Disentangling water sources in a gypsum plant community. Gypsum crystallization water is a key source of water for shallow-rooted plants

Laura de la Puente1,*, Juan Pedro Ferrio2 and Sara Palacio1,*

1Departamento Biodiversidad y Restauración, Instituto Pirenaico de Ecología, Consejo Superior de Investigaciones Científicas, Avenida Nuestra Señora de la Victoria, 16, Jaca, ES-22700, Spain and 2Unidad de Recursos Forestales, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Av. Montañana 930, Zaragoza, ES-50059, Spain

*For correspondence. E-mail delapuente@ipe.csic.es

Received: 19 May 2021 Returned for revision: 3 August 2021 Editorial decision: 13 August 2021 Accepted: 17 August 2021 Electronically published: 18 August 2021

• Background and Aims Gypsum drylands are widespread worldwide. In these arid ecosystems, the ability of different species to access different water sources during drought is a key determining factor of the composition of plant communities. Gypsum crystallization water could be a relevant source of water for shallow-rooted plants, but the segregation in the use of this source of water among plants remains unexplored. We analysed the principal water sources used by 20 species living in a gypsum hilltop, the effect of rooting depth and gypsum affinity, and the interaction of the plants with the soil beneath them.

• Methods We characterized the water stable isotope composition, δ2H and δ18O, of plant xylem water and related it to the free and gypsum crystallization water extracted from different depths throughout the soil profile and the groundwater, in both spring and summer. Bayesian isotope mixing models were used to estimate the contribution of water sources to plant xylem sap.

• Key Results In spring, all species used free water from the top soil as the main source. In summer, there was segregation in water sources used by different species depending on their rooting depth, but not on their gypsum affinity. Gypsum crystallization water was the main source for most shallow-rooted species, whereas free water from 50 to 100 cm depth was the main source for deep-rooted species. We detected plant–soil interactions in spring, and indirect evidence of possible hydraulic lift by deep-rooted species in summer.

• Conclusions Plants coexisting in gypsum communities segregate their hydrological niches according to their rooting depth. Crystallization water of gypsum represents an unaccounted for, vital source for most of the shallow-rooted species growing on gypsum drylands. Thus, crystallization water helps shallow-rooted species to endure arid conditions, which eventually accounts for the maintenance of high biodiversity in these specialized ecosystems.

Key words: Water sources, hydrological niche, drought, gypsum crystallization water, plant community, root depth, gypsum affinity, water stable isotopes.

INTRODUCTION

Plant species from arid and semi-arid ecosystems have adapted to water scarcity by different mechanisms of water uptake and use. An important strategy is the segregation in hydrological niches, which makes possible the coexistence of different plant species in stable and diverse communities (Ehleringer et al., 1991; Filella and Peñuelas, 2003; Araya et al., 2011; Silvertown et al., 2015, Palacio et al., 2017). Hydrological niche segregation has often been found in several ecosystems affected by drought, such as Mediterranean shrublands and forests (Filella and Peñuelas, 2003; Moreno-Gutierrez et al., 2012; del Castillo et al., 2016), deserts (Ehleringer et al., 1991; Schachtschneider and February, 2010) or seasonal tropical forests (Liu et al., 2010; Brum et al., 2019; Ding et al., 2021). Different traits related to changes in root architecture and rooting depth allow divergent water use strategies and the partition of this scant resource among coexisting plants (Donovan and Ehleringer, 1994; Moreno-Gutierrez et al., 2012). Water from precipitation present in the topsoil favours nutrient availability and microbial processes, using this pool preferentially during the growth period (Caldwell et al., 1998; Querecta et al., 2021). However, during drought, roots should access deeper soil layers, sometimes even reaching the water table, where water availability is more stable (Ehleringer et al., 1991; Ryel et al., 2008, 2010; Rempe and Dietrich, 2018). These deeper water pools are normally used to maintain transpiration during periods of limited growth (Votlas et al., 2015). Many plants have developed dimorphic root systems with both superficial and deep roots, and the different water potential between dry shallow layers and wet deep layers can lead to hydraulic lift (Bauerle et al., 2008; Prieto et al., 2012). This is a widespread process in semi-arid environments consisting of the passive movement of water from deeper layers to upper layers by roots (Prieto et al., 2010, 2012). Through hydraulic lift, plants can act as ‘bioirrigators’ to neighbouring plants, hence increasing their chances of survival and, ultimately, the coexistence of diverse communities (Jackson et al., 2000; Bayala and Prieto,
Assessing the functional importance of contrasting soil water pools and their spatial and temporal variation is necessary to evaluate how climate change and land use may affect the ecohydrology of vegetation and the dynamics of plant communities (Ehleringer et al., 1991; Oerter and Bowen, 2019; Dwivedi et al., 2020). Understanding the mechanisms by which different plant species take up and partition water resources in arid and semi-arid conditions is crucial to unravel the processes supporting plant coexistence in dryland communities (Dodd et al., 1998; Peñuelas et al., 1999).

Gypsiferous soils, i.e., soils with high (>40 %) gypsum (CaSO₄·2H₂O) content (Casby-Horton et al., 2015), are frequently present in drylands, being widespread around the world (FAO, 1990; Verheye and Boyadgiev, 1997). Together with the arid conditions, these soils have low water retention (Herrero and Porta, 2000) and, consequently, water is a major limiting factor for plants growing on gypsum soils. Some studies, however, found better water availability in summer in gypsum soils than in surrounding non-gypsum soils (Meyer and García-Moya, 1989; Escudero et al., 2015). This observation is further supported by the discovery of crystalline gypsum water as a source for plants and other organisms during the dry period (Palacio et al., 2014b, 2017; Huang et al., 2020). Gypsum contains water in its crystalline structure, which represents up to 20.8 % of its weight. Under certain conditions of vapour pressure and temperature (from 42 °C in pure gypsum, Marshall et al., 1964), gypsum could dehydrate, changing into bassanite (the hemihydrate: CaSO₄·0.5H₂O) or into anhydrite (CaSO₄) (Van’t Hoff et al., 1903; Freyer and Voigt, 2003; Ossorio et al., 2014). In addition, it has been demonstrated that this phase transformation can be induced by some micro-organisms, leading to anhydrite re-precipitation (Huang et al., 2020). There is evidence of a large use of crystallization water by the gypsum endemic plant H. squamatum, and it has been suggested that its use could be extended to other shallow-rooted species in gypsum plant communities (Palacio et al., 2014b, 2017; Henschel et al., 2019). However, it is still unknown up to what point plants coexisting in the same plant community show different abilities to retrieve crystallization water, and thus whether the use of this water pool is a relevant factor defining hydrological niches in gypsum plant communities.

Gypsiferous soils also show particular chemical and physical properties, which could constrain the development of plant life (Escudero et al., 2015). Plant roots have to cope with a high penetration resistance (Poch and Verplancke, 1997; Moore et al., 2014, Sánchez-Martín et al., 2021) and morphological transitions of the soil due to dissolution–precipitation sequences of gypsum (Casby-Horton et al., 2015). In addition, most of these soils have a low nutrient supply caused by their low organic matter content and cation exchange capacity, and their saturation in Ca and S (Moore et al., 2014; Casby-Horton et al., 2015). Despite these limitations, gypsum soils host highly diversified floras, rich in endemic and highly specialized species (Moore et al., 2014) which have been the subject of a more in-depth study from only a few years ago (Escudero et al., 2015).

Plant species growing on gypsiferous soils can be classified into two groups depending on their affinity for gypsum: gypsophiles, which only grow on gypsiferous soils and often have substrate-specific physiological strategies (Palacio et al., 2007; Escudero et al., 2015; Cera et al., 2021); and gypsovags, which are non-exclusive to gypsum soils (i.e. also grow off gypsum) and frequently display stress-tolerant strategies (Palacio et al., 2007; Bolukbasi et al., 2016). Gypsophiles have shown a range of mechanisms to detoxify the excess of Ca and SO₄ considering their leaf elemental composition, whereas gypsovags would follow an avoidance strategy, reducing the absorption of these compounds (Palacio et al., 2007, 2014a; Merlo et al., 2019; Cera et al., 2021). Thus, if obtaining the crystallization water from gypsum is related to its dissolution (Huang et al., 2020) and, consequently, the release of Ca and sulfate ions, gypsophiles could be more prone to using this water than gypsovags.

Tracing water movement in the soil and plants is possible using the natural variations of stable isotopes of hydrogen (¹H) and oxygen (¹⁸O) in water molecules. This widely used method, extensively applied in hydrology and ecophysiology, allows evaluation of the result of several processes without disrupting the natural behaviour of the elements in the system (Meisner et al., 2014; Penna et al., 2018). Water phase changes (vapour–liquid–solid) explain most of the isotopic variability, as the heavier isotopes have a lower mobility (Dawson et al., 2002). The water sources acquired by plants can be determined with the following premises (1) alternative water pools must be isotopically distinct and (2) there is no isotopic fractionation during water uptake. In dry environments, the first assumption is generally fulfilled: due to evaporative fractionation, upper soil layers often become enriched in the heavy isotopes ¹H and ¹⁸O, thus being distinguishable from deeper soil layers or groundwater (Barnes and Allison, 1988; Dawson and Ehleringer, 1998). With regard to the second assumption, fractionation during water uptake is considered negligible in most plants (Dawson et al., 2002, and references cited therein), with the exception of some coastal wetland species (Lin et al., 1993) and certain woody xerophytes (Ellsworth and Williams, 2007). Nevertheless, different authors have reported discrepancies between source and stem water, attributed to different causes, e.g. heterogeneity in the soil (Barbeta et al., 2020), stem evaporation during periods of limited water flow (Dawson and Ehleringer 1993; Martín-Gómez et al., 2015) or sampling artefacts (Marshall et al., 2020).

The purpose of this study was to analyse the distribution of water sources among the 20 main dominant plant species in a hill top gypsum community. We characterized the variation in the isotopic composition of water along the soil profile and evaluated the effect of species rooting depth and affinity for gypsum soils on their water use in both spring and summer. We also analysed how plants interacted with the soil beneath them. Considering plant water uptake patterns, we hypothesized that: (1) shallow-rooted, gypsum-exclusive species will preferentially use crystallization water from gypsum in summer, whereas shallow-rooted, non-exclusive species will be restricted to the (scarce) free water available in the topsoil. Conversely, deep-rooted species, regardless of gypsum affinity, will rely mainly on the use of deep soil water and/or groundwater during summer drought. Considering plant–soil interactions, we also hypothesized that (2) deep-rooted species will interact with
the shallow soil, lifting water up from deeper soil layers (hence performing hydraulic lift).

**MATERIALS AND METHODS**

**Study area and species**

We conducted field sampling on a gypsum hill in the Middle Ebro Depression, Zaragoza province, North-East Spain (1°37′52.5″N, 0°41′23.7″W, 287 m a.s.l.) The main component of the soil in this region is gypsum (63.4 %), with thin outcrops of marls and clays (Quirantes, 1978). The climate is semi-arid and highly seasonal (Palacio et al., 2007). Mean annual temperature is 14.9 °C, average annual rainfall is 313.5 mm, which falls mainly during spring and autumn, and evapotranspiration is around 1200 mm, so plants experience intense drought during summer months. An important proportion of the soil surface in the upper part of the gypsum hill is bare or coated with a biological crust dominated by cyanobacteria, lichens and mosses (Concostrina-Zubiri et al., 2014). The plant community is dominated by sub-shrubs such as Helianthemum squamatum, with some taller shrubs, such as Gypsophila struthium subsp. hispanica or Ononis tridentata (Braun-Blanquet and Bolos, 1987).

We selected 20 dominant perennial plant species to represent the community living at the top of the hill, where stress conditions are most severe (Hodgson et al., 1994; Guerrero Campo et al., 1999; Casby-Horton et al., 2015). These representative species included different life forms (woody vs. herbaceous), root depths, affinity for gypsum soils and taxonomic families. We considered species with roots >1 m deep to be deep-rooted species, and the rest were considered to be shallow-rooted (Guerrero-Campo, 1998; Table 1).

**Plant and soil sampling**

Field sampling for isotope analyses was performed in the rainy spring (24–25 April, 2018) and in the dry summer (7–8 August, 2018), after a long rainless period. On each sampling date, we harvested the main stems (including the root crown) of five individuals of each species. We selected vigorous, medium-sized individuals located at least 5 m away from each other. To minimize the risk of stem water evaporation and to maximize the representativeness of xylem water as an indicator of the main water sources used by plants, we harvested between 06.30 and 10.00 h (solar time). In this time frame, we expect maximum transpiration rates and low evaporative demand to prevent stem dehydration (Grammatikopoulos et al., 1995; Martín-Gomez et al., 2017). In herbaceous species, the root collar was used as a proxy for non-enriched source water (Barnard et al., 2006). In woody species, the bark and phloem were removed with a knife to avoid contamination with phloem water and organic compounds present in living cells and/or the bark (Ehleringer and Dawson, 1992; see Fig. 1D). Two soil samples were collected underneath each plant at two different depths: 10 and 20 cm, (approx. ± 2 cm) avoiding the intrusion of roots in the samples (see Fig. 1C). In addition, to capture variation in soil water isotopic composition throughout the soil depth, three profiles 1 m deep were dug underneath the bare soil on each sampling date (see Fig. 1B). Soil samples were collected at 12 different depths (approx. ± 2 cm): 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 cm. In spring, we gathered two extra samples from a small temporal creek upwelling in the saline depression downhill, representative of the groundwater. At the time of sampling, the water formed a small temporal creek, easily distinguished from rain puddles. Immediately after harvest, water, stem and soil samples were placed in airtight sealed

**Table 1. Main characteristics of the study species.**

| Id  | Species                                                      | Root depth | Maximum root depth | Gypsum affinity | Stem | Family       |
|-----|--------------------------------------------------------------|------------|--------------------|-----------------|------|--------------|
| Fu.er | Fumana ericifolia Wallr.                                     | Shallow    | 1–2                | Gypsophag       | Woody| Cistaceae    |
| Ge.sc | Genista scorpius L.DC                                       | Deep       | 3                  | Gypsophag       | Woody| Fabaceae     |
| Gy.hi | Gypsophila struthium L. subsp. Hispanica (Willk.) G. López | Deep       | 3                  | Gypsophile      | Woody| Caryophyllaceae |
| He.hi | Helianthemum kirtum (L.) Mill                              | Shallow    | 1–2                | Gypsophag       | Woody| Cistaceae    |
| He.ma | Helianthemum marifolium (L.) Mill                           | Shallow    | 1                  | Gypsophag       | Woody| Cistaceae    |
| He.sq | Helianthemum squamatum (L.) Pers.                           | Shallow    | 2                  | Gypsophile      | Woody| Cistaceae    |
| He.sy | Helianthemum syriacum (Jacq.) Dum.Cours.                    | Shallow    | 2                  | Gypsophag       | Woody| Cistaceae    |
| He.st | Helichrysum stoechas (L.) Moench subsp. stoechas            | Shallow    | 2                  | Gypsophag       | Woody| Asteraceae   |
| He.fr | Herniaria fruticosa L.                                      | Shallow    | 2                  | Gypsophile      | Woody| Caryophyllaceae |
| Ko.va | Koeleria vallesiana (Honcken) Gaudin subsp. vallesiana       | Shallow    | 1–2                | Gypsophag       | Herbaceous | Poaceae     |
| Le.su | Lepidium subalatum. L.                                      | Shallow    | 2                  | Gypsophile      | Woody| Brassicaceae |
| Li.sf | Linum suffruticosum L.                                      | Shallow    | 2–3                | Gypsophag       | Woody| Linaceae     |
| Li.fr | Lithodora fruticose (L.) Griseb.                            | Shallow    | 2–3                | Gypsophag       | Woody| Boraginaceae |
| Ma.fr | Matthiola fruticula (Loefl. ex L.) Maire subsp. fruticulosa | Shallow    | 1–2                | Gypsophag       | Woody| Brassicaceae |
| On.tr | Ononis tridentata L.                                         | Deep       | 3                  | Gypsophile      | Woody| Fabaceae     |
| Ro.of | Rosmarinus officinalis L.                                   | Deep       | 3                  | Gypsophag       | Woody| Lamiaceae    |
| St.of | Stipa ovifera Breist.                                       | Deep       | 3                  | Gypsophag       | Herbaceous | Poaceae     |
| Te.ca | Tetradium capitatum L. subsp. capitatum                     | Shallow    | 1–2                | Gypsophag       | Woody| Lamiaceae    |
| Th.ti | Thymelaea tinctoria (Pouret) Endl. subsp. tinctoria          | Deep       | 3                  | Gypsophag       | Woody| Thymelaeaceae |
| Th.vu | Thymus vulgaris L.                                           | Shallow    | 2                  | Gypsophag       | Woody| Lamiaceae    |

Maximum root depth: 1, 25–50 cm; 2, 50–100 cm; 3, > 100 cm (Guerrero-Campo, 1998).
tubes (Duran GL18), immediately frozen with dry ice and kept frozen until distillation in the lab.

**Water extraction**

Xylem and soil water were extracted by cryogenic vacuum distillation (Ehleringer and Dawson, 1992), adapted as described in Palacio et al. (2014b). Spring samples were extracted at the Laboratory of Silviculture of the Universitat de Lleida (Lleida, Spain) and summer samples were extracted with the same procedure at the laboratory of the Instituto Pirenaico de Ecología (IPE-CSIC, Zaragoza, Spain). Sample tubes were placed in a heated silicone oil bath, and connected with Ultra-Torr unions (Swagelok Company, Solon, OH, USA) to a vacuum system (approx. 10⁻² mbar) including U-shaped water traps in series that were cooled with liquid nitrogen. Eight lines were installed. After an extraction time of 90 min for plant and soil samples (West, 2006; Meisner, 2014), captured water was transferred into screw-capped 2 mL vials, and stored at 4 °C until isotope analysis. Xylem water was distilled at 130 °C, whereas gypsum soils were distilled in two steps: first at 35 °C, and then at 130 °C to separate free and crystallization water and ensure almost complete dehydration of gypsum (Freyer and Voigt, 2003; Palacio et al., 2014b). Between the first and second distillation, sample tubes were kept in a desiccator with silica gel to avoid any re-hydration with ambient moisture, which could contaminate the next extraction water. Distilled samples were completely dried in the oven for 24 h at 60 °C. The samples were weighed before and after each distillation and after oven-drying to measure water content and confirm complete distillation.

**Stable isotope analyses**

Oxygen and hydrogen isotope composition (δ¹⁸O and δ²H) were determined by cavity ring-down spectroscopy (CRDS). For spring samples, the analyses were performed at the Serveis Científico-Tècnics (Universitat de Lleida), using a Picarro L2120-i with vaporizer A0211 (Picarro, Santa Clara, CA, USA). Summer samples were analysed at the scientific services of the Instituto Pirenaico de Ecología (CSIC), using a Picarro L2130-i with vaporizer A0211. The estimated precision was 0.10 % for δ¹⁸O and 0.40 % for δ²H. Deuterium excess was calculated according to Dansgaard (1964), as the divergence from the Global Meteoric Water Line: Dex = δ²H – 8 × δ¹⁸O. Where appropriate, we applied the post-processing correction to manage the organic contamination of the samples. After describing the magnitude of contamination with the software PostProcess ChemCorrect™ v1.2.0, the H₂¹⁸O, HD²O and H₂O peaks, filtered by the spectral features of organic compounds, were converted to organic-corrected δ¹⁸O and δ²H by applying a formula using device-specific factory calibration values (see Martin-Gómez et al., 2015 for details).
**Statistical analyses**

Changes in soil water content and in the isotopic composition of water along soil profiles, as well as δ²H–δ¹⁸O bi-plots with soil water and xylem sap isotopic compositions were visualized using ggplot2 in R (Wickham, 2016). Soil water content was calculated from sample weights before and after water extractions. Variation in the isotopic composition along the soil profiles was analysed to characterize potential deep water sources for plants and locate the evaporation front in both seasons. To identify the possible sources of deep soil water for plants, we defined soil depths > 20 cm with homogeneous isotopic composition of free soil water that markedly differed from other depths in the soil (Fig. 2). Transition depths with intermediate and highly variable soil water isotopic composition were not included in the model, so that alternative sources could be clearly differentiated. For this reason, water isotopic values at 30 and 40 cm depth were removed from the set of sources (see Fig. 2 and Supplementary data Fig. S1). Considering the results for the characterization of soil water along the soil profile (see Figs 2 and 3), we could differentiate four potential water sources for plants (see below). This characterization of sources was the simplification of a preliminary, seven-source model (see below).

Differences among study species and sampling dates in xylem water isotopic composition (δ¹⁸O and δ²H) and deuterium excess were evaluated using residual maximum likelihood (REML) analysis with the lmer function from the lme4 package in R (Bates et al., 2015). Models were run separately for each water isotope, ²H, ¹⁸O and deuterium excess. General models included species nested within family as a random factor to account for potential phylogenetic bias, and gypsum affinity (two levels: gypsophile and gypsovag), water pool (two levels: free and crystallization) and root depth (two levels: shallow and deep) as fixed factors. Separate models were run for each season to explore differences between species with different gypsum affinity and root depth in each season for each isotope. Shapiro–Wilk normality test (Royston, 1995) and Levene test for homogeneity of variances (Noguchi and Gel, 2010; Gastwirth et al., 2009) were used to check the normal distribution and homoscedasticity of residuals. Residuals were visually checked using the DHARMa package (Hartig, 2021). When interactions were significant, groups were analysed with post-hoc Tukey HSD tests using the lsmeans package (Russell, 2016).

The relative contribution of different water sources to xylem sap was estimated using Bayesian mixing models for stable
isotopic data with the package MixSIAR (Stock et al., 2018). This procedure estimates the proportion of source contributions to a mixture. The model used as ‘consumers’ the isotope values of xylem water in each individual (\(\delta^2\)H and \(\delta^{18}\)O). For ‘sources’, alternative models were run with different grouping of sources in order to select those that best described and simplified the potential water sources for plants. The Mix-SIAR model, that had better accuracy and so explained better the contribution of the sources to the xylem of plants, was run with seven different sources for each species: free soil and crystallization water from 10 and 20 cm, free and crystallization water from the ‘deep soil’ (50–100 cm combined) and groundwater. Values for 10 cm and 20 cm soil depth included one replicate per individual plant, whereas values from deeper soil were averaged across the three soil profiles. This model was simplified a posteriori by the addition of the contributions of each source into four simplified sources: (1) ‘crystal water’, i.e. gypsum crystallization water from the soil underneath the plants and deep soil, as they clearly departed from free water, and had a comparatively small variation along the soil profile. It was calculated by the addition of the contributions to the xylem of plants of all three crystallization water sources initially considered. (2) ‘Shallow free’, i.e. free water in the shallow soil (until approx. 20 cm depth), represented by free water extracted from soil collected underneath each plant owing to the better replication. It was calculated as the addition of the contribution to the xylem of the free water at 10 and 20 cm. (3) ‘Deep soil free’, i.e. free water in the deep soil (between 50 and 100 cm depth). (4) The water table (i.e ‘groundwater’), not modified from the output in the Bayesian model. The contribution of the water sources to the species separated by their root depth was calculated by the addition of the contributions of the different sources to the composition of the xylem water of the different species in each rooting depth group.

The effect of plant species on the isotopic composition of the soil beneath was considered by assessing the significance of between- and within-group variations in the isotopic composition of the soil collected under each individual. Effects were analysed separately for each isotope (\(\delta^2\)H, \(\delta^{18}\)O and deuterium excess) and season. To account for interspecific differences in the isotopic composition of soil water, we ran linear models using the lm function (Chambers, 1992). Specific models were run with REML using the lmer function (lme4 package) to assess differences for the fixed factors: ‘gypsum affinity’, ‘root depth’ and their interaction with the same random term structure as in xylem water comparisons. To assist in the interpretation of plant–soil interactions, e.g. to visually identify evidence of hydraulic lift, the isotopic compositions of the xylem water and the water extracted from the soil beneath the plants were visually compared using the ggplot2 package. All statistical analyses were run in R 4.0.0. (R Core Team, 2020).

RESULTS

Water source characterization along soil profiles

\(\delta^2\)H and \(\delta^{18}\)O composition of free soil water showed more homogeneous values in spring than in summer (Fig. 2A, B), mainly due to the spatial heterogeneity of soil water evaporative enrichment and the location of the evaporation front at slightly different positions among the three different soil profiles. In spring, water in shallow soil layers showed more negative values of both \(\delta^2\)H and \(\delta^{18}\)O than water in the deep soil (Fig. 2A, B), which corresponded to very negative values from a recent rain event in April 2018 (Supplementary data Table S1). No evaporation front was observed in spring, whereas, in summer, the evaporation front in the bare soil was located at approx. 15 cm depth, showing an abrupt change from isotopically depleted values of \(\delta^2\)H and \(\delta^{18}\)O at 5–10 cm, typical of water vapour, to highly enriched values at 15–20 cm (Fig. 2A, B). Below 20 cm, \(\delta^2\)H and \(\delta^{18}\)O became more negative with depth, until they stabilized from 40–50 cm to 80–90 cm depth, with a slight increase from 90 to 100 cm (Fig. 2A, B). In both seasons, the \(\delta^2\)H and \(\delta^{18}\)O of gypsum crystallization water showed a similar pattern with depth (between 5 and 60 cm). Values were more positive in the upper soil layers, presumably due to the re-crystallization of gypsum with more evaporated...
In spring, this progressive depletion with depth continued until 100 cm, whereas, in summer, a small increase in isotopic signatures was observed between 70 and 90 cm, together with larger variability among profiles.

In spring, deuterium excess of free water was rather homogeneous throughout the soil profile (Fig. 2C), becoming slightly negative in the top layer (5 cm) and in the deepest layers (60–100 cm). Conversely, deuterium excess of free water in summer showed large variations, following an opposite pattern to that in \( \delta^2 \text{H} \) and \( \delta^{18} \text{O} \) that indicates strong evaporative enrichment of soil water in the upper soil layers (Fig. 2C). For crystallization water, deuterium excess in both seasons became less negative with depth, further indicating re-crystallization of gypsum with more evaporated water in the top soil layers.

We found much higher free water content in the shallow soil layers in spring than in summer (Fig. 2D). In spring, we observed relatively uniform free water content in the soil profile until 60–70 cm, where soil water content decreased in the vicinity of the underlying bedrock. In summer, we observed severe soil desiccation in shallow soil layers and higher water content with depth, until reaching layers next to the bedrock, where the soil water content decreased again. The content of crystallization water retrieved is related to the gypsum content in the soil which was homogeneous through most of the soil profile in summer. Nevertheless, we found more variability in the upper layers in spring (Fig. 2D).

Regarding the position of the water sources and the xylem of plants in the bi-plot showing \( \delta^2 \text{H} \) vs. \( \delta^{18} \text{O} \), we observed the segregation of crystallization and free water, and the clustering of the xylem sap of shallow-rooted plants with crystallization water during summer. This is compatible with an important use of this water source by these species during drought (see below). Free water from the first 20 cm in the soil (collected underneath the plants) showed values typical of water vapour (Fig. 3; Supplementary data Fig. S2). Contrastingly, free water collected underneath the bare soil, which retained more water, showed values of evaporated water (Fig. 2; see Supplementary data Fig. S2). These could be due to the biological and physical crust formed in the bare soil that decreases evaporation (Escudero et al., 2015) and/or to the more intense exploitation of the scarce free water from the soil beneath them by plants. Further, many of the isotopic values of shallow-rooted species with a high gypsum water contribution in their xylem sap cannot be solely explained by an eventual evaporation within the stem (see Supplementary data Fig. S3).

### Analysis of factors explaining differences among plants in their xylem isotopic composition

Both season and rooting depth had a significant effect on the isotopic composition of the xylem water of the target species. Conversely, the affinity for gypsum soils did not show a significant effect on xylem water composition, indicating that plants did not use different water sources according to this factor. Three main groups could be identified based on their xylem water composition: the first group included all species in spring, whereas the second and third groups included summer values for shallow-rooted and deep-rooted species, respectively (Fig. 4; Supplementary data Table S2). Differences in the isotopic composition of the xylem water of plants were highly significant between seasons, as well as for the interaction between season and root depth. In spring, \( \delta^2 \text{H} \) and \( \delta^{18} \text{O} \) had more negative values than in summer, and more positive deuterium excess, but the xylem sap isotopic composition did not show significant differences due to species rooting depth. In summer, however, deep-rooted species had more negative values than shallow-rooted species (Fig. 4; Supplementary data Table S3). Overall, these results indicate that in spring all plants in the community used similar water pools, whereas in summer plants used different water sources, depending on their rooting depth, and irrespective of gypsum affinity.

### Contribution of different water sources to the xylem water of plants

Estimation of the most likely sources of water used by plant species by Bayesian models revealed that, in spring, all plants used a large proportion of free water from the shallow soil (estimated using 10–20 cm underneath the plants). However, in summer, crystallization water from gypsum was the main source for shallow-rooted species, whereas deep soil water (50–100 cm) was the main source for deep-rooted species (Fig. 5: Supplementary data Fig. S4). In spring, we also detected a moderate contribution of groundwater (16 % for deep-rooted and 13 % for shallow-rooted), particularly in the deep-rooted Ononis tridentata, Gypsophila hispanica and Genista scorpius, and the shallow-rooted Teucrium capitatum, Hernaria fruticosa and Fumana ericifolia (Supplementary data Fig. S4).

In summer, the main source of water for shallow-rooted plants was crystallization water (59 %), irrespective of species affinity for gypsum soils. In addition, 30 % of the water used by shallow-rooted plants was free soil water from deeper layers (50–100 cm; Fig. 5; Supplementary data Figs S4 and S5). Deep-rooted species in summer mainly used free water from the deeper soil layers (52 %), but crystallization water still accounted for 32 % of the water used by these plants (Fig. 5; Supplementary data Figs S4 and S5).

### Soil–plant interaction

In spring, soil underneath the plants (10–20 cm depth) showed significant species-specific variations in \( \delta^2 \text{H} \) and \( \delta^{18} \text{O} \) for both free and crystallization water, and in deuterium excess for crystallization water (Table 2). We also found significant differences among species in summer, for free water \( \delta^{18} \text{O} \) and deuterium excess (Table 2). In summer, we did not find significant effects of either rooting depth, gypsum affinity or their interaction on the isotope composition of soil free water collected beneath plants (Supplementary data Table S4). Free water isotopic composition of the shallow soil beneath some of the deep-rooted species (G. scorpius, G. hispanica, Rosmarinus officinalis and Thymelaea tinctoria) during summer was similar to their xylem water isotopic composition for \( \delta^2 \text{H} \), and closer to that of deep soil layers than in other species, providing indirect evidence of hydraulic lift by these
Fig. 4. Seasonal variation in the isotopic composition of xylem water, according to root depth (spring, left panels; summer, right panels). $\delta^2$H and $\delta^{18}$O isotope composition and deuterium excess are shown. Different letters indicate significant differences after Tukey’s post-hoc analyses across root depth and season ($P < 0.05$). $F$-ratios and $P$-values show differences in the xylem sap between plants with distinct root depth, in models run separately for each season. Black boxes indicate gypsophiles and grey boxes gypsovags.
species. However, the $\delta^{18}$O composition of the soil was consistently more negative than the xylem isotopic composition of plants (Supplementary data Fig. S6).

### DISCUSSION

Our results agree with previous studies that demonstrate the role for summer drought as a structuring factor in plant communities growing on gypsum drylands (Palacio et al., 2017). Hydrological niche segregation differentiates functional strategies between shallow-rooted species, dominant in these communities, and deep-rooted plants. This spatial segregation could have consequences on plant community assembly, promoting diverse plant communities whose variable response to soil moisture decrease enhances their stability under arid conditions (Peñuelas et al., 2011; Silvertown et al., 1999, 2015).

We identified gypsum crystallization water as a crucial component of the water balance in gypsum drylands. Water held in the crystalline structure of gypsum was the most important water source for almost all shallow-rooted species and a highly relevant water source for deep-rooted species during summer drought. Our results demonstrate that gypsum crystallization water is widely used by plants, irrespective of their affinity for gypsum soils. Contrary to our predictions, both gypsum-endemic and non-endemic species (gypsophiles and gypsovags) with shallow roots used gypsum crystallization water as the preferential water source during summer. The uptake mechanisms that make such use possible remain undescribed. The similar isotopic composition of gypsum crystallization water in both seasons agrees with the notion that continuous processes of gypsum dissolution–precipitation take place during the year, involving both precipitation and more evaporated free soil water (Fig. 2; Van Driessche et al., 2012). It is known that the temperature for pure gypsum dehydration can be decreased by some ionic solutions (Gázquez et al., 2017). Recent findings indicate that some micro-organisms can dissolve gypsum rock by secreting organic acids, retrieving crystallization water under extreme xeric conditions (Huang et al., 2020). We suggest that plant roots and their associated micro-organisms could similarly be altering gypsum to mine its crystalline water. This is supported by several previous studies providing evidence on the ability of plants and their associated micro-organisms to exude organic acids and other compounds that alter the substrate where they grow (Bashan et al., 2002; Puente et al., 2004; Chaparro et al., 2013; Lebre et al., 2017). However, detailed analyses on the specific compounds that plants could be secreting to the gypsum soil, and their potential effect on the thermodynamic equilibrium among gypsum phases, are lacking.

Other studies identified groundwater as the main water source enabling the maintenance of activity during drought for deep-rooted species (Salvucci and Entekhabi, 1995; Fan et al., 2017; Koirala et al., 2017; Palacio et al., 2017). In contrast, our results indicated water from 50–100 cm depth (i.e. 'rock
moisture’, Rempe and Dietrich, 2018) as the main water source in summer for deep-rooted species in the studied community. Although its dynamics and hydraulic properties have not yet been explored in detail (Dwivedi et al., 2020), this crucial source of water probably came from precipitation that passed through unsaturated weathered bedrock until reaching the groundwater (Oshun et al., 2016; Rempe and Dietrich, 2018). Despite the isotopic composition of groundwater and deep soil water being very similar in summer, for consistency between the spring and summer models, we kept the same water sources in the Bayesian models for both seasons. The model choice for the deep free water instead of groundwater could probably be due to its higher variability and higher probability area. Although we cannot untangle the use of these sources by plants during summer, groundwater did not outflow in the creek located under the study hill during summer (L. de la Puente, pers. obs.), being sited >10 m deep from the top of the hill. Consequently, considering the plants’ position at the top of a hill and their observed (relatively limited) root length, deep soil free water seems a more plausible source of water for these plants than groundwater. Plants may also show a preference for rock moisture over groundwater, as happens with large trees that take advantage of the oxygenated conditions of the weathered bedrock (Zwieniecki, and Newton, 1996; Graham et al., 2010; Liu et al., 2014; Hahn et al., 2020). Interestingly, our results show that not only deep-rooted species, but also some relatively shallow-rooted species (Teucrium capitatum, Linum suffruticosum and Lithodora fruticosa), were mainly using free water from the deeper soil during summer (Supplementary data Fig. S2). The maximum rooting depths of these species is between 50 and 100 cm depth (Guerrero-Campo, 1998), with actual rooting depth being sensitive to reach free water (Hodge, 2004; Fan et al., 2017). Nevertheless, we cannot rule out the possibility that these plants could also be using free water from slightly shallower layers, i.e. 30–40 cm deep, which had not included in the Bayesian models due to its variability and slight similarity with water from 20 cm depth. In any case, the use of free water by these species could be favoured through the segregation of water sources between coexisting shallow-rooted species to mitigate competition. Further approaches comprising experimental manipulation of resources or models to determine the processes that stabilize community composition best would be required to ascertain these possibilities (Stoll and Weiner, 2000; Silvertown et al., 2015).

Another explanation for the use of deep soil water from relatively shallow rooted plants might be the hydraulic lift by some deep-rooted species during summer. The species Genista scorpius, Gypsophila struthium subsp. hispanica, Rosmarinus officinalis and Thymelaeae tinctoria showed similar δ2H isotopic values between the shallow soil beneath them and their xylem composition (Supplementary data Fig. S4). This indicated water drawing up from the deeper soil, which could also be available to neighbouring shallow-rooted species. According to previous studies considering the composition of just one of the water stable isotopes (δ2H or δ18O) (Dawson, 1993; Ludwig, 2003; Durand et al., 2007) to prove this phenomenon, we could have indirect evidence of hydraulic lift in the dry season in our system. Nevertheless, further investigations including information on the water used by shallow-rooted plants located close to deep-rooted species potentially drawing up water are required to prove the influence of hydraulic lift by deep-rooted plants on neighbouring shrubs (Filella and Peñuelas, 2003).

We observed a significant effect of plant species on the isotopic composition of the free water from the soil beneath them in spring, when plants were using water available in the shallowest soil layers (10–20 cm). This suggests that the micro-environment created under plants is species specific and is able to modify soil water conditions. In summer, we observed an effect of the species on the δ18O composition and deuterium excess of free shallow water, but not for free water δ2H. This could be due to a pore scale isotope heterogeneity in the water soil caused by water surface interaction effects (Penna et al., 2018) or to the differences in the relative contribution of equilibrium and kinetic effects during evaporative enrichment for δ18O and δ2H, which cause different sensitivity to environmental variables (Craig and Gordon, 1965; Cappa et al., 2003). Recent meta-analysis on the environmental drivers of leaf water isotopic composition revealed that δ2H is more related to the isotopic composition of source water and atmospheric vapour, whereas δ18O seems to be more responsive to relative humidity of the air (Cuntz and Cernusak, 2020). Extrapolating these processes to the soil, it is reasonable to expect more homogeneous δ2H isotopic values in the soil during summer, whereas δ18O isotopic values would be more variable owing to the different soil microminiature during evaporative enrichment underneath each species.

Conclusions

To conclude, our results prove that during drought there is a partitioning of water sources among coexisting species, which segregated the species’ hydrological niche by root depth, but not by gypsum affinity. In this plant community living on the top of a gypsum hill, crystallization water of gypsum represents a vital source for most of the shallow-rooted species during summer, and allows them to survive the arid conditions, forming diverse communities. Rock moisture arises as the main water source for deep-rooted species during drought. However, our results show that all species in the community are able to use crystalline gypsum water during the summer drought period, indicating a hidden water pool important for life in gypsum drylands. Hence, we strongly recommend that gypsum crystallization water is included as a potential source in water balance studies dealing with ecosystems developed on gypsum soils, which span >200 Mha in all continents (Eswaran and Gong, 1991).

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: precipitation isotopic values in Zaragoza. Table S2: results of GLMM analysing the effects of the root depth, affinity for gypsum soils, season and their interaction on the isotopic composition of the xylem water of plants. Table S3: results of GLMMs analysing the effects of root depth, affinity for gypsum soils and their interaction on the isotopic composition of the xylem water of plants in spring and...
summer. Table S4: results of GLMMs analysing the effects of root depth and gypsum affinity on the isotopic composition of the soil free water underneath plants. Figure S1: δ2H and δ18O biplot for xylem values and sources including free and crystallization water between 30 and 40 cm deep. Figure S2: summer water content in the first 10 and 20 cm of the bare soil and soil underneath the plants. Figure S3: isotopic composition of the soil up to 50 cm and of the shallow rooted plants in summer. Figure S4: contribution of the four main water sources to the xylem water of each species in both seasons. Figure S5: contributions of seven different water pools included in Bayesian stable isotope mixing models to the xylem of deep- and shallow-rooted species in both seasons. Figure S6: summer isotopic values of free water underneath plants and their xylem water.

ACKNOWLEDGEMENTS

We thank A. Cera and G. Montserrat-Martí for help during fieldwork; M. Perez-Serrano for assistance with cryogenic distillations; J. Rodríguez-Arévalo and S. Castaño for providing isotope data for precipitation collected by the REVIP (CEDEX-AEMET); AEMET OpenData service (https://opendata.aemet.es/) for providing access to meteorological data; Centro Clínico Veterinario Jaca, for supplying liquid nitrogen necessary for distillations; and J. I. Queutejela for useful comments on earlier versions of this manuscript.

FUNDING

This work was supported by the Spanish Government [MICINN (Ministerio de Ciencia e Innovación), CGL2015-71360-P and PID2019-111159GB-C31] and by European Union’s Horizon 2020 [H2020-MSCA-RISE-777803 GYPWORLD]. L.P. was funded by a fellowship FSE (Fondo Social Europeo)-Aragón 2014-2020 of the Gobierno de Aragón, Spain; J.P.F. was supported by Reference Group H09_20R (Gobierno de Aragón, Spain); J.C.A. was supported by a Ramón y Cajal Fellowship [MICINN, RYC-2013-14164].

CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw RNA-Seq data generated in this study are available from the corresponding author upon request.

LITERATURE CITED

Araya YN, Silvertown J, Gowing DJ, McConway KJ, Peter Linder H, Midgley G. 2011. A fundamental, eco-hydrological basis for niche segregation in plant communities. New Phytologist 189: 253–258.

Barbeta A, Gimeno TE, Clavé I, et al. 2020. An explanation for the isotopic offset between soil and stem water in a temperate tree species. New Phytologist 227: 766–779.

Barnard RL, de Bello F, Gilgen AK, Buchmann N. 2006. The δ18 O of root crown water best reflects source δ18 O in different types of herbaceous species. Rapid Communications in Mass Spectrometry 20: 3799–3802.

Barnes CJ, Allison GB. 1988. Tracing of water movement in the unsaturated zone using stable isotopes of hydrogen and oxygen. Journal of Hydrology 100: 143–176.

Bashan Y, Li CY, Lebsky VK, Moreno M, De-Bashan LE. 2002. Primary colonization of volcanic rocks by plants in arid Baja California, Mexico. Plant Biology 4: 392–402.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1–48.

Bauerle TL, Richards JH, Smart DR, Eissenstat DM. 2008. Importance of internal hydraulic redistribution for prolonging the lifespan of roots in dry soil. Plant, Cell & Environment 31: 177–186.

Bayala J, Prieto I. 2020. Water acquisition, sharing and redistribution by roots: applications to agroforestry systems. Plant and Soil 453: 17–28.

Bolukbasi A, Kurt L, Palacio S. 2016. Unravelling the mechanisms for plant survival on gypsum soils: an analysis of the chemical composition of gypsum plants from Turkey. Plant Biology 18: 271–279.

Braun-Blanquet J, de Bolos 0. 1987. Las comunidades vegetales de la Depresión del Ebro y su dinamismo. Reeditado 1987. Delegación de Medio Ambiente, Ayuntamiento de Zaragoza. Ins Spanische übersetzte Neuauflage des 1957 erschienenen: Les groupement.

Brum M, Vadéboncoeur MA, Ivanov V, et al. 2019. Hydrological niche segregation defines forest structure and drought tolerance strategies in a seasonal Amazon forest. Journal of Ecology 107: 318–333.

Caldwell MM, Dawson TE, Richards JH. 1998. Hydraulic lift: consequences of water efflux from the roots of plants. Oecologia 113: 151–161.

Cappa CD, Hendricks MB, DePaolo DJ, Cohen RC. 2003. Isotopic fractionation of water during evaporation. Journal of Geophysical Research 108: 4525.

Casby-Horton S, Herrero J, Rolong SA. 2015. Gypsum soils – their morphology, classification, function, and landscapes. Advances in Agronomy 130: 231–290.

del Castillo J, Comas C, Voltas J, Ferro JP. 2016. Dynamics of competition over water in a mixed oak–pine Mediterranean forest: spatio-temporal and physiological components. Forest Ecology and Management 382: 214–224.

Cera A, Montserrat-Martí G, Ferro JP, Drenovsky R, Palcio S. 2021. Gypsum-exclusive plants accumulate more leaf S than non-exclusive species both in and off gypsum. Environmental and Experimental Botany 182: 104294.

 Chambers JM. 1992. Linear models. In: Chambers JM, Hastie TJ, eds. Statistical models in S. Pacific Grove, CA: Chapman & Hall.

 Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM. 2013. Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One 8: e55731.

 Concostrina-Zubiri L, Martínez I, Rabasa SG, Escudero A. 2014. The influence of environmental factors on biological soil crust: from a community perspective to a species level approach. Journal of Vegetation Science 25: 503–513.

 Craig H, Gordon IL. 1965. Deuterium and oxygen 18 variations in the ocean and marine atmosphere. In: Tongiorgi E, ed. Stable isotopes in oceanographic studies and paleotemperatures. Pisa, Italy: Laboratorio di Geologia Nucleare, 9–130.

 Cuntze M, Cermusak LA. 2020. The dominant environmental driver of leaf water stable isotope enrichment differs for H-2 compared to O-18. In: 22nd EGU General Assembly Conference Abstracts.

 Dansgaard W. 1964. Stable isotopes in precipitation. Tellus 16: 436–468.

 Dawson TE. 1993. Hydraulic lift and water use by plants: implications for water balance, performance and plant–plant interactions. Oecologia 95: 565–574.

 Dawson TE, Ehleringer JR. 1993. Isotopic enrichment of water in the ‘woody’ tissues of plants: implications for plant water source, water uptake, and other studies which use the stable isotopic composition of cellulose. Geochimica et Cosmochimica Acta 57: 3487–3492.

 Dawson TE, Ehleringer JR. 1998. Plants, isotopes and water use: a catchment-scale perspective. In: Kendall C, McDonnell JJ, eds. Isotope tracers in catchment hydrology. Amsterdam: Elsevier, 165–202.

 Dawson TE, Lambelli S, Plambeock AH, Templer PH, Tu KP. 2002. Stable isotopes in plant ecology. Annual Review of Ecology and Systematics 33: 507–559.
Ding Y, Nie Y, Chen H, Wang K, Querejeta JI. 2021. Water uptake depth is coordinated with leaf water potential, water-use efficiency and drought vulnerability in karst vegetation. New Phytologist 229: 1339–1353.

Dodd MB, Lauenroth WK, Welker JM. 1998. Differential water resource use by herbaceous and woody plant life-forms in a shortgrass steppe community. Oecologia 117: 504–512.

Donovan LA, Ehleringer JR. 1994. Water stress and use of summer precipitation in a Great Basin shrub community. Functional Ecology 8: 289–297.

Durand JL, Baria T, Ghesquiere M, et al. 2007. Ranking of the depth of water extraction by individual grass plants, using natural 18O isotope abundance. Environmental and Experimental Botany 60: 137–144.

Dwivedi R, Eastoe C, Knowles JF, et al. 2020. Vegetation source water identification using isotopic and hydrometric observations from a subhumid mountain catchment. Ecohydrology 13: e2167.

Ehleringer JR, Dawson TE. 1992. Water uptake by plants: perspectives from stable isotope composition. Plant, Cell & Environment 15: 1073–1082.

Ehleringer JR, Phillips SL, Schuster WS, Sandquist DR. 1989. Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In: Ehleringer JR, Hall AE, Farquhar GD, eds. Stable isotopes and plant carbon–water relations. New York: Academic Press Inc., 497–510.

Lin GH, Sternberg L, Ehleringer JR, Hall AE, Farquhar GD. 1993. Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In: Ehleringer JR, Hall AE, Farquhar GD, eds. Stable isotopes and plant carbon–water relations. New York: Academic Press Inc., 497–510.

Marshall JD, Cuntz M, Beyer M, Dubbert M, Kuehnhammer K. 2020. Borehole equilibration: testing a new method to monitor the isotopic composition of tree xylem water in situ. Frontiers in Plant Science 11: 358.

Marshall WL, Shusler R, Jones EV. 1964. Aqueous systems at high temperatures XIV. Solubility and thermodynamic relationships for CaSO 4 in NaCl–H 2 O solutions from 40° to 200°C. 0 to 4 Molal NaCl. Journal of Chemical & Engineering Data 9: 187–191.

Martin-Gómez P, Barbeta A, Voltas J, et al. 2015. Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources? New Phytologist 207: 914–927.

Matsumura Y, Serrano I, Ferriero GJ. 2017. Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecohydrology. Tree Physiology 37: 511–522.

Meisner M, Köhler M, Schwendenmann L, Hüscher D, Dyckmans J. 2014. Soil water uptake by trees using water stable isotopes (δ 2 H and δ 18 O) – a method testing for soil moisture, texture and carbonate. Plant and Soil 376: 327–335.

Merlo ME, Garrido-Becerra JA, Mata JF, et al. 2019. Threshold ionic content for defining the nutritional strategies of gymnosperms. Ecological Indicators 97: 247–259.

Meyer SE, García-Moya E. 1989. Plant community patterns and soil moisture regime in gymnosperm grasslands of north central Mexico. Journal of Arid Environments 16: 147–155.

Moore MJ, Mata JF, Douglas NA, Olvera HF, Ochozera H. 2014. The ecology, assembly, and evolution of gymnospermea communities. In: Rajakaruna N, Boyd RS, Harris TB, eds. Plant ecology and evolution in harsh environments. Hauppauge, NY: Nova Science Publishers, 97–128.

Moreno-Gutiérrez C, Dawson TE, Nicolás E, Querejeta JI. 2012. Isotopes reveal contrasting water use strategies among coexisting plant species in a Mediterranean ecosystem. New Phytologist 196: 489–496.

Noguchi K, Gel YR. 2010. Combination of Levene-type tests and a finite-intersection method for testing equality of variances against ordered alternatives. Journal of Nonparametric Statistics 22: 897–913.

Oerter EJ, Bowen GJ. 2019. Spatio-temporal heterogeneity in soil water stable isotopic compositions and its hydroecological implications in semiarid ecosystems. Hydrological Processes 33: 1724–1738.

Oshun J, Dietrich WE, Dawson TE, Fung I. 2016. Dynamic, structured heterogeneity of water isotopes inside hillslopes. Water Resources Research 52: 164–189.

Ossorio M, Van Driessche AES, Pérez P, García-Ruiz JM. 2014. The gypsum–anhydrite paradox revisited. Chemical Geology 386: 16–21.
de la Puente et al. — Water sources in a gypsum plant community

Palacio S, Escudero A, Montserrat-Martí G, Maestro M, Milla R, Albert MJ. 2007. Plants living on gypsum: beyond the specialist model. *Annals of Botany* **99**: 333–343.

Palacio S, Aitkenhead M, Escudero A, Montserrat-Martí G, Maestro M, Robertson AJ. 2014a. Gypsofile chemistry unveiled: Fourier transform infrared (FTIR) spectroscopy provides new insight plant adaptations to gypsum soils. *PLoS One* **9**: e107285.

Palacio S, Azorín J, Montserrat-Martí G, Ferrio JP. 2014b. The crystallization water of gypsum rocks is a relevant water source for plants. *Nature Communications* **5**: 4660.

Palacio S, Montserrat-Martí G, Ferrio JP. 2017. Water use segregation among plants with contrasting root depth and distribution along gypsum hills. *Journal of Vegetation Science* **28**: 1107–1117.

Penna D, Hopp L, Scandellari F, Palacio S, Montserrat-Martí G, Ferrio JP, Aitkenhead M, Escudero A, Montserrat-Martí G, Maestro M, Peñuelas J, Filella I, Terradas J. *Poch* RM, Verplancke H. Prieto I, Kikvidze Z, Pugnaire FI. Puente ME, Bashan Y, Li CY, Lebsky VK. Querejeta JI, Ren W, Prieto I. Russell VL. Royston P. R Core Team. Rempe DM, Dietrich WE.

: 1107–1117. 28 hills. *Journal of Vegetation Science* **28**: 4660.

tion water of gypsum rocks is a relevant water source for plants. *Nature* **4660**.

Robertson AJ. 2014 *Annals of Botany* **99**: 333–343.

terrestrial ecosystem water fluxes using hydrogen and oxygen stable iso-

*Biogeosciences* **15**: 6399–6415.

Peñuelas J, Filella I, Terradas J. 1999. Variability of plant nitrogen and water use in a 100-m transect of a subdesertic depression of the Ebro valley (Spain) characterized by leaf 813C and 815N. *Acta Oecologica* **20**: 119–123.

Peñuelas J, Terradas J, Lloret F. 2011. Solving the conundrum of plant species coexistence: water in space and time matters most. *New Phytologist* **189**: 5–8.

Poch RM, Verplancke H. 1997. Penetration resistance of gypsiferous hori-

zons. *European Journal of Soil Science* **48**: 535–543.

Prieto I, Kikvidze Z, Pugnaire FI. Puente ME, Bashan Y, Li CY, Lebsky VK. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colon-

ization and weathering of igneous rocks. *Plant and Soil* **6**: 333–343.

*Plant Biology* **6**: 629–642.

*Estudio sedimentológico y estratigráfico del Terciario con-

siderado en las cuencas fluviales del río Ebro, y del río Segre.* *Provincial, Zaragoza.*

Querejeta JI, Ren W, Prieto I. 2021. Vertical decoupling of soil nutrients and water under climate warming reduces plant cumulative nutrient uptake, water-use efficiency and productivity. *New Phytologist* **230**: 1378–1393.

R Core Team. 2020. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Rempe DM, Dietrich WE. 2018. Direct observations of rock moisture, a hidden component of the hydrologic cycle. *Proceedings of the National Academy of Sciences, USA* **115**: 2664–2669.

Royston P. 1995. Remark AS R94: a remark on algorithm AS 181: the W-test for normality. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* **44**: 547–551.

Russell VL. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software* **69**: 1–33.

Ryel RJ, Ivans CY, Peek MS, Leffler AJ. 2008. Functional differences in soil water pools: a new perspective on plant water use in water-limited ecosystems. *Progress in Botany* **69**: 397–723.

Ryel RJ, Leffler AJ, Ivans C, Peek MS, Caldwell MM. 2010. Functional differences in water-use patterns of contrasting life forms in Great Basin steppelands. *Vadose Zone Journal* **9**: 548–560.

Salvucci GD, Entekhabi D. 1995. Hillslope and climatic controls on hydro-

logic fluxes. *Water Resources Research* **31**: 1725–1739.

Sánchez-Martin R, Querejeta JI, Voltas J, et al. 2021. Plant’s gypsum af-

finity shapes responses to specific edaphic constraints without limiting re-

sponses to other general constraints. *Plant and Soil* **1–13**.

Schachtschneider K, February EC. 2010. The relationship between fog, floods, groundwater and tree growth along the lower Kuiseb River in the hyperarid Namib. *Journal of Arid Environments* **74**: 1632–1637.

Silvertown J, Dodd ME, Gowing DJ, Mountford JO. 1999. Hydrologically defined niches reveal a basis for species richness in plant communities. *Nature* **400**: 61–63.

Silvertown J, Araya Y, Gowing D. 2015. Hydrological niches in terrestrial plant communities: a review. *Journal of Ecology* **103**: 93–108.

Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX. 2018. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* **6**: e5096.

Stoll P, Weiner J. 2000. A neighbourhood view of interactions among indi-

vidual plants. In: Dieckmann U, Law R, Metz JAJ, eds. *The geometry of ecological interactions: simplifying spatial complexity*. Cambridge: Cambridge University Press, 11–27.

Van Driessche AES, Benning LG, Rodríguez-Blanco JD, Ossorio M, Bots P, García-Ruiz JM. 2012. The role and implications of bassanite as a stable precursor phase to gypsum precipitation. *Science* **336**: 69–72.

Van’t Hoff JH, Armstrong EF, Hinrichsen W, Weigert F, Just G. 1903. Gypsum and anhydrite. *Physics and Chemistry of the Atmosphere* **45**: 259–306.

Verheye WH, Boyadgiev TG. 1997. Evaluating the land use potential of gypsiferous soils from field pedogenic characteristics. *Soil Use and Management* **13**: 97–103.

Voltaes J, Lucabaugh D, Chambel MR, Ferrio JP. 2015. Intraspecific vari-

ation in the use of water sources by the circum-Mediterranean conifer *Pinus halepensis*. *New Phytologist* **208**: 1031–1041.

West AG, Patrickson SJ, Ehleringer JR. 2006. Water extraction times for plant and soil materials used in stable isotope analysis. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute. *Research in Mass Spectrometry* **20**: 1317–1321.

Wickham H. 2016. Programming with ggplot2. In: *ggplot2*. Cham: Springer, 241–253.

Zwieniecki MA, Newton M. 1996. Seasonal pattern of water depletion from soil-rock profiles in a Mediterranean climate in southwestern Oregon. *Canadian Journal of Forest Research* **26**: 1346–1352.


