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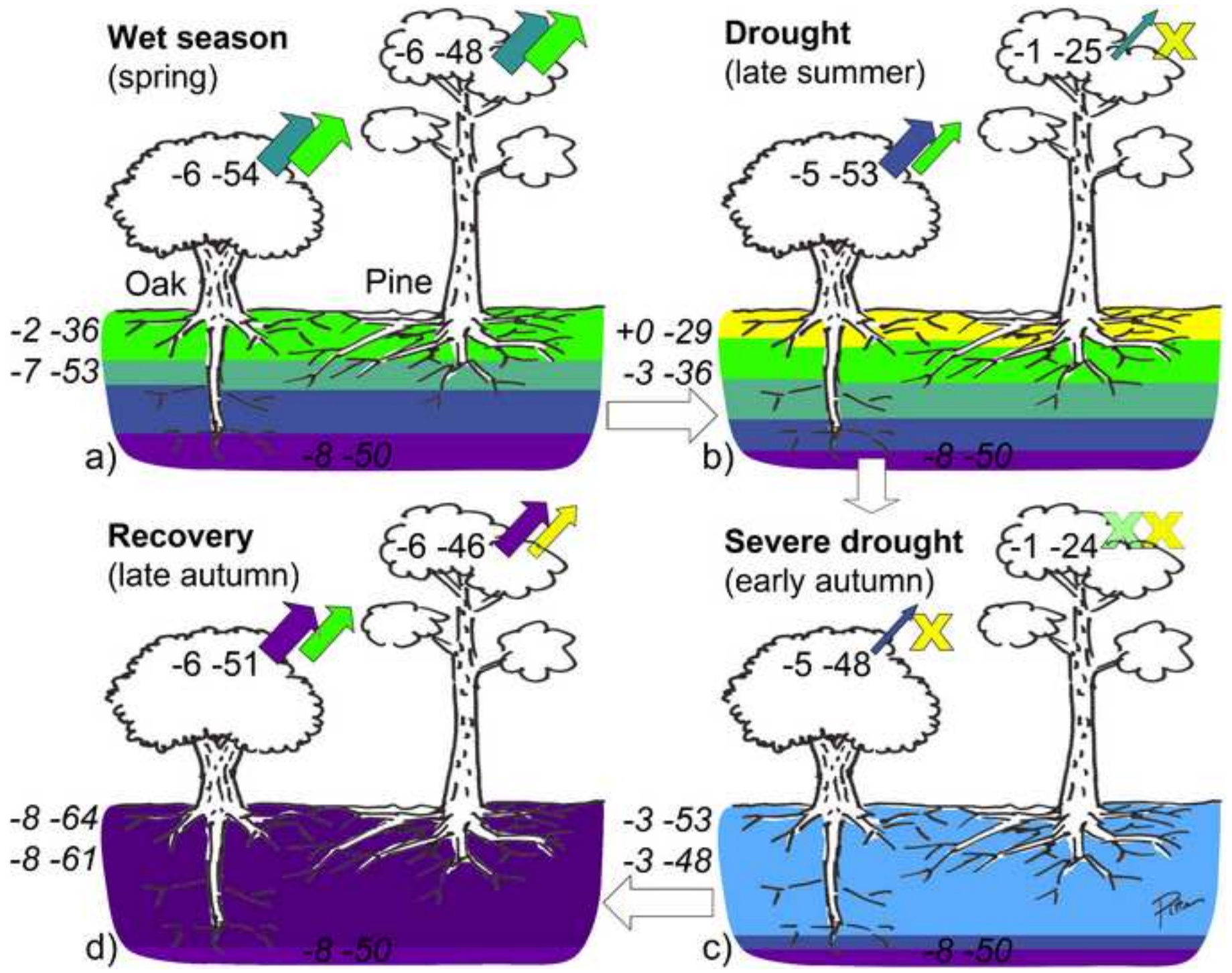
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**Dynamics of competition over water in a mixed oak-pine Mediterranean forest:
spatio-temporal and physiological components**

Jorge del Castillo, Carles Comas, Jordi Voltas, Juan Pedro Ferrio

Highlights

- We studied water-use interactions in a holm oak-Aleppo pine mixed forest
- Stable isotopes revealed species-specific seasonal patterns of water uptake
- Two levels of interaction emerged: spatial and physiological (dynamic)
- Intra-specific interactions were more dynamic than inter-specific interactions



1 **Dynamics of competition over water in a mixed oak-pine Mediterranean forest:**
2 **spatio-temporal and physiological components**

3

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20

21 **Abstract**

22 Understanding inter- and intra-specific plant interactions and competition over water is
23 challenging because of the lack of effective approaches for accessing and monitoring
24 root distribution and activity. In this context, stable isotopes are excellent eco-
25 hydrological tracers that allow characterizing the dynamics of water uptake patterns in
26 trees and shrubs. Here, we studied biotic interactions for water uptake between two
27 typical Mediterranean tree species, Aleppo pine (*Pinus halepensis*) and holm oak
28 (*Quercus ilex*), coexisting in a mixed forest. We measured stable isotope composition
29 ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) of xylem water in all trees found in the studied stand during one
30 growing season, covering an exceptionally long summer drought and subsequent
31 recovery. We applied point-process statistics together with stand density information to
32 evaluate tree-to-tree interactions for water use. In pines, we observed a clear uncoupling
33 between soil and xylem water isotope composition after two months of persistent
34 drought. Conversely, the isotope composition of xylem water in oaks tracked observed
35 changes in the soil during the first two months of drought, but began to depart from soil
36 values after three months. These results suggest that during drought the oaks were able
37 to keep active for longer using alternative soil water sources, not available for the pines.
38 Point-process statistics revealed more positive isotope compositions at distances below
39 4-6 m, but only between con-specific individuals (i.e. pine-pine, oak-oak). These intra-
40 specific responses were first seen in the pines (after two months of drought) and
41 subsequently in oaks (after three months), coinciding with the onset of soil-xylem
42 uncoupling for each species. On the other hand, the isotope composition of individual
43 oaks decreased with increasing neighbor pine density, but increased in response to oak
44 density. Conversely, the pines showed more positive values with increasing oak density.
45 Our results suggest that the use of shallow water in oaks is limited by the presence of

46 pines, which force them to shift to deep-soil water use, whereas pines have more
47 restricted access to deep water in the presence of oaks, leading to more positive isotope
48 values. According to the dynamics of interaction patterns, we conclude that inter-
49 specific differences in pine-oak mixed forests hold two components: a static, spatial
50 component determined by root distribution, and a dynamic, physiological component
51 related to water uptake capacity within the soil profile.

52

53 **Keywords:**

54 plant-to-plant interactions; point patterns; stable isotopes; *Pinus halepensis*; *Quercus*

55 *ilex*; ecohydrology

56

57 **1. Introduction**

58 The composition and dynamics of forest systems in the Mediterranean basin are likely
59 to undergo substantial changes due to the predicted increase in frequency of drought and
60 heatwaves for the next decades (IPCC 2013). In this region, subtle variations in water
61 availability may have profound effects at the ecosystem level (Bellot et al. 2004; Filella
62 and Peñuelas 2003; Zavala et al. 2000). A typical situation in coastal ranges along the
63 western Mediterranean basin is the formation of pine-oak mixed forests (Gil et al. 1996;
64 Klein et al. 2013). Mediterranean pines, as water-saving, drought-avoiding species,
65 shares space and resources with drought-tolerant, less conservative species such as
66 evergreen oaks (Lookingbill and Zavala 2000; Zavala et al. 2011, 2000). Traditionally,
67 pine-oak mixed forests have been considered as transient states in ecological succession
68 towards an oak-dominated climax vegetation (Rivas-Martínez 1987). However,
69 increasing evidence points towards niche segregation as a mechanism determining the
70 long-term persistence of these communities (Zavala et al. 2000; Zavala and Zea 2004).
71 Yet, it is a matter of debate whether facilitation or competition define species co-
72 existence (Díaz-Sierra et al. 2010; Maestre et al. 2005; Zavala and Bravo de la Parra
73 2005), and how resource scarcity could promote or inhibit these two processes (Brooker
74 *et al.* 2008; Craine and Dybzinski 2013). Recent studies have shown that water scarcity
75 increases competition for water in mixed forests (Grossiord et al. 2014), reducing the
76 potential benefits of species co-existence (Jucker et al. 2014). Conversely, other works
77 show evidence of facilitation processes in water-limited environments, e.g. hydraulic lift
78 by deep-rooted species favoring neighbor shallow-rooted species (Prieto et al. 2012;
79 Rodríguez-Robles et al. 2015). So far, studies based on large-scale gradients do not
80 provide a clear answer regarding the ecological effects of water availability on
81 Mediterranean communities (see e.g. Grossiord et al. 2014). In this regard,

82 complementary, stand-based studies are needed considering the highly dynamic nature
83 of water uptake patterns, which may cause an alternation between competition and
84 facilitation processes during the growing season.

85 Understanding the dynamics of inter- and intra-specific belowground interactions and
86 competition for water is challenging because of the lack of effective approaches for
87 accessing and monitoring root distribution and water uptake in trees (Maeght et al.
88 2013; Rewald and Leuschner 2009). In this context, stable isotopes constitute a
89 powerful tool to examine short-term variability in plant water uptake (see e.g. Barbeta et
90 al. 2014; David et al. 2007; Eggemeyer et al. 2008; Filella and Peñuelas 2003; Moreno-
91 Gutiérrez et al. 2012; Palacio et al. 2014; Voltas et al. 2015). In a recent work (Comas et
92 al. 2015), we applied point-process statistics to examine the spatial patterns of stable
93 isotope composition of xylem water in a mixed stand of Aleppo pine (*Pinus halepensis*
94 Mill.) and holm oak (*Quercus ilex* L.) during summer drought. We did not identify
95 inter-specific interactions and, therefore, we concluded that these species occupy
96 distinct niches for water uptake, at least under conditions of limited water availability.
97 However, we determined neither to what extent these conclusions held throughout the
98 growing cycle nor the underlying mechanisms for this apparent niche segregation.

99 In the present study, we hypothesize that inter-specific interactions for water uptake in
100 pine-oak mixed stands are determined by two components: 1) a static, spatial
101 component related to constitutive differences between species (i.e. due to contrasting
102 root distribution); and 2) a dynamic component triggered by water scarcity and linked to
103 differential (physiological) drought responses. Therefore, the main aim of this study is
104 to determine whether clear spatial niche segregation within the soil precludes any inter-
105 specific interaction throughout the growing season, or rather there is prevalence of a
106 more dynamic physiological component, leaving room for competition and/or

107 facilitation mechanisms under certain conditions. For this purpose, we characterized the
108 temporal evolution of spatial interactions for water uptake during one growing season in
109 a mixed pine-oak stand, integrating information from stable isotope composition in soil
110 and xylem water, remote-sensing and other physiological indicators.

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115 **2. Material and Methods**

116 **2.1 Site description**

117 The study area was a mixed forest of holm oak and Aleppo pine located in the Montsant
118 range, northeastern Iberian Peninsula (41° 19' 47.3" N, 0° 50' 2.6" E, 750 m a.s.l). The
119 site is close to the optimal distribution range for both species (topo-climatic suitability
120 index > 0.8; <http://www.opengis.uab.cat/IdoneitatPI/>). The climate in the region is
121 Mediterranean temperate with continental tendency, with a mean annual precipitation of
122 515 mm and mean annual temperature of 12.3 °C. It is characterized by a dry and a
123 relatively warm summer (summer precipitation of 90 mm, mean temperature of 20.9
124 °C) and wet spring and autumn seasons. The forest stand originated from natural
125 regeneration of pine and oak in a former agricultural terrace which, according to the age
126 of the oldest individuals, was abandoned more than 100 years ago. Understory
127 vegetation is relatively scarce and current regeneration of both species is rare.
128 According to USDA soil taxonomy (Soil Survey Staff 2010), soil is a loamy-skeletal,
129 carbonatic, termic, active calcic pachic haploxeroll, with soil depths ranging from 20 cm
130 to 50 cm approximately.

131

132 **2.2 Remote sensing and meteorological data**

133 Meteorological data was obtained from the nearest automatic agro-meteorological
134 station (Ulldemolins, 4 km), maintained by the *Servei Meteorològic de Catalunya*. The
135 station provides a continuous record of precipitation (*P*), air temperature, humidity,
136 global radiation and wind speed, as well as a set of derived variables, including hourly
137 estimations of Penman-Monteith-FAO reference Evapotranspiration (*ET_o*) (following
138 Allen et al. 1998). We estimated daily changes in soil water storage (*SWS*, in mm) using
139 a simple water balance model (Botey et al. 2011), which assumes additive increments

140 during wet days ($P \geq ET_o$) until reaching maximum soil water storage (SWS_{max}), and
141 exponential decay during dry days ($P < ET_o$):

$$142 \quad P_i \geq ET_{o_i} \Rightarrow SWS_i = SWS_{i-1} + P_i - ET_{o_i} \quad (1)$$

$$143 \quad P_i < ET_{o_i} \Rightarrow SWS_i = SWS_{i-1} \times e^{-(ET_{o_i} - P_i) / SWS_{max}} \quad (2)$$

$$144 \quad SWS_i \geq SWS_{max} \Rightarrow SWS_i = SWS_{max} \quad (3)$$

145 Where SWS_i and SWS_{i-1} stand for SWS at days i and $i-1$, respectively, and ET_{o_i} and P_i
146 are daily accumulated values of ET_o and P at day i . The model was run assuming a
147 SWS_{max} of 150 mm, as reported at the catchment level (Candela et al. 2012), including
148 calculations with SWS_{max} of 100 mm and 200 mm as a sensitivity test. SWS after the first
149 recharge period recorded (April 2008) was set to SWS_{max} , and subsequently calculated
150 on a daily basis along the studied period. It should be noted that this model does not
151 account for stomatal limitations of transpiration under increasing drought and, therefore,
152 it is expected to overestimate soil drying in drought-adapted forest species.

153 An independent estimate of changes in soil water availability was obtained from remote
154 sensing data. Seasonal changes in soil water content (SWC, in m^3/m^3) were derived
155 from data acquired from the ESA's Soil Moisture and Ocean Salinity (SMOS) mission,
156 available at <http://www.smos-bec.icm.csic.es>; the dataset is a high resolution (1×1 km)
157 soil moisture delayed product, which contains two layers per day, corresponding to
158 SMOS ascending (6 A.M.) and descending (6 P.M.) passes. Maps are obtained using a
159 downscaling algorithm, which combines the brightness temperature measurements from
160 ESA-SMOS with Land Surface Temperature and Normalized Difference Vegetation
161 Index (NDVI) data from Terra/Aqua MODIS.

162 To assess species-specific vegetation responses during the study period, we compiled
163 biweekly spectral variations of the NDVI from the MODIS Land Subsetted Products,
164 available at the MODIS web service (2014). We collected data from five pixels ($250 \times$

165 250 m) per species, selected among pine-dominated and oak-dominated plots (>80% of
166 species coverage, visually determined from satellite images) within a radius of 8 km
167 from the study site.

168

169 ***2.3 Sample collection and tree mapping***

170 Sampling took place in a rectangular plot with clear boundaries (24 × 37 m) having a
171 strong slope (15-22%) facing west (X-axis), and a gentle slope (3-7%) facing south (Y-
172 axis). Stand density (dbh > 10 cm) was 874 stems ha⁻¹ (pines) and 560 stems ha⁻¹ (oaks).
173 Oak stems from the same stool were considered as a single individual; hence the
174 individual oak density was 370 stools ha⁻¹. Tree position was determined using a high
175 resolution GPS technology (GeoExplorer 6000 Series Handheld, Trimble Navigation
176 Limited, California, USA). The distribution of individuals was found to be totally at
177 random for both species (Comas et al. 2015).

178 We performed six sampling campaigns during one seasonal cycle (from spring 2011 to
179 spring 2012) including all plot individuals (33 oaks and 78 pines). Sampling dates were
180 May 26th, July 20th, September 9th, October 19th, November 18th (2011) and March 27th
181 (2012) (Table 1). For each sampling time, xylem samples were collected from sun-
182 exposed twigs of the upper third of the crown. Xylem was separated from the bark and
183 immediately frozen with dry ice in air-tight tubes for subsequent water extraction by
184 cryogenic distillation, as detailed in Martín-Gómez et al. (2015). Soil samples were also
185 collected from six different points in the plot at two depths: "upper soil" (2-10 cm, A
186 horizon) and "lower soil" (5 cm above the maximum soil depth, ranging between 20 and
187 50 cm, B horizon). Sampling points were determined at random in each sampling time,
188 but attempting to be representative of the spatial heterogeneity within the plot. For a
189 subset of ten representative individuals per species, we also performed two additional

190 campaigns in August 21st 2013 and July 23rd 2015 (Table 1) in which soil and xylem
191 water records were complemented with xylem water potential determined in the same
192 twigs using a pressure chamber (Scholander et al. 1965). Aims of these additional
193 samplings were 1) to assess inter-specific differences in water potential and 2) to
194 determine at the species level whether those trees apparently suffering less water stress
195 (i.e. higher water potential) were those having access to deeper soil water (i.e. lower
196 isotope composition).

197

198 ***2.4 Water isotope analysis***

199 Hydrogen and oxygen isotope ratios of xylem and soil water were determined using a
200 Picarro L2120i analyzer coupled to a high-precision A0211 vaporizer (Picarro Inc.,
201 Santa Clara, California), and expressed in delta (δ) notation (‰) relative to VSMOW
202 (i.e. isotopic composition of oxygen, $\delta^{18}\text{O}$, and hydrogen, $\delta^2\text{H}$). The estimated
203 precision, based on the repeated analysis of four reference water samples, was 0.10‰
204 and 0.40‰ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively. The potential presence of organic
205 contaminants was checked using the post-processing software Picarro Chem-Correct
206 1.2.0, giving in most cases negative results. Nevertheless, for consistency we applied
207 the post-processing correction described in Martín-Gómez et al. (2015) to all samples.
208 In agreement with the low level of contamination, we found a very strong correlation
209 between corrected and uncorrected values ($r^2=0.995$ for $\delta^{18}\text{O}$; $r^2=0.979$ for $\delta^2\text{H}$, $N=$
210 680), with 92% of the samples showing differences lower than 0.4‰ for $\delta^{18}\text{O}$ and 4‰
211 for $\delta^2\text{H}$.

212

213

214 **2.5 Assessment of evaporative processes in soil and xylem water**

215 Evaporation processes lead to enrichment in both isotopes, but cause greater changes in
216 $\delta^{18}\text{O}$ than in $\delta^2\text{H}$. As a result, isotopic values increase along an evaporation line having
217 a flatter slope than the meteoric water in the $\delta^{18}\text{O}$ - $\delta^2\text{H}$ scatterplot. In order to evaluate
218 potential evaporation processes in xylem and soil water we plotted $\delta^2\text{H}$ against $\delta^{18}\text{O}$
219 records, which were compared with the pattern of meteoric (precipitation) water (Craig
220 1961). Under a particular set of environmental conditions, water samples from the same
221 meteoric origin should move along the same evaporation line, whereas distinct
222 evaporation lines would indicate different origins.

223 As a reference, we used the Global Meteoric Water Line (GMWL), which represents the
224 relationship between the two isotopes in global precipitation ($\delta^2\text{H} = 10 + 8 \times \delta^{18}\text{O}$; Craig
225 1961), and a Local Meteoric Water Line (LMWL) built from precipitation records
226 (period 2000-2012) from Tortosa, the nearest station (*ca.* 60 km from the study site) of
227 the *Red Española de Vigilancia de Isótopos en Precipitación* (REVIP,
228 [http://www.cedex.es/cedex/lang_castellano/organismo/centylab/ceta/lineas/07_revip.ht](http://www.cedex.es/cedex/lang_castellano/organismo/centylab/ceta/lineas/07_revip.htm)
229 [m](http://www.cedex.es/cedex/lang_castellano/organismo/centylab/ceta/lineas/07_revip.htm)).

230

231 **2.6 Data analysis**

232 **2.6.1 Analysis of covariance**

233 As preliminary step, a mixed model analysis of covariance (ANCOVA) was applied
234 aimed at identifying spatial anisotropic effects in the isotopic compositions of oxygen
235 and hydrogen. In particular, xylem isotopic records were subjected to ANCOVA
236 considering the fixed effect of species (pine, oak) and the linear variation along the X
237 and Y axes of the two-dimensional space (covariates). The ANCOVA also accounted
238 for heterogeneity of regression slopes at the species level following X and Y directions

239 to check for differential systematic variation in the response variables ($\delta^{18}\text{O}$ and $\delta^2\text{H}$),
 240 i.e. spatial anisotropic effects. In the case of anisotropy in the marginal distribution of
 241 stable isotopes, anisotropic effects may be incorporated in a mark correlation function
 242 (see subsection 2.6.2 on spatial statistics) by allowing differential variation for stable
 243 isotopes along a directional component. However, this approach is not mathematically
 244 trivial; instead, we used an easier correction of anisotropic effects by considering the
 245 residuals of these variables (marks) after assuming a deterministic, species dependent
 246 linear trend across the X- or Y-axis in accordance with the outcome of the ANCOVA.
 247 In this way the resulting pattern of residuals would not be affected by a possible linear
 248 trend, ensuring isotropy. Additionally, the mixed model ANCOVAs for either $\delta^{18}\text{O}$ or
 249 $\delta^2\text{H}$ allowed for heterogeneity of residual variances at the species level, which was
 250 checked by means of a log likelihood ratio test (Verbeke and Molenberghs 2000).

251

252 *2.6.2 Assessment of tree-to-tree interactions: point-process statistics*

253 For each sampling time, the mark correlation function $k_m(r)$ was used to analyze the
 254 spatial point patterns of xylem water isotopes ($\delta^{18}\text{O}$, $\delta^2\text{H}$) associated to each tree
 255 position (Stoyan and Stoyan 1994). This was done separately for oaks and pines. The
 256 mark correlation function $k_m(r)$ describes the spatial structure of marks ($\delta^{18}\text{O}$, $\delta^2\text{H}$) and
 257 an estimator of this function can be written via:

$$258 \quad \hat{k}_m(r) = \frac{1}{2\pi r \hat{\lambda}^2 |A|} \sum_{(x_1, m_1), (x_2, m_2) \in \varphi_m} \frac{m_1 m_2 \kappa(\|x_1 - x_2\| - r)}{e(x_1, \|x_1 - x_2\|) \hat{g}(r) \hat{\mu}^2} \quad (4)$$

259 for a forest stand A with area $|A|$, where φ_m is a marked point pattern, $\hat{\mu}$ is an
 260 estimator of the expectation of $m_1 m_2$, and m_1 is the mark value for tree 1 (say) and x_1
 261 its spatial location, $\hat{\lambda}$ is an estimator of the point intensity, $\kappa(\cdot)$ is the Epanechnikov

262 kernel function, \sum^{\neq} stands for the summation over all pairs such that $x_1 \neq x_2$ and $e(\cdot)$
 263 is the Ripley's factor (Ripley, 1976) to correct for edge effects. When we have $k_m(r) > 1$
 264 this means that the product of marks (i.e. isotope values) between pairs of individuals
 265 with inter-tree distance r is higher than the plot average; conversely, $k_m(r) < 1$ means
 266 that the product of marks is lower than the plot average. Following the outcome of the
 267 ANCOVA (Supplementary Material, Table S1), we corrected for mark anisotropic
 268 effects using the residuals of the species-dependent linear trend through the X-axis.
 269 These residuals were subsequently scaled to avoid negative values. The resulting
 270 marked point pattern therefore ensures isotropy.

271 To characterize the spatial interactions between oaks and pines a derivation of the mark
 272 correlation function proposed by Penttinen et al. (1992) was adopted. Here the point
 273 pattern consists of two distinct tree species together with a mark associated to each tree
 274 position. Penttinen et al. (1992) defined an estimator of this cross-mark correlation
 275 function through:

$$276 \quad \hat{k}_m^{12}(r) = \frac{1}{2\pi r \hat{\lambda}_1 \hat{\lambda}_2 |A|} \sum_{(x_1, m_1) \in \varphi_{m_1}, (x_2, m_2) \in \varphi_{m_2}} \frac{m_1 m_2 \kappa(\|x_1 - x_2\| - r)}{e(x_1, \|x_1 - x_2\|) \hat{g}_{12}(r) \hat{\mu}_{12}^2} \quad (5)$$

277 where φ_{m_i} is the marked point pattern for class 1 (say), $\hat{\lambda}_1$ its point intensity, $\hat{\mu}_{12}$ is an
 278 estimator of μ_{12} (i.e. the expectation of $m_1 m_2$, marks from classes 1 and 2) and
 279 $\hat{g}_{12}(r)$ stands for the cross-pair correlation functions. The interpretation of $\hat{k}_m^{12}(r)$ is
 280 similar to that of $k_m(r)$. It indicates positive correlations for the spatial pattern when
 281 $\hat{k}_m^{12}(r) > 1$, whilst $\hat{k}_m^{12}(r) < 1$ implies negative dependencies. For the Epanechnikov
 282 kernel function, we chose the bandwidth to be equal to $c/\sqrt{\lambda}$, where typically
 283 $c = 0.1-0.2$ (here $c = 0.2$, as suggested by Stoyan et al. 1994).

284 Testing for spatial independence of marked point patterns of oaks or pines was based on
285 a *random marking* approach (Illian et al. 2008). We simulated 199 marked point
286 patterns assuming a random marking approach (marks, isotope composition, are located
287 at random over the fixed tree locations) and an estimator of the aforementioned
288 correlation functions was obtained for each point pattern. We rejected the null
289 hypothesis (marks are spatially uncorrelated) if the resulting (empirical) estimator of
290 this correlation function lay outside the fifth largest and/or smallest envelope values
291 obtained from the set of simulated functions with a significant level of
292 $\alpha = 2 \times 5 / (199 + 1) = 0.05$. For the point pattern consisting of both species together with
293 a mark associated to each tree position we assumed *random labelling* of tree species
294 over the fixed point positions, and then over this new bivariate point pattern, we
295 considered a *random marking* approach to generate independent bivariate marked point
296 configurations (see Penttinen et al. 1992).

297 For the statistical analysis of point patterns we used the package *Spatstat* available in
298 the R software environment (R Development Core Team, 2007).

299

300 *2.6.3 Assessment of density-related interactions*

301 As a complementary spatial approach we examined the effect of local variations in
302 stand density (accumulated basal area, BA, $\text{m}^2 \text{ha}^{-1}$) on the isotope composition of
303 xylem water. To estimate local density, we determined the accumulated BA of all
304 individuals included in a 5 m-radius around each tree, considering separately the BA for
305 each species. Relationships between water isotopes and local density were tested using
306 pair-wise correlations. The large number of simultaneous correlations, testing for these
307 relationships across samplings, called for *p*-value adjustments controlling the false
308 discovery rate (or expected proportion of errors among the rejected hypotheses). To this

309 end, we used a linear step-up adjustment as described by Benjamini and Hochberg
310 (1995). To correct for plot border effects, we calculated weighted correlation
311 coefficients, with a weight ranging from 0 to 1, and proportional to the distance to the
312 border. Assuming a minimum inter-tree distance of *ca.* 0.5 m, all trees located at 4.5 m
313 or more from the border were assigned a weight of 1, then linearly decreasing for
314 individuals at shorter distances (up to weight=0 at distance=0). The same calculations
315 were done for individual density (trees ha⁻¹), as well as applying different radius for
316 determining local density (4 and 6 m). We also tested correlations without correction for
317 border effects, as well as more restrictive corrections (e.g. removing all samples at 3, 4
318 or 5 m from the border). In all cases, we obtained comparable results (not shown).
319

320 **3. Results**

321 ***3.1 Site conditions and NDVI during the 2011-2012 season***

322 The growing season of 2011-2012 was exceptionally dry (Figure 1a). Summer was
323 moderately warm (21.6°C), but accumulated summer precipitation was four-fold lower
324 than average (22 mm). Drought started late in the season (June-July were relatively
325 wet), but extended for three months, until the short but intense rainy season between
326 late October and mid November (175 mm). This was followed by a moderately cool but
327 very dry winter (accumulated precipitation between mid November 2011 and mid
328 March 2012 was only 9 mm).

329 The peak in precipitation during spring 2011 (Figure 1a) translated into near-optimum
330 soil water availability, as reflected in both water-balance and remote-sensing estimates
331 (SWS and SWC, respectively, Figure 1b). However, warm temperatures contributed to
332 reduce soil water availability in early summer as compared to spring (Figure 1b). At this
333 moment, NDVI of oaks and pines started to drift apart, with pines showing lower values
334 (Figure 1c). As drought progressed during late summer (September 2011), NDVI of
335 oaks declined to values close to those of pines. Drought further increased during early
336 autumn (October 2011), when precipitation was nearly absent, leading to a clear
337 reduction in soil water availability (Figure 1b). This was associated with a sharp decline
338 in NDVI (*ca.* 15%) for both species (Figure 1c). At this moment, oaks and pines
339 showed a similar annual minimum NDVI. With the arrival of autumn-winter
340 precipitation (November 2011) NDVI values increased by 30%, reaching their annual
341 maximum. From this time on, a long winter to early spring drought period caused a
342 progressive decline in water availability (Figure 1b). After the first rains in March 2012,
343 we only found a slight recovery in NDVI (Figure 1c).

344

345 ***3.2 Seasonal changes in the isotopic composition of soil and xylem water***

346 We found strong seasonal variations in soil water isotope composition ($\delta^{18}\text{O}$, $\delta^2\text{H}$;
347 Figure 2). Water in the upper soil was generally more enriched than in the lower soil, in
348 agreement with the expected evaporative enrichment within the soil profile. The only
349 exceptions coincide with the two extremes of soil water availability: October 2011
350 (severe drought), and November 2011 (soil water recharge). Remarkably, from
351 September to October 2011, as drought progressed, soil water became isotopically
352 depleted, particularly in $\delta^2\text{H}$ (Figure 2b). Lower soil water was generally more enriched
353 than groundwater, except during the soil recharge event in November 2011 and, for $\delta^2\text{H}$
354 only, in the dry October 2011 (Figure 2b).

355 We also observed species-specific seasonal patterns in xylem water $\delta^{18}\text{O}$ and $\delta^2\text{H}$
356 (Figure 2). During the wet spring and early summer (May-July 2011), isotopic values of
357 xylem water overlapped in both species, falling mostly within the range of lower soil
358 water. However, as the dry season progressed (September-October 2011) we found a
359 clear divergence in isotopic signatures between species. During this period, the xylem
360 water of Aleppo pine became isotopically enriched, whereas the holm oak remained in
361 the range of lower soil water. During drought recovery (November 2011), the isotopic
362 signatures of pines and oaks showed a sharp depletion, but they were still slightly
363 enriched, as compared to soil water. Conversely, after the next significant rain event
364 (March 2012) both species showed more depleted values than the lower soil.

365

366 ***3.3 Seasonal changes in soil and xylem evaporation water lines***

367 The isotopic composition of soil and xylem water showed, in most sampling times, a
368 clear deviation from the Global and Local Meteoric Water Lines (Figure 3). Xylem
369 water in both species followed the soil evaporation line during the wet spring and early

370 summer (May-July 2011; Figure 3a, 3b), as well as during the two water recharge
371 periods (November 2011 and March 2012; Figure 3e, 3f). However, during the long-
372 lasting drought (September-October 2011; Figure 3c, 3d) the xylem water of Aleppo
373 pine departed from the current soil evaporation line, remaining along the evaporation
374 line of soil and xylem values of the previous sampling time (July 2011; Figure 3b).
375 Conversely, in September 2011 (Figure 3c) the xylem water in holm oak tracked soil
376 changes, moving to the new evaporation line of soil water. However, in October 2011
377 (Figure 3d) the xylem water in holm oak began to be decoupled from soil values, and
378 followed a evaporation line similar to that of soil in the previous sampling time
379 (September 2011; Figure 3c).

380 The two field samplings in which xylem water potential was measured were
381 representative of a mild (August 2013) and a dry-warm summer (July 2015)
382 respectively (see Table 1). In August 2013 (mild summer), the isotope composition of
383 xylem water in both species followed the evaporation line of soil water, although
384 showing more depleted values, suggesting access to deeper water reservoirs (Supporting
385 information, Figure S1a). Conversely, we found a decoupling between xylem and soil
386 water records in July 2015 (dry-hot summer), particularly strong for Aleppo pine
387 (Figure S1b).

388

389 ***3.4 Seasonal changes in inter and intra-specific spatial dependencies***

390 The mark correlation function $k_m(r)$ for xylem water isotopes informed about water use
391 inter-dependencies as a function of distance between tree pairs. In autumn and winter it
392 usually showed products of marks higher than the plot average ($k_m(r) > I$) at short inter-
393 tree distance, irrespective of the species, decreasing progressively to values around
394 unity at about 4–5 m (Figure 4). Although individual values were often non-significant

395 (i.e. they did not lie outside the fifth largest envelope), overall they indicate that
396 neighboring trees showed a consistent trend towards larger (i.e. more enriched) isotopic
397 values than expected under lack of inter-dependencies for water-use (Figure 4 c,d,e, and
398 f). This result can be interpreted as a preferential use of topsoil water for trees separated
399 up to 4–5 m, especially in September (initial drought, Figure 4c) for Aleppo pine and
400 November (drought recovery, Figure 4e) for holm oak. Conversely, in late spring and
401 early summer we could not detect clear patterns of tree-to-tree dependencies for stable
402 isotopes. Notably, we did not find any consistent inter-specific spatial interaction
403 according to the bivariate mark correlation function for $\delta^{18}\text{O}$ (Supporting information,
404 Figure S2). The univariate and bivariate mark correlation functions for $\delta^2\text{H}$ followed
405 similar patterns to those observed for $\delta^{18}\text{O}$ (Supporting information, Figure S3).
406 Besides tree-to-tree interactions based on mark correlation functions, we also
407 characterized the effects of local density on the isotope composition of xylem water. For
408 Aleppo pine, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ were not significantly correlated with BA of neighbor pines
409 (Fig. 5a). Conversely, we found consistent increases in both isotopes in response to
410 higher BA of surrounding oaks, although only significant in June and September
411 (Figure 5a). On the other hand, the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of oaks were positively correlated with
412 con-specific local density (oak BA, Figure 5b), whereas they were negatively correlated
413 with the local density of pines (pine BA, Figure 5b).

414

415 *3.5 Water potential and its relation to stable isotopes in xylem water*

416 At the summer campaigns of 2013 and 2015 oaks and pines did not differ significantly
417 in mean water potential, but the former showed a more plastic response (mean and
418 range, for oaks and pines, respectively: -2.5 MPa [-3.5 -1.5], -2.5 MPa [-2.9 -2.0]). At
419 the mild summer of 2013 oaks showed a significantly higher water potential (-2.1 MPa)

420 than at the drier summer of 2015 (-2.9 MPa), whereas the pines did not show significant
421 differences between sampling times. We did not find any significant relationship
422 between water potential and the surrounding local tree density or basal area (data not
423 shown). The relationship between water isotopes and xylem water potential provided
424 contrasting trends across species and years (Figure S4). For pines (Figures S4a,c), we
425 found a strong negative association between xylem water potential and water isotopes at
426 summer of 2013 ($r=-0.83$, $p=0.005$ and $r=-0.89$, $p<0.001$; for $\delta^{18}\text{O}$ and $\delta^2\text{H}$,
427 respectively), but not at summer of 2015. Conversely, for oaks we only found a weak
428 negative association between water potential and isotope composition in holm oak at
429 summer of 2015 (only for $\delta^2\text{H}$; $p=0.051$) (Figure S4d).

430

431 **4. Discussion**

432 ***4.1 Stable isotope composition reflects seasonal dynamics of water uptake***

433 As summarized in Figure 6, *Q. ilex* and *P. halepensis* used similar sources of water
434 during the wet season, and shifted to distinct water sources during the drought period,
435 returning back to a similar source in the following wet season. Similarly, other studies
436 have found larger inter- and intra-specific differences in water uptake during the dry
437 season (Filella & Peñuelas, 2003; Flanagan *et al.*, 1992; Grossiord *et al.*, 2016; Voltas *et*
438 *al.*, 2015). The observed seasonal dynamic also agrees with other studies concluding
439 that *Q. ilex* uses a larger amount of deeper soil layers and groundwater in summer than
440 during wet periods (Barbeta *et al.* 2015). David *et al.* (2007) also pointed out that the
441 deep root system of *Q. ilex* (up to 13 m) allows this species to access groundwater,
442 therefore remaining physiologically active during summer drought. Conversely, pine
443 species tend to rely more on shallow soil moisture during summer drought (Flanagan *et*
444 *al.*, 1992; Voltas *et al.*, 2015). Hence, the observed inter-specific differences in isotope
445 compositions of xylem water could be attributed, at least partially, to a different root-
446 system distribution (Eggemeyer *et al.* 2008; Filella and Peñuelas 2003; Klein *et al.*
447 2013). *In-situ* observations from road-cuts around the study site are consistent with such
448 distinct rooting pattern between species (Supplementary Material, Figure S5).

449 Figure 6 also depicts the key underlying processes explaining the observed seasonal
450 changes in soil and xylem water isotope composition. During spring and early summer
451 (Figure 6a), pines and oaks showed similar $\delta^{18}\text{O}$ and $\delta^2\text{H}$ to that found in the soil,
452 suggesting that both species were actively taking up water from soil water pools. The
453 persistence of favorable soil water conditions is also supported by both modeled and
454 satellite-derived data, together with the sustained (yet not optimum) values of NDVI
455 during this period. During late summer, after two months of drought, the soil dried out

456 and strong inter-specific differences emerged (Figure 6b). Surprisingly, soil water $\delta^{18}\text{O}$
457 and $\delta^2\text{H}$ did not merely showed an evaporative gradient, but also the soil evaporative
458 line shifted downwards (i.e. more depleted $\delta^2\text{H}$, compare Figure 3b and 2c). In the
459 absence of significant precipitation events during this period, we cannot attribute these
460 changes to the arrival of new water. Instead, this sudden shift in soil values is likely due
461 to the 'uncover' of the isotopic signature of a more tightly-bound soil water fraction,
462 once the most mobile water pools had been exhausted (Tang and Feng 2001). This
463 water fraction would be originated during soil re-hydration phases in autumn-winter,
464 thus having a distinct meteoric source as compared to the most recent mobile fraction
465 (Tang and Feng 2001). Indeed, precipitation during this period shows strongly depleted
466 isotopic values in the region (Díaz-Teijeiro et al. 2009), which would explain the
467 observed depletion in soil water as soil dries and tightly-bound water becomes the
468 dominant water pool. Besides, recent studies have shown isotopic depletion of water
469 adsorbed to organic and mineral matrices, particularly under low water contents, a
470 process that may also contribute to isotopic depletion of soil water after long drought
471 periods (Chen et al. 2016; Oerter et al. 2014). Interestingly, these changes in soil water
472 were tracked by the oaks, whereas pines showed more enriched xylem water than soil
473 water, hence following the evaporation line of early-summer soil values (compare
474 Figure 3b and 2c). This suggests that the tightly-bound water pool was still available for
475 oaks, but not for pines. This is in agreement with the typical isohydric response of
476 Aleppo pine, which results in a higher water potential threshold for limiting stomatal
477 conductance (and hence transpiration) under drought than the oaks (Klein et al. 2014a;
478 Klein et al. 2013). In this regard, the isotopic enrichment in xylem water of pines from
479 July to September would be a consequence of evaporative enrichment in the stem,

480 which has been associated to water stagnation during periods of restricted sap flow
481 (Dawson and Ehleringer 1993; Palacio et al. 2014).
482 As drought progressed (Figure 6c), we found a new shift in soil water towards ^2H -
483 depleted values, which again was likely associated to the increasing contribution of
484 tightly-bound water and the aforementioned fractionation processes (Chen et al. 2016;
485 Oerter et al. 2014; Tang and Feng, 2001). Xylem water of pines showed generally little
486 change, but at this time the oaks did not track well soil changes, suggesting that tightly-
487 bound soil water was no longer available after three months of severe drought, even for
488 drought-tolerant oaks. The situation of severe water stress for both species is also
489 supported by the minimum values of soil water content and NDVI reached by the end of
490 October (Figures 1b and 1c, respectively). In particular, NDVI values were in range
491 with the lowest values of the historical satellite record (2002-2013) found in summer
492 2003 and 2004 for pines and oaks, respectively (data not shown). This situation reverted
493 after the first significant rains occurred in late autumn (Figure 6d), when xylem water
494 $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of both species moved in the direction of the new soil isotope values.

495

496 ***4.2 Inter and intra-specific interactions for water during drought and recovery***

497 The larger isotopic enrichment found in pines at closer inter-tree distances suggests
498 intra-specific competition for water. Moreno-Gutiérrez et al. (2015) reported similar
499 effects between *P. halepensis* and understory shrubs, with shrubs becoming more
500 enriched in their xylem water values as they were located closer to pines. Considering
501 the evaporative gradient in the soil, the enriched values at short distances (<5 m) could
502 be due to a promotion of shallower roots under competition as a way to capture more
503 efficiently short precipitation pulses. However, as discussed above, the xylem water
504 enrichment of pines in September went beyond soil values, following the evaporative

505 line of previous soil records. Although we cannot rule out differences in root
506 distribution, the highly dynamic response of pines in terms of their isotope values,
507 together with the observed isotopic uncoupling between xylem and soil water, suggests
508 that the main effect of intra-specific competition for this species was a large reduction in
509 transpiration rates, which resulted in high evaporative enrichment in stems (Dawson et
510 al. 1993; Palacio et al. 2014).

511 In agreement with Comas et al. (2015), mark-correlation functions did not show clear
512 inter-specific patterns of interaction for water uptake. However, density-related effects
513 unveiled that this was due to the opposing effects of inter-specific competition for pines
514 and oaks, as a consequence of their different strategies to use belowground resources
515 (Jucker et al. 2014). In this regard, we observed that a high oak density was associated
516 with more positive values in Aleppo pine under moderate drought (Figure 4a), whereas
517 a high pine density caused a depletion in holm oak isotope composition, particularly
518 strong under severe drought and subsequent recovery (Figure 4b). A possible
519 explanation is that holm oak individuals were forced to further develop its deep-root
520 system as a consequence of competition with pines, whereas Aleppo pine would be
521 confined to shallower soil layers in the presence of neighbor oaks.

522 Conversely, a high oak density increased the isotope composition of oak xylem water,
523 pointing to the use of shallower soil layers and/or reduced transpiration rates as a result
524 of intra-specific competition. In support of this explanation, Sardans et al. (2004)
525 reported that seedlings of holm oak increased the allocation of biomass to the roots only
526 if they co-existed with Aleppo pine, but not in the presence of conspecific seedlings.
527 Similarly, other studies have shown that shrubs with much shallower root systems than
528 holm oak can compete for water with this species during drier than usual periods (Rolo
529 and Moreno 2011). This apparent enhancement of spatial segregation under competition

530 is also consistent with the steady inter-specific responses in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ along the
531 study period. This contrasts with the highly dynamic intra-specific response found in
532 Aleppo pine, as expected if driven mainly by physiological processes (i.e., stomatal
533 closure). Interestingly, intra-specific trends in holm oak were more consistent over time
534 than in the case of Aleppo pine, and the period of soil-xylem isotopic uncoupling was
535 also much shorter. Therefore, both intra- and inter-specific competition effects in holm
536 oak appeared to be largely dominated by a spatial component, i.e. related to different
537 root distribution. Nevertheless, during the drought recovery (November 2011), we
538 found the strongest tree-to-tree interaction among all oak individuals. This indicates that
539 those oak trees with closer con-specific neighbors were not able to rapidly capture the
540 new water available, contrary to what would be expected if intra-specific competition
541 were only driven by changes in root distribution. The shallower root system, together
542 with the faster soil water depletion in the vicinity of other oaks, likely contributed to a
543 longer effective drought period for these oak individuals, causing larger hydraulic
544 limitations and, hence, slower recovery (see e.g. Barbeta et al. 2015).

545

546 ***4.3 Niche segregation in pine-oak mixed forests: keys for long-term coexistence***

547 In line with results of the seasonal survey, the water potential of pines was positively
548 correlated with stable isotopes during the mild summer of 2013, hence suggesting
549 phenotypic plasticity in the use of water sources (Voltas et al. 2015). However, this was
550 not the case in the drier year of 2015, when the water potential of Aleppo pine was
551 lower and less variable, and all trees approached the reported threshold for stomatal
552 closure (between -2.0 and -2.8 MPa; see e.g. Klein *et al.*, 2011; Klein *et al.*, 2013;
553 Schiller & Cohen, 1995; Tognetti *et al.*, 1997). On the other hand, the high water
554 potential and depleted isotope values of oaks during 2013 point to the access to deep

555 water reservoirs where water is more easily available, as has been reported for other
556 deep-rooting species (Filella & Peñuelas, 2003; Flanagan *et al.*, 1992; Grossiord *et al.*,
557 2016). This situation contrasts with the low water potentials reached in 2015, when we
558 found more enriched isotopic values and a weak negative association between both
559 traits, indicative of higher water stress. Despite the two species showed no significant
560 differences in water potential, the oaks showed a larger plasticity, and reached lower
561 values (up to -3.5 MPa), in agreement with their lower threshold for stomatal limitation
562 (between -3.0 and -4.0 MPa, see e.g. David *et al.*, 2007; Lansac *et al.*, 1994; Terradas &
563 Savé, 1992; Tognetti *et al.*, 1998). In line with our findings, Klein *et al.* (2013) reported
564 a more sustained water use along the year by oaks in mixed stands of *P. halepensis* and
565 *Quercus calliprinos* (another Mediterranean evergreen oak). Bellot *et al.* (2004)
566 obtained similar results on the physiological performance of *Quercus coccifera* (a deep-
567 rooted shrub), which was unaffected by the presence of *P. halepensis*, unlike other
568 shallow-rooted shrubs that suffered from increasing water competition from pines, but
569 mainly during the periods of greater water availability (i.e. when pines are
570 physiologically more active).

571 ***4.4 Increasing 'water conflicts' as a threat for Mediterranean mixed forests***

572 Our study does not support the prediction of some ecological models stating that
573 facilitation mechanisms are fostered by increasing resource scarcity (see e.g. Brooker *et*
574 *al.* 2008, and references therein), but exemplifies a limiting environment in which none
575 of the dominant species appears to benefit clearly from the other. Previous studies on
576 co-existing species have shown evidence of facilitated water-uptake in shallow-rooted
577 species growing near deep-rooted species (Prieto *et al.* 2012; Rodríguez-Robles *et al.*
578 2015). If this were the case, we would expect pines growing in the vicinity of oaks to
579 show similar (i.e. more negative) isotope values to those of the 'nurse' oaks, but the

580 opposite was actually the case. Hence, our results suggest that under conditions of high
581 stand density and long-lasting drought, the demand from trunk refilling in oaks might
582 impede the release of water into shallow soil layers, leaving little margin for inter-
583 specific hydraulic facilitation. Here, the inability of pines to compete with oaks for
584 belowground resources and tightly-bound water may reduce the former species' fitness
585 if drought increases in severity and duration, leading to longer low-activity periods and,
586 potentially, causing carbon starvation and mortality (Moreno-Gutiérrez et al. 2012, but
587 see Klein et al. 2014a). In fact, at least seven pines (8%) died in the stand during the two
588 years following this drought event. On the other hand, it might be argued that the rapid
589 uptake of precipitation water by *P. halepensis* (Klein et al. 2014b) would increase the
590 sensitivity of *Q. ilex* to drought events, as groundwater levels could become more easily
591 exhausted in the long-term. In this regard, a recent study in a nearby oak-dominated
592 forest indicated that *Q. ilex* exhausted groundwater reserves during the summer-autumn
593 of 2011 following a long-term experimental drought, leading to oak mortality (Barbeta
594 et al. 2015). Contrary to this, however, we did not detect oak mortality in the studied
595 stand, suggesting that the expected negative effect of water withdrawal by pines could
596 be partially compensated for by reduced soil evaporation due to soil shadowing (Raz-
597 Yaseef et al. 2010).

598

599 **5. Conclusions**

600 Our results indicate that Aleppo pine and holm oak shared the same hydrological niche
601 during the wet season, but shifted to distinct water sources during the summer drought
602 period. Inter-specific interactions for plant water uptake were dominated by an
603 enhancement of spatial niche segregation along the soil profile under competition, with
604 Aleppo pine taking up more water from shallow soil layers, and holm oak relying more
605 on deep water. Both species, however, showed similar intra-specific responses, with
606 closer con-specific individuals showing more enriched values. Although we cannot
607 completely rule out changes in root distribution, a physiological, dynamic component
608 associated with limited transpiration and soil-xylem uncoupling seems a key factor
609 determining intra-specific competition for water uptake. In agreement with their
610 contrasting water use strategies, competition effects occurred early in the dry season for
611 Aleppo pine, whereas in holm oak they were mainly visible after long-lasting drought
612 events, and also during drought recovery.

613

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623

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812

813 **Supplementary Material**

814

815 Table S1. Mixed model analysis of covariance (ANCOVA) for $\delta^{18}\text{O}$ in six different
816 sampling times during the season 2011-2012.

817

818 Table S2. Mixed model analysis of covariance (ANCOVA) for $\delta^2\text{H}$ in six different
819 sampling times during the season 2011-2012.

820 Figure S1. Isotope composition of oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$) of water in pines,
821 oaks, and soil profiles during the two additional campaigns in summer 2013 and 2015.

822

823 Figure S2. Seasonal evolution of the bivariate (pine vs. oak) mark correlation function
824 for oxygen isotope composition ($\delta^{18}\text{O}$).

825

826 Figure S3. Seasonal evolution of the univariate (pine, oak) and bivariate (pine vs. oak)
827 mark correlation function for hydrogen isotope composition ($\delta^2\text{H}$).

828

829 Figure S4. Relationship between xylem water potential and the isotope composition of
830 oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$) in water extracted from twig xylem of pines (a) and
831 oaks (b). Black squares, August 2013; gray diamonds, July 2015.

832

833 Figure S5. Pictures exemplifying the typical coarse-root distribution of Aleppo pine and
834 holm oak, as observed in road cuts around the study site.

835

836 Appendix A1. Full dataset.

837

838 **Figure captions**

839

840 Figure 1. Physiological and environmental variables of the study site during the 2011-
841 2012 seasonal sampling: a) daily mean temperature (T) and precipitation (P); b) soil
842 water content (SWC) derived from ESA-SMOS remote-sensing data for the pixel
843 including the study site (1×1 km), and soil water storage (SWS), modeled from daily
844 precipitation and Penman-Monteith-FAO reference Evapotranspiration (ET_o), and
845 assuming three values of maximum SWS (100, 150 and 200 mm); c) vegetation index
846 (NDVI) derived from MODIS remote-sensing data from nearby pixels (250×250 m),
847 representative of pine-dominated and oak-dominated stands (mean and standard error of
848 5 pixels per species). Dashed vertical lines indicate the six sampling dates.

849

850 Figure 2. Seasonal evolution of the average isotope composition of a) oxygen ($\delta^{18}\text{O}$)
851 and b) hydrogen ($\delta^2\text{H}$) in water extracted from twig xylem of pines and oaks (dark red
852 triangles and light blue circles, respectively), and from the upper (ca. 2-10 cm) and
853 lower (20-50 cm) soil layers (dark grey and white diamonds, respectively). Horizontal
854 dashed lines indicate groundwater values from a nearby fountain.

855

856 Figure 3. Relationship between isotope composition of oxygen ($\delta^{18}\text{O}$) and hydrogen
857 ($\delta^2\text{H}$) in precipitation, water extracted from twig xylem of pines and oaks, and from the
858 upper (ca. 2-10 cm) and lower (20-50 cm) soil layers. Panels are organized clockwise in
859 chronological order to facilitate the comparison between contiguous sampling times.
860 Crossed circles, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of precipitation for each sampling date (isotope values
861 from the REVIP station in Tortosa, size proportional to precipitation in Ulldemolins);
862 dark red triangles, pine; light blue circles, oak; white and dark grey diamonds, lower

863 and upper soil, respectively. Solid and dashed lines indicate, respectively, the global
864 (GMWL, solid) and local (LMWL, dashed) meteoric water lines.

865

866 Figure 4. Seasonal evolution of the univariate (pine, oak) mark correlation function for
867 oxygen isotope composition ($\delta^{18}\text{O}$). Red and blue lines indicate the pine and oak
868 correlation functions, respectively. Dashed lines indicate the confidence envelopes for
869 the function, resulting from 199 random simulations. Panels are organized clockwise in
870 chronological order.

871

872 Figure 5. Seasonal patterns of pair-wise correlation coefficients between isotope
873 composition of oxygen ($\delta^{18}\text{O}$, empty symbols) and hydrogen ($\delta^2\text{H}$, filled symbols) for
874 each individual, against accumulated basal area (BA) in a 5 m radius. a) Aleppo pine
875 values against pine and oak BA; b) holm oak values against pine and oak BA. Triangles
876 and circles stand for correlations with pine and oak BA, respectively. Dashed grey lines
877 indicate $p=0.05$ threshold for significance. Asterisks denote the adjusted p -value for
878 each correlation ($\delta^2\text{H}/\delta^{18}\text{O}$): * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

879

880 Figure 6. Interpretation of observed seasonal changes in the isotope composition ($\delta^{18}\text{O}$,
881 $\delta^2\text{H}$) of soil and xylem water in oaks and pines during the growing season of 2011. The
882 size of the two arrows on top of the trees denote the estimated range of transpiration
883 rates for each species; a cross denotes stomatal closure. Colors indicate different
884 isotopic signatures: the warmer the color the more enriched isotope values (i.e. from
885 violet to blue, green and yellow). Numbers indicate average $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in upper and
886 lower soil layers, xylem water of each species, and groundwater. In spring and early

887 summer (a), soil water is easily available throughout the soil profile. Both species are
888 fully active and show similar isotope signatures, reflecting soil values. In late summer
889 (b), after two months of drought, the soil profile shows the strongest evaporative
890 gradient, with more enriched values in the upper layers. Oaks maintain a substantial
891 transpiration, tracking changes in soil isotope values, whereas most pines show more
892 enriched xylem water, following the evaporation line of early-summer soil values. This
893 suggests an uncoupling between soil and xylem water in pines due to limited
894 transpiration. In early autumn (c), after three months of severe drought, xylem water
895 isotope composition in both species is generally uncoupled from soil values. During the
896 soil water recharge phase, in late autumn (d), xylem water in both species moves in the
897 direction of new soil isotope values.

Table 1. Summary of meteorological variables during the 30/60 days preceding each sampling date. SWS, modeled soil water storage (maximum storage=150 mm); P, accumulated precipitation; ET_0 , accumulated Penman-Monteith reference evapotranspiration; T_{max} , mean daily maximum temperature.

Sampling date	SWS (mm)	30 days			60 days		
		P (mm)	ET_0 (mm)	T_{max} (°C)	P (mm)	ET_0 (mm)	T_{max} (°C)
26/05/2011	46	39	136	23.5	53	236	21.7
20/07/2011	20	17	172	29.0	44	250	26.9
09/09/2011	4	3	152	30.6	9	303	29.1
19/10/2011	2	1	93	24.7	1	229	26.9
18/11/2011	145	161	37	15.7	162	130	20.2
27/03/2012	101	61	77	15.7	63	120	11.9
21/08/2013	14	7	157	29.4	27	321	28.8
23/07/2015	8	4	182	31.9	18	326	28.8

Figure 1
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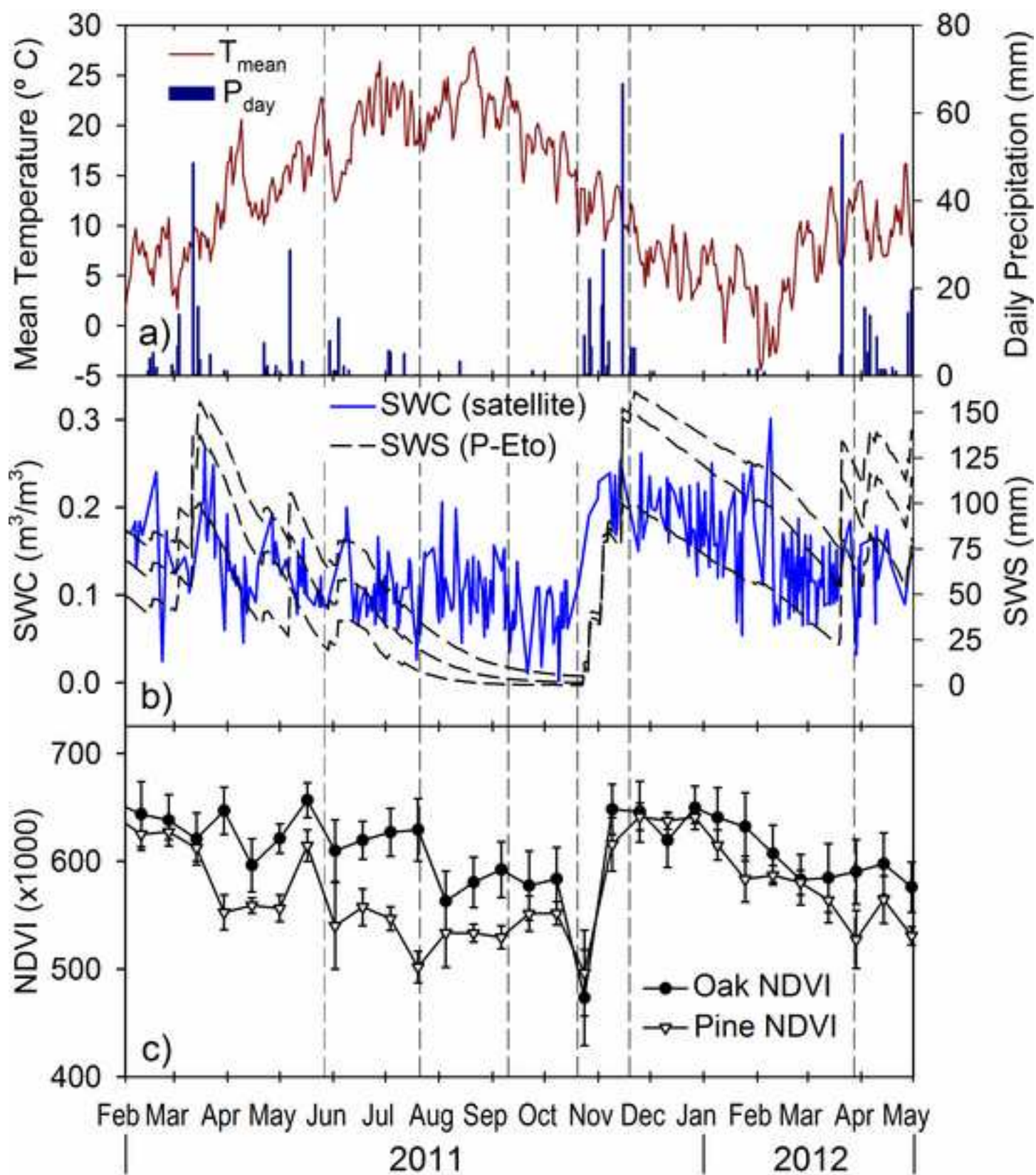


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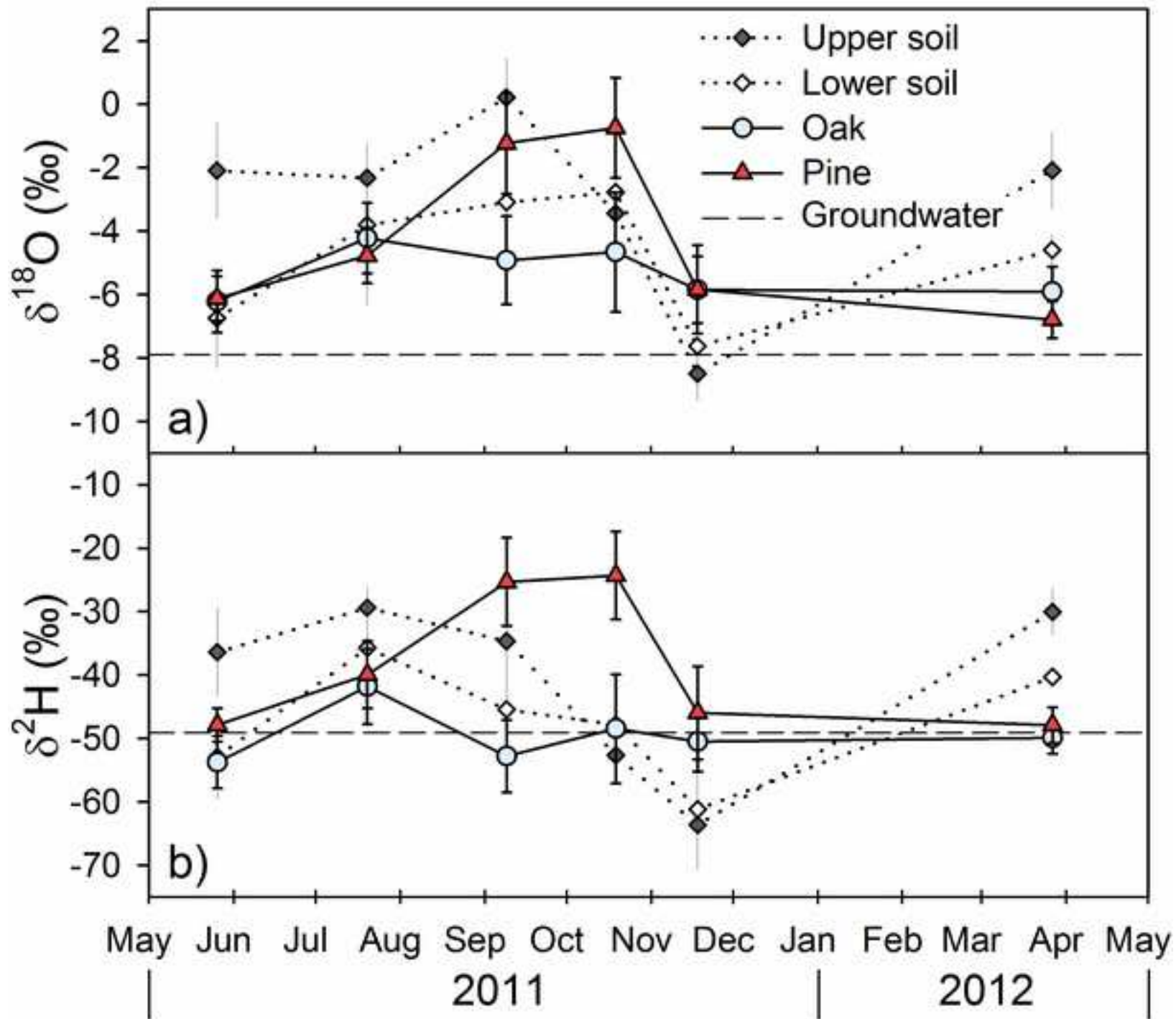


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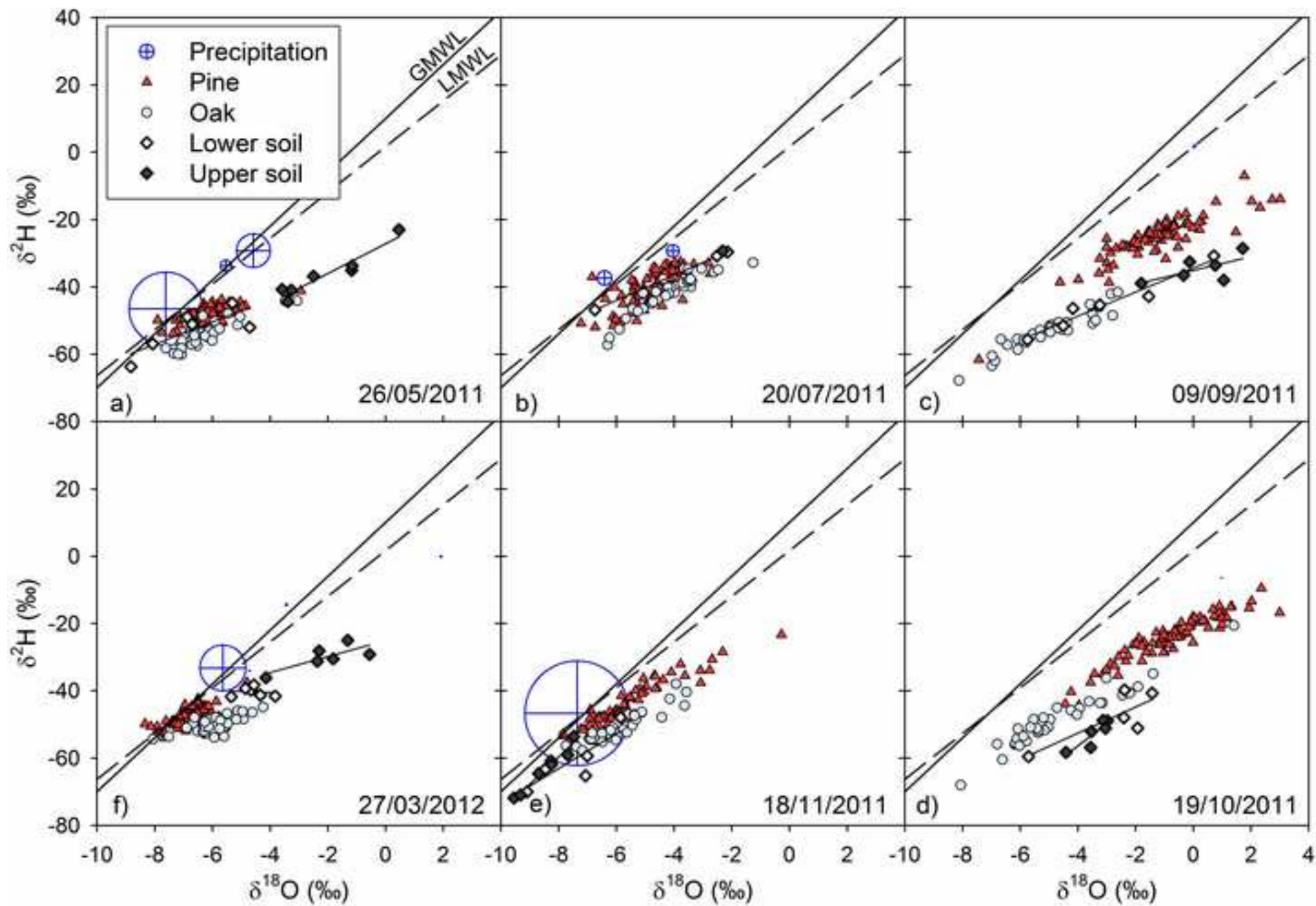


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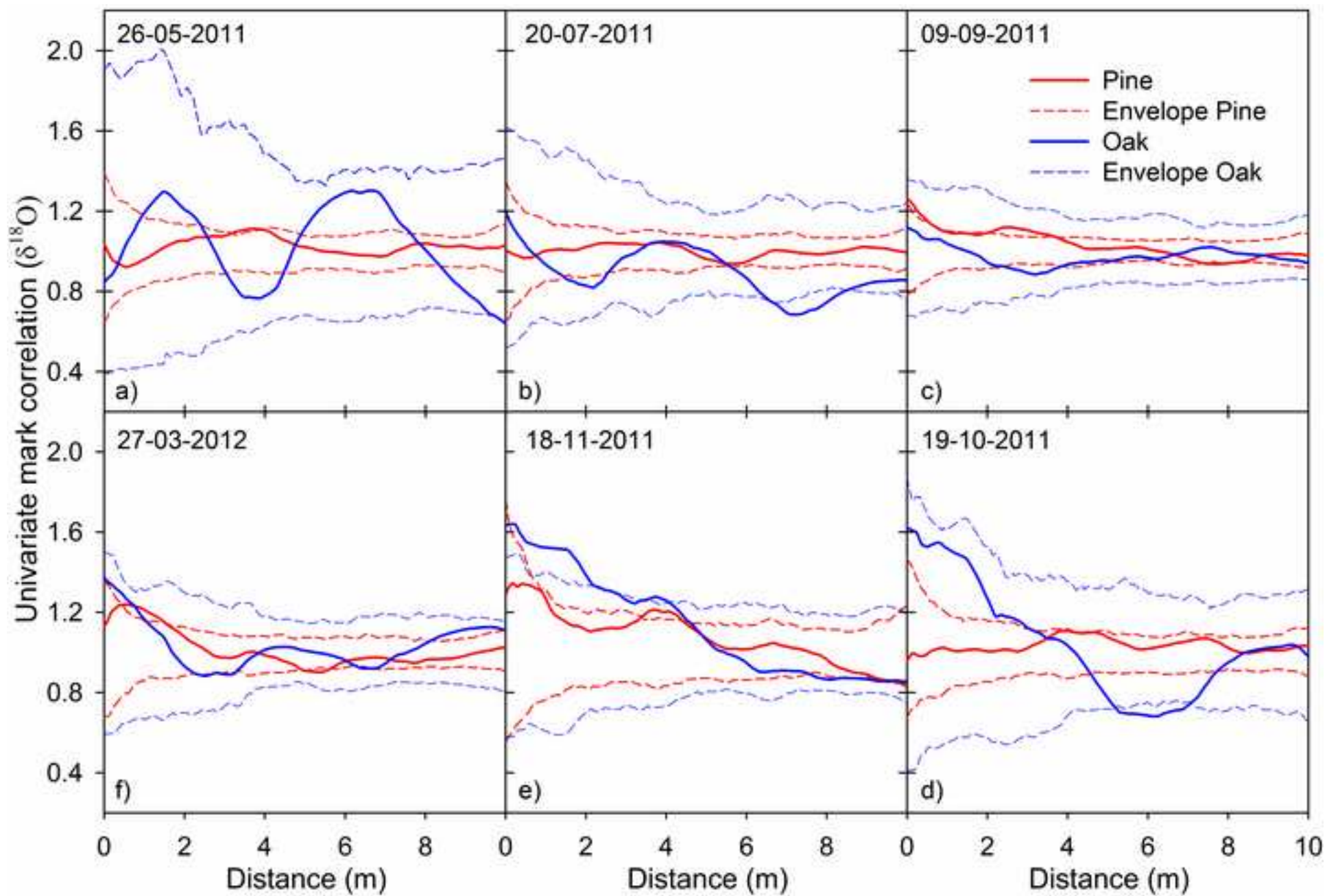


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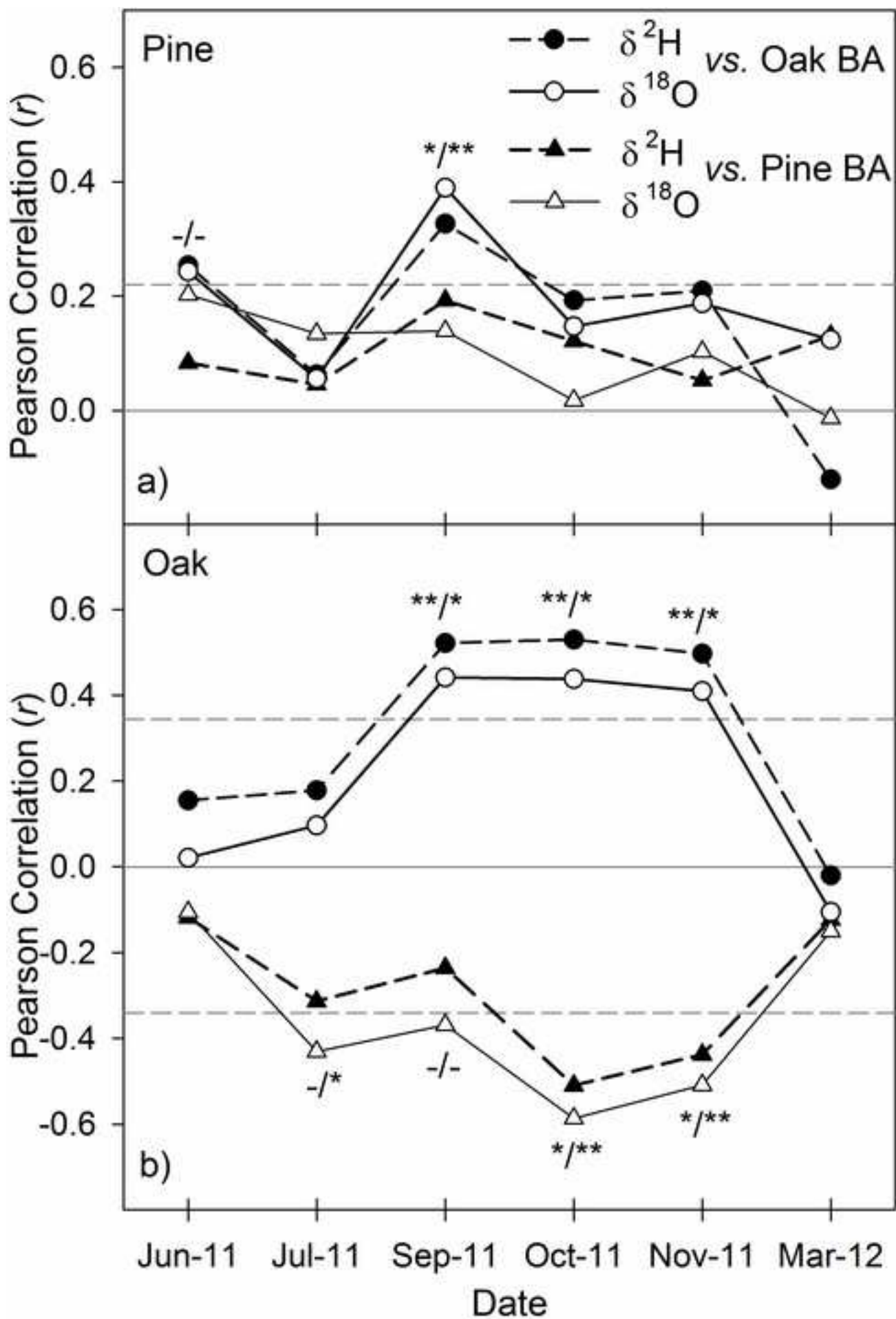
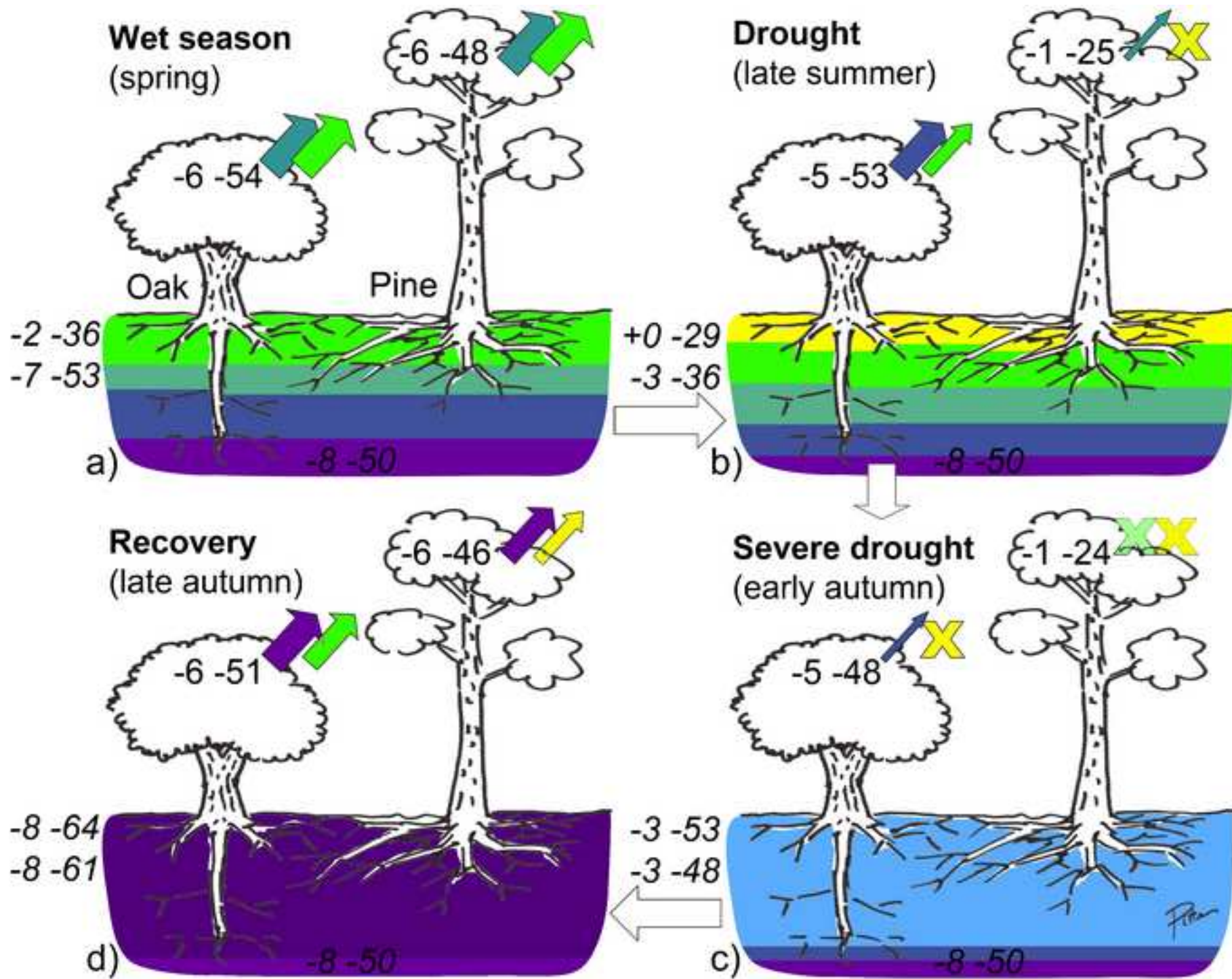


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