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5 **Water source partitioning among plant functional types in a semi-arid** 6 **dune ecosystem**

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17

18 **Abstract**

19 **Question(s):** The aim of this work was to characterize the main water sources used by the
20 woody plant community of a semi-arid coastal dune ecosystem and to enlighten the relevance
21 of soil water partitioning. We ask: Do the seasonal water sources used by plants differ
22 between functional types? Does the seasonal drought promote shifts towards deeper soil
23 layers in all plants? Does greater use of deep soil water enables the maintenance of a more
24 favorable plant water status? Do water-sources-use strategies mirror overall drought
25 strategies?

26 **Location:** Semi-arid coastal dune system in Doñana Biological Reserve, southwest Spain.

27 **Methods:** Fourteen coexisting woody species with contrasting functional traits were
28 considered. We analyzed each plant's xylem water oxygen isotope composition and
29 compared it to water sources (soil water at 10, 30, 50 cm, rain and groundwater), both in

30 spring and dry summer. Bayesian isotope mixing models were used to estimate the proportion
31 of each water source used by plants. We tested the influence of different (categorical) traits
32 (growth form, water requirement, leaf longevity and leaf form) on the patterns of plants'
33 water-source-use. We evaluated the relationships between proportions of deep soil water use
34 and ecophysiological parameters related to water economy (leaf $\delta^{13}\text{C}$ and water-index).

35 **Results:** A greater similarity between species' water-source-use was found in periods of
36 higher water availability (spring). Contrastingly, during the dry season, a high inter-specific
37 differentiation was observed. All four functional traits showed a significant effect on water
38 sources used in summer. Accordingly, species clustered in five water-use functional groups,
39 exploring water from shallower to deeper soil layers. Under strong drought conditions, the
40 highest use of shallow soil layers was observed in narrow-leaved xerophytic shrubs, while
41 evergreen xerophytic trees and evergreen hygrophytic species showed a higher use of deep
42 soil layers. Greater use of deep soil layers in the dry season was linked to a better seasonal
43 maintenance of plant water status.

44 **Conclusions:** Coexisting plant functional types segregated along a wide spectrum of
45 contrasting water-source use under extremely dry conditions, evidencing great soil water
46 partitioning. Relevant seasonal water-use shifts towards deeper soil layers were observed,
47 but not in all functional types. Furthermore, we confirmed that the water-source-use behavior
48 accompanied broader strategies of drought resistance.

49

50 **key-words:** soil water partitioning; water-sources segregation; coexistence; groundwater
51 use; Mediterranean; functional groups; sandy coastal ecosystem; dry conditions; stable
52 isotope mixing model; water-use shifts; drought

53

54 **running head:** segregation in water-sources used by woody plants

55

56 **nomenclature:** Castroviejo et al. 1986–2012

57

58

60 **Introduction**

61 The function, performance and, ultimately, survival of plant species living in dry ecosystems
62 depend significantly on water availability as well as on the ability to use the available water
63 (Austin et al. 2004; Grossiord et al. 2017; Palacio et al. 2017). In semi-arid systems,
64 interspecific (belowground) competition for water defines plant communities (Fowler 1986;
65 Eissenstat & Caldwell 1988; Casper & Jackson 1997; Schenk & Jackson 2002). Nevertheless,
66 different species can coexist by using different ranges and proportions of resources
67 (Silvertown et al. 1999, 2015; Filella & Peñuelas 2003; Valladares et al. 2015; Palacio et al.
68 2017). Hence, temporal and spatial (vertical) water partition is expected to represent an
69 important process in these seasonal dry ecosystems. This is especially relevant in sandy soils
70 with low water retention (Veneklaas & Poot 2003; Alessio et al. 2004). When exposed to an
71 extreme decrease in soil water availability and in the absence of rain, dune woody plants will
72 need to rely on another water source and/or readjust root system and water uptake to new
73 water levels. In fact, under drought, water adjustments can and must be made, otherwise the
74 impacts will be detected in plant performance and eventually in plant dieback (Nepstad et al.
75 2007; Padilla & Pugnaire 2007; Allen et al. 2010; Barbeta et al. 2015).

76 There is evidence of water sources use segregation between growth forms in several
77 environments (Silvertown et al. 1999, 2015; Nippert & Knapp 2007; Barbeta & Peñuelas
78 2017). In fact, an important differentiation in Mediterranean communities is established
79 between tree species with deep roots, with more constant water and nutrient resources, and
80 shrubby species with shallow roots, which use episodic rainwater and associated nutrient
81 uptake (Moreno-Gutiérrez et al. 2012). Water-use under drought will determine the degree
82 to which a plant experiences a given hydric variation, with deep-rooted species being
83 possibly buffered from drought to a greater extent than shallow-rooted species (Nepstad et
84 al. 1994, 2007; Oliveira et al. 2005; West et al. 2012). Besides growth form, other key
85 functional traits can play a role in water-sources use patterns (Leng et al. 2013). Leaf
86 phenology is one of them. Studies in Mediterranean scrub, desert savannas, and tropical dry
87 forests have shown that evergreen species shift water uptake from shallow water to deep
88 water in the dry season (Jackson et al. 1999; Hasselquist et al. 2010; Esquivias et al. 2014;

89 Ellsworth & Sternberg 2015; Grossiord et al. 2017). As an adaptation for water absorption
90 and for protection against water deficit, leaf shape can also affect water-use patterns of plants
91 (Wright et al. 2006; Antunes et al. 2018). Additionally, an important functional syndrome
92 related with water-use is plant water requirement. Species can explore different niches
93 according to their relative requirement of soil moisture; hygrophytes will grow in a moist
94 habitat and xerophytes are adapted to survive in an environment with little water (Ackerly
95 2004). This classification has been pointed out as key in differentiating semi-arid
96 Mediterranean coastal dune plants' physiological patterns (Zunzunegui et al. 2005, 2009,
97 2011).

98 Although the basis for niche segregation by rooting depth in plant communities have been
99 studied (Silvertown et al. 1999, 2015; Filella & Peñuelas 2003; Araya et al. 2011; Peñuelas
100 et al. 2011; Palacio et al. 2017) there are water-sources use differences between plants that
101 still have been overlooked. Attempts to understand mechanisms underlying plant responses
102 to drought have led to the emergence of distinct hydraulic and water strategies among
103 coexisting species (McDowell et al. 2008; West et al. 2012; Skelton et al. 2015; Pivovarov
104 et al. 2016). However, when exploring plant water strategies, specific quantification of water
105 sources used by plants is not usually used. This is an important piece of the puzzle since it
106 can be linked to the ability to explore different water sources (and in some cases buffering
107 the drought effect). Furthermore, it can clarify soil water partitioning (i.e. differentiation of
108 water-uptake depth) among species. Because partitioning of water based solely on
109 differences in rooting depth does not explain patterns of water uptake for many species
110 (Jackson et al. 1999; Oliveira et al. 2005; Nippert & Knapp 2007; Ellsworth & Sternberg
111 2015), quantifying the relative contribution of different water sources to the composition of
112 the xylem water is utmost relevant. One valuable approach to do so is through Bayesian stable
113 isotope mixing models (Parnell et al. 2013; Stock et al. 2016; Palacio et al. 2017).

114 The aim of this work was to characterize the woody community seasonal water source use
115 and enlighten the relevance of segregation in the sources of water used by woody species in
116 a semi-arid coastal dune ecosystem. Through an isotopic approach, and considering fourteen
117 species of widely contrasting functional traits, we explored: (i) which are the main water
118 sources used by plants under contrasting water availability conditions, (ii) the influence of
119 functional traits on patterns of water-sources use, (iii) the seasonal water-source-use variation
120 and possible shifts towards deeper soil layers in drier conditions, (iv) the effect of water-

121 sources used on plant water status, and (v) the link between water-use functional groups and
122 plants' drought strategies.

143

144 **Methods**

145 Study site and plant species

146 The study was conducted at a semi-arid Mediterranean coastal dune ecosystem at Biological
147 Reserve of Doñana, southwest of Spain (AI=0.47) (UNEP 1992; [http://www.cgiar-](http://www.cgiar-csi.org/data/global-aridity-and-pet-database)
148 [csi.org/data/global-aridity-and-pet-database](http://www.cgiar-csi.org/data/global-aridity-and-pet-database)) (Fig. S1). It showed a typical western
149 Mediterranean climate, with a total annual rainfall (August 2012 – August 2013) of 566.1
150 mm (weather station at RBD: 36°59'19"N; 6°26'35"W, Fig. S1). During the dry season, the
151 well-drained sand, underlying the sandy plant community in Doñana scrub, exacerbates soil
152 drying and water limitation. Soil water content, measured at 10 cm depth, was reduced by
153 88% from spring to summer, reaching very low values in the dry season (Fig. S1).

154 The sand mantle is mostly covered by Mediterranean scrub vegetation with local patches of
155 the original *Juniperus* and *Quercus* woodlands (Muñoz-Reinoso & García Novo 2005). To
156 represent the dominant vegetation of the studied sandy ecosystem, we selected fourteen
157 woody plant species, with contrasting functional traits. The categorical functional traits
158 considered were: growth form (GF), water requirements (WR), leaf longevity (LL) and leaf
159 form (LF) (Table 1). The following classes within each trait were considered: GF – shrub or
160 tree; WR – xerophytic, mesophytic or hygrophytic; LL – summer semi-deciduous, evergreen,
161 aphyllous; LF – narrow, broadleaf, needle, scale, spike/aphyllous. The sampled species and
162 their functional classification (based on (Díaz Barradas et al. 1999; Zunzunegui et al. 2005;
163 Zunzunegui et al. 2011; Castroviejo 2012) are described in Table 1. To better characterize
164 the woody community at landscape level, and encompass eventual local heterogeneity, we
165 consider a study area covering the prevalent dune formations (of ~7 km²). Eighteen sampling
166 plots (20 m x 20 m), separated by at least 200 m, were distributed throughout the study area.
167 In each plot, we sampled 3 to 4 species, belonging to contrasting functional types. We assured
168 that, within each plot, species of: both GF classes, at least two WR classes (except in one
169 plot), at least two LL classes, and at least two LF classes were sampled (Table S1). When

170 possible, we sampled three individuals per species within plot (replicates), resulting in a total
171 of 180 (adult) individuals sampled in each sampling date (Table 1, Table S1).

172

173 Analysis of xylem and sources water $\delta^{18}\text{O}$

174 To access the water being used by plants, lignified woody stems of each plant individual
175 selected were collected in two contrasting water conditions: spring (humid) and summer (dry)
176 (Fig. S1).

177 To determine available water sources for the roots, water samples were collected from soil,
178 rain and groundwater. Rainwater was sampled on one pluviometer equipped with liquid
179 paraffin to prevent evaporation (at 36°59'19"N, 6°26'35"W). We considered the rainwater
180 collected over the 2 weeks prior to stem sampling. Groundwater samples were collected in
181 piezometers or wells reaching the water table on the two periods of stem sampling. Mean
182 depth to water table was 2.8 and 3.9 m in spring and summer sampling dates, respectively.
183 Soil samples were collected at 3 depths: 10 cm, 30 cm and 50 cm in each sampling plot with
184 3 replicates per depth, on the same days of stem sampling. Having in mind the extreme
185 conditions of Mediterranean dune sites, especially in summer, soil and twig samples were
186 immediately stored in airtight vials, sealed with a cap and parafilm and placed in a cool ice
187 chest until they could be stored. All samples were kept in the fridge until isotopic analysis.

188 Water from xylem and soil samples was extracted using a custom-made cryogenic vacuum
189 distillation system housed at SIAF (Ehleringer & Dawson 1992; West et al. 2007). All
190 samples were kept at 4°C until isotopic analysis. When the isotopic fraction of the available
191 water sources (soil water, rain, and groundwater) is known, an analysis of the oxygen isotopic
192 composition of xylem water provides information about the water sources being used by the
193 plant at the time of study (Ehleringer & Dawson 1992; Dawson 1993; Dawson & Pate 1996).
194 We used stem water $\delta^{18}\text{O}$ values (rather than δD) as a measure of plant water sources because
195 xerophytic plants might be able to discriminate against deuterium during soil water uptake
196 by roots (Ellsworth & Williams 2007). Oxygen stable isotope ratio analyses were performed
197 by headspace equilibration, on an Isoprime (Micromass, UK) SIRMS, coupled on continuous
198 flow mode to a Multiflow (Micromass, UK) auto-sampler and sample equilibration system.
199 Analytical uncertainty is <0.1‰.

200 Soil water at 10, 30 and 50 cm depth and groundwater are from now on named SW10, SW30,
201 SW50 and GW respectively.

202

203 Quantification of water sources used by plants

204 The relative contribution of different water sources to the composition of the xylem water
205 was estimated by Bayesian stable isotope mixing models using the graphical user interface
206 and model framework MixSIAR for R (Parnell et al. 2013; Stock et al. 2016). In our study,
207 the model used as input the individual isotope values of xylem water $\delta^{18}\text{O}$ of each plant
208 ('mixture' raw data), all sources described in water sources (mean plus standard deviation)
209 and a matrix for potential fractionation (set to 0). We calculated the water use proportion in
210 the two seasons and in each plot separately, using an individual-based model to obtain
211 separate estimates for each of the plants. We ran models using the individual plant ('ID'
212 within the 'plot') values of xylem water $\delta^{18}\text{O}$, the sampling plot water sources mean (plus
213 standard deviation) and the 'process only (n=1)' error structure option. We set the Markov
214 Chain Monte Carlo to 'long' or 'very long' model run, until the diagnostics were satisfying.
215 Spring precipitation was (prior) aggregated with SW10 because their isotopic composition
216 was similar. Thus, spring mixing model ran with only 4 sources (Precipitation+SW10, SW30,
217 SW50 and GW). Summer mixing model ran with the 4 sources sampled (SW10, SW30,
218 SW50 and GW), since it didn't rain in the two weeks prior to stem sampling (Fig. S2). Details
219 of extremely enriched xylem water samples are given in Appendix 1.

220

221 Physiological parameters

222 The following ecophysiological traits were measured in all plants (n=180) (Table 1, Table S1)
223 in both spring and summer sampling dates (Fig S1).

224 *Leaf carbon isotope composition ($\delta^{13}\text{C}$)*

225 Leaf $\delta^{13}\text{C}$ is a good proxy of leaf-level intrinsic water use efficiency (WUEi), which is given
226 by the ratio between leaf net photosynthetic rate (A) and g_s (Farquhar et al. 1989; Dawson et
227 al. 2002). Mature leaves (2-10, depending on leaf size) were collected from each plant. The
228 bulk leaf samples were dried at 60 °C for at least 48 h, and milled to fine powder in a ball
229 mill (Retsch MM 2000, Germany) for isotopic analysis. Leaf $\delta^{13}\text{C}$ was determined by
230 continuous flow isotope ratio mass spectrometry (CF-IRMS) on a Sercon Hydra 20-22

231 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector,
232 Italy) elemental analyser. Uncertainty of the isotope ratio analysis, calculated using values
233 from 6 to 9 replicates of secondary isotopic reference material interspersed among samples
234 in every batch analysis, was $\leq 0.1\%$.

235 *Reflectance water index*

236 We used a nondestructive optical method, based on the reflectance of light by an intact leaf.
237 Spectral reflectance was measured using a UniSpec Spectral Analysis System (PP Systems,
238 Haverhill, Massachusetts, USA) and carried out in 6 full expanded leaves per plant. The mean
239 value of the 6 measurements per plant was considered to calculate:

240 Water Index (WI) = $R900/R970$, where $R900$ and $R970$ are the reflectance at wavelengths of
241 900 nm and 970 nm (Peñuelas et al. 1997). This index is highly related with plant water
242 content, so it can be used as a proxy for water status in the plant (Claudio et al. 2006).

243 Additionally, the plant water content (PWC) was calculated as: $PWC(\%) = -620 + 684 * WI$
244 (following Peñuelas et al. (1997)).

245

246 Statistical analysis

247 To explore the influence of different traits on the patterns of plants' water-source use, firstly
248 we performed Principal Component Analyses (PCA) based on all individuals information
249 (n=180), using (i) the relative contribution of the water sources to xylem water per season
250 (Fig. S3ab), and (ii) the seasonal differences of the relative contribution of the water sources
251 (spring to summer variation, i.e. summer minus spring values) (Fig.S3c). Positive values
252 represent an increase of the proportions of water-source use from spring to summer, and
253 negative values a decrease from spring to summer. Secondly, we tested if these water-source-
254 use patterns were dependent on growth form (GF), water requirements (WR), leaf longevity
255 (LL) or leaf form (LF). For that, based on the individual scores of PCAs' first axis (PC1), we
256 performed an analysis of variance (factorial ANOVA) to test the main effects of the four
257 categorical functional traits (GF, WR, LL and LF) on: (i) the water used by plants in spring
258 ($PC1_{spring} \sim GF+WR+LL+LF$), (ii) the water used by plants in summer
259 ($PC1_{summer} \sim GF+WR+LL+LF$), and (iii) the water-sources-use seasonal variation (summer
260 minus spring) ($PC1_{diff} \sim GF+WR+LL+LF$). Lastly, we explored the differences of water-
261 sources used between plant functional groups. To do so, we used a priori functional

262 classification for each plant as the concatenation of the four trait variables
263 (GF&WR&LL&LS). We performed Tukey HSD post-hoc multiple comparisons of means to
264 test differences in water-sources-use between the a priori functional groups (PC1~FG_{4traits};
265 with PC1_{spring}, PC1_{summer} and PC1_{diff} as dependent variables). Then, we used the grouping
266 resulting from the post-hoc information to define the new (a posteriori) functional groups of
267 water-source use.

268 We further tested the relationships between the proportions of deep soil use (i.e. the
269 proportion of water from deeper than 0.5 m soil layers used by plants, i.e. % of GW use) and
270 the physiological parameters (leaf $\delta^{13}\text{C}$ and WI in spring and summer, and seasonal variation
271 of leaf $\delta^{13}\text{C}$ and PWC) through Pearson correlations (for the community and per *a posteriori*
272 functional group). We also explored the differences in the physiological parameters (leaf
273 $\delta^{13}\text{C}$ and WI) and in the seasonal variation of leaf $\delta^{13}\text{C}$ and PWC among the functional groups
274 by performing an analysis of variance (ANOVA) and post-hoc Tukey HSD. Preliminary
275 analyses were performed to ensure that there was no violation of statistical assumptions.

276 All statistical analyses were performed in R 3.2.3 (R Core Team, 2015).

277

278 **Results**

279 A greater similarity between species' water-source use was found in periods of higher water
280 availability (spring). In this season, we only found a significant effect of growth form on
281 plants' water-source use ($F = 173.63$, $p < 0.0001$, Table 2). The composition of xylem water
282 of shrubs was better explained by precipitation mostly present at SW₁₀, while water used by
283 trees was mostly from soil layers between 30 and 50 cm (Fig. 1a). At this period, some
284 individuals of the xerophytic tree *J. phoenicea* were using a high percentage of groundwater
285 (>60%) (Fig. 1a). During the dry season (summer), we observed a high range of water sources
286 used, from shallower to deeper soil layers, depending on plant functional traits. Growth form,
287 water requirements, leaf longevity, and leaf form showed a significant effect on the sources
288 of water used in summer (Table 2). After testing differences in water-source use, the
289 following new functional groups were defined: xerophytic shrubs with narrow leaves (*NXs*),
290 semi-deciduous mesophytic shrubs (*sMs*), aphyllous/spike or semi-deciduous broad-leaf
291 shrubs (*Os*), evergreen hygrophytes (*eH*) and evergreen xerophytic trees (*eXt*) (Fig. S4b).
292 These functional types acquired water from different soil depths, with their reliance on deeper

293 soil water increasing in the order of: $NXs < Os < sMs < eH < eXt$ (Fig. 1c). Hence, the highest
294 use of shallow soil layers was observed in xerophytic shrubs with narrow leaves. Evergreen
295 xerophytic trees (eXt) were the functional group that showed a higher use of deep soil layers
296 (Fig. 1c). *Erica scoparia*, an eH shrub species, showed a wide range of water-source use
297 under dry conditions (Fig 1b).

298 Although we found no seasonal differences in overall community water-source use, plants
299 presented different seasonal variation in water sources' used depending on particular
300 functional traits (Fig. 2, Fig S4c). Similarly to summer, species' seasonal adjustments in
301 water-sources were influenced by growth form, water requirements, leaf longevity and leaf
302 form (Table 2). Accordingly, the following functional groups were identified: NXs , Os , sMs ,
303 eXt , eHt and eHs (Fig. 2, Fig. S4). Each of these functional groups showed a distinct pattern
304 of seasonal (spring to summer) water-source-use variation (Fig. 2). The highest seasonal
305 variation towards groundwater table in summer was observed in evergreen hygrophytic
306 shrubs and trees (eHt by substituting SW50 and eHs by substituting SW10). Contrastingly,
307 NXs showed the lowest seasonal variation towards deeper soil layers (Fig. 2), and, unlike
308 other shrubs, a very high use of shallow soil layers in the dry period (Fig. 1).

309 Lower use of water from deep soil layers (i.e. greater use of shallow soil layers) showed to
310 be related with lower values of water use efficiency ($\delta^{13}C$) and higher values of plant water
311 status (WI) in spring (Fig. 3a, Fig. S5a). In this period eXt plants showed the highest values
312 of mean $\delta^{13}C$ (higher stomatal control) and lower values of WI (Fig. 3a). Contrastingly, in
313 summer, the high reliance on water from deep soil layers of eXt and eH implied a higher
314 mean WI (Fig. 3b). However, no significant correlations were observed for $\delta^{13}C$ patterns (Fig
315 3b, Fig. S5a). Although a high intra-group variability was observed, there was a better
316 seasonal maintenance of plant water content values (positive spring to summer variation) in
317 the eXt and eHt groups (accompanied by low mean seasonal enrichment of leaf $\delta^{13}C$) (Fig.
318 3c). Thus, higher water uptake depth during the dry sampling was positively correlated with
319 a summer increase of water content (Fig. 3c, Fig. S5b).

320

321

322 **Discussion**

323 Our results showed evidence of great soil water partitioning under water limited conditions.
324 Although overall woody community seasonally maintained the mean use of water sources,
325 relevant water- uptake depth adjustments were made by different functional groups. Thus,
326 coexisting plant functional types in this semi-arid Mediterranean ecosystem segregate along
327 a wide spectrum of contrasting water source use under extremely dry conditions.

328

329 *Water sources partitioning under drought*

330 At periods of high rainwater availability at shallow soil layers (spring), a higher similarity of
331 water sources use between species was found. Contrastingly, in the dry season, we observed
332 segregation in the sources of water used by woody species in a semi-arid dune ecosystem
333 (Fig. 1). Even though water sources-use differentiation among functional types, a mechanism
334 to reduce competition for limited resources and contribute to patterns of coexistence in
335 woody community, has been long supposed in this ecosystem, empirical verification has been
336 lacking. Based on differences of summer water uptake depth among species, we were able to
337 identify five distinct water-sources-use strategies, from a strong reliance on soil water from
338 the upper layer to water uptake predominately from deeper soil layers (Table 3). Thus, our
339 results supported a soil water partitioning among several functional types in response to
340 changes in water content (Jackson et al. 1999; Filella & Peñuelas 2003; Nippert & Knapp
341 2007; Palacio et al. 2017).

342 In periods of higher water availability, shrubby species (regardless being evergreen or semi-
343 deciduous and hygrophytic or xerophytic) explore shallow soil layers where precipitation is
344 available, and trees explore slightly deeper soil layers, where moisture is still high in spring.
345 Although, under this wetter conditions, individuals of *J. phoenicea* seems to make a greater
346 use [consistent with previous water use studies in similar species that pointed to deeper
347 rooting system of *Juniper sp.* (West et al. 2007, 2008; Grossiord et al. 2017)], most species
348 were mostly dependent on <50 cm soil layers. These results point to the importance of water
349 from upper soil layers in spring and a benefit of rainfall pulses for several species (as also
350 seen in Williams & Ehleringer 2000, Chesson et al. 2004 and Grossiord et al. 2017). When
351 water is not limiting, several species can exploit the same water resource, and commonly an
352 increased competition among coexisting species for available soil water exists without great
353 implications to plants performance (Fig. 3).

354 Species differences in water uptake depth developed during the dry summer, a seasonal shift
355 also observed previously in other dry ecosystems (West et al. 2007; Saha et al. 2009;
356 Grossiord et al. 2017; Palacio et al. 2017). During the drier period, when precipitation is very
357 scarce or absent, despite similar growth form, the xerophytic shrubs show reliance on water
358 in surface soil layers, while evergreen hygrophytic shrubs explored deeper soil layers
359 (exhibiting greater shifts of water sources used in response to reduced water availability)
360 (Fig. 2). Thus, growth form was not the only trait influencing plants' water-source use under
361 summer dry conditions. Water requirements, leaf longevity and leaf form (particularly in
362 xerophytic shrubs) were shown to be important traits for water sources explored. We inferred
363 a common strategy among xerophytic shrubs with narrow leaves, competing for water in
364 surface soil layers even during periodic drought. In agreement with other studies (Álvarez-
365 Cansino et al. 2010; Moreno-Gutiérrez et al. 2012), we showed that this functional group
366 tended not to use water from deep layers of soil nor groundwater as a main water source
367 throughout the seasons, showing a particularly distinct signature of xylem water. Evergreen
368 hygrophytic shrubs and xerophytic tree species, by contrast, explored deeper soil layers,
369 possibly avoiding competition at surface layers when this water resource is limiting and
370 exploring a more available water source. Although a considerable intra-specific variability
371 in seasonal shifts was observed, both studied evergreen xerophytic trees responded to
372 (seasonal) precipitation reduction in a similar way, which is not in agreement with other
373 *Juniperus-Pinus* studies (Grossiord et al. 2017; but see Williams & Ehleringer 2000).
374 Furthermore, the individual short-term (seasonal) changes in water-uptake depth point to the
375 existence of an active dual root system in these functional groups. A dimorphic root system
376 is considered a major advantage in water limited habitats, allowing plants to explore water
377 from different soil layers, to take advantage of any rain event and of water from a deep soil
378 profile (Ehleringer & Dawson 1992; Dawson & Pate 1996; Rossatto et al. 2012; Barbeta et
379 al. 2015). Although it has been shown that under these conditions, once shallow soils become
380 dry, trees may switch water access and subsequently garner water from deep sources, we
381 shown that also certain shrub species can do this water sources use shifts in this ecosystem.

382

383 *Linking water sources use to drought strategies*

384 The summer shifts in water source use towards deeper soil layers can be seen as a way to
385 explore a more stable water supply, thus supporting evergreen trees and hygrophytes water

386 demand and seasonal maintenance of their water statuses (Fig. 3). We confirmed that, not only
387 important differences in water sources use exist, but that they are linked to general strategies
388 to cope with water stress in this ecosystem. The observed water use trends agreed well with
389 the previously observed seasonal changes in water potential and leaf physiology (Zunzunegui
390 et al. 2005, 2011). This indicates an important relationship between the depth of water
391 extraction and leaf physiological traits in this semi-arid Mediterranean ecosystem.
392 Xerophytic shrubs with narrow leaves (mostly semi-deciduous species, but including the
393 evergreen *C. album*), that showed a distinct water-source-use in our study, were previously
394 considered as poikilohydric-type, with a pronounced decrease in water potential and a severe
395 drought stress (Zunzunegui et al. 2005, 2011). The absence of a summer shift to deeper soil
396 layers denote a low capacity of this functional group to change to more available water
397 sources, which forces them to a great morpho-physiological specialization targeted to greater
398 photosynthetic activity and water status in the peak of growing season (spring) (Fig. 3). This
399 is an adaptive trait for small-sized shallow rooted plant species heavily dependent on
400 fluctuating shallow soil water pool that is subjected to rapid evaporation (Schwinning &
401 Ehleringer 2001; Alessio et al. 2004; Saha et al. 2009; Moreno-Gutiérrez et al. 2012; Sardans
402 & Peñuelas 2013). The summer semi-deciduous mesophytic shrub *H. halimifolium* was
403 previously assigned to the center of an avoidance-tolerance gradient, pointed out as a species
404 of wide plasticity and with different roots' depth depending on the topographic position
405 (Zunzunegui et al. 2002, 2009, 2011; Wegener et al. 2015). This corroborates with our
406 observation of high intra-specific variability of summer water sources (and low mean
407 seasonal water use variation) and the observed high adjustment in water use efficiency
408 during dry season. Consistent with the previously defined conservative water-use strategy of
409 evergreen sclerophyll species in hygrophytic sites within this ecosystem (Zunzunegui et al.
410 2011), we observed a summer maintenance of water status of hygrophytes. The higher
411 proportion of water obtained from deeper soil layers during the dry season observed for the
412 studied evergreen hygrophytic species seems to allow the avoidance of severe water stress,
413 and further support the presence of turgid leaves throughout the year (Merino et al. 1995;
414 Zunzunegui et al. 2011). Thus, we demonstrated that, similarly to other studies, the depth of
415 soil water uptake is related to broader water-use strategies, and associated with plant habit,
416 leaf phenology and growing period (West et al. 2008; Moreno-Gutiérrez et al. 2012;
417 Schwendenmann et al. 2015; Palacio et al. 2017). Further, we have shown that the same

418 fundamental tradeoff between growth capacity and low-resource tolerance that separates life
419 forms (Chapin et al. 1993) is found within what is commonly considered to be one plant
420 functional type. The degree of separation or aggregation between species based on water-
421 sources-use strategies was related to a differential response to environmental water variation
422 and their water-use strategies. Additionally, species may exhibit multiple strategies,
423 purportedly to reduce competition and facilitate resource acquisition (Schenk 2006;
424 Valladares & Sánchez-Gómez 2006; Angert et al. 2009; Moreno-Gutiérrez et al. 2012;
425 Pivovarov et al. 2016). Thus, the functional groups identified in our study end up exhibiting
426 a trade among traits, each contributing to alleviate a part of the plant stress. Their water
427 strategies result, therefore, from a combination of leaf level and water uptake depth
428 adjustments under drought.

429 *In conclusion*, unlike spring, a high inter-specific differentiation in water source use was
430 observed during the dry. In the studied ecosystem, under extremely dry conditions, coexisting
431 plant functional types segregated along a wide spectrum of water source use. Woody species
432 clustered in five water-sources-use functional groups, exploring soil water from shallower to
433 deeper soil layers. This segregation in water-sources use was observed not only in different
434 growth forms, but also among species with different water requirements, leaf longevity and
435 leaf form characteristics. The highest use of shallow soil layers was observed in narrow-
436 leaved xerophytic shrubs, while evergreen xerophytic trees and evergreen hygrophytic
437 species showed a higher use of deep soil layers. This contrasting (and potentially
438 complementary) water source use should foster plant species coexistence through temporal
439 and spatial segregation of the resource that is most limiting to vegetation (i.e. soil moisture)
440 in this ecosystem. Accordingly, relevant seasonal shifts in the water sources used towards
441 deeper soil layers were observed in summer, but not in all functional types. These water
442 uptake depth adjustments were associated with a more favorable seasonal maintenance of
443 plant water status. Furthermore, our observations confirmed that the water-sources-use
444 behavior accompanied broader strategies of drought resistance.

445 It is noteworthy to mention that the high intra-specific variability within summer season
446 showed by particular functional groups indicates that other fine-scale
447 (hydrological/environmental) factors can be playing an important role on their water-sources-
448 use patterns (Araya et al. 2011; Voltas et al. 2015). Thus, further work is needed to better

449 understand functional groups' and overall community water use and strategies along
450 ecohydrological gradients in these semi-arid ecosystems.

451

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464

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645

646 SUPPORTING INFORMATION

647 **Fig S1.** Study site: sampling points and climatic conditions.

648 **Fig. S2** Isotopic composition ($\delta^{18}\text{O}$) of xylem water of the studied species and of the water
649 sources considered at spring and summer (dry) season.

650 **Fig S3.** Principal Component Analyses considering: (a) spring water-use, (b) summer water-
651 use and (c) seasonal variation of water-use.

652 **Fig S4.** Functional groups classification, based on grouping of trait-categories, considering:
653 (a) spring water-use, (b) summer water-use and (c) seasonal variation of water-use.

654 **Fig S5.** Relationships between physiological parameters ($\delta^{13}\text{C}$ and plant water index) and the
655 proportion of deep soil (>50 cm deep) water use (%).

656 **Table S1.** Plants, functional types and species sampled by plot.

657 **Appendix 1.** Xylem water $\delta^{18}\text{O}$ isotopic enrichment of narrow-leaved xerophytic shrub
658 species in summer.

659

660 **Table 1.** Species, species' family, code, number of sampling individuals in each sampling date (*n*)
661 and traits considered: growth form (GF), water requirements (WR), leaf longevity (LL) and leaf form
662 (LF). Classes considered were: GF – shrub (shrub) or tree (tree); WR – xerophytic (xero), mesophytic
663 (meso) or hygrophytic (hygr); LL – summer semi-deciduous (semd), evergreen (ever), aphyllous
664 (aphy); LF – narrow (narr), broadleaf (brle), needle (need), scale (scal), spike/aphyllous (aphy).
665 Classification based on Díaz Barradas et al. (1999), Muñoz-Reinoso & García Novo (2005),
666 Zunzunegui et al. (2005, 2011) and Castroviejo (2012).

Species	Family	code	GF	WR	LL	LF	<i>n</i>
<i>Cistus libanotis</i>	Cistaceae	Cl	shru	xero	semd	narr	3
<i>Cistus salviifolius</i>	Cistaceae	Cs	shru	hygr	semd	brle	3
<i>Corema album</i>	Ericaceae	Ca	shru	xero	ever	narr	8
<i>Erica scoparia</i>	Ericaceae	Es	shru	hygr	ever	narr	33
<i>Halimium calycinum</i>	Cistaceae	Hc	shru	xero	semd	narr	14
<i>Halimium halimifolium</i>	Cistaceae	Hh	shru	meso	semd	brle	42
<i>Juniperus phoenicea</i>	Cupressaceae	Jp	tree	xero	ever	scal	24
<i>Lavandula stoechas</i>	Lamiaceae	Ls	shru	xero	semd	brle	6
<i>Phillyrea angustifolia</i>	Oleaceae	Pa	shru	hygr	ever	brle	4
<i>Pinus pinea</i>	Pinaceae	Pp	tree	xero	ever	need	23
<i>Quercus suber</i>	Fagaceae	Qs	tree	hygr	ever	brle	5
<i>Rosmarinus officinalis</i>	Lamiaceae	Ro	shru	xero	semd	narr	9
<i>Stauracanthus genistoides</i>	Fabaceae	Sg	shru	xero	aphy	aphy	3
<i>Ulex australis</i>	Fabaceae	Ua	shru	xero	aphy	aphy	3

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670 **Table 2.** Results of the variance analysis (ANOVA) conducted to test the effect of growth form (GF),
671 water requirement trait (WR), leaf longevity (LL) and leaf form (LF) on spring water-use, summer
672 water-use and seasonal variation of water-use. Bold represents significant effects ($p < 0.01$).

Effect	df	Spring WU			Summer WU			Seasonal WU variation		
		SS	<i>F</i>	<i>p</i>	SS	<i>F</i>	<i>p</i>	SS	<i>F</i>	<i>p</i>
GF	1	215.55	173.63	<2e-16	66.68	44.50	3.4e-10	26.03	15.17	1.4e-4
WR	2	2.85	1.15	0.32	76.85	25.65	1.8e-10	38.37	11.18	2.7e-5
LL	2	6.96	2.80	0.06	27.68	9.24	1.5e-4	16.81	4.90	0.009
LF	3	8.01	2.15	0.10	29.57	6.58	3.1e-4	21.40	4.16	0.007

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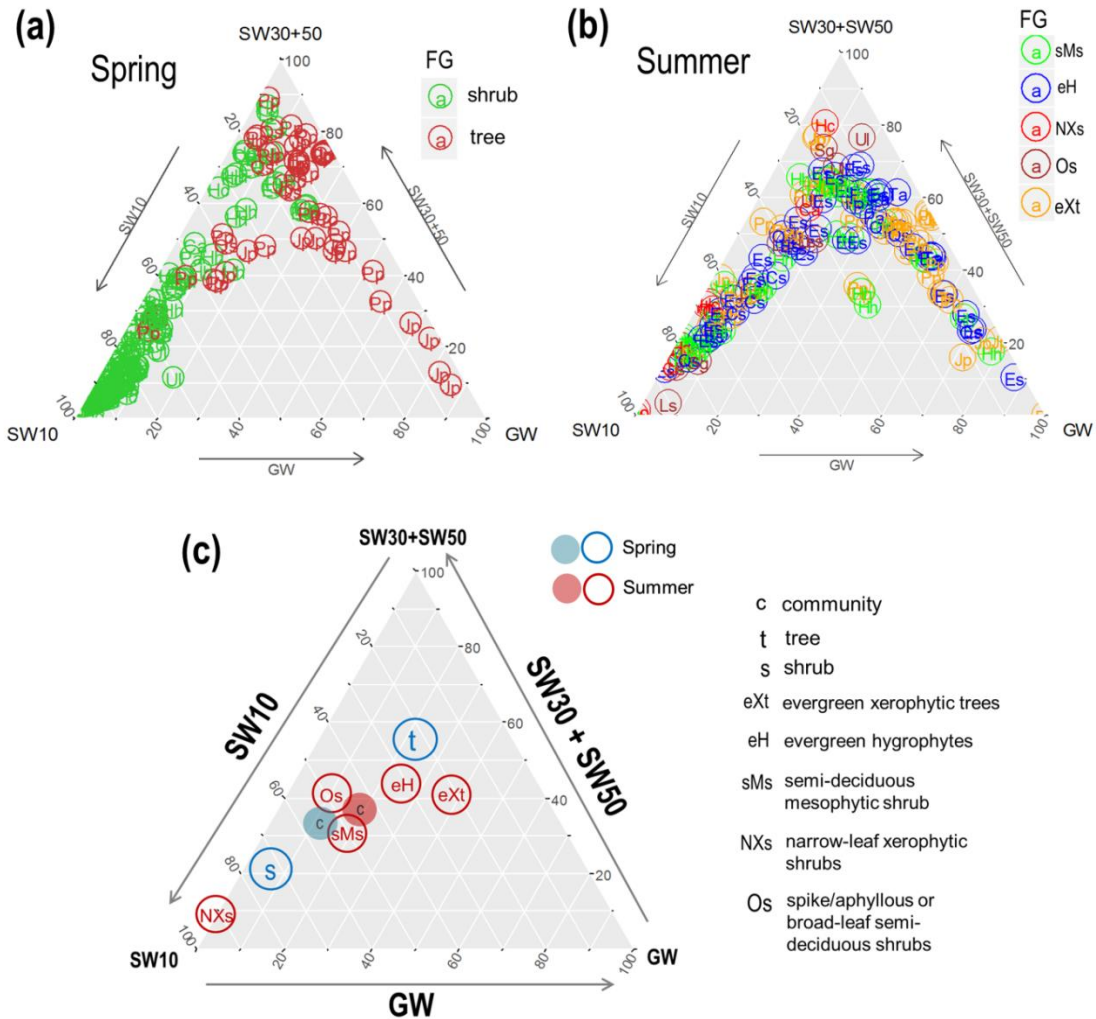
678

679 **Table 3.** Functional classification of the studied species based on water-use patterns. Species code as in Table
 680 1. A priori functional groups (FG) defined as a concatenation of the four trait categories considered in this
 681 study [Leaf form (LF), leaf longevity (LL), water requirements (WR) and growth form (GF)]. For classification
 682 name, the first letter of each trait' classes (Table 1) was used. A posteriori FGs were defined considering the
 683 comparison analysis of water-use (WU) patterns between *a priori* FGs and respective grouping, for spring,
 684 summer and WU seasonal variation (see Fig. S4). An overview of the mean water use and seasonal water
 685 uptake shifts made by each a posteriori WU FG is also provided: the mean water sources used by the different
 686 FGs ranged from shallow to deep soil layers as indicated by dashed arrow; seasonal water uptake shifts are
 687 shown as up- or downward arrows representing the direction of water uptake changes (e.g. downward arrows
 688 indicate seasonal changes to deeper soil layers). See Fig. 2 for functional groups' quantitative water-use in
 689 spring and summer and Fig. 3 for quantitative water-use seasonal variation.

Species	<i>a priori</i> FG	<i>a posteriori</i> FG			Description	Mean		
		Spring WU	Summer WU	WU seasonal variation		sources used	seasonal WU shifts	
Cl	NsXs					shallow		
Hc	NsXs					↓		
Ro	NsXs		NXs	NXs	narrow-leaved xerophytic shrubs		↑	
Ca	NeXs							
Cs	BsHs	shrub						
Ls	BsXs		Os	Os	aphyllous/spike or semi-deciduous broad- leaf shrubs		↓	
Sg	AaXs							
Ua	AaXs							
Hh	BsMs		sMs	sMs	semi-deciduous mesophytic shrub		↔	
Es	NeHs							↓↓↓
Pa	BeHs		eH	eHs	evergreen hygrophytic shrubs		↓	
Qs	BeHt			eHt	evergreen hygrophytic tree	↓↓		
Pp	NEeXt	tree						
Jp	SeXt		eXt	eXt	evergreen xerophytic trees	deep	↓↓	

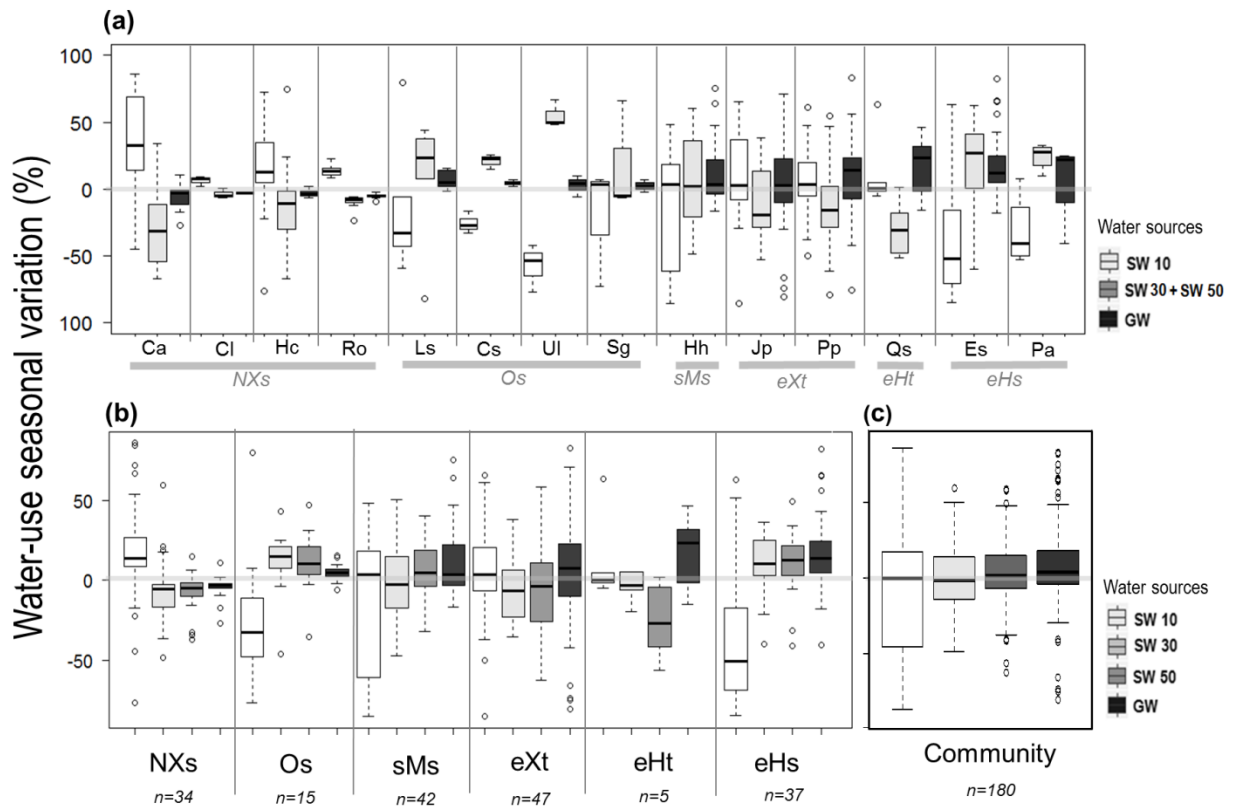
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693 **Fig. 1.** Water sources proportion used by plants in spring and summer. Four main water sources
 694 considered: soil water at 10cm, 30 and 50cm (SW10, SW30, SW50) and groundwater (GW). In
 695 spring, SW10 is also representing rain water. SW30 was aggregated with SW50 (posteriorly to mixing
 696 model calculation), and represented as SW30+SW50. Species grouping and functional classification
 697 was done considering the significant effects of functional traits on water sources used and the
 698 comparison between prior classified plant groups (see Table 3 and Fig. S4ab). **(b)** Spring individual
 699 water source proportion used (%) by the water-use FGs identified in this season (see Table 3): trees
 700 (red) and shrubs (green). Inner labels represent species name (see species code in Table 1). **(c)**
 701 Summer individual water source proportion used by the FGs identified in this season (see Table 3).
 702 Inner labels represent species name (see species code in Table 1). **(c)** Spring (blue) and summer (red)
 703 mean of water source proportion used (%) by the FGs defined for the two seasons (unfilled circle, see
 704 Table 3) and overall community (filled circle).
 705



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707 **Fig. 2.** Water-use seasonal variation (from spring to summer, %) considering: (a) species, (b)
 708 functional groups and (c) overall woody community. Positive values represent higher values in
 709 summer compared to spring, and negative values lower values in summer compared to spring. Water
 710 sources considered were SW10, SW30, SW50 and GW (white to dark grey, as described in inner
 711 legend). (a) Species represented as code name described in Table 1. For figure simplification, SW30
 712 was aggregated with SW50, and represented as SW30+SW50 in this panel. Grey horizontal thick line
 713 below species code indicates the species included in the grouping of (b). (b) Functional groups'
 714 definition was done considering the significant effects of functional traits on seasonal variation of
 715 water use and the comparison between prior classified plant groups (see Table 3 and Fig. S4c):
 716 xerophytic shrubs with narrow leaves (*NXs*), semi-deciduous broad-leaf or aphyllous/spiky shrubs
 717 (*Os*), semi-deciduous mesophytic shrubs (*sMs*), evergreen xerophytic trees (*eXt*), evergreen
 718 hygrophytic tree (*eHt*) and evergreen hygrophytic shrubs (*eHs*). Number of individuals considered
 719 (*n*) represented by functional group.

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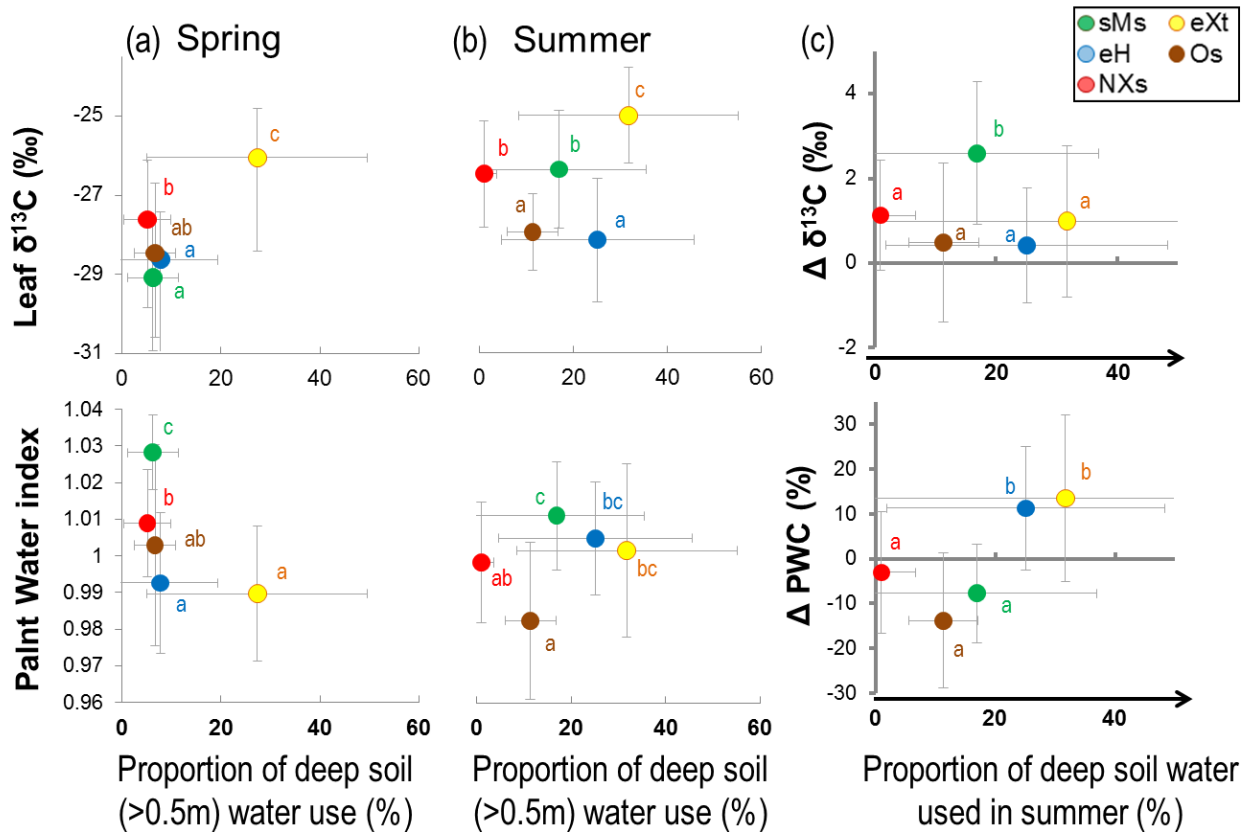
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728 **Fig. 3.** Physiological parameters ($\delta^{13}C$ and plant water index) and the proportion of deep soil (>50
 729 cm deep) water use (%) in (a) spring and (b) summer. (c) Seasonal variation (summer minus spring
 730 values) of the physiological parameters ($\delta^{13}C$ ‰ and plant water content %) with the proportion of
 731 deep soil water (>50 cm deep) used by plants in the summer (%). Each functional type is represented
 732 by a different color as indicated in the inner legend: xerophytic shrubs with narrow leaves (NXs,
 733 n=34), semi-deciduous broad-leaf or aphyllous/spiky shrubs (Os, n=15), semi-deciduous mesophytic
 734 shrubs (sMs, n=42), evergreen xerophytic trees (eXt, n=47), evergreen hygrophytes (eH, n=42). Dots
 735 represent mean values per functional type and bars represent their standard deviation. Significant
 736 differences between functional types are denoted with different letters (* $p < 0.05$).

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