyses (see details below).

On the same date, we sampled three soil profiles down to 1 m of depth using a hand auger of 4 cm of diameter. Soil cores were sampled from the centre of each selected plot. Soil cores were separated into 10 different portions, corresponding to different soil depth intervals (0–5, 5–10, 10–15, 10–20, 20–30, 30–40, 40–50, 50–65, 65–80 and 80–100cm, see Figure S1), which were stored in separate sealable bags and maintained in a cooler. Within the same day, we transported all soil and plant samples in coolers to the laboratory, where they were kept at –20°C, except for the leaf material which was stored at 4°C and processed the day after field sampling. No permissions were required to carry out the fieldwork.

2.2 | Trait measurement and isotopic analyses

We extracted all water contained in plant stems and soil samples using a cryogenic vacuum extraction line (2-hr extraction time at 100°C and 10 millitorr vacuum pressure; Ehleringer et al., 2000). Stem and soil water contents were calculated based on sample weights before and after the extraction. Extracted water samples were shipped to the Center for Stable Isotope Biogeochemistry of the University of California (Berkeley, USA) for isotopic analyses. Specifically, we determined oxygen and hydrogen stable isotopes ratios in both stem and soil water by isotopic ratio mass spectrometry (IRMS), through a Thermo Gas Bench II and a hot chromium reactor unit (H/Device™), interfaced to a Thermo Delta V Plus mass spectrometer. Hydrogen ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) isotopic composition are expressed in ‰ notation relative to the standard V-SMOW (Vienna standard Mean Ocean Water), according to the equation:

$$\delta^2\text{H} (\delta^{18}\text{O}) = 1,000 \times (R_{\text{sample}}/R_{\text{standard}}) - 1,$$

where R_{sample} and R_{standard} represent the ratio of the heavy to light isotope ($^2\text{H}/^1\text{H}$ or $^{18}\text{O}/^{16}\text{O}$) of the sample and the standard, respectively (Dawson et al., 2002). As it is considered a good indicator of evaporative isotopic fractionation processes (Craig & Gordon, 1965; Gat, 1996), we estimated water deuterium excess (Dansgaard, 1964) as:

$$d - \text{excess} = \delta^2\text{H} - 8 \times \delta^{18}\text{O}.$$

The Local Meteoric Water Line (LMWL) representing the isotopic composition of precipitation for Central Spain was obtained from Díaz-Teijeiro et al. (2009):

$$\delta^2\text{H} = 12.40 + 8.49 \times \delta^{18}\text{O}.$$

We selected five mature leaves of each sampled individual to calculate SLA. First, we scanned the leaves and measured leaf area (LA). Thereafter, we dried them at 60°C for 48hr to assess the dry weight (DW). SLA (cm^2/g) was calculated as LA/DW . For the leaf isotopic analyses, we ground the dried leaves into a fine powder using a ball mill, and samples were weighted and encapsulated in tin ($\delta^{13}\text{C}/\delta^{15}\text{N}$, 4 mg) or silver capsules ($\delta^{18}\text{O}$, 0.3 mg). Leaf samples were shipped to the Centre for Stable Isotope Biogeochemistry of the University of California (Berkeley, USA) for analysis. Measurements of leaf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and leaf C and N concentrations (%), were carried out by continuous flow (CF) dual isotope analysis using a CHNOS Elemental Analyser interfaced to an IsoPrime100 mass spectrometer. Long-term external precision for C and N isotope determinations is $\pm 0.10\%$ and $\pm 0.20\%$, respectively. Leaf $\delta^{18}\text{O}$ was measured in CF using an Elemental PYRO Cube interfaced to a Thermo Delta V mass spectrometer. Long-term external precision for IAEA-V-9 (cotton cellulose) is $\pm 0.20\%$. Isotopic composition values are expressed in delta notation (‰) relative to the international standard (V-SMOW for $\delta^{18}\text{O}$, V-PDB for ^{13}C). Leaf P and K concentrations (%) were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Elemental Iris Intrepid II XDL) at CEBAS-CSIC.

2.3 | Phylogenetic tree construction

We sequenced the 24 species using the barcoding locus *rbcl*. First, we collected leaves from three individuals of each species in the same study site. Then, we air-dried and stored 20mg of leaf material in silica gel. DNA extraction was carried out using the DNeasy Plant Mini Kit (QIAGEN). The *rbcl* barcode was amplified in a 25 μl reaction with 2 μl of DNA and 23 μl mix reaction composed by 2.5 μl of Taq buffer with 2mM MgCl_2 , 1 μl of dNTP Mix (0.4mM), 1.25 μl of reverse and forward primer and 1.25U Taq DNA Polymerase (Biotools). PCR amplification was performed on a S1000 Thermal Cycler (Bio Rad). Primers and PCR conditions are provided in Table S1. Amplified PCR products were purified using the ExoSap purification kit® (USB Corporation), and sequenced by MACROGEN (Seoul, Korea and Amsterdam, Netherlands).

Consensus sequences were assembled using Sequencher 4.1.4 software (Gene Codes Corporation) and aligned with MAFFT online v. 7, then checked manually with Mesquite version 2.6. Sequences were used to build a phylogenetic tree by maximum likelihood methods using the R package PHANGORN (Schliep, 2011), using the GTR+G+I model and 100 bootstrap replicates (Violle et al., 2011).

2.4 | Statistical analysis

To estimate the proportion of different water sources used by each species, we used a Bayesian mixed modelling approach (*run_model* function, MixSIAR package, Stock et al., 2018). Given the growing uncertainties related to the widespread $\delta^2\text{H}$ depletion observed in plant stem water relative to soil water sources (Barbeta et al., 2020, 2022; Chen et al., 2021; de la Casa et al., 2021), we first assessed whether plant water values presented a significant $\delta^2\text{H}$ depletion compared with the soil isotopic values (Figure 1 and Figure S2 for individual values). As the two regression lines corresponding to plant and soil water values showed different slopes, we carried out the Bayesian model using only the $\delta^{18}\text{O}$ data. Researchers have traditionally reduced the number of sources through aggregation to improve model inference (e.g. Ben-David et al., 1997; Stock et al., 2018). Thus, we selected three soil intervals as main water sources: shallow (5–30cm), intermediate (30–50cm) and deep (50–100cm). However, since two water sources (30–50 and 50–100cm) resulted to be highly correlated ($R = 0.84$, Figure S3), we carried out an ‘a posteriori’ aggregation, that is, after running the mixing model, of the two sources, combining them into a single water source pool

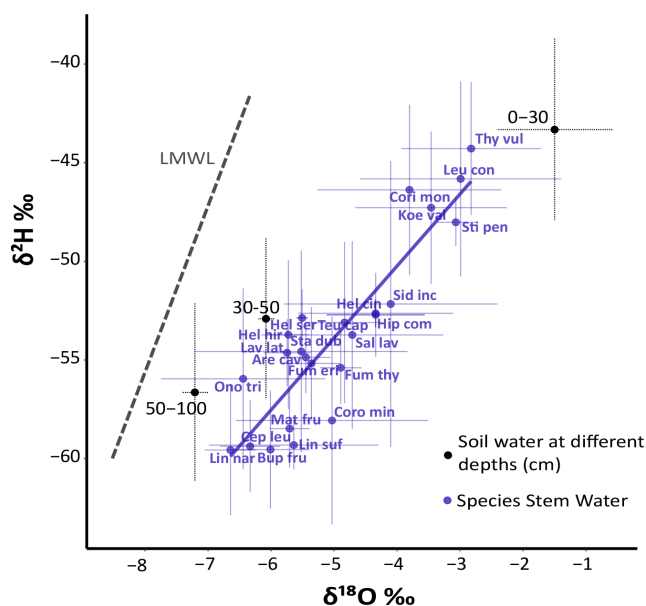


FIGURE 1 Regression line of plant stem water isotopic composition ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) and the Local Meteoric Water Line (LMWL). The points represent mean values ($\pm\text{SD}$) of 24 coexisting plant species and soil water isotopic composition at the three different depth intervals used in the mixed Bayesian model

(*combine_sources* function, *MixSIAR* package), as suggested by Stock et al. (2018). We calculated the mean and standard errors for the $\delta^{18}\text{O}$ values of soil water at each soil depth interval from the three plots. The calculated means ($\pm\text{SD}$) were included as water source data input in the model, while the raw stem $\delta^{18}\text{O}$ values of the 72 individuals were considered as the 'consumers' data input. We run the model setting manually the number of iterations, burn-in and thinning to 500,000, 200,000 and 300,000, respectively. Model diagnosis was performed via the Gelman–Rubin and Geweke tests (Stock & Semmens, 2013). Therefore, we finally obtained proportions of water sources used by each study species from two different soil depth intervals (shallow 0–30 and deep, 30–100 cm).

We assessed whether plant traits related to water-use strategy, that is, stem water $\delta^{18}\text{O}$, leaf $\delta^{18}\text{O}$ and leaf $\delta^{13}\text{C}$, were coordinated with leaf N and SLA by carrying out a Principal Components Analyses (*prcomp* function, *STATS* package) considering mean species values. SLA and leaf N concentration were selected because they are the main representative traits of the LES. We selected the stem water $\delta^{18}\text{O}$ for the PCA because it is considered a more reliable proxy of water source utilisation compared with stem water $\delta^2\text{H}$ (Barbeta et al., 2020, 2022; Chen et al., 2021). When necessary, variables were log-transformed to adjust for normality, and all were scaled before running the analysis. We carried out the PCA (phyIPCA hereafter) including the phylogenetic distance among species to account for the evolutionary history of our study species (method = BM, i.e.

Brownian Motion; *phyl.pca* function, *PHYTOOLS* package). We also run the same PCA without including phylogeny to assess for potential discrepancies between the two outputs.

Finally, to assess whether the plant water- and/or nutrient-use strategy affected species abundances, we tested the existence of correlation (Kendall's correlations; *cor.test* function, *STATS* package) between the species scores in the first two axes of both PCAs, with and without phylogeny, and the species abundance in the plant community. Species abundance data were collected in a previous study where we fully mapped the same plant community (for more details, see Illuminati et al., 2021). Specifically, we considered both the cover (%) and the number of individuals as indicators of the species dominance and abundance at the community level (Table S2).

3 | RESULTS

3.1 | Vertical soil water isotopic gradient and ecohydrological niche segregation among species

Soil water content ranged from a minimum of 3.66% ($SD = \pm 0.70$) in the topsoil layer (0–5 cm), to a maximum of 11.38% ($SD = \pm 4.67$) in the deepest soil layer (80–100 cm; Figure 2). We found a steep gradient in the isotopic composition of water along the soil profile, with major differences between sampled layers in the first 0–30 cm of soil depth

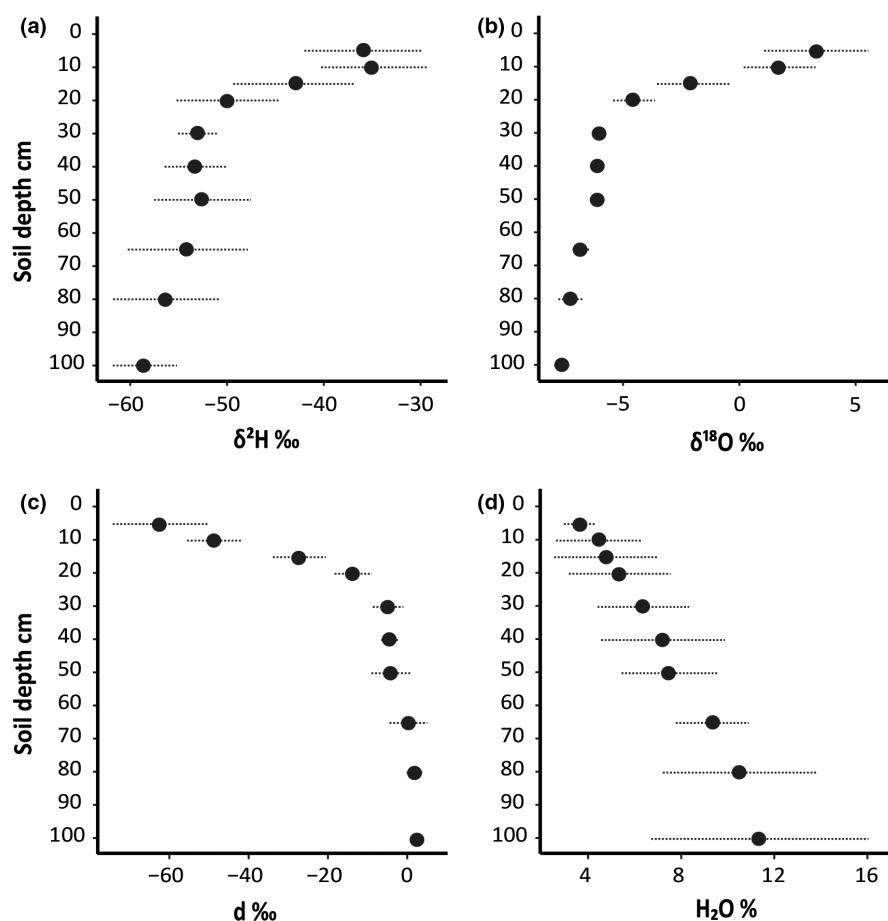


FIGURE 2 Steep gradients with depth (from 5 to 100 cm) of soil water $\delta^2\text{H} \text{‰}$ (a), $\delta^{18}\text{O} \text{‰}$ (b), d-excess ‰ (i.e. deuterium excess), (c) and soil water content % (d). Mean ($\pm\text{SD}$) are represented and calculated as the mean of three replicate soil samples (one soil sample from each of the three different plots)

(Figure 2a,b). Both soil water $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values became more negative with depth along the soil column, whereas d-excess values were most negative near the surface and increased with depth (Figure 2c). While standard deviations of water $\delta^2\text{H}$ partially overlapped among soil layers, standard deviations of water $\delta^{18}\text{O}$ were smaller and most of the soil layers presented a clearly distinct isotopic composition (Figure 2a,b). The observed patterns indicate strong evaporative isotopic fractionation of soil water stored in the upper layers, which progressively decreased with depth. The slope of the linear model relating the isotopic composition of soil water across soil samples was flatter than that of the LMWL, which further indicates strong evaporative enrichment of water stored in upper soil layers (Figure 1).

The 24 coexisting species displayed large and clinal differences in stem water isotopic composition ($\delta^2\text{H}$, $\delta^{18}\text{O}$, d-excess) and water content (Figure S4). The Bayesian model calculations (Figure 3) showed that the 24 coexisting species used a median of 37% of shallow soil water (0–30 cm) and 63% of deep soil water (30–100 cm). Still, water source proportions varied considerably between species, with several species, that is, *Leuzea conifera*, *Thymus vulgaris*, *Stipa pennata*, *Koeleria vallesiana*, *Coris monspeliensis*, *Sideritis incana*, relying more on water stored in the shallow topsoil layer (0–30 cm) than in deeper layers. Indeed, shallow soil water use by plants ranged widely from a minimum of 6.4% for *Linum narbonense*, to a maximum of 68% for *Leuzea conifera*.

3.2 | Species variation in leaf nutrient and isotopic traits

The 24 coexisting species showed a wide range of variation in functional leaf traits related to water- and nutrient-use strategies. Interspecific variability in foliar carbon investment (SLA) and leaf N, P and K concentrations (Figure 4; Figure S5) was remarkably

high. Species mean SLA values ranged from 50.61 to 133.74 cm²/g; while species mean leaf N, P and K concentrations ranged from 1.16% to 4.54%, 0.02% to 0.06% and 0.14 to 0.97%, respectively. Species mean leaf $\delta^{18}\text{O}$ values ranged from 28.22 to 40.15 ‰, leaf $\delta^{13}\text{C}$ ranged from –30.16 to –26.48 ‰, and leaf $\delta^{15}\text{N}$ ranged from –4.67 to 0.67 ‰, suggesting large differences in stomatal regulation, water-use efficiency and nitrogen acquisition mechanisms among coexisting species (Figure 4; Figure S6).

3.3 | Coordination between LES, water-use traits and species abundance

The first axis of the phyIPCA explained 43% of the total variance in key plant traits, while PC2 and PC3 explained an additional 24% and 18%, respectively (Figure 5; Table S3). The first axis largely reflected species variation along the LES, with species showing higher SLA and leaf N values representing a more acquisitive carbon- and nutrient-use strategy in one side, and species with lower SLA and leaf N representing a more conservative strategy at the opposite side (Table S3). Reinforcing this interpretation of the first phyIPCA axis as a LES gradient, we observed significant (or marginally significant) correlations of this axis with both leaf P ($R = -0.31$, $p = 0.04$) and leaf K concentrations ($R = -0.27$, $p = 0.07$), which are known to be related to the LES. Interestingly, stem water $\delta^{18}\text{O}$ also loaded heavily on the first PCA axis ($R = 0.59$; Table S3), indicating greater utilisation of deep soil water in species with more conservative nutrient-use strategies along the LES.

The second phyIPCA axis was largely related to leaf-level water-use traits, with heavy loadings of both leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Table S3). Species exhibiting higher leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values representing a water-saver strategy with tighter stomatal regulation of transpiration

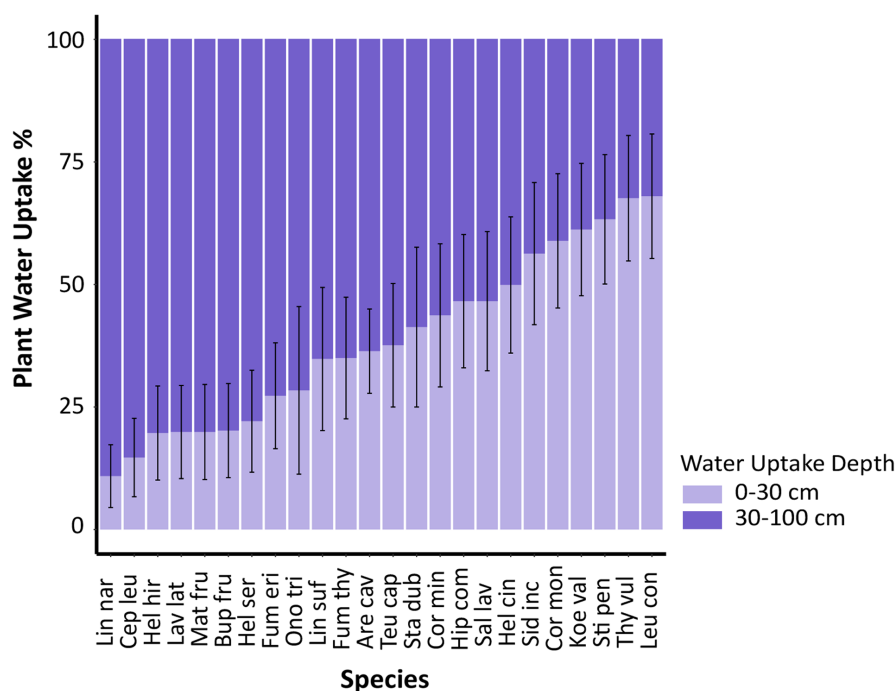


FIGURE 3 Proportions (%) of two different water sources (0–30 and 30–100 cm of depth), and their standard deviations, estimated by the mixed Bayesian model, captured by 24 species coexisting in the same plant community corresponding to the moment of vegetative peak

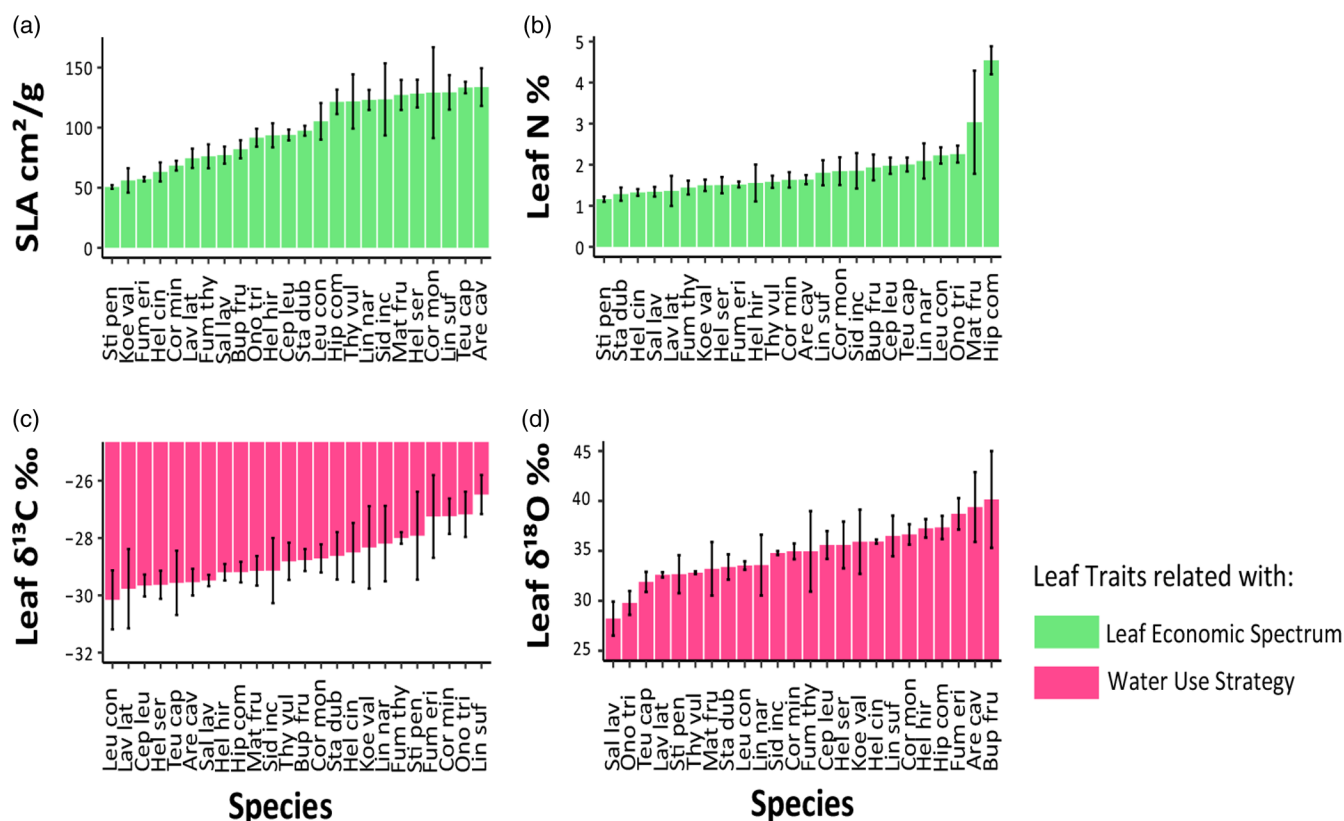


FIGURE 4 Variation of SLA (cm²/g) (a), leaf N (%) (b) and leaf isotopic traits (δ¹³C and δ¹⁸O, (c) and (d), respectively) across the 24 coexisting species

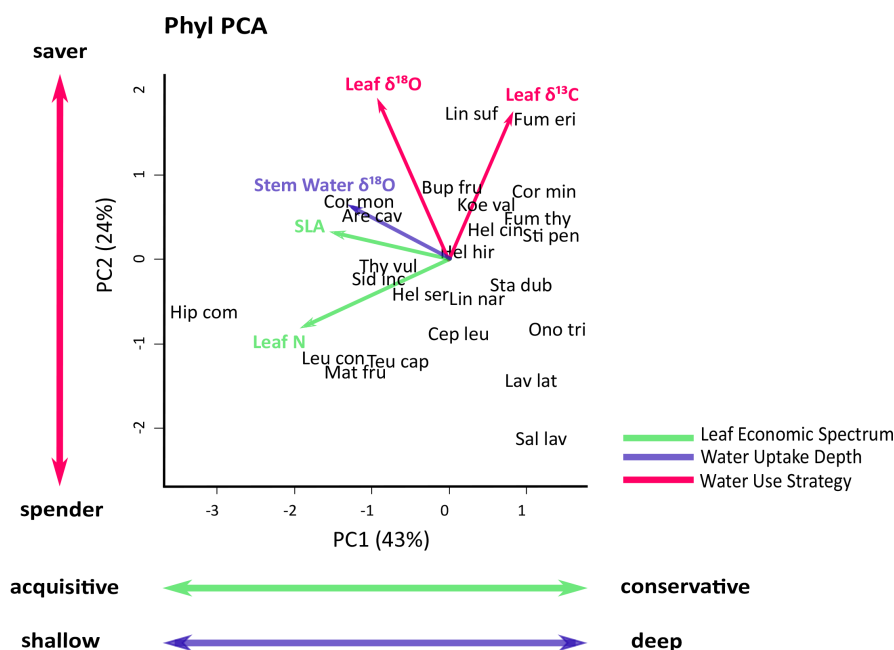


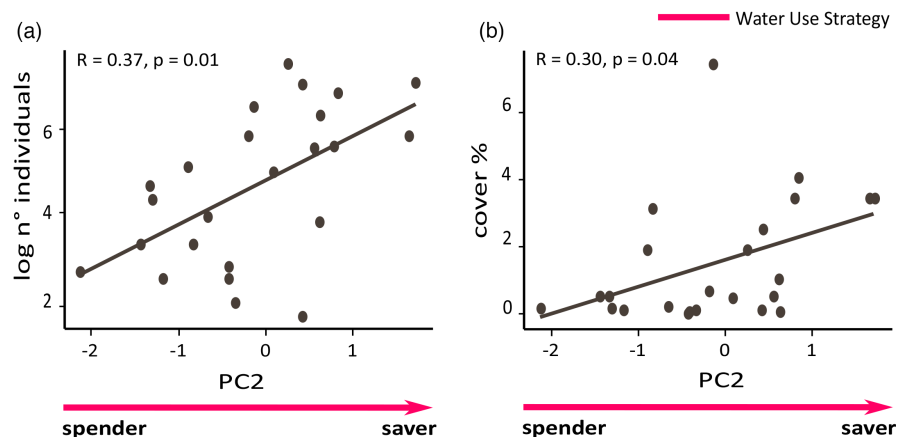
FIGURE 5 Biplot of the two first axes of the phylPCA, where phylogenetic distances between species were considered and the species mean values were used as the input variables. The first phylPCA axis represents species variation both along the leaf economic spectrum and in water uptake depths. The second phylPCA axis depicts species variation along a leaf-level water-use strategy gradient ranging from water-saver to water-spender. Blue arrow indicates water uptake depth (shallow/deep); red arrow indicates leaf-level water-use strategy and stomatal regulation (water-spender/water-saver); green arrow indicates carbon- and nutrient-use strategy along the leaf economic spectrum (conservative/acquisitive)

and higher intrinsic water-use efficiency plotted on one side of the PC2 axis. Species with lower leaf δ¹³C and δ¹⁸O values representing a more water-spender strategy with high stomatal conductance and low intrinsic water-use efficiency plotted on the opposite side of the second phylPCA axis. Results of the PCA carried out without including phylogeny were rather similar to the phylPCA, although an

important discrepancy emerged in the coordination of stem water δ¹⁸O with the second PCA axis, instead of with the first PCA axis (Figure S7; Table S4).

Species abundances, that is, both the cover and the number of individuals were significantly correlated to the species scores along the second phylPCA axis ($R = 0.30$ and 0.37 ; $p = 0.04$ and 0.01 ,

FIGURE 6 Kendall's correlation tests carried out between the PC2 axis of the phylPCA, representing the continuum from a water-spender to a water-saver strategy, and the species abundances in the plant community [number of individuals (a) and the cover % (b)]



respectively; Figure 6), indicating dominance by species with water-saver strategies. We did not find any correlation of species abundances with their scores along the first phylPCA axis. Interestingly, different results were found when considering the PCA carried out without phylogeny. The only marginally significant correlation observed in this case was that between the first axis and the species cover ($R = 0.27$; $p = 0.06$), suggesting that the plant community was dominated by species with a conservative LES strategy.

4 | DISCUSSION

4.1 | Ecohydrological niche segregation and variation in leaf functional traits

Coexisting perennials in this semi-arid shrubland showed a marked vertical soil water partitioning and ecohydrological niche segregation during the phenological peak. The pattern observed of heavy deep water use by many species is similar to that found in other drylands where deep root systems are widespread (Schenk & Jackson, 2002). Nevertheless, several species such as *Leuzea conifera*, *Thymus vulgaris*, *Coris monspeliensis* and the grasses *Stipa pennata* and *Koeleria vallesiana* relied more heavily on shallow soil water sources (0–30 cm) than on deeper soil water pools (30–100 cm; Figure 3).

Silvertown et al. (2015) found that ecohydrological niche segregation is widespread and occurs in a broad range of vegetation types, from drylands to tropical forests, although most of this evidence relied on a relatively small number of species per vegetation type. Our results showed that a clear complementary pattern in the exploitation of the most limiting resource (i.e. soil water) exists when a diverse array of coexisting species is considered. Indeed, an evaluation of all the species coexisting in a plant community is necessary to assess if ecohydrological niche segregation explains fine-scale species coexistence and assembly (Silvertown, 2004). To our knowledge, few previous works (e.g. Palacio et al., 2017; Sohel et al., 2021) have reported such well-structured and distinct water niche segregation among multiple coexisting species within the same plant community at fine spatial scales.

The large variability in leaf morphological (SLA) and nutrient (N, P and K) traits (Figure 4; Figure S5) encountered among the 24

coexisting species indicated a high functional diversity regarding carbon- and nutrient-use strategy along the LES (Wright et al., 2004). This was further evidenced by the large interspecific variation in leaf $\delta^{15}\text{N}$ values (Figure S6), which largely reflects different nitrogen acquisition mechanisms related to the presence, or lack thereof, of different root-microbe symbiotic associations (Craine et al., 2009). The highest leaf $\delta^{15}\text{N}$ values were found in non-mycorrhizal or facultative-mycorrhizal species, whereas leaf $\delta^{15}\text{N}$ values around zero were found in N_2 fixing species (*Fabaceae*). Obligate mycorrhizal species (arbuscular and/or ectomycorrhizal) showed negative leaf $\delta^{15}\text{N}$ values, with ectomycorrhizal species in particular showing the most negative values of all (Figure S6; Brundrett, 2009; Craine et al., 2009).

Alongside the wide variation in LES traits related to carbon- and nutrient-use strategy, the species presented large variability in leaf isotopic traits ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) linked to stomatal regulation and leaf-level water-use efficiency. This indicated the coexistence of species with sharply contrasting water-use strategies within the same plant community, ranging from water-saver to water-spender species (Moreno-Gutiérrez et al., 2012; Prieto et al., 2018).

4.2 | Linking water-use strategy, the leaf economic spectrum and species' abundances

Despite the remarkably large interspecific variability along the LES, most species plotted on the conservative side of the carbon- and nutrient-use strategy gradient in the multidimensional trait space (see phylPCA in Figure 5). Dominance of conservative carbon- and nutrient-use strategies has been previously predicted and reported in several nutrient-poor dryland plant communities (Chapin et al., 1993; Wright et al., 2004; Reich, 2014). Interestingly, stem water $\delta^{18}\text{O}$ also loaded substantially on (and covaried with) the LES axis, indicating that species on the resource conservative side of the LES primarily used water stored in deeper soil layers with more temporally stable moisture content. Conversely, species with more acquisitive nutrient-use strategies along the LES used a much greater proportion of water stored in shallower soil layers that are richer in nutrients but are exposed to sharper fluctuations

in moisture content (Figures 1 and 5). It is worth to note that species using isotopically enriched water stored in the shallower soil layers during spring may not necessarily be characterised by a shallow root system. Indeed, several species may have dimorphic root systems which can capture water from different soil depths (e.g. Dawson & Pate, 1996; Filella & Peñuelas, 2003), according to the fluctuating soil moisture conditions in upper soil layers which are subjected to sharp seasonal variations in arid and semi-arid environments (e.g. Schwinning & Ehleringer, 2001), but also depending on other important factors such as plant phenology (Reynolds et al., 2004; Ryel et al., 2008).

Greater use of water stored in nutrient-rich topsoil layers may favour a more acquisitive nutrient-use strategy along the LES, particularly after rainfall pulses that enhance nutrient mineralisation, solubilisation and uptake by roots in the fertile topsoil (Huxman et al., 2004; Schwinning & Sala, 2004). Our findings are in agreement with the two-pool hypothesis postulated by Ryel et al. (2008), according to which the shallow soil water pool, also named 'growth' pool (rich in nutrients), would be preferentially exploited during the main resource acquisition and growth period, that is, in the wet season. The shallow water pool would therefore be the milieu where high interspecific competition dynamics would take place (Ryel et al., 2008; Schenk, 2008), and thus species characterised by acquisitive nutrient-use strategies may gain an advantage over more conservative species. The deep water pool, also termed the 'maintenance' water pool, would instead represent a water source used by those perennial species which do not senesce but maintain physiological activity, even if low, during drought periods (Ryel et al., 2008; Schenk, 2008). In agreement with this hypothesis, some

recent studies (e.g. Kulmatiski et al., 2017, 2020) comparing trees and grasses have indeed shown that nitrogen and water uptake may in part be spatially decoupled in trees, with a preference for shallow nutrient-rich layers for nitrogen uptake.

Conversely, the species relying primarily on water stored in deeper and nutrient-poor soil layers may have more limited access to essential nutrients like nitrogen, which may be necessarily coupled with a more conservative nutrient-use strategy along the LES (Figure 5). Moreover, root access to deeper and more stable soil water pools may favour longer leaf life spans in conservative species by buffering them against severe drought stress during extended rainless spells in drylands, which would be in agreement with the LES theory and predictions (Wright et al., 2004).

Our findings suggest a probably inevitable trade-off and co-ordination between soil water uptake depth and LES traits in Mediterranean semi-arid shrubland communities, which may also occur in other nutrient-poor dryland ecosystems (Figure 7). Previous pioneer works, such as Walter's two-layer hypothesis describing hydrological segregation between coexisting trees and grasses in dry savannas, and later extended to other arid and semi-arid environments, may have implicitly suggested this potential trade-off (Ryel et al., 2008; Walter, 1939; Ward et al., 2013). Even though water source partitioning was generally interpreted as the result of different rooting depths and water-use strategies between woody vegetation and grasses, the hydrological niche segregation observed in these studies may have also implied different nutrient-use strategies. Indeed, nutrient-use strategies have been shown to vary strongly between different life-forms, especially between woody and non-woody species (e.g. Díaz et al., 2016).

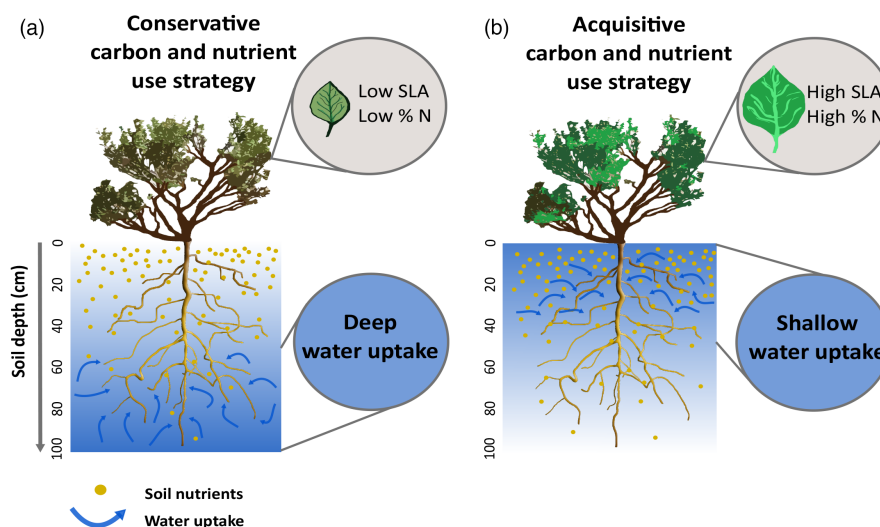


FIGURE 7 Conceptual model of the proposed trade-off between leaf-level carbon- and nutrient-use strategy (leaf economic Spectrum) and water uptake depth in drought-prone environments. Panel (a) shows the coupling between a more conservative carbon and nutrient strategy at the leaf level (low SLA and leaf nitrogen content) and a deeper water uptake pattern. Deeper soil layers are poorer in nutrients but reliably store more water during prolonged rainless periods. Panel (b) represents the coupling between a more acquisitive carbon- and nutrient-use strategy (high SLA and leaf nitrogen content) and a shallower water uptake pattern, which enhances acquisition of dissolved nutrients from the fertile topsoil layer after rainfall pulses. Dryland soils typically exhibit a steep vertical gradient in nutrient distribution, as nutrient availability to roots is highest in topsoil layers but steeply declines with depth (Jobbágy & Jackson, 2001; Querejeta et al., 2021; Ryel et al., 2008, 2010)

In addition, coexisting species appeared scattered along a second orthogonal gradient related to leaf-level water-use strategies (Figure 5). The second axis of the phyIPCA explained 24% of the variance and showed strong loadings of both leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, indicating that it was primarily related to stomatal behaviour (Barbour et al., 2000). According to previous studies conducted in Mediterranean ecosystems (e.g. Moreno-Gutiérrez et al., 2012; Prieto et al., 2018), this second axis segregated species with tight stomatal regulation of transpiration and high water-use efficiency (water-savers) from species with loose stomatal regulation, low water-use efficiency and more profligate water use (water-spenders). In sub-humid Mediterranean shrublands located in southern France, Prieto et al. (2018) found a tight coordination between plant traits related to the LES (SLA and leaf N) and traits related to leaf-level water-use strategy (leaf $\delta^{13}\text{C}$ and leaf $\delta^{18}\text{O}$) converging along a single main PCA axis. Our results partly agree with this pattern, as leaf $\delta^{13}\text{C}$ also showed substantial loading on the first axis of the phyIPCA (Table S3), indicating that species on the conservative side of the LES generally show higher WUEi than their neighbour nutrient-acquisitive species. However, the heavy loadings of both leaf $\delta^{13}\text{C}$ and leaf $\delta^{18}\text{O}$ along a distinct second dimension (phyIPCA axis 2) that was orthogonal to the LES (phyIPCA axis 1) in the multi-trait space suggested a separation between the leaf-level water-use continuum and the LES continuum in our semi-arid shrubland.

Interestingly, we found an unexpected discrepancy between the PCA results accounting (or not) for species phylogeny and relatedness (Figure 5; Figure S7). When evolutionary history was not considered in the PCA, stem water $\delta^{18}\text{O}$ was tightly coordinated with leaf $\delta^{13}\text{C}$ and leaf $\delta^{18}\text{O}$. This agrees with previous studies (e.g. Ding et al., 2021; Moreno-Gutiérrez et al., 2012), in which plant species with a water-saver strategy exploited deeper soil water sources, whereas species with a more profligate water-use strategy preferentially used shallower soil water sources. This finding highlights how phylogeny strongly affects the observed coordination between plant traits. We suggest that the apparent coordination of leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with stem water $\delta^{18}\text{O}$ along the same PCA axis may be due to common evolutionary history that is probably connected with the existence of strong ecological filters, rather than to the existence of a true universal trade-off between these functional traits. Similarly, a recent study (Zhou et al., 2021) showed how variation in traits related to water-use strategy was greatly influenced by phylogeny and that certain traits were more strongly variable in certain clades compared to others. It is thus critically important to consider the potential role played by evolutionary history when exploring the coordination and trade-offs among multiple functional traits.

Finally, we observed that the species in this Mediterranean shrubland were rather scattered and evenly distributed along the leaf-level water-use gradient (phyIPCA axis 2, Figure 5). Whereas most of the species in the plant community clearly showed a conservative carbon- and nutrient-use strategy along the LES (low SLA, low N content) and strong reliance on deep soil water sources, there were nearly as many water-spender as water-saver species along the phyIPCA second axis. Species dominance (cover %), but not abundance (number

of individuals), was negatively associated, even if only marginally, to the first axis of the PCA carried out without phylogeny, indicating a link between a nutrient-conservative strategy and species performance. However, when we constrained this PCA by adding phylogenetic relatedness, we found that the first axis, mostly related with the nutrient-use strategy, became unrelated to species dominance. Instead, species dominance and abundance were significantly correlated with the second phyIPCA, which was related to the water-use strategy. These differences support the notion that evolutionary history significantly affects community assembly processes at ecological time-scale. Our findings may also suggest that the coexistence of species with sharply contrasting water-use strategies may represent a key driver of plant community assembly in drylands where water is the most limiting resource. Given the dominance of conservative carbon- and nutrient-use strategies along the LES in nutrient-poor drylands, species also presenting a more conservative water-use strategy at leaf-level would be expected to further improve their whole-plant resource-use efficiency (Reich, 2014), and therefore their dominance and abundance within the plant community.

The remarkably wide diversity of plant functional traits and resource-use strategies encountered in this species-rich semi-arid plant community suggests that an efficient and exhaustive acquisition and exploitation of the limiting soil resources available across the whole soil profile may reduce competition and may be ruling the assembly of this community (e.g. Peñuelas et al., 2011; Escudero & Valladares, 2016). Vertical soil water niche segregation and complementarity among coexisting species would be expected to enhance the primary productivity and drought resistance and resilience of water-limited Mediterranean shrublands. Moreover, the presence of a few species with acquisitive LES strategy and/or profligate water-use pattern may enhance soil resource capture and utilisation during the short water and nutrient pulses after rainfall, thereby helping maximise overall plant community productivity in semi-arid shrublands largely dominated by water-saver species. In particular, water-spender species with acquisitive nutrient-use strategy may be capable of achieving more efficient exploitation of water (and dissolved nutrients) stored in topsoil, which is a relatively ephemeral resource pool that is rapidly lost to unproductive direct soil evaporation in dryland ecosystems.

In conclusion, we provide evidence of strong vertical ecophysiological niche segregation among coexisting species in a diverse Mediterranean shrubland. Water source partitioning among neighbours likely enhances complementarity and decreases competition for water, the most critical limiting resource in drylands. Water uptake depth was coordinated with LES traits across species, with nutrient-conservative species relying more heavily on deep soil water sources, and nutrient-acquisitive species using a greater proportion of shallow water from the fertile topsoil layer, although the latter could be either shallow-rooted species or species with dimorphic root systems that may also be capable of using deep water sources during the dry summer season. To our knowledge, this is the first study reporting a strong trade-off and coordination between soil water uptake depth and the leaf economics spectrum in nutrient-poor dryland plant communities.

Moreover, leaf-level water-use pattern represented a second distinct functional dimension in multi-trait space, and we encountered a significant link between tighter stomatal regulation, higher water-use efficiency and greater species cover and abundance in semi-arid shrublands. Finally, this study highlights the need to account for the evolutionary history and phylogenetic relationships among coexisting species when assessing functional traits trade-offs defining whole-plant resource-use strategies.

AUTHORS' CONTRIBUTIONS

A.I., J.I.Q., A.E. and S.M. planned and designed the research; A.I. and B.P. conducted the fieldwork; A.I. and J.I.Q. conducted the data statistical analyses and interpretation; A.I. wrote the manuscript extensively aided by all other authors.

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CONFLICT OF INTEREST

None of the authors have conflicts of interest for this article.

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ORCID

Angela Illuminati  <https://orcid.org/0000-0003-2313-1639>

José Ignacio Querejeta  <https://orcid.org/0000-0002-9547-0974>

Beatriz Pías  <https://orcid.org/0000-0002-1136-8914>

Adrián Escudero  <https://orcid.org/0000-0002-1427-5465>

Silvia Matesanz  <https://orcid.org/0000-0003-0060-6136>

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