



Spatial and seasonal patterns of water use in Mediterranean coastal dune vegetation

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Abstract

Aims This paper examines the water dynamics of a coastal dune plant community, addressing spatial and seasonal variations. We aimed to detect the patterns of water use by plants at the community level according to their distribution across a coastal dune gradient from beach to inland.

Methods Five sites were established: upper beach, embryo-dune, slack, foredune, and inland. Eight perennial species were collected seasonally to analyse the isotopic composition and water potential. Soil water samples at 3 depths, groundwater, and atmosphere, were obtained to determine plant water sources.

Results The species from Inland and foredune plant communities, *Retama*, *Juniperus*, and *Helichrysum*, showed the most stable isotopic signal throughout the year. On the contrary, the species most abundant on the upper beach, embryo-dune, and slack

(*Ammophila*, *Achillea*, and *Polygonum*) showed the highest variability. Water deficit decreased the dependence on shallow and mid-soil layers along the beach-inland gradient. Beach and embryo-dune sites showed less negative leaf water potential values than the other positions in the dune gradient.

Conclusions Three factors mark the proportion of water sources used for vegetation in coastal vegetation: community composition, distance to the sea, and seasonality. Coastal dune vegetation exhibited a species-specific response in water uptake that was modified by its location on the gradient. From upper beach to inland, the plant communities showed a slight progressive increase in the use of water from deeper layers. This pattern was similar and overlapped with the wet to dry seasonal pattern.

Keywords Coastal dunes · Leaf water potential · Soil water · Stable isotopes · Water-uptake strategies · Water sources · Water deficit

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Introduction

Coastal dunes are classic ecotones occurring on the boundary between land and sea, where the distribution of vegetation follows a strong environmental gradient of salt water spray and sand burial (Moreno-Casasola 1986; Hesp 1990; Wilson and Sykes 1999; Stallins 2001; Kim and Yu 2009). In Mediterranean coastal dune ecosystems, hot and dry summers

represent for this vegetation stress factors that add to those mentioned above; especially as seasonal water deprivation is aggravated by the low field water capacity of sandy soils, and by the likelihood of eventual ocean water intrusion (Sternberg and Swart 1987). In sandy soils, compared to other mechanisms (indirect recharge from runoff), aquifer recharge occurs mainly through direct infiltration of precipitation (Schmidt et al. 2011). Variations in precipitation cause alterations in the structure and function of plant communities (Greaver and Sternberg 2010).

Different studies have provided evidence of the differential use of water by terrestrial vegetation in coastal dunes (Greaver and Sternberg 2006, 2007, 2010; Antunes et al. 2018a, b, 2019); therefore, plants living in coastal environments may extract water from different available sources, rainwater, groundwater, fog, ocean water, or their mixtures. Few works have focused on vegetation water uptake on Mediterranean coastal dunes (Valentini et al. 1992; Alessio et al. 2004; Antunes et al. 2018a, b).

Spatial and seasonal variations in water uptake and differences between species have also been studied from different perspectives (Dawson and Pate 1996; Pivovarov et al. 2016; Ding et al. 2021). Nevertheless, there are few studies focused on water uptake dynamics across environmental gradients and the differences in the response among co-occurring species to the annual hydrological cycle.

The water-uptake strategy is a significant plant trait in dry or seasonally dry type environments that determines plant survival (Dawson and Pate 1996). Furthermore, different water uptake patterns between species are useful strategies to reduce competition for water in dry soils (Mooney et al. 1980; Verweij et al. 2011). Understanding the water uptake pattern of plant roots is important to improve our knowledge about the responses of plants to hydrological conditions and is particularly significant in water-limited habitats. Besides, in addition to seasonal fluctuations in soil moisture conditions (as happens in Mediterranean areas), fluctuations in water availability across short gradients must also be considered (Oliveira et al. 2005; West et al. 2012).

Previous studies have shown that a mere root distribution is not sufficient to identify plant root uptake strategies (Verweij et al. 2011, Tron et al. 2015; Nehemy et al. 2021). Stable isotopes used as tracers are an effective tool in the determination

of water uptake patterns by plants (Sternberg and Swart 1987; Dawson and Ehleringer 1993; Penna et al. 2018). The relative abundance of ^{18}O vs ^{16}O (expressed as $\delta^{18}\text{O}$) in xylem sap can be effectively used for differentiating the specific origins of the water taken up (Flanagan and Ehleringer 1991; Dawson et al. 2002; Barbeta et al. 2018; Amin et al. 2020) since depending on its source water usually exhibits specific isotopic signatures, $^{18}\text{O}/^{16}\text{O}$ ratios (Craig 1961; Dansgaard 1964).

Soil water evaporation causes enrichment in the heavy isotopes (^{18}O , ^2H) in the remaining soil water (Allison et al. 1983), which is more pronounced in the superficial layers of the soil than in the deep layers. Accordingly, plants with roots exploring deep layers of the soil will present a lower and depleted isotopic signal in the heavy isotope, indicating little evaporated water. On the contrary, plants with shallow roots that therefore explore the surface layers of the soil will be subjected to greater evaporation and will present a higher and enriched isotopic signal (Querejeta et al. 2007; Nie et al. 2011). However, this difference in isotopic signals as a function of depth of soil layers is dependent on environmental conditions such as soil water content, temperature, or soil porosity (Sprenger et al. 2016). After a few days of dryness and with high evaporative demand, these differences will be more pronounced, while under high humidity or abundant rainfall, this effect decreases and can even be reversed.

Another aspect to consider is the vulnerability of coastal dunes to the effects of climate change. An increase in mean sea level in association with climate variation could alter the spatial distribution of dune plant species (Mendoza-González et al. 2013). The erosion of coastal dune systems will increase as sea level rises (Feagin et al. 2005; Ranasinghe et al. 2012) and since vegetation is essential in the stabilization of dunes, it is crucial to better understand the response of dune vegetation to changes in water availability. Climate model simulations foresee that the Mediterranean region will increase in dryness and warming, especially in summer (reduction in precipitation overpassing -25–30% and warming overpassing 4–5 °C) (Giorgi and Lionello 2008). As a response to current climate change, many species have shifted their geographic ranges, seasonal activities, and abundances (IPCC 2014). Knowledge of plant water uptake strategies is useful to know how species will be affected by

changes in water availability and soil water resources under the predicted climate change.

Since an environmental gradient from the ocean to the inland exists and assuming seasonal differences along the year in the Mediterranean climate (mainly due to precipitation), we hypothesised a changing pattern in vegetation's water use both in space and time. Specifically, our starting hypotheses are: 1) the source of water used by dune vegetation varies seasonally, 2) there is a zonal distribution of the species across the beach-inland gradient according to water use strategy, 3) the water uptake strategy is species-specific but modulated by their distribution across the beach-inland gradient.

Taking all this into account, the main objective of this study was to assess whether the water uptake pattern of dune plants is species-specific or, in contrast, is modulated by the spatial gradient from the upper beach to the inland and seasonal water availability. We attempted to answer the following research questions: I) How do the main water sources of dune plants change over the seasons according to water availability? II) Is there a water-uptake pattern at the community level related to species distribution across the coastal dune gradient from the beach to the inland? III) Is the water uptake strategy species-specific and independent of the spatial distribution of plant dunes across the beach-inland gradient?

Materials and methods

Study site and species

Research work was carried out on the El Rompido spit (Lepe, Huelva, 37°12'N, 7°07'W), South-West Spain. El Rompido spit is a sandy bar that extends for some 12 km parallel to the coastline, at the Piedras river estuary. It is 300 to 700 m wide and comprises dune ridges separated by tidal swales and salt marshes. The soil is fine sand with <3% of fine particles (silt + clay). It is a very poor soil, with an organic matter content of 1 to 2.6 mg g⁻¹ established at depths of 5 to 10 cm. Soil pH is alkaline, 9.5 (due to the high carbonate content of 4–7 mg CaCO₃) and the conductivity is low (<100 μS cm⁻¹) (Muñoz-Vallés et al. 2015).

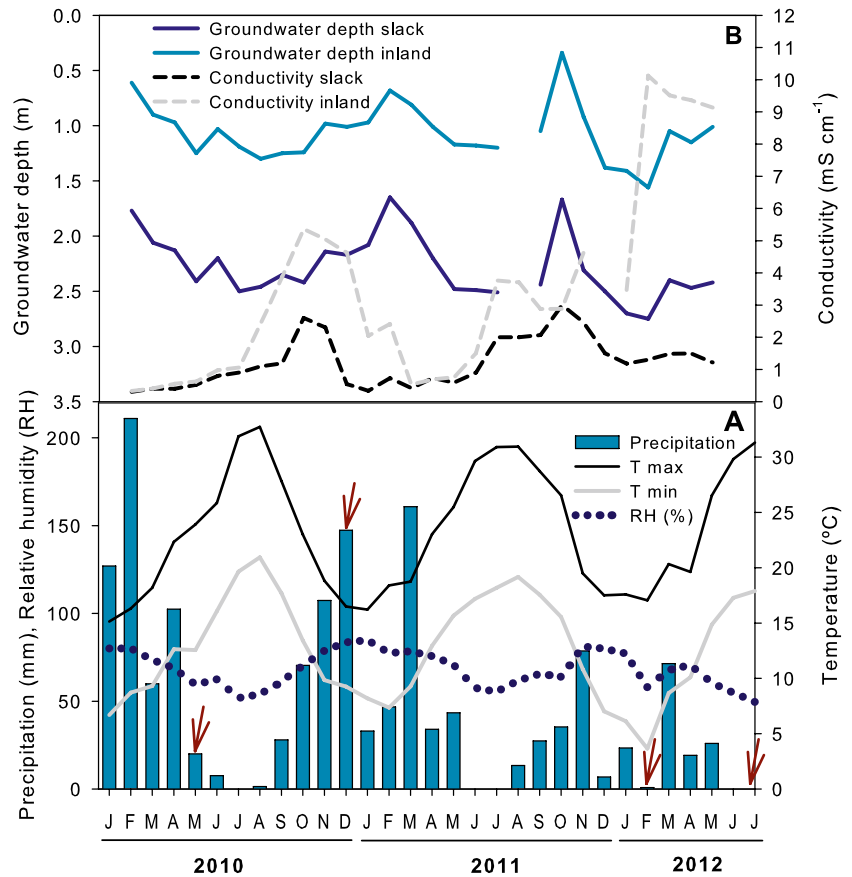
The vegetation of El Rompido dunes is well described by Muñoz-Vallés et al. (2015). In the

upper beach, the vegetation is sparse and composed of *Polygonum maritimum*, *Cakile maritima*, *Elymus farctus*, *Pancratium maritimum* and *Euphorbia paralias*, among others. On embryo-dunes *Ammophila arenaria*, *Achillea maritima* and *Euphorbia paralias* are present. On foredune, the vegetation is dominated by *A. arenaria* and other species such as *Eryngium maritimum*, *Artemisia campestris* subsp. *maritima* and *Crucianella maritima*. Finally, on the back of the dunes, inland, the plant community is dominated by the leguminous shrub *Retama monosperma* and other shrub species such as *A. campestris* subsp. *maritima*, *Helichrysum italicum* subsp. *picardii* and *Thymus carnosus*. This multi-aged and well-developed shrub community represents the late-successional stage of the coastal dune vegetation, where more woody species become established because of the more stable areas at the back of the dunes.

The climate in the study area is Mediterranean with Atlantic influence. The average annual temperature and rainfall are 18.1° C and 490 mm, respectively, including a long dry and warm period from May to September (30-year record, from 1971 to 2000; data from Huelva Meteorological Station, AEMET). We used monthly precipitation and mean temperature information from a meteorological station located 12 km away (Lepe, Huelva, Spain) (Fig. 1A). The precipitation pattern was wetter than usual during the periods 2009–2010 and 2010–2011, being 880.6 mm and 684.6 mm respectively. These values represent 80% and 40% over the 30-year average (490 mm). Nonetheless, the period 2011–2012 was exceptionally dry, with an annual precipitation of 289.4 mm, 41% below the average.

The study was conducted in May (spring) and December (autumn) 2010, selected as warm and cold dates respectively, of a wet year. These two sampling months would be representative of the most favourable periods of the year for Mediterranean vegetation and of a hydrologically optimal year. The sampling periods of February (winter) and July (summer) 2012, respectively the coldest and warmest months of the year, would represent the periods of the year with the greatest stress on vegetation in the Mediterranean climate, and in this case, accentuated by being a dry year. According to Ellsworth and Sternberg (2015), the natural inter and intraseasonal variations cannot be captured by single measurements, especially in seasonal climates. For that reason, we chose several

Fig. 1 Monthly precipitation and mean temperature (A) and conductivity and depth of groundwater (B) during the study period (from July 2010 to August 2012). Arrows indicate sample periods



measurements per species, dune site and season, in the four seasons distributed in two hydrological years.

Beach-inland gradient: dune profiles and vegetation pattern

According to the topography of the dune and proximity to the ocean, sampling plots were set at the following sites: upper beach, embryo-dune crest, slack, foredune crest and inland depression (hereafter beach, embryo-dune, slack, foredune and inland, respectively) (Fig. 2). The beach and inland sites were the closest and farthest points to the ocean, and marked the extremes of a hydrological gradient across the dunes. To determine the dune profile, we established three parallel transects starting at the mean high-tide point and ending at the inland depression (each transect around 80 m long, perpendicular to the dune line, and separated 100 m from each other). Topographic measurements were taken

every meter with an optical theodolite to determine the difference in height between points. Along the dune system gradient, 1 × 1 m plots were set every metre to determine plant species distribution. In every vegetation plot, the presence-absence of every species was registered (Table 1).

Based on their abundance on dune communities, we selected eight perennial species distributed across the gradient of the dune system from the upper beach to the inland (Table 1): *Polygonum maritimum* L. (Polygonaceae), *Euphorbia paralias* L. (Euphorbiaceae), *Achillea maritima* (L.) Hoffmanns & amp; Link (Asteraceae), *Ammophila arenaria* (L.) Link (Poaceae), *Artemisia campestris subsp. maritima* (DC.) Arcang. (Asteraceae), *Helichrysum italicum* (Roth) G. Don (Asteraceae), *Retama monosperma* (L.) Boiss (Fabaceae) and *Juniperus oxycedrus subsp. macrocarpa* (Sm.) Ball (Cupressaceae). Hereinafter, we will refer to the species by the genus.

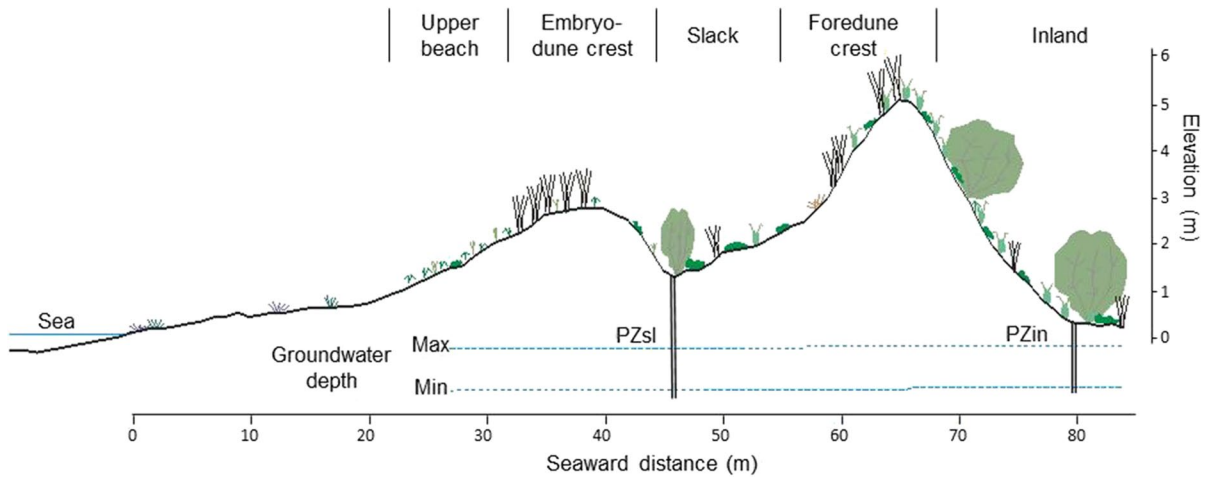


Fig. 2 Representative topographic profile across one of the three sampling transects (middle transect), indicating groundwater depth (dashed lines), piezometer locations (PZsl: Slack

and PZin: Inland), and oceanic front position. The dashed line indicates the maximum and minimum depths of the water table

Groundwater level and salinity

Groundwater (GW) depth and salinity, through its electrical conductivity (EC, $mS\ cm^{-1}$), were measured monthly using a water level indicator (KLL mini, Seba Hydrometrie, Kaufbeuren, Germany) and a conductivity meter (HI 9835, Hanna Instruments, Woonsocket, USA). To reach the GW, we installed two piezometers (polyvinyl chloride tubes with an outside diameter of 90 mm), one in the slack (PZsl) and another one in the inland site (PZin). The buried end of the tubes were covered with a permeable polyethylene fabric piece to avoid sand filling. The groundwater level was taken with the ground surface as a reference so that PZsl always appeared deeper than

PZin due to topography (see Fig. 2). The water level in the piezometer is assumed to be the same as that of the phreatic level.

Water source sampling

Plant water sources in adult plants were determined through the oxygen isotopic composition of xylem water ($\delta^{18}O$) and the possible water sources. ($n=6-9$ plants per species and site in spring, summer, and autumn, $n=5-6$ plants in winter). Bayesian mixing models were used to compare xylem $\delta^{18}O$. Comparing these values with those obtained from GW and soil at different depths, we could determine the origin of water used by plants for every species, site, and

Table 1 Study plant species frequency (%) per zone across the three transects along the dune system gradient

	DUNE SECTIONS	Upper beach	Embryo dune	Slack	Fore dune	Inland
	Zone length range(m)	7–15	10–14	12–17	13	16–29
LF	Total number of plots	22	24	29	26	45
PH	<i>Polygonum maritimum</i>	18.2				
PH	<i>Euphorbia paralias</i>	18.2	12.5		3.9	
PH	<i>Achillea maritima</i>	9.1	29.5	6.9		
TG	<i>Ammophila arenaria</i>	4.5	37.5	17.2	42.3	2.2
S	<i>Artemisia campestris</i>		16.5	62.1	42.3	37.8
S	<i>Helichrysum italicum</i>			13.8	57.7	82.2
LS	<i>Retama monosperma</i>			5.4	7.7	24.4
LS	<i>Juniperus oxycedrus</i>			0.9	0.4	1.0

Life forms (LF) are denoted as PH: Perennial herb, TG: Tussock grass, LS: Large shrub, and S: Shrub. G-test of goodness-of-fit showed that abundance among the five positions differed significantly for every species ($P < 0.05$)

season utilizing MixSIAR. Soil, GW, and rainwater are possible water sources for plants available in the soil profile that vertically mix.

Groundwater samples were extracted seasonally from the two piezometers mentioned above using a pump. The water samples were kept refrigerated in double cap polyethene bottles sealed with parafilm until analysed to prevent evaporation and isotopic fractionation.

Soil samples were collected seasonally at three different depths (topsoil: 10, mid soil: 25, and deep soil: 50 cm) in each site and in the three transects (three replicates per depth). The samples were stored in screw-cap glass vials, following the same procedure as plant samples. Soil samples were also collected in polyethene bags to measure soil water content in each site at the three depths. The samples were cleaned of plant materials and weighted before and after oven-dried for 48 h at 100°C to calculate the gravimetric water content (% g g⁻¹). We sampled the first 50 cm because, in the case of the studied sandy soils, most root biomass is concentrated in the upper layers (75% of the root biomass is located in the upper 37.5 cm, Martínez et al. 1998). We also took into account that evaporation fractionation is generally limited to the upper 0.3 m of the soil (Sprenger et al. 2016) and that according to Amin et al. (2020), water uptake mainly occurs at a superficial layer of 30–50 cm depth in this type of climate.

In this study, rainwater has not been considered a potential water source. Rainwater always mixes with soil water stored during previous rain events before being taken up by roots and is often segregated in space and time even before being mixed in the soil or for groundwater recharge (Evaristo et al. 2015). Although under certain conditions, water can be absorbed through leaves or even bark during rain events, these takings are very low compared to transpiration in arid ecosystems (Cavallaro et al. 2020). However, rainwater was collected from two pluviometers installed on purpose on the sampling site to know its values for soil water. To prevent evaporation, a 5-mm layer of liquid paraffin was added to the pluviometer collector.

Atmospheric water can be another important source of moisture as some of these species have leaf morphological structures, which facilitate dew uptake. Seasonally, we collected atmospheric water

(as either vapour or small water droplets) at dawn by pulling air through a dry-ice-cooled glass condenser (following Helliker et al. 2002).

Plant material sampling

We collected xylem samples seasonally in the morning ($n=6-9$ samples per species and site in spring and autumn 2010 and summer 2012, $n=5-6$ samples in winter 2012). Samples from small-size species could include more than one individual. For the isotopic analysis of xylem water, leafless, lignified and mature stem fragments (rhizomes in the case of *Ammophila*) were cut and directly preserved in screw cap glass vials, sealed with parafilm, and kept refrigerated during transport to the laboratory, where they were frozen until extraction of xylem water.

Water extraction and isotopic analysis

The water from the soil and plant samples was extracted employing a custom-made cryogenic vacuum distillation system at the Stable Isotopes and Instrumental Analysis Facility (SIIAF), Centro de Ecología, Evolução e Alterações Ambientais (CE3C), Universidade de Lisboa (Lisbon, Portugal). The guidelines of Ehleringer and Osmond (1989), Ehleringer and Dawson (1992) and West et al. (2006) were followed. In summer, we could not obtain enough water from several soil samples from 10 and 25 cm deep due to the excessive dryness of the soil. Consequently, the mean of the soil samples of the five sites was used for the MixSIAR analyses and the figures in summer.

According to Ellsworth and Williams (2007), during plant water uptake, $\delta^2\text{H}$ may fractionate in xylem water samples in species adapted to saline or xeric environments. Hence, we used only $\delta^{18}\text{O}$ to detect water sources in plants.

The abundance of the heavy isotope was expressed in delta notation (δ) in parts per thousand (‰) as:

$$\delta^{18}\text{O}_{\text{sample}}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

where R_{sample} and R_{standard} are the molar ratios of heavy to light isotopes of the sample and the international standard (Vienna Standard Mean Ocean

Water, VSMOW). Oxygen stable isotope ratio analyses were performed at SIAF by headspace equilibration on an Isoprime (Micromass, UK) SIRMS, coupled in continuous flow mode to a Multiflow (Micromass, UK) auto-sampler and sample equilibration system. The materials used as reference were Medium Natural Water (Elemental Microanalysis Ltd, UK; $\delta^{18}\text{O}_{\text{V-SMOW}} = -10.18 \pm 0.2\text{‰}$) and Zero Natural Water (Elemental Microanalysis Ltd, UK; $\delta^{18}\text{O}_{\text{V-SMOW}} = 0.56 \pm 0.23\text{‰}$), regularly checked against IAEA-VSMOW and IAEA-GISP (Coleman and Meier-Augenstein 2014). The analytical precision was $< 0.1\text{‰}$.

Leaf water potential

In order to evaluate the water status of vegetation, leaf water potential was monitored in the study species ($n=9$ measures per species and site in spring 2010, autumn 2010 and summer 2012, $n=5-6$ measures in winter 2012). Pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential values were measured in the field on freshly excised terminal shoots through a pressure chamber (Scholander et al. 1965; modified by Manofrigido, Portugal). All samples were measured immediately after cutting. Samples for Ψ_{pd} were collected and measured from 5:30 a.m. to 7:30 a.m. while samples for Ψ_{md} were collected within an hour around noon.

Xylem water potential is an important indicator of the plant water status and reflects a balance between root water uptake and weather conditions (Bhaskara and Ackerly 2006). We measured the leaf water potential of vegetation to assess the relationship between water-source use and plant water status. Thus, we hoped to know how the rooting strategy influences the seasonal plant water status by integrating data of vegetation distribution, hydrology, and ecophysiology.

Midday water potential stands for the maximum water deficit that xylem and leaves may undergo (Pockman and Sperry 2000; Ackerly 2004), whereas predawn water potential shows the recovery capacity of every species during the night.

Statistical analyses

Data of perennial species in every site were analysed with a row-by-column contingency test (G-test of

goodness-of-fit) to detect eventual statistical differences in species frequency among sites (following the method by Causton 1988).

Two-way and one-way nested MANOVAs were carried out to compare the differences in leaf water potential (Ψ_{pd} and Ψ_{md}), xylem oxygen isotopic composition ($\delta^{18}\text{O}$) and relative contributions of soil water sources to vegetation uptake (top%, mid%, deep%) across the beach-inland gradient in each season. To determine how these variables differed across the beach-inland gradient, season and position were considered as a fixed factor and species as a random factor nested within the position where the plants were collected. Pairwise differences were tested using posthoc Tukey tests. Spearman's correlations between oxygen isotopic composition, water potential variables (predawn and midday water potential) and percentage of contribution to sources were performed to examine the influence of plant water sources on plant water status. All statistical analyses were conducted using SPSS 26 software package (Chicago, IL, USA). To achieve normality, the variables were transformed by $\ln(\Psi_{\text{pd}}$ and $\Psi_{\text{md}})$ or square root ($10 + \delta^{18}\text{O}$, top%, mid%, deep%).

The most likely contribution of water sources to coastal dune vegetation uptake was estimated using the Bayesian mixing model MixSIAR (Stock et al. 2018) which have been recommend for determining plant water sources (Wang et al. 2019). MixSIAR is a model framework in R (<https://github.com/brianstock/MixSIAR>) that allows creating and running Bayesian mixing models to analyse the uncertainties in biotracer data (in this study, the tracers were based on the $\delta^{18}\text{O}$ values on the xylem). The model used $\delta^{18}\text{O}$ values of the xylem water of individual ('mixture or consumers', raw data of the eight dune species separately), the water sources described in the methods (mean values), and the discrimination factor (which for water uptake is set as 0). MixSIAR incorporates source and discrimination (fractionation) uncertainty to assign the posterior probability distributions of source contributions to a mixture. We followed an a priori aggregation approach (Phillips et al. 2005) so that the combined sources were similar, but also that they had some biological meaning. Accordingly, we combined deep soil and GW sources (hereafter-deep soil) to reduce the number of sources and obtain less diffuse solutions (Phillips et al. 2005). The low isotopic values recorded for atmospheric

water, $-12.2‰ \pm 0.23$, compared to the xylem water values indicated that this water source, apparently, did not have an effect on the isotopic composition of the plants, so it was discarded from the statistical analysis. In summary, we analysed separately the four study seasons and narrowed down water sources to three (topsoil, mid soil and deep soil + GW). We set the Markov Chain Monte Carlo (MCMC) to 5000 000 burn-in sizes. We used Gelman–Rubin and Geweke diagnostics to assess the convergence of the model. Gelman confidence intervals close to 1 and <1.05 indicate model convergence, while Geweke diagnostics is a standard Z-scores based on the equality on two parts of the Markov chains. At convergence, the means of the chains should be the same, $\leq 5\%$ of variables in each chain outside of ± 1.96 (Stock and Semmens 2016). In our study, the convergence was satisfied with a number of iterations 'very long' model (1 000 000 chain length) in the four seasons.

Results

Dune topographic profiles and plant species distribution

The maximum heights of the dune topography of the transects were respectively, 5, 4.5 and 3.8 mamsl (Fig. 2), the highest point being the foredune crest of the middle transect. As shown in Table 1, the frequency of species was statistically different between sites. The main contrast among areas was the higher vegetation cover on the inland site.

The lowest values of soil water content were recorded in July, ranging from 0.1% in the topsoil to 1.7% in the deep soil, while the highest values were recorded in winter, ranging from 2.1% in the topsoil to 6.9% in the deep soil.

Groundwater

The groundwater level usually followed the precipitation pattern (Fig. 1A), where rainy periods implied higher water levels (1.7 m and 0.4 m, respectively, in slack and inland) and dry periods, lower levels (2.7 m and 1.7 m respectively in slack and inland). EC followed an inverse pattern concerning GW levels, with increasing values when water table and precipitation diminished and vice versa (Fig. 1B). EC was usually

lower in PZsl than in PZin. During the dry period 2011–2012, EC in PZin increased more than twice compared to previous years, reaching 10.1 mS cm^{-1} in winter. On the contrary, EC in PZsl followed the common pattern of the previous year, reaching the highest level (2.5 mS cm^{-1}) at the end of summer.

Species-level: plants and water sources $\delta^{18}\text{O}$

The $\delta^{18}\text{O}$ value of ocean water remained between $1.1‰$ in spring and summer to $0‰$ in winter (Fig. 3). The $\delta^{18}\text{O}$ value of rainwater was more enriched during the warm season (spring = $-3.4‰$) than during the cold seasons (autumn = $-5.4‰$, winter = $-5.3‰$), which is consistent with precipitation records expected in the cold season, in winter (Máguas et al. 2011). No rain was collected in summer. The isotopic composition of GW showed small differences throughout the study period, although they presented neither a temporal (spring = $-5.1‰$, autumn = $-4.9‰$, winter = $-3.9‰$, summer = $-3.7‰$) nor a spatial pattern (PZsl = $-4.5‰$, PZin = $-4.3‰$, Fig. 3). The isotopic composition of the atmospheric water at dawn ranged from -11.6 in summer to -12.7 in winter.

The different $\delta^{18}\text{O}$ mean values of xylem water indicated a clear effect of seasonality on the isotopic signal of the water sources used by dune vegetation. Summer was the season having the most variable xylem $\delta^{18}\text{O}$ signature, ranging from $+11.5‰$ in *Ammophila* plants growing in the embryo-dune to depleted values of $-3.8‰$ in *Retama* and *Ammophila* plants from the foredune and inland (Fig. 3C). On the contrary, in autumn, $\delta^{18}\text{O}$ was similar throughout the soil profile and with low variability among sites or species, ranging from $-2.3‰$ in *Ammophila* plants from the beach to $-5.98‰$ in *Retama* plants from the foredune and inland (Fig. 3B).

The species with the most stable isotopic signal throughout the year, *Retama*, *Juniperus* and *Heli-chrysum*, were more abundant in inland and fore-dune, while the species with the highest variability, *Ammophila*, *Achillea* and *Polygonum*, were more abundant in the upper beach, embryo-dune and slack (Table 1).

Two-way nested ANOVA showed significant differences in $\delta^{18}\text{O}$ among seasons ($P < 0.001$ in both cases). Fore-dune and inland sites were different from the rest of the positions on the gradient, while the

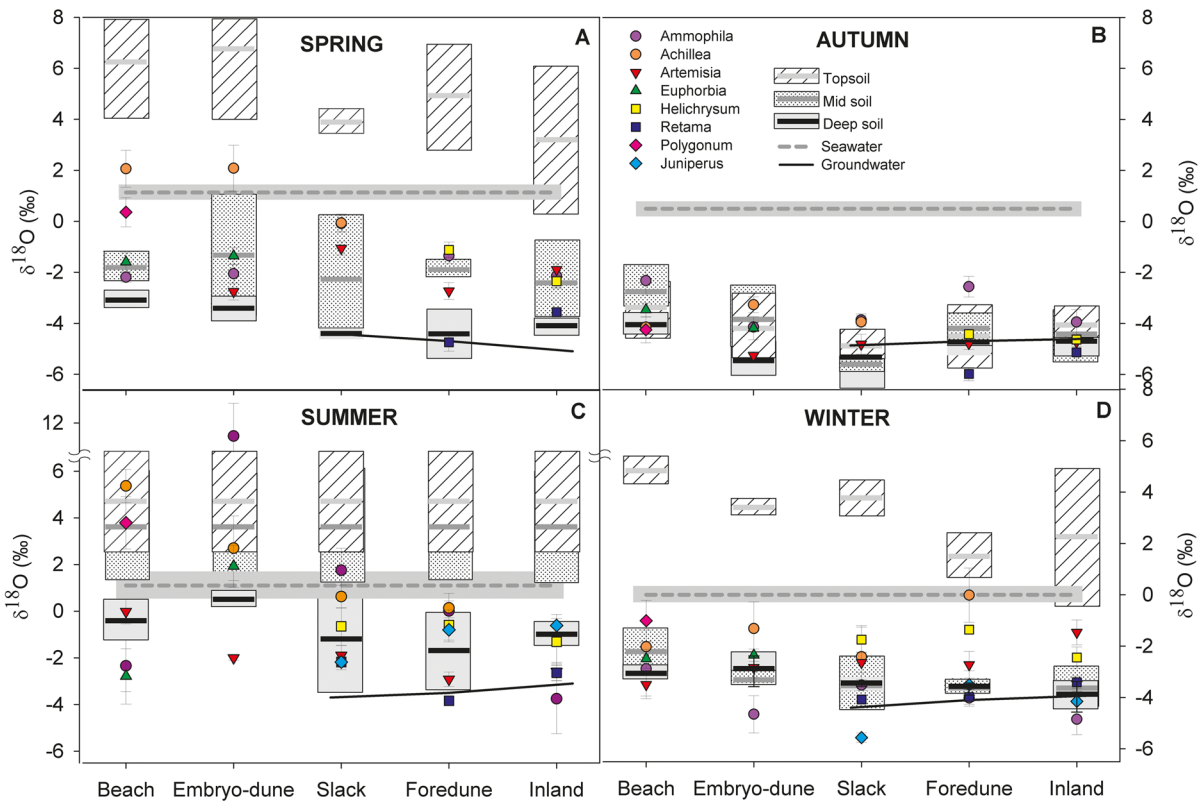


Fig. 3 Seasonal $\delta^{18}\text{O}$ of xylem water and potential water sources across the dune system gradient including, soil water at three depths, groundwater, and ocean water. Spring: **A**; Autumn: **B**; Winter: **C**; Summer: **D**. Significant differences are shown in Table 2. The grey boxes indicate the standard

deviation of the different water sources. Abbrev: Poly: *Polygonum maritimum*; Eup: *Euphorbia paralias*; Ach: *Achillea maritima*; Amm: *Ammophila arenaria*; Art: *Artemisia campestris*; Heli: *Helichrysum italicum*; Ret: *Retama monosperma*; Jun: *Juniperus oxycedrus*

four seasons were significantly different from each other (Table 2).

MixSIAR results at community level

Topsoil water did not represent a relevant water source for the vegetation in any season of the year, nor in any gradient position, except for slack and fore-dune plants in autumn and winter, for which it could account for up to 20% of the uptake (Fig. 4). Deep soil water appears to contribute to water uptake in plant communities across the entire beach-inland gradient in a relevant way (Beach 47%, Embryo-dune 47%, slack 62%, fore-dune 65% and inland 74%), although significant differences were found among sites ($P=0.001$, Fig. 4). Water uptake from deeper layers increased with water deficit and following the beach-inland gradient. At

the community level, deep soil water use increased seasonally from the wet to the dry seasons by 15% (mean of the five sites), while the mean increase of deep water from the beach to the inland accounted for 27%.

Slack and embryo-dunes were the sites with the highest differences between seasons (Fig. 4). In contrast, the lowest seasonal differences in water sources was recorded in the beach inland sites (Fig. 4). In all the analyses, the confidence intervals of Gelman diagnostics were lower than the estimated factor of 1.01 ($0 > 1.01$). Similarly in Geweke diagnostics $< 5\%$ of the variables were ± 1.96 , except in May (where the second chain had 9.2% of the variables ± 1.96) and November (where the second chain was 10%). In our mixing models, the largest uncertainty was obtained in autumn (Fig. 4) (with a variability of 11% in topsoil, 22% in mid soil, and 22% for deep soil) and the

Table 2 Two-way nested MANOVA for the effects of site (species nested to site) and season on xylem sap ($\delta^{18}\text{O}$), leaf water potential (Ψ_{pd} and Ψ_{md}), and contributions (%) of soil water to vegetation uptake across the dune system

Variables	Season			Site			Season*Site			Species(Site)			
	df	F	P	df	F	P	df	F	P	df	F	P	
MANOVA	12	25.7	0.001	36	5.8	0.001	9	107.2	0.001	63	15.3	0.001	
$\delta^{18}\text{O}$	3	118.0	0.001	4	12.10	0.001	12	5.2	0.40	21	9.1	0.001	
LWPdp	3	2137	0.001	4	43.9	0.001	12	18.4	0.001	21	18.4	0.001	
LWPmd	3	239.4	0.001	4	49.5	0.001	12	2.5	0.003	21	26.5	0.001	
Topsoil %	3	26.1	0.001	4	3.48	0.014	12	2.5	0.008	21	1.4	0.173	
Mid soil %	3	10.3	0.001	4	4.89	0.002	12	0.75	0.689	21	0.9	0.530	
Deep soil %	3	10.3	0.001	4	4.568	0.003	12	1.24	0.272	21	1.3	0.232	
Tukey results by site							Tukey results by season						
Site	$\delta^{18}\text{O}$	Ψ_{pd}	Ψ_{md}	Top	Mid	Deep	Season	$\delta^{18}\text{O}$	Ψ_{pd}	Ψ_{md}	Top	Mid	Deep
Beach	A	A	A	A	A	A	Autumn	A	A	A	B	C	A
Embryo-dune	A	A	A	A	A	A	Winter	B	B	B	A	AB	B
Slack	A	B	B	A	AB	AB	Spring	C	C	C	A	A	B
Foredune	B	B	B	A	B	AB	Summer	D	D	D	A	BC	B
Inland	B	B	C	A	B	B							

lowest in summer (mean variability between 5 and 10%).

MixSIAR results at species level

The MixSIAR results by species (Figs. 5 and 6), allowed us to identify the water uptake strategies of

the study species. The contribution of topsoil water to plant water uptake was significant only in the wet seasons for some species, *Achillea* (16.5%), *Helichrysum* (15.4%), *Euphorbia* (10.9%), *Polygonum* (10.9%), *Artemisia* (10.2) and *Ammophila* (6.4%). Mid soil is a relevant source of water throughout the year for *Polygonum* (53%), *Achillea* (52%), *Ammophila* (43%)

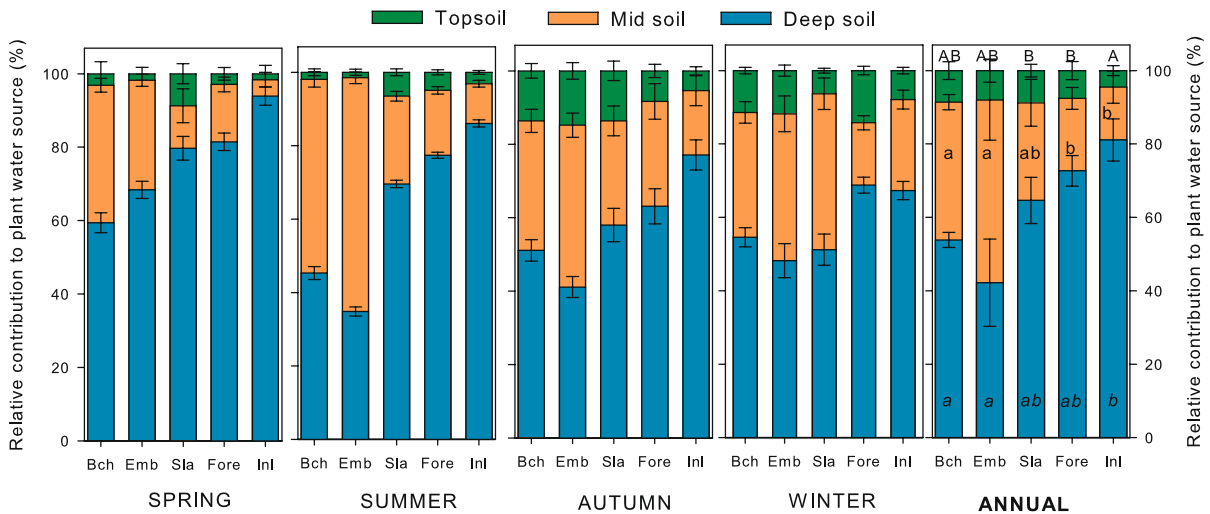


Fig. 4 Annual and seasonal relative contributions in the percentage of water sources used by vegetation across the dune system gradient from MixSIAR Bayesian mixing models (upper beach: Bch; embryo-dune: Emb; slack: Sla; fore-

dune: Fore; inland: Inl). Letters denote significant differences between sites and error bars represent standard errors. Confidence intervals of Gelman diagnostics should be <1.05 and were lower than 1.01 for all the variables (0 > 1.01)

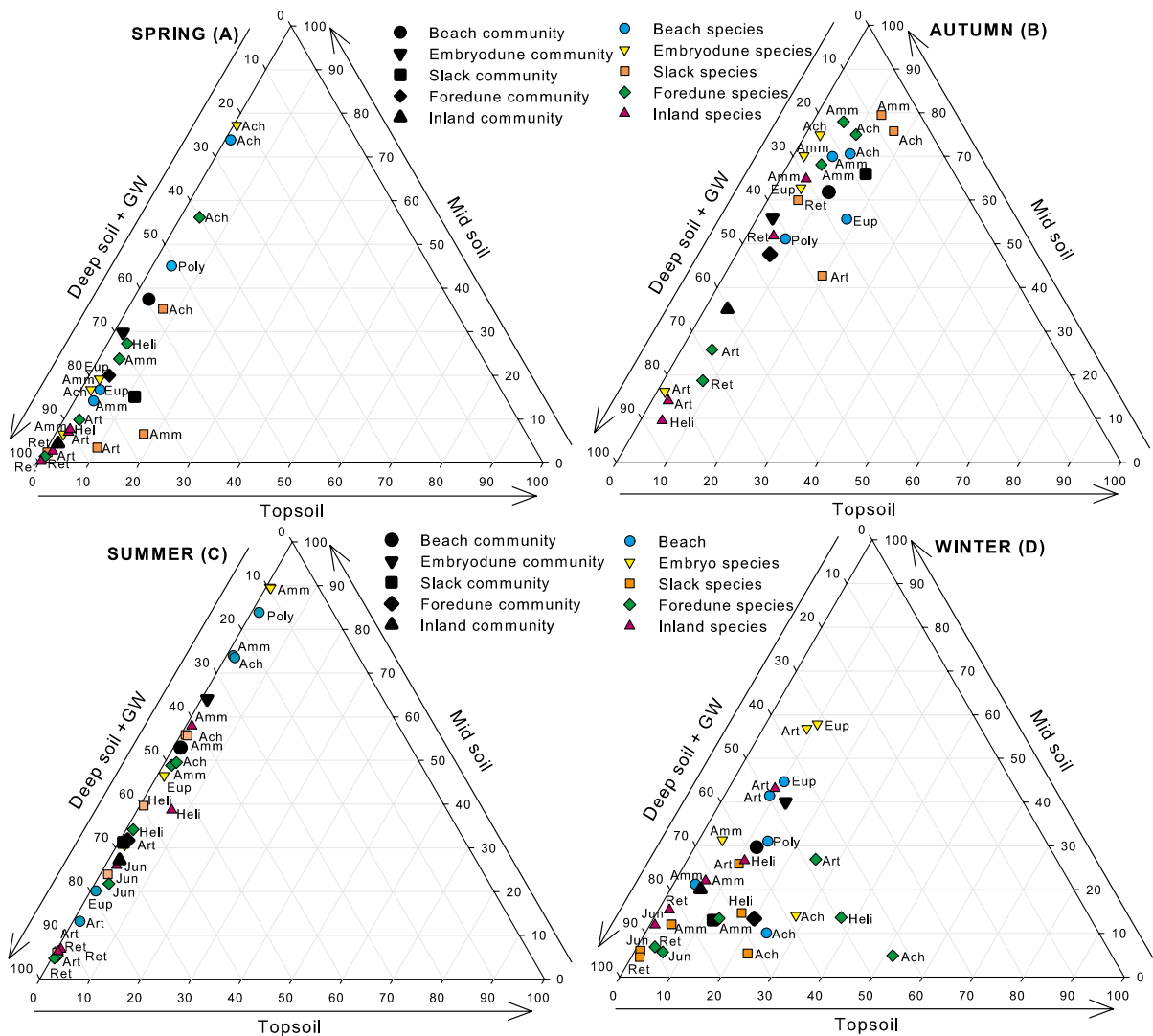


Fig. 5 Seasonal contributions in percentage of water sources used by study species and by sites across the dune system gradient from MixSIAR Bayesian mixing models. Confidence intervals of Gelman diagnostics should be <1.05 and were lower than 1.01 for all the variables ($0 > 1.01$). Abbrev: Poly:

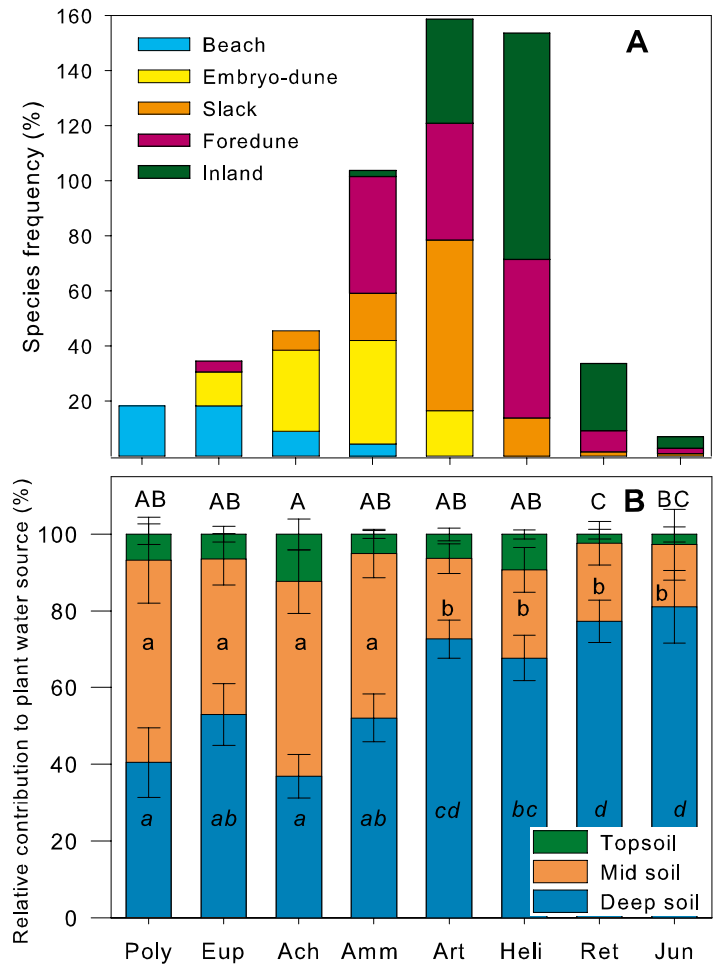
and *Euphorbia* (40%), all of them, species characteristic of the beach and embryo-dune sites. *Retama* and *Juniperus* were the species more dependent on deep soil layers (89% and 81% respectively) and both more abundant in the inner positions of the gradient. Additionally, the beach species *Polygonum* and *Achillea* displayed the greatest annual fluctuation, indicating a high level of plasticity in water uptake (Fig. 5). In

Polygonum maritimum; Eup: *Euphorbia paralias*; Ach: *Achillea maritima*; Amm: *Ammophila arenaria*; Art: *Artemisia campestris*; Heli: *Helichrysum italicum*; Ret: *Retama monosperma*; Jun: *Juniperus oxycedrus*

contrast, the foredune and inland species, *Juniperus* and *Helichrysum*, presented the lowest variability.

All species (except *Polygonum*, which only grew on the beach) slightly increased the use of water from mid and deep layers of the soil from the upper beach to inland (Fig. 7). In this regard, a significant correlation was detected between the proportion of deep soil water absorbed by plants and their position in the gradient ($R^2=0.376$, $P<0.001$).

Fig. 6 **A** Study plant species frequency (%) per zone across the dune system gradient. **B** Annual contributions of water sources (%) used by the study species from MixSIAR Bayesian mixing models (results obtained from data of water oxygen isotopes of plants, soil layers at three depths, and groundwater). Letters denote significant differences between species in the annual contributions to water uptake at that soil depth and error bars represent standard errors. Confidence intervals of Gelman diagnostics should be < 1.05 and were lower than 1.01 for all the variables ($0 > 1.01$). Abbrev: Poly: *Polygonum maritimum*; Eup: *Euphorbia paralias*; Ach: *Achillea maritima*; Amm: *Ammophila arenaria*; Art: *Artemisia campestris*; Heli: *Helichrysum italicum*; Ret: *Retama monosperma*; Jun: *Juniperus oxycedrus*



Water potential

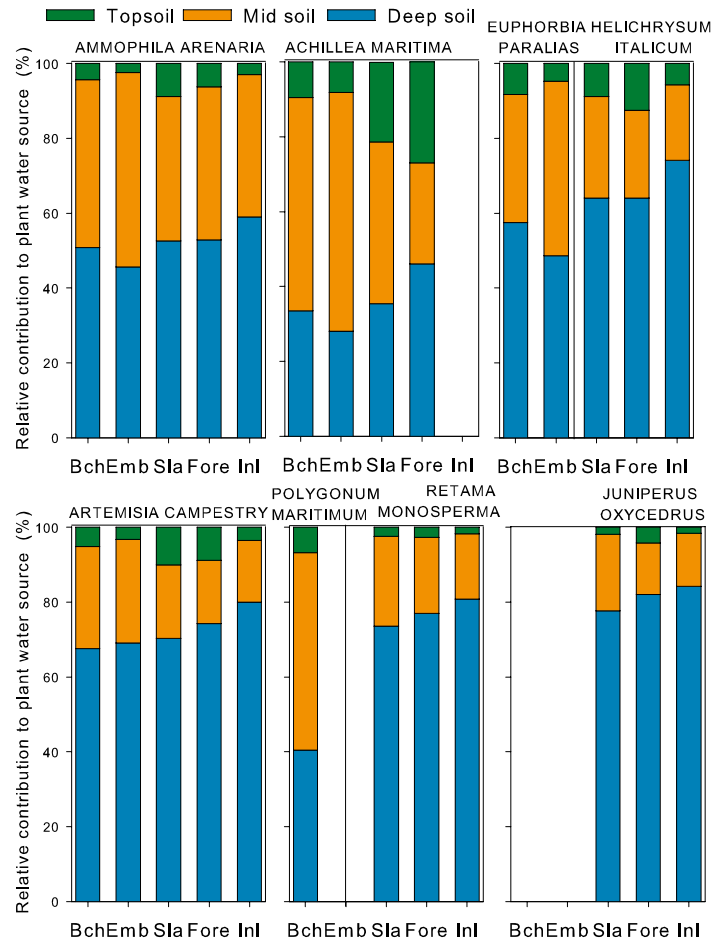
Two-way nested ANOVA showed that differences in Ψ_{pd} and Ψ_{md} among seasons and sites were significant (Table 2, $P < 0.001$ in both cases). Beach and embryo-dune sites, with the less negative values, were different from the rest of the positions in the dune gradient. Regarding annual differences, the four seasons were significantly different from each other. The highest leaf water potential, both at predawn and midday were recorded in autumn ($\Psi_{pd} = -0.09$ MPa, $\Psi_{md} = -0.93$ MPa) and the lowest in summer ($\Psi_{pd} = -1.25$ MPa, $\Psi_{md} = -1.75$ MPa) (Fig. 8).

Concerning species, the lowest Ψ_{md} values and the broadest annual fluctuations were reached in *Juniperus* (-5.8 MPa and 5.37 MPa), *Retama* (-2.6 MPa and 2.47 MPa), and *Ammophila* (-2.1 MPa and 1.94 MPa). In contrast, *Polygonum* and *Euphorbia* were the

species with the least negative water potentials (-1.3 MPa and -1.4 MPa respectively) and the smallest annual fluctuation (1.25 MPa). *Juniperus* was also the species with the lowest Ψ_{pd} values (-0.44 MPa, Fig. 8).

Focusing on all sites and seasons together, the correlation between $\delta^{18}\text{O}$ and water potentials, both mid-day and predawn, was negative so that water potential became more negative when $\delta^{18}\text{O}$ was more enriched ($R^2 = 0.496$ for $\delta^{18}\text{O}-\Psi_{pd}$ and $R^2 = 0.608$ for $\delta^{18}\text{O}-\Psi_{md}$, $P < 0.004$, Fig. 9A, B). However, giving attention only to the summer, the $\delta^{18}\text{O}-\Psi$ relationship observed was inverse: a positive correlation existed from beach to inland since Ψ decrease was positively associated with $\delta^{18}\text{O}$ depletion, ($R^2 = 0.827$ $\delta^{18}\text{O}-\Psi_{pd}$ $P < 0.03$ and $R^2 = 0.648$ $\delta^{18}\text{O}-\Psi_{md}$; $P < 0.1$). Furthermore, a positive relationship was detected between leaf water potential (Ψ_{pd} and Ψ_{md}) and topsoil water uptake proportion (both $P < 0.001$) throughout the year.

Fig. 7 Annual contributions, in percentage, of water sources used by the species across the dune system gradient from MixSIAR Bayesian mixing models. Confidence intervals of Gelman diagnostics should be < 1.05 and were lower than 1.01 for all the variables ($0 > 1.01$). Abbrev.: upper beach: Bch; embryo-dune: Emb; slack: Sla; foredune: Fore; inland: Inl)



Discussion

In Mediterranean ecosystems, the origin of the water used by vegetation is expected to vary seasonally in a complex way (Antunes et al. 2018a, b). Accordingly, in the present study water-uptake strategy and water status of the coastal dune plants communities shifted from wet to dry seasons and across the beach-inland gradient. We found both temporal and spatial patterns, but also, our data showed that coastal vegetation exhibited a species-specific response in water uptake. Our data indicate that no single source contributes to all species, but individual species track with specific sources and that this species-specific response is modified by its location in the gradient from the upper beach to inland. There are numerous pieces of evidence in other studies that niche segregation of water uptake sources is a process that varies at the species level at the ecosystem scale (Brinkmann

et al. 2019). However, in our study, this species-specific water uptake pattern is modulated by their location, since the species showed a slight progressive increase in the proportion of the use of water from the deeper layers from the upper beach area to the inland dunes (Fig. 7).

Isotopic signal: plants and water sources

The potential water sources absorbed by plants changed seasonally and locally depending on the species. According to the xylem $\delta^{18}\text{O}$ data (Fig. 3), in spring and summer, the species closer to the ocean, showed more inter-species variability (7.2‰) of water sources than inland species (3.1‰), as pointed out by the high diversity of xylem $\delta^{18}\text{O}$ figures in the species of beach and embryo-dune sites. This higher variance of $\delta^{18}\text{O}$ signature in plants growing in the proximity of the ocean denotes a diversified water

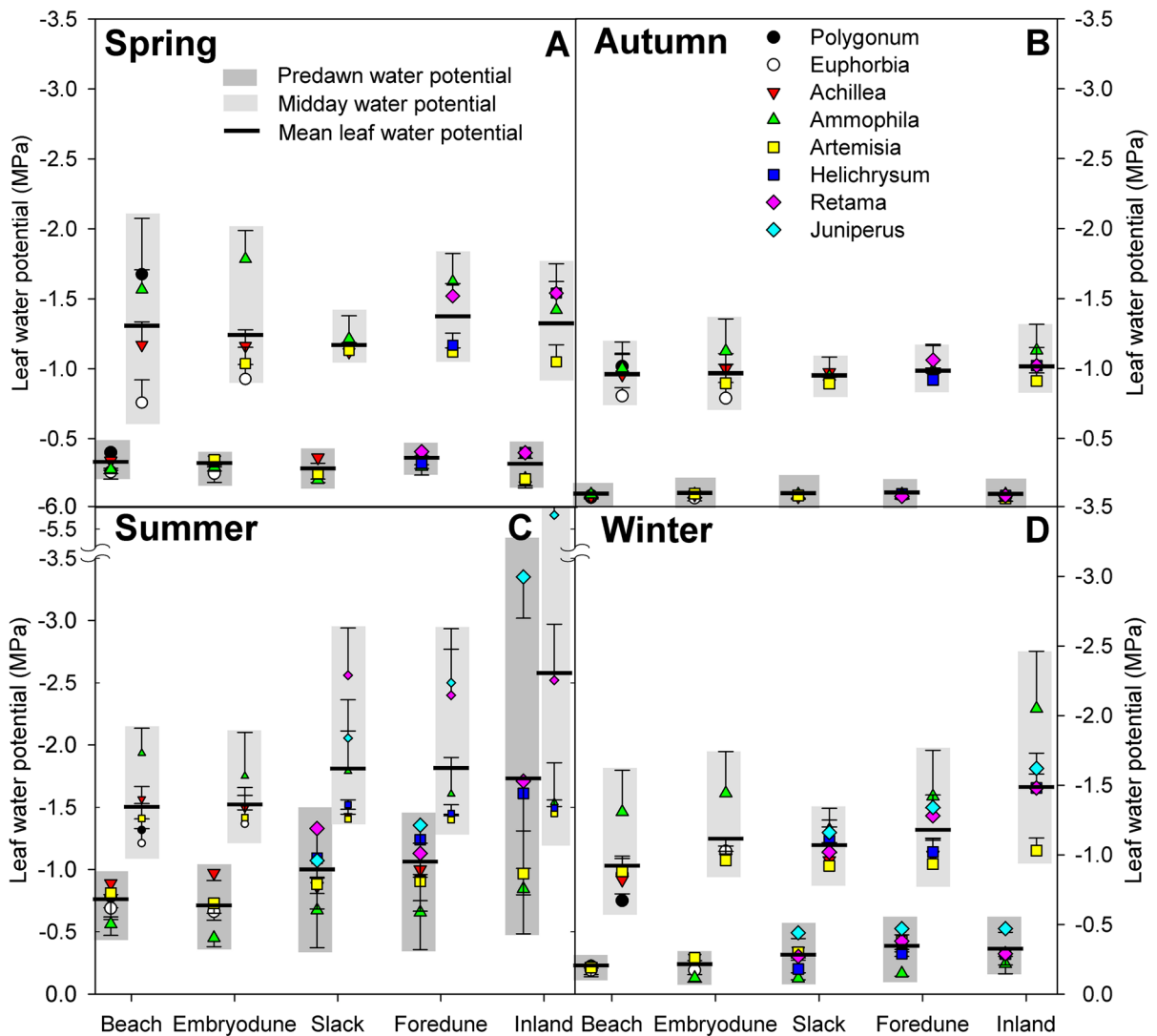


Fig. 8 Predawn and midday leaf water potential across the dune system gradient in the four study periods (spring: **A**; autumn: **B**; summer: **C**; winter: **D**). Significant differences between sites for predawn and midday leaf water potential are shown in Table 2

uptake strategy among species, with species depending mainly on top and mid-layers (*Achillea*, *Ammophila*, *Polygonum*) and species relying more on mid and deep layers (*Artemisia*, *Euphorbia*). In the case of *Artemisia*, this outcome is consistent with previous studies in other species of this genus (*A. gmelinii* and *A. tridentata*) that have described their capacity of exploring deep soil layers, to switch uptake to shallower soil layers when water is sufficient, or even to supply water to upper soil layers at night (hydraulic lift), (Richards and Caldwell 1987; Lü et al. 2017; Wang et al. 2017).

On the contrary, the assemblage of species growing inland (foredune and inland sites) showed a narrower range of water sources in spring and summer. The mean $\delta^{18}\text{O}$ signature of the xylem water in these sites was lower and matched with deep soil water and groundwater signatures, indicating that plants restrict their water uptake to deeper layers. These data suggest that dune plant communities may diversify their water uptake strategy, where some species living in areas closer to the sea can uptake water from upper soil horizons more successfully than those of the inland community, which would share more similar water uptake strategies.

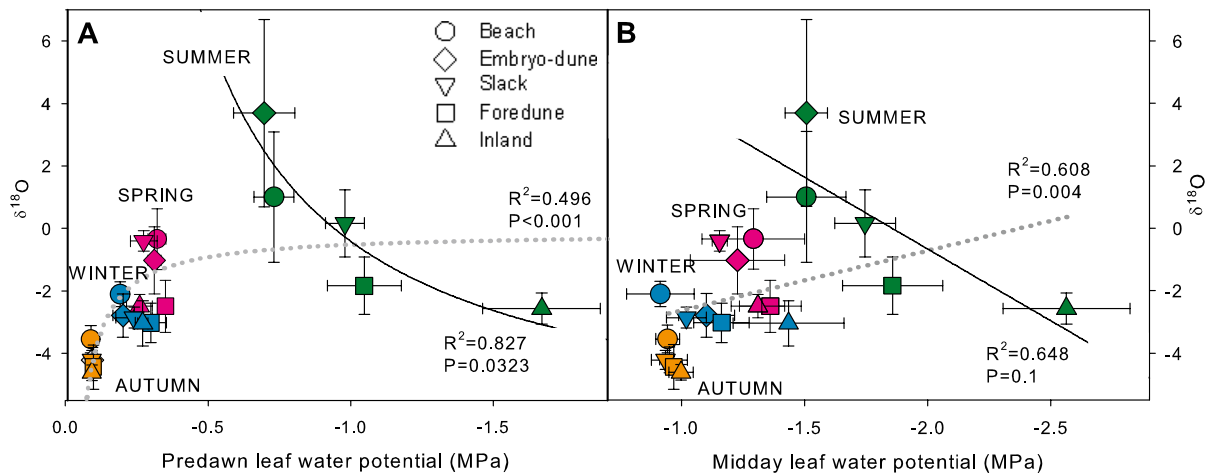


Fig. 9 Stem xylem water $\delta^{18}\text{O}$ and predawn (A) and midday (B) leaf water potential linear regression across the dune system gradient in the four study periods (autumn in light grey,

winter in black, spring in dark grey, summer in white). Points are seasonal means for each site. Global annual regression in grey dashed line and summer regression in black line

Regarding the $\delta^{18}\text{O}$ positive values recorded in spring and especially in summer and considering that, they matched the isotopic composition of soil water, our data support that these plants use soil water that is highly evaporated due to high temperatures. Conversely, it is worth mentioning the positive $\delta^{18}\text{O}$ values observed in plants of *Ammophila* from embryo-dune sites in summer, $11.5 \pm 2.92\text{‰}$, as the isotopic composition of soil water at no depth (nor groundwater) could explain the isotopic signature of the xylem water. Therefore, other factors should explain these enriched $\delta^{18}\text{O}$ values, such as bark evaporation, exchange of evaporated leaf water with xylem water, a decline in the sap flow rate through reverse flow, uptake of dew water, or mixing of phloem and xylem water (Dawson and Ehleringer 1993; Gan et al. 2003; Alessio et al. 2004; Cernusak et al. 2005; Ellsworth and Williams 2007; Ellsworth and Sternberg 2015; Palacio et al. 2014). Although it is problematic to assess the fractionation processes involved without combining data of ^2H excess or $\delta^2\text{H}$ with the enriched ^{18}O values recorded, according to our results, the probable explanation for the enriched $\delta^{18}\text{O}$ values in this perennial grass might be fractionation in cuticular evaporation and redistribution of the enriched water (Dawson and Ehleringer 1993; Eller et al. 2013; Martín-Gómez et al. 2016; Poca et al. 2019). In this regard, the high $\delta^{18}\text{O}$ data combined with the lowest Ψ_{md} values of beach and embryo-dune *Ammophila*

plants would support the explanation mentioned above of high evaporation at leaf level, which would allow the water potential to drop to those low values (Pivovarov et al. 2016).

Water uptake pattern

We are aware that the isotopic composition of xylem water is modified by the heterogeneity in physical and physiological processes in soils and plants and the complexity of the hydrological systems that involved plant water uptake (Penna et al. 2018; von Freyberg et al. 2020; Beyer and Penna 2021). As a result, mixing models may have associated a large uncertainty that must be taken into account when interpreting analysis results.

Even so, the MixSIAR results obtained are consistent with the different strategies across the beach-inland gradient defined by $\delta^{18}\text{O}$. In this way, the species most abundant in the inner position of the gradient (*Retama* and *Juniperus*) were the most dependent on deep water, while the most abundant in beach and embryo dune sites (*Polygonum* and *Achillea*), used the largest proportion of shallow soil water. This spatial segregation in water uptake could be related to life form, as the two beach species are perennial herbs (*Chamaephytes*), while the two inland species are large shrubs (*Phanerophytes*). This would be in agreement with the two-layer hypothesis stated

by Walter (1939) in savannas (revised by Ward et al. 2013) or the niche partitioning in other ecosystems (Weltzin and McPherson 1997; Ward et al. 2013). According to this model, grasses and herbs predominantly utilize water from the upper soil layers, while woody plants rely on deeper soil layers beyond the reach of grasses (Weltzin and McPherson 1997; Wang et al. 2017), especially during the dry season (Dawson and Pate 1996; Antunes et al. 2018a, b, c). Nevertheless, the spatial segregation in water uptake would be related not only to root distribution, but also to environmental conditions, water availability, and also to physiological and hydraulic traits of plants.

Even though we observed a species-specific water uptake pattern, this was not a fixed trait, but rather, it varied in response to the beach-inland gradient and seasonality. As a result, both factors modulated the specific response of the target species. In this way, the species increased deep soil water dependence following the beach-inland gradient and with the increasing aridity as the summer period began. At the community level, the spatial gradient will play a more relevant role in modifying the species-specific water use strategy than the seasonal gradient. This trend can be explained in two ways. First, higher water competition sites due to the greater vegetation cover (Table 1) in the inland and secondly by the mitigation of water stress by the ocean spray in beach and embryo-dune sites.

Regarding seasonality, through the wet seasons, recent rainwater is mixed within the upper soil layers so plant roots acquired water mainly from these soil layers during this wet period (Dawson and Pate 1996, Amin et al. 2020). As the shallower soil layers dried out during dry seasons, the dependence on deep layers increased, as suggested through the more negative $\delta^{18}\text{O}$ values in xylem water and higher contributions to the water uptake of a mixture of mid and deep soil layers in spring and summer.

This shift to deeper soils for water uptake following the beach-inland gradient (Fig. 7) and the increasing aridity (Fig. 5) is in line with the observations of other authors. Accordingly, they reported that the depth of water extraction by plants is dynamic and plants could shift water uptake from shallow to deep soil layers as water availability decreased (Ellsworth and Sternberg 2015; Pivovarov et al. 2016; Antunes et al., 2019; Barbata and Peñuelas 2017). Furthermore, the plasticity in the root system is a major

factor for plant acclimation to water deficit (Kano et al. 2011; Kano-Nakata et al. 2011; 2013). Our finding suggests an intracommunity variation in water uptake depending on the environmental conditions, (as in Barbata et al. 2015; Voltas et al. 2015; Antunes et al. 2018c, 2019) in our study case, water availability and proximity to the sea. Consequently, plants in the entire spatial gradient presented small differences and the water uptake pattern was not only conditioned by its availability, but also by vegetation root distribution (Yadav et al. 2009; Ellsworth and Sternberg 2015).

Water potential and water-uptake strategies

Leaf water potential is an important variable of plant water status since it indicates the tolerance to water deficit of plants to maintain physiological activity (Bhaskara and Ackerly 2006). In our study, the inland vegetation relied primarily on deep soil water, which should lead to better physiological performance, but conversely, the summer Ψ_{pd} and Ψ_{md} and the winter Ψ_{md} of some inland plants were the lowest in the whole spatial gradient. The relationship between Ψ and $\delta^{18}\text{O}$ across the community of dune plants showed a different pattern in summer compared to the rest of the year. In summer, the most negative Ψ values were associated with the most depleted $\delta^{18}\text{O}$ values for xylem water, suggesting deeper water sources.

The dependence on more reliable water sources (eg groundwater or deep soil layers) is normally associated with less negative water potentials. In fact, the relationship $\delta^{18}\text{O}$ - Ψ usually behaves negatively so that plants with favourable water status show more depleted ^{18}O signatures (Jackson et al. 1999; Otieno et al. 2006; Martín-Gómez et al. 2017), whereas the enriched isotopic values (because of isotopic fractionation during high evaporation rate) correlate with values of water potential more negative, as we found in the whole annual cycle (Fig. 9).

Nevertheless, under water-deficit conditions, the opposite pattern can also be found: Even though plants extract water from progressively deeper soil horizons as summer drought advances, the lowest Ψ can be measured under these conditions. This is the case of Pivovarov et al. (2016), who proved that deeper soil water uptake is important to allow mid-day Ψ to achieve more negative values. Zunzunegui et al. (2018) found the same water-use strategy in

argan trees (*Argania spinosa*): under drought conditions, trees extracted water resources from deeper soil layers and the most depleted $\delta^{18}\text{O}$ values were coupled to the lowest Ψ_{pd} . Overall, we show that combining physiological measurements with traditional isotope tracing can reveal mechanistic insights into plant responses to changing environmental conditions (Nehemy et al 2021),

In summer, when plants from foredune and inland sites were the ones using the highest proportion of deep soil water, they were the ones with the lowest water potentials. Differences among sites could be attributed to differences in the water-use strategy. The response of the lowest- Ψ in GW-dependent plants might be explained by the hypothesis thrown by Poole and Miller (1975, 1978), who stated that deep-rooted plants were more sensitive to water stress. In this sense, studying a Mediterranean shrub species, Zunzunegui et al. (2000) found that *Halimium halimifolium* populations with shallow soil water dependence responded better to drought than groundwater-dependent ones. Furthermore, Antunes et al. (2018a) found that plants that were more dependent on groundwater, when subjected to severe water deficit must readjust their root architecture and water extraction strategies, which would involve physiological adjustments, to survive the drop in the water table.

In autumn and winter, the physiological responses of the foredune and inland plants were significantly different. Even though plant species of both sites were the same (Table 1), Ψ_{pd} and Ψ_{md} were lower in the inland site plants. This different behaviour in the same species but different positions of the gradient could be explained by differences in root access to deeper soil layers and more stable water sources as GW. The fact that GW becomes brackish in a dry year would affect phreatophytic vegetation, less used to salty water than plants living closer to sea. The reason could be in the distance and accessibility to GW, with plants from inland using groundwater with high EC, whereas plants in the foredune crest also use water from deep soil layers, less saline. Groundwater salinization was proposed to have occurred in this same area in a previous study (Esquivias et al. 2014), probably due to ocean water intrusion. This is consistent with the high EC and $\delta^{18}\text{O}$ values recorded in GW in the present study in the inland site during low rainfall seasons, as a positive relationship exists between $\delta^{18}\text{O}$ and EC (Esquivias et al. 2014). According to

the seasonal dynamics of GW in the area, the only possible freshwater source is rainfall infiltration into the water table. The GW in both piezometers was not homogeneous the EC in PZsl being lower than in PZin, thus implying a differential phreatic recharge. Infiltration of precipitation from both embryo-dune and foredune would be the main source for the PZsl, whereas ocean water infiltration would be more remarkable in the PZin when precipitation is scarce. Further research is needed to explore the role of seawater in coastal dunes vegetation.

To summarize, the water-source dynamic of Mediterranean coastal dune vegetation changes according to spatial and temporal patterns. It can be seen that during water scarcity, the water sources used by vegetation along the beach-inland gradient are less dependent on shallow and mid soil layers; in addition, this response pattern is similar and overlaps with the effect of the beach-inland gradient. It is noteworthy that the vegetation from lower zones, slack and inland, maintains a marked dependence on deep water (from soil or GW) throughout the year, especially in the case of inland vegetation. In contrast, the zones closest to the beach shift water sources seasonally from top to mid soil and deep soil layers. Antunes et al. (2019) studying coastal vegetation found that in dry periods (no precipitation and a deeper phreatic level) vegetation generally relied on deeper soil horizons and presented a greater water status evenness. Furthermore, Bermúdez and Retuerto (2014) demonstrated the diversity in dune vegetation as species coexisting in the front dune exhibited a wide variation in relevant functional traits concerning water use.

Concluding remarks

Our results indicate that three factors mark the proportion of use of the water sources available for vegetation in coastal vegetation: the species composition of the community, distance to the sea and seasonality (precipitation and temperature). The interactions between species-specific strategies with spatial gradients and weather dynamics that are identified here will address how dune ecosystems will be affected by future scenarios of global change. The proximity to the beach is of primary relevance in determining the structure and function of Mediterranean coastal dune vegetation, especially during dry and warm seasons and even more in dry years. Predictions on global

climate change point out that drought periods will be longer and more severe (Giorgi 2006; Sheffield and Wood 2008) thus increasing stressing factors for dune vegetation. It will be useful to identify a threshold of plant community survival through the quantification of each species tolerance to drought and its location on the beach-inland gradient.

The present study indicates that a decrease in precipitation could put at risk the continuity of dune vegetation through two mechanisms: altering root distribution and altering community composition. Maintenance of the perennial species of coastal dune vegetation is critical, as they are usually the most important species in the building of dunes, binding of sediments and reduction of erosion (Hesp 2002; Feagin et al. 2005). Furthermore, this perennial vegetation provides permanent coverage of the dunes, and hence its eventual loss would certainly increase erosion rates (Gracia et al. 2018). Our study reveals that plant species closer to the sea would respond better to water scarcity than the inland community. Therefore, species from the upper beach and the embryo-dune would expand due to climate change previsions. The results presented reveal new insights into how coastal ecosystems may be affected by changes in precipitation patterns as a result of climate change.

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Author’s contributions MZ and JBGF conceived the study; MPE, MZ and JBGF were responsible for methodology and data collection; MPE and MZ conducted laboratory work, data analysis and manuscript writing. All authors made critical contributions to the draft.

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Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate All authors have been informed and agree to participate

Articles that report the results involving humans and/or animals Not applicable

Conflicts of interest There are no conflicts of interest or competing interest.

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References

- Ackerly D (2004) Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecol Monogr* 74:25–44. <https://doi.org/10.1890/03-4022>
- Alessio GA, De Lillis M, Brugnoli E, Lauteri M (2004) Water sources and water-use efficiency in Mediterranean coastal dune vegetation. *Plant Biol* 6:350–357. <https://doi.org/10.1111/j.1438-8677.2011.00531.x>
- Allison G, Barnes C, Hughes M, Leaney F (1983) Effect of climate and vegetation on oxygen-18 and deuterium profiles in soils. *Isot Hydrol*: 105–123
- Amin A, Zuecco G, Geris J, Schwendenmann L, McDonnell JJ, Borga M, Penna D (2020) Depth distribution of soil water sourced by plants at the global scale: A new direct inference approach. *Ecology* 13:e2177
- Antunes C, Díaz-Barradas MC, Zunzunegui M, Vieira S, Pereira Â, Anjos A, Correia O, Pereira MJ, Máguas C (2018a) Contrasting plant water-use responses to groundwater depth in coastal dune ecosystems. *Funct Ecol* 32:1931–1943. <https://doi.org/10.1111/1365-2435.13110>
- Antunes C, Díaz-Barradas MC, Zunzunegui M, Vieira S, Máguas C (2018b) Water source partitioning among plant functional types in a semi-arid dune ecosystem. *J Veg Sci* 29:671–683. <https://doi.org/10.1111/jvs.12647>
- Antunes C, Chozas S, West J, Zunzunegui M, Diaz-Barradas MC, Vieira S, Máguas C (2018c) Groundwater drawdown drives ecophysiological adjustments of woody vegetation in a semi-arid coastal ecosystem. *Glob Change Biol* 24:4894–4908. <https://doi.org/10.1111/gcb.14403>
- Antunes C, Silva C, Máguas C, Joly CA, Vieira S (2019) Seasonal changes in water sources used by woody species in a tropical coastal dune forest. *Plant Soil* 437:41–54. <https://doi.org/10.1007/s11104-019-03947-9>

- Barbeta A, Peñuelas J (2017) Relative contribution of groundwater to plant transpiration estimated with stable isotopes. *Sci Rep* 7:10580. <https://doi.org/10.1038/s41598-017-09643-x>
- Barbeta A, Ogée J, Peñuelas J (2018) Stable-Isotope Techniques to Investigate Sources of Plant Water. In: Sánchez-Moreiras AM, Reigosa MJ, *Advances in Plant Ecophysiology Techniques*. Springer, Cham, pp. 439–456
- Barbeta A, Mejía-Chang M, Ogaya R, Voltas J, Dawson TE, Peñuelas J (2015) The combined effects of long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. *Glob Change Biol* 21:1213–1225. <https://doi.org/10.1111/gcb.12785>
- Bermúdez R, Retuerto R (2014) Together but different: Co-occurring dune plant species differ in their water and nitrogen-use strategies. *Oecologia* 174:651–663. <https://doi.org/10.1007/s00442-013-2820-7>
- Beyer M, Penna D (2021) On the spatio-temporal under-representation of isotopic data in ecohydrological studies. *Front Water* 3:643013. <https://doi.org/10.3389/frwa.2021.643013>
- Bhaskara R, Ackerly DD (2006) Ecological relevance of minimum seasonal water potentials. *Physiol Plant* 127:353–359. <https://doi.org/10.1111/j.1399-3054.2006.00718.x>
- Brinkmann N, Eugster W, Buchmann N, Kahmen A (2019) Species-specific differences in water uptake depth of mature temperate trees vary with water availability in the soil. *Plant Biol* 21:71–81. <https://doi.org/10.1111/plb.12907>
- Causton DR (1988) *An introduction to vegetation analysis: principles, practice and interpretation*. Springer, London
- Cavallaro A, Silleta LC, Pereyra DA (2020) Foliar water uptake in arid ecosystems-seasonal variability and ecophysiological consequences. *Oecologia* 193:37–348. <https://doi.org/10.1007/s00442-020-04673-1>
- Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. *Tree Physiol* 25:129–146. <https://doi.org/10.1093/treephys/25.2.129>
- Coleman M, Meier-Augenstein W (2014) Ignoring IUPAC guidelines for measurement and reporting of stable isotope abundance values affects us all. *Rapid Commun Mass Sp* 28:1953–1955. <https://doi.org/10.1002/rcm.6971>
- Craig H (1961) Isotopic variations in meteoric waters. *Science* 133:1702–1703. <https://doi.org/10.1126/science.133.3465.1702>
- Dansgaard W (1964) Stable isotopes in precipitation. *Tellus* 16:436–468. <https://doi.org/10.1111/j.2153-3490.1964.tb00181.x>
- 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. *Geochim Cosmochim A* 57:3487–3492
- Dawson TE, Pate JS (1996) Seasonal Water Uptake and Movement in Root Systems of Australian Phraeatophytic Plants of Dimorphic Root Morphology: A Stable Isotope Investigation. *Oecologia* 107:13–20. <https://doi.org/10.1007/BF00582230>
- Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable Isotopes in Plant Ecology. *Annu Rev Ecol Syst* 33:507–559. <https://doi.org/10.1146/annurev.ecolsys.33.020602.095451>
- 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 Phytol* 229:1339–1353. <https://doi.org/10.1111/nph.16971>
- Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds). *Plant Physiological Ecology Field Methods and Instrumentation*. Chapman & Hall, London, UK. pp 281–300. <https://doi.org/10.1007/978-94-009-2221-1>
- Ehleringer JR, Dawson TE (1992) Water uptake by plants: perspectives from stable isotope composition. *Plant Cell Environ* 15:1073–1082. <https://doi.org/10.1111/j.1365-3040.1992.tb01657.x>
- Eller CB, Lima AL, Oliveira RS (2013) Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasiliensis* (Winteraceae). *New Phytol* 199:151–162. <https://doi.org/10.1111/nph.12248>
- Ellsworth PZ, Sternberg LSL (2015) Seasonal water use by deciduous and evergreen woody species in a scrub community is based on water availability and root distribution. *Ecohydrology* 8:538–551. <https://doi.org/10.1002/eco.1523>
- Ellsworth PZ, Williams DG (2007) Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant Soil* 291:93–107. <https://doi.org/10.1007/s11104-006-9177-1>
- Esquivias MP, Zunzunegui M, Díaz Barradas MC, Álvarez-Cansino L (2014) The role of water use and uptake on two Mediterranean shrubs’ interaction in a brackish coastal dune ecosystem. *Ecohydrology* 7:783–793. <https://doi.org/10.1002/eco.1401>
- Evaristo J, Jasechko S, McDonnell J (2015) Global separation of plant transpiration from groundwater and streamflow. *Nature* 525:91–94
- Feagin RA, Sherman DJ, Grant WE (2005) Coastal erosion, global sea-level rise, and the loss of sand dune plant habitats. *Front Ecol Environ* 3:359–364. [https://doi.org/10.1890/1540-9295\(2005\)003\[0359:CEGSRA\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2005)003[0359:CEGSRA]2.0.CO;2)
- Flanagan LB, Ehleringer JR (1991) Stable isotopes composition of stem and leaf water: applications to the study of plant water use. *Funct Ecol* 5:270–277. <https://doi.org/10.2307/2389264>
- Gan KS, Wong SC, Yong JWH, Farquhar GD (2003) Evaluation of models of leaf water ¹⁸O enrichment using measurements of spatial patterns of vein xylem water, leaf water and dry matter in maize leaves. *Plant Cell Environ* 26:1479–1495. <https://doi.org/10.1046/j.1365-3040.2003.01070.x>
- Giorgi F (2006) Climate change hot-spots. *Geophys Res Lett* 33:1–4. <https://doi.org/10.1029/2006GL025734>
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. *Glob Planet Chang* 63:90–104. <https://doi.org/10.1016/j.gloplacha.2007.09.005>
- Gracia AC, Rangel-Buitrago N, Oakley JA, Williams A (2018) Use of ecosystems in coastal erosion management. *Ocean*

- Coast Manag 156:277–289. <https://doi.org/10.1016/j.ocecoaman.2017.07.009>
- Greaver TL, Sternberg L da SL (2006) Linking marine resources to ecotonal shifts of water uptake by terrestrial dune vegetation. *Ecology* 87:2389–2396. [https://doi.org/10.1890/0012-9658\(2006\)87\[2389:lmrtes\]2.0.co;2](https://doi.org/10.1890/0012-9658(2006)87[2389:lmrtes]2.0.co;2)
- Greaver TL, Sternberg L da SL (2007) Fluctuating deposition of ocean water drives plant function on coastal sand dunes. *Glob Change Biol* 13:216–223. <https://doi.org/10.1111/j.1365-2486.2006.01287.x>
- Greaver TL, Sternberg L da SL (2010) Decreased precipitation exacerbates the effects of sea level on coastal dune ecosystems in open ocean islands. *Glob Change Biol* 16:1860–1869. <https://doi.org/10.1111/j.1365-2486.2010.02168.x>
- Helliker BR, Roden JS, Cook C, Ehleringer JR (2002) A rapid and precise method for sampling and determining the oxygen isotope ratio of atmospheric water vapor. *Rapid Commun Mass Sp* 16:929–932. <https://doi.org/10.1002/rcm.659>
- Hesp PA (1990) Ecological processes and plant adaptations on coastal dunes. *J Arid Environ* 21:165–191. [https://doi.org/10.1016/S0140-1963\(18\)30681-5](https://doi.org/10.1016/S0140-1963(18)30681-5)
- Hesp PA (2002) Foredunes and blowouts: initiation, geomorphology and dynamics. *Geomorphology* 48:245–268. [https://doi.org/10.1016/S0169-555X\(02\)00184-8](https://doi.org/10.1016/S0169-555X(02)00184-8)
- IPCC 2014, Climate Change (2014) Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Meyer LA (eds.)]. IPCC, Geneva, Switzerland, 151 pp
- Jackson RB, Moores LA, Hoffmann WA, Pockman WT, Linder CR (1999) Ecosystem rooting depth determined with caves and DNA. *Ecology* 96:1137–11392. <https://doi.org/10.1073/pnas.96.20.11387>
- Kano M, Inukai Y, Kitano H, Yamauchi A (2011) Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. *Plant Soil* 342:189–196. <https://doi.org/10.1007/s11104-010-0675-9>
- Kano-Nakata M, Inukai Y, Wade LJ, Siopongco JDLC, Yamauchi A (2011) Root development, water uptake, and shoot dry matter production under water deficit conditions in two CSSLs of rice: functional roles of root plasticity. *Plant Prod Sci* 14:329–339. <https://doi.org/10.1626/ppls.14.307>
- Kano-Nakata M, Gowda VRP, Serraj AHR, Inukai Y, Fujita D, Kobayashi N, Suralta RR, Yamauchi A (2013) Functional roles of the plasticity of root system development in biomass production and water uptake under rainfed lowland conditions. *Field Crop Res* 144:288–296. <https://doi.org/10.1016/j.fcr.2013.01.024>
- Kim D, Yu K (2009) A conceptual model of coastal dune ecology synthesizing spatial gradients of vegetation, soil, and geomorphology. *Plant Ecol* 202:135–148. <https://doi.org/10.1007/s11258-008-9456-4>
- Lü T, Zhao X, Gao X, Pan Y (2017) Soil water use strategy of dominant species in typical natural and planted shrubs in loess hilly region. *Chin J Plant Ecol* 41:175–185. <https://doi.org/10.17521/CJPE.2016.0253>
- Máguas C, Rascher KG, Martins-Loução A, Carvalho P, Pinho P, Ramos M, Correia O, Werner C (2011) Responses of woody species to spatial and temporal groundwater changes in coastal sand dunes systems. *Biogeosciences* 8:1591–1616. <https://doi.org/10.5194/bg-8-3823-2011>
- Martínez F, Merino O, Martín A, García Martín D, Merino J (1998) Belowground structure and production in a Mediterranean sand dune shrub community. *Plant Soil* 201:209–216
- Martín-Gómez P, Serrano L, Ferrio JP (2016) Short-term dynamics of evaporative enrichment of xylem water in woody stems: implications for ecophysiology. *Tree Physiol* 37:511–522. <https://doi.org/10.1093/treephys/tpw115>
- Martín-Gómez P, Aguilera M, Pemán J, Gil-Pelegrín E, Ferrio JP (2017) Contrasting ecophysiological strategies related to drought: the case of a mixed stand of Scots pine (*Pinus sylvestris*) and a submediterranean oak (*Quercus subpyrenaica*). *Tree Physiol* 37:1478–1492. <https://doi.org/10.1093/treephys/tpx101>
- Mendoza-González G, Martínez ML, Martínez-Gordillo D, Rojas-Soto OR, Vázquez G, Gallego-Fernández JB (2013) Environmental niche modelling of coastal dune plants and its future potential distribution in response to climate change and sea level rise. *Glob Change Biol* 19:2524–2535. <https://doi.org/10.1111/gcb.12236>
- Mooney HA, Gulmon SL, Rundel PW, Ehleringer JR (1980) Further observations on the water relations of *Prosopis tamarugo* of the northern Atacama desert. *Oecologia* 44:177–180. <https://doi.org/10.1007/BF00572676>
- Moreno-Casasola P (1986) Sand movement as a factor in the distribution of plant communities in a coastal dune system. *Vegetatio* 65:67–76. <https://doi.org/10.1007/BF00044876>
- Muñoz-Vallés S, Cambrolle J, Gallego-Fernández JB (2015) Effect of soil characteristics on plant distribution in coastal ecosystems of SW Iberian Peninsula sand spits. *Plant Ecol* 216:1551–1570. <https://doi.org/10.1007/s11258-015-0537-x>
- Nehemy MF, Benettin P, Asadollahi M, Pratt D, Rinaldo A, McDonnell JJ (2021) Tree water deficit and dynamic source water partitioning. *Hydrol Process* 35. <https://doi.org/10.1002/hyp.14004>
- Nie YP, Chen HS, Wang KL, Tan W, Deng PY, Yang J (2011) Seasonal water use patterns of woody species growing on the continuous dolostone outcrops and nearby thin soils in subtropical China. *Plant Soil* 341:399–412. <https://doi.org/10.1007/s11104-010-0653-2>
- Oliveira RS, Bezerra L, Davidson EA, Pinto F, Klink CA, Nepstad DC, Moreira A (2005) Deep root function in soil water dynamics in cerrado savannas of Central Brazil. *Funct Ecol* 19:574–581. <https://doi.org/10.1111/j.1365-2435.2005.01003.x>
- Otieno DO, Kurz-Besson C, Liu J, Schmidt MWT, Vale-Lobo do R, David TS, Siegwolf R, Pereira JS, Tenhunen JD, (2006) Seasonal variations in soil and plant water status in a *Quercus suber* L. Stand: Roots as determinants of tree productivity and survival in the Mediterranean-type ecosystem. *Plant Soil* 283:119–135. <https://doi.org/10.1007/s11104-004-7539-0>
- Palacio S, Azorín J, Montserrat-Martí G, Ferrio JP (2014) The crystallization water of gypsum rocks is a relevant water source for plants. *Nat Commun* 5:4660. <https://doi.org/10.1038/ncomms5660>

- Penna D, Hopp L, Scandellari F, Allen ST, Benettin P, Beyer M, Geris J, Klaus J, Marshall JD, Schwendenmann L, Volkmann THM, von Freyberg J, Amin A, Ceperley N, Engel M, Frentress J, Giambastiani Y, McDonnell JJ, Zucco G, Llorens P, Siegwolf RTW, Dawson TE, Kirchner JW (2018) Ideas and perspectives: Tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes - challenges and opportunities from an interdisciplinary perspective. *Biogeosciences* 15:6399–6415. <https://doi.org/10.5194/bg-15-6399-2018>
- Phillips DL, Newsome SD, Gregg JW (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia* 144:520–527. <https://doi.org/10.1007/s00442-004-1816-8>
- Pivovarov AL, Pasquini SC, De Guzman ME, Alstad KP, Stemke JS, Santiago LS (2016) Multiple strategies for drought survival among woody plant species. *Funct Ecol* 30:517–526. <https://doi.org/10.1111/1365-2435.12518>
- Poca M, Coomans O, Urcelay C, Zeballos SR, Bodé S, Boeckx P (2019) Isotope fractionation during root water uptake by *Acacia caven* is enhanced by arbuscular mycorrhizas. *Plant Soil* 441:485–497. <https://doi.org/10.1007/s11104-019-04139-1>
- Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *Am J Bot* 87:1287–1299. <https://doi.org/10.2307/2656722>
- Poole DK, Miller PC (1975) Water relations of selected species of chaparral and coastal sage communities. *Ecology* 56:1118–1128. <https://doi.org/10.2307/1936151>
- Poole DK, Miller PC (1978) Water related characteristics of some sclerophyll shrubs in central Chile. *Oecologia Plantar* 13:289–299
- Querejeta JJ, Estrada-Medina H, Allen MF, Jimenez-Osornio JJ (2007) Water source partitioning among trees growing on shallow karst soils in a seasonally dry tropical climate. *Oecologia* 152:26–36. <https://doi.org/10.1007/s00442-006-0629-3>
- Ranasinghe R, Callaghan D, Stive MJ (2012) Estimating coastal recession due to sea level rise: beyond the Bruun rule. *Clim Change* 110:561–574. <https://doi.org/10.1007/s10584-011-0107-8>
- Richards JH, Caldwell MM (1987) Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. *Oecologia* 73:486–489. <https://doi.org/10.1007/BF00379405>
- Schmidt A, Santos IR, Burnett WC, Niencheski F, Knöller K (2011) Groundwater sources in a permeable coastal barrier: Evidence from stable isotopes. *J Hydrol* 406:66–72. <https://doi.org/10.1016/j.jhydrol.2011.06.001>
- Scholander PF, Hammer HT, Bradstreet ED, Hemmingen EA (1965) Sap pressure in vascular plants. *Science* 148:339–346. <https://doi.org/10.1126/science.148.3668.339>
- Sprenger M, Leistert H, Gimbel K, Weiler M (2016) Illuminating hydrological processes at the soil-vegetation-atmosphere interface with water stable isotopes. *Geophys* 54:674–704. <https://doi.org/10.1002/2015RG000515>
- Stallins JA (2001) Soil and vegetation patterns in barrier-island dune environments. *Phys Geogr* 22:79–98. <https://doi.org/10.1080/02723646.2001.10642731>
- Sternberg LSL, Swart PK (1987) Utilization of freshwater and ocean water by coastal plants of Southern Florida. *Ecology* 68:1898–1905. <https://doi.org/10.2307/1939881>
- Stock BC, Semmens BX (2016) MixSIAR GUI User Manual, version 3.1. <https://github.com/brianstock/MixSIAR/>. <https://doi.org/10.5281/zenodo.47719>. Accessed 20 Feb 2022
- 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. <https://doi.org/10.7717/peerj.5096>
- Tron S, Bodner G, Laio F, Ridolfi L, Leitner D (2015) Can diversity in root architecture explain plant water use efficiency? A modelling study. *Ecol Modell* 312:200–210. <https://doi.org/10.1016/j.ecolmod.2015.05.028>
- Valentini R, Scarascia Mugnozza GE, Ehleringer JR (1992) Hydrogen and carbon isotope ratios of selected species of a Mediterranean macchia ecosystem. *Funct Ecol* 6:627–631. <https://doi.org/10.2307/2389955>
- Verweij RJT, Higgins SI, Bond WJ, February EC (2011) Water sourcing by trees in a mesic savanna: Responses to severing deep and shallow roots. *Environ Exp Bot* 74:229–236. <https://doi.org/10.1016/j.envexpbot.2011.06.004>
- Volta J, Lucabaugh D, Chambel MR, Ferrio JP (2015) Intraspecific variation in the use of water sources by the circum-Mediterranean conifer *Pinus halepensis*. *New Phytol* 208:1031–1041. <https://doi.org/10.1111/nph.13569>
- von Freyberg J, Allen ST, Grossiord C, Dawson TE (2020) Plant and root-zone water isotopes are difficult to measure, explain, and predict: some practical recommendations for determining plant water sources. *Methods Ecol Evol* 11:1352–1367. <https://doi.org/10.1111/2041-210X.1346>
- Walter H (1939) Grasland, Savanne und Busch der arideren Teile Afrikas in ihrer o'kologischen Bedingtheit. *Jahrb Wiss Bot* 87:750–860
- Wang J, Fu B, Lua N, Zhang L (2017) Seasonal variation in water uptake patterns of three plant species based on stable isotopes in the semi-arid Loess Plateau. *Sci Total Environ* 609:27–37. <https://doi.org/10.1016/j.scitotenv.2017.07.133>
- Wang J, Lu N, Fu B (2019) Inter-comparison of stable isotope mixing models for determining plant water source partitioning. *Sci Total Environ* 666:685–693. <https://doi.org/10.1016/j.scitotenv.2019.02.262>
- Ward D, Wiegand K, Getzin S (2013) Walter's two-layer hypothesis revisited: back to the roots! *Oecologia* 172:617–630. <https://doi.org/10.1007/s00442-012-2538-y>
- Weltzin JF, McPherson GR (1997) Spatial and temporal soil moisture resource partitioning by trees and grasses in a temperate savanna, Arizona USA. *Oecologia* 112:156–164. <https://doi.org/10.1007/s004420050295>
- West AG, Patrickson SJ, Ehleringer JR (2006) Water extraction times for plant and soil materials used in stable isotope analysis. *Rapid Commun Mass Sp* 20:1317–1321. <https://doi.org/10.1002/rcm.2456>
- West AG, Dawson TE, February EC, Midgley GF, Bond WJ, Aston TL (2012) Diverse functional responses to drought in a Mediterranean-type shrubland in South Africa. *New Phytol* 195:396–407. <https://doi.org/10.1111/j.1469-8137.2012.04170.x>

- Wilson JB, Sykes MT (1999) Is zonation on coastal sand dunes determined primarily by sand burial or by salt spray? A test in New Zealand dunes. *Ecol Lett* 2:233–236. <https://doi.org/10.1046/j.1461-0248.1999.00084.x>
- Yadav BK, Mathur S, Siebel MA (2009) Soil moisture dynamics modelling considering the root compensation mechanism for water uptake by plants. *J Hydrol Eng* 14:913–922. [https://doi.org/10.1061/\(ASCE\)HE.1943-5584.0000066](https://doi.org/10.1061/(ASCE)HE.1943-5584.0000066)
- Zunzunegui M, Díaz Barradas MC, García Novo F (2000) Different phenotypic response of *Halimium halimifolium* in relation to groundwater availability. *Plant Ecol* 148:165–174. <https://doi.org/10.1023/A:1009847628243>
- Zunzunegui M, Boutaleb S, Díaz Barradas MC, Esquivias MP, Valera J, Jáuregui J, Tagma T, Ain-Lhout F (2018) Reliance on deep soil water in the tree species *Argania spinosa*. *Tree Physiol* 38:678–689. <https://doi.org/10.1093/treephys/tpx152>

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