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**Increased root investment can explain the higher survival of seedlings of ‘mesic’
Quercus suber than ‘xeric’ *Quercus ilex* in sandy soils during a summer drought**

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23 **Abstract**

24 In Mediterranean-type ecosystems, drought is considered the main ecological filter for
25 seedling establishment. The evergreen oaks *Quercus ilex* and *Q. suber* are two of the most
26 abundant tree species in the Mediterranean Basin. Despite their shared evergreen leaf
27 habit and ability to resist low soil water potentials, traditionally, it has been suggested
28 that *Q. ilex* is better suited to resist dry conditions than *Q. suber*. In this study, we
29 examined how seedlings of *Q. ilex* and *Q. suber* grown in sandy soils responded to
30 different levels of water availability using natural dry conditions and supplemental
31 watering. Specifically, we estimated survival and water status of seedlings and explored
32 the role of acorn mass and belowground biomass in seedling performance. To our
33 surprise, *Q. suber* was better able to survive the summer drought in our experiment than
34 *Q. ilex*. Nearly 55 % of the *Q. suber* seedlings remained alive after a two-month period
35 without rain or supplemental water, which represents almost 20 % higher survival than
36 *Q. ilex* over the same period. At the end of the dry period, the surviving seedlings of *Q.*
37 *suber* had strikingly higher water potential, potential maximum quantum yield of PSII
38 (F_v/F_m) and stomatal conductance than those of *Q. ilex*. Acorn mass was associated with
39 the probability of survival under dry conditions, however, it did not explain the
40 differences in survival or water status between the species. In contrast, *Q. suber* had a
41 higher root ratio and root:shoot ratio than *Q. ilex* and these traits were positively
42 associated with predawn leaf water potential, F_v/F_m , stomatal conductance and survival.
43 Taken together, our results suggest that the higher relative investment in roots by *Q. suber*
44 when growing in a sandy acidic substrate allowed this species maintain better
45 physiological status and overall condition than *Q. ilex*, increasing its probability of
46 survival in dry conditions.

47 Keywords: drought tolerance, drought avoidance, root ratio, root depth, seed size, seed
48 mass, oaks

49 **Introduction**

50 Drought is one of the most important selective factors in nature; affecting plant
51 establishment, long-term survival, growth and reproduction (Malhi and Wright 2004,
52 Sheffield and Wood 2008, Allen et al. 2010, McDowell et al. 2011, Ramírez-Valiente et
53 al. 2010, 2015, Asner et al. 2016). Recently, severe and prolonged seasonal droughts
54 inducing dieback events have been increasingly reported across many ecosystems
55 globally (Malhi and Wright 2004, Sheffield and Wood 2008, Allen et al. 2010, McDowell
56 et al. 2011, Asner et al. 2016). Seedlings are particularly vulnerable to water stress
57 (Cavender & Bazzaz 2000, Mediavilla and Escudero 2004), and their ability to cope with
58 water shortage is critical to the long-term sustainability of drought-prone ecosystems
59 (Lloret et al. 1999, Padilla and Pugnaire 2007). Under a scenario of increasing drought,
60 understanding trees' and particularly seedlings' responses to water limitation is critical
61 for forest conservation and management (Engelbrecht et al. 2007, Bonan 2008, Feeley et
62 al. 2011, Choat et al. 2012, Anderegg et al. 2016).

63 In Mediterranean-type ecosystems, drought is considered the main ecological
64 filter for seedling establishment (Larcher 2000, Fotelli et al. 2000, Ackerly 2004, Arosa
65 et al. 2015). The evergreen oaks *Quercus ilex* and *Q. suber* are two of the most abundant
66 tree species in the Mediterranean Basin (EUFORGEN database
67 www.euforgen.org/species, Caudullo et al. 2017). They are adapted to withstand seasonal
68 drought through their small leaf lamina, thick leaves, low shoot water potential at turgor
69 loss point, low cuticular transpiration and high resistance to drought-induced cavitation
70 when compared with more mesic oaks (Kerstiens 1996, Tyree and Cochard 1996,
71 Corcuera et al. 2002, Vilagrosa et al. 2003, Quero et al. 2006, Montserrat-Martí et al.
72 2009, Peguero-Pina et al. 2016). These species also have deep and well-developed root
73 systems that allow them to access groundwater (David et al. 2004, Lubczynski and

74 Gurwin 2005, Kurz-Besson et al. 2006), and close their stomata at lower water potentials
75 than other congener species from more mesic areas (Corcuera et al. 2002, Mediavilla and
76 Escudero 2003, Vilagrosa et al. 2003). However, despite their shared evergreen leaf habit
77 and ability to withstand low water potentials, these two species have different and
78 contrasting distribution ranges. For example, in the Iberian Peninsula, where both species
79 are present, *Q. ilex* is the dominant tree in the drier and cooler areas inland, whereas the
80 calcifugous *Q. suber* is mainly restricted to the south-western region; characterized by a
81 wetter and warmer climate and acidic soils (Díaz-Fernández et al. 1995, Jiménez et al.
82 1996). The high sensitivity of *Q. suber* to low temperatures (Aranda et al. 2005), contrasts
83 with *Q. ilex* which has developed an efficient leaf photo-protection strategy to cope with
84 low temperatures in winter (García-Plazaola et al. 1999). There are some areas where
85 both species occur and occasionally hybridise (Belahbib et al. 2001, Soto et al. 2007,
86 Burgarella et al. 2009) (Fig. 1). It is typically suggested that their geographical
87 distributions reflect differences in the species' response to a combination of abiotic
88 factors including water availability, temperature and soil type (Cavender-Bares et al.
89 2005, David et al. 2007).

90 Dry conditions are traditionally thought to suit *Q. ilex*, particularly *Q. ilex* spp.
91 *rotundifolia*, better than *Q. suber* (David et al. 2007): in part, this is because of its higher
92 LMA, leaf and mesophyll thickness (Mediavilla and Escudero 2004, Peguero-Pina et al.
93 2017). Likewise, *Q. ilex* is more resistant to xylem embolism than *Q. suber* (Tyree and
94 Cochard 1996). In contrast, it has been hypothesized that *Q. suber* should displace *Q. ilex*
95 in more mesic Mediterranean areas because of its higher carbon assimilation and growth
96 rates (Quero et al. 2006, 2008). However, there are some inconsistencies in the results of
97 studies comparing the two species in terms of growth potential and drought resistance.
98 For instance, Peguero-Pina et al. (2017) reported that *Q. ilex* subsp. *rotundifolia* and *Q.*

99 *suber* had similar carbon assimilation rates when water was not a limiting factor for
100 seedlings. On the other hand, Corcuera et al. (2002) found that *Q. suber* and *Q. ilex* subsp.
101 *rotundifolia* had a similar water potential at the turgor loss point and maximum bulk
102 modulus of elasticity, which would confer on both species similar tolerance to moderate
103 water stress. Surprisingly, none of the studies comparing *Q. suber* and *Q. ilex* subsp.
104 *rotundifolia* have examined how drought affects fitness-related traits such as survival.

105 We devised a field experiment to test how seedlings of *Q. ilex* and *Q. suber*
106 respond to different levels of water availability, using natural conditions and
107 supplemental watering to create differential soil-moisture treatments during summer
108 drought. In particular, this allowed us to evaluate the potential role of investment in roots
109 for the water status, photosynthetic potential and ultimately survival of the oak seedlings.
110 Allocation to below-ground organs is considered a drought avoidance mechanism that
111 allows plants to increase their probability of survival during dry periods (Lloret et al.
112 1999, Markesteijn and Poorter 2009). Several studies have found a higher root biomass
113 ratio (i.e. the biomass invested in roots compared to the total biomass), or root-shoot ratio
114 (i.e. the biomass invested in roots compared to the aboveground biomass), to increase
115 survival in oaks (Villar-Salvador et al. 2004, 2012). Analyses of soil and leaf water
116 potentials have demonstrated that deep soil layers can supply most of the water required
117 by *Q. suber* during summer drought (Otieno et al. 2006). Likewise, isotope analyses have
118 shown that mature trees of *Q. ilex* obtain most of their water from deep soil layers during
119 dry periods (Barbeta and Peñuelas 2017). These studies suggest that root investment
120 might be critical in allowing both *Q. ilex* and *Q. suber* to cope with water stress during
121 prolonged droughts. In addition, seed mass is also considered a key trait determining the
122 successful recruitment of seedlings, particularly in dry environments (Leishman and
123 Westoby 1994, Seiwa 2000, Baraloto et al. 2005, Rodríguez-Calcerrada et al. 2011). The

124 importance of seed mass lies in the influence that it exerts on traits such as above- and
125 belowground growth during seedling establishment under both competitive and resource-
126 limiting conditions (Moles and Westoby 2004). Positive associations between seed mass
127 and survival have previously been reported in *Q. ilex* and *Q. suber* (Gómez, 2004,
128 Ramírez-Valiente et al. 2009), likewise an acorn-size growth relationship (Quero et al.
129 2008), but the mechanisms mediating such a pattern have not yet been elucidated in these
130 species.

131 In this study, our specific research questions were: Do seedlings of *Q. ilex* and *Q.*
132 *suber* differ in their response to natural vs supplemental precipitation regimes in terms of
133 survival, growth, root ratio, photosystem II (PSII) efficiency and stomatal conductance?
134 Are acorn mass, root ratio, PSII efficiency and stomatal conductance associated with
135 survival under dry conditions?

136 Since *Q. ilex* is usually found in more xeric areas than *Q. suber*, we expected that
137 *Q. ilex* would have higher survival under dry conditions than *Q. suber*, whereas the latter
138 would have higher growth rates under mesic conditions (Quero et al. 2006, David et al.
139 2007, Peguero-Pina et al. 2017). Previous studies reveal that *Q. ilex* has a greater capacity
140 for osmotic adjustment and consequently tolerates low soil water potential better than *Q.*
141 *suber* (drought tolerance strategy) (e.g. Salleo and Lo Gullo 1990, Tognetti et al. 1996,
142 Villar-Salvador et al. 2004, Otieno et al. 2006, Castro-Díez and Navarro 2007). Less-
143 consistent differences between these species have been reported in drought avoidance
144 mechanisms such as allocation patterns and stomatal conductance (e.g. González-
145 Rodríguez et al. 2011).

146 **Material and Methods**

147 *Seed collection and experimental design*

148 In winter 2006, we collected acorns from *Q. suber* and *Q. ilex* stands in an area located
149 in Central Spain (40° 20' N, 7° 00' W), where both species coexist (Fig. 1). The climate
150 in this area is typically Mediterranean and soils are dystric and eutric cambisols (Díaz-
151 Fernández et al. 1995, Jiménez et al. 1996). Twenty open-pollinated trees (ten per species)
152 were sampled. Sampled trees were each separated by more than 150 m in order to avoid
153 kinship structures (Soto et al. 2007). Seeds were stored at 4°C. Most viable seeds
154 germinated at this temperature before the synchronous sowing in May 2007. A common-
155 garden experiment was established in a field site at the *Centro Nacional de Recursos*
156 *Genéticos Forestales* (CNRGF) growth facilities (40°25'N, 3°44'W). The soils in the
157 experimental plot and surrounding area where both species naturally coexist are dystric
158 and eutric cambisols with a pH between 6.0 and 7.0 (Martín-Albertos et al. 1998). In the
159 plot, the analyses revealed that the soil has sandy loam texture, 2 % of organic matter in
160 the top layer and very low content of carbonates (Table S1 for more details). The
161 experimental design consisted of two randomized complete blocks. Each block was
162 divided into two subplots where watering treatments were later implemented. Between 2
163 and 37 seeds per family per subplot (depending on availability) were directly sown on a
164 grid at 25-×-25-cm spacing ($n = 1120$). Once emerged, seedlings were kept under well-
165 watered conditions (20-30 % soil water content) and ambient temperature (average $T =$
166 19.6°C) for 60 days after sowing. At the end of this period, two watering treatments were
167 applied for another 60 days. In the *well-watered treatment*, we watered every two days.
168 In the *dry treatment*, no water was given after the first 60 days. Soil water content was
169 monitored every 3-7 days over the experiment at four depths: 20, 30, 40 and 60 cm, using

170 TDR probes (Time Domain Reflectometry, Trime®IT IMKO, Germany) (see Fig S1 and
171 results for details). There was no rainfall during the watering treatments.

172

173 *Trait measurements*

174 Due to the logistical limitations, we randomly selected 211 seedlings from five families
175 per species (3-17 plants/family/species/treatment depending on availability) to monitor
176 growth, survival, allometry and physiology traits. Stem height, basal diameter and the
177 number of leaves were measured at the beginning and at the end of the watering
178 treatments (i.e. 0 and 60 days after the start of the watering treatments). We used these
179 variables to estimate initial (M_{initial} at $t = 0$ days; T_{initial}) and final biomass (M_{final} at $t = 60$
180 days; T_{final}) with allometric equations developed from a subsample of plants harvested at
181 the end of the experiment ($n = 36$ plants per species). Absolute growth rate (AGR g day^{-1})
182 was calculated as $(M_{\text{final}} - M_{\text{initial}})/(T_{\text{final}} - T_{\text{initial}})$. Relative growth rate ($\text{mg g}^{-1} \text{day}^{-1}$) was
183 calculated as $\text{RGR} = [\log_e(M_{\text{final}}) - \log_e(M_{\text{initial}})]/(T_{\text{final}} - T_{\text{initial}})$.

184 Twenty days after the start of the watering treatments ($t = 20$), dark- and light-
185 acclimated chlorophyll fluorescence measurements were performed on leaves of the 211
186 selected seedlings using FMS-2 (Hansatech Instruments Ltd, Norfolk, UK).
187 Measurements were repeated on two further occasions at intervals of 15 days ($t = 35$ and
188 $t = 50$). Dark measurements were made predawn or very early in the morning using dark-
189 adaptation clips. Light measurements were made on leaves receiving full sun on clear
190 sunny days at midday, when the irradiance was highest. We measured F_m and F_0 , F_m' and
191 F_s . F_m is the maximal chlorophyll fluorescence measured during a 0.8 s pulse of saturating
192 white light, and F_0 is the minimal chlorophyll fluorescence measured during exposure to
193 a weak modulated measuring beam. F_m' and F_s are the maximal and steady-state
194 chlorophyll fluorescence measured during actinic illumination, respectively. For further

195 analysis, we calculated: $F_v/F_m = (F_m - F_0)/F_m$, which is the potential maximum quantum
196 yield of PSII in a dark adapted leaf, and $\Phi_{PSII} = (F_m' - F_s)/F_m'$, the effective quantum yield
197 of PS II in an irradiated leaf, which is related to its CO₂ fixation rate and growth rates in
198 oaks (Cavender-Bares and Bazzaz 2004).

199 On the same three dates (t = 20, t = 35 and t = 50), we measured stomatal
200 conductance (g_s) in each plant of the five selected families per species. We made two
201 measurements per leaf (which were averaged prior to statistical analyses), and measured
202 at two time intervals during the day, early in the morning and at midday. Stomatal
203 conductance was measured with a leaf porometer (SC-1; Decagon Devices, Pullman,
204 WA, USA).

205 We also monitored survival on the same dates as above. At t = 50, we measured
206 pre-dawn water potential (Ψ_{PD}) in the surviving plants. At the end of the experiment (t =
207 60), we harvested a randomly-selected subsample of plants within each treatment to
208 estimate total biomass and investment in roots, leaves and stems ($n = 36$ plants per
209 species). All plant parts were dried in an oven at 65°C. Root ratio (also known as, root
210 mass fraction), leaf and stem ratios (i.e., each as a fraction of total dry biomass) were
211 calculated. The root:shoot ratio [root biomass/(stem + leaf biomass)] was also estimated.

212

213 *Statistical analyses*

214 A linear mixed model was used for statistical analyses of growth traits, allometric traits
215 and leaf water potential. Species and Treatment were fixed-effect factors, Block and
216 Family were nested within Species as random-effect factors and acorn mass was a
217 covariate.

218 The model equation for these traits was:

$$219 Y_{ijklp} = \mu + S_i + T_j + F(S)_{ik} + B_l + ST_{ij} + TF(S)_{ijk} + M_p + E_{ijklp}$$

220 where Y_{ijklp} is the observed value for the variable considered in the p -th individual of the
 221 k -th open-pollinated family from the i -th species in the j -th treatment into the l -th block;
 222 μ is the overall mean; S_i is the fixed effect of the i -th species; T_j is the fixed effect of the
 223 j -th treatment; $F(S)_{ik}$ is the random effect of the k -th family nested within the i -th species;
 224 B_l is the random effect of the l -th block; ST_{ij} is the fixed effect of the interaction between
 225 species i and treatment j , $TF(S)_{ijk}$ is the random effect of the interaction between treatment
 226 j and family k nested within species i ; M_p is the value of acorn mass for individual p and
 227 E_{ijklp} is the residual error term.

228 This linear mixed model was amended for F_v/F_m and Φ_{PSII} to include Date as an
 229 additional fixed-effect factor because measurements were made on three different dates,
 230 making the model a split-plot-in-time. The mixed model for g_s also included time-of-day
 231 as a fixed-effect factor because this trait was measured in the morning and at midday. The
 232 model equations for F_v/F_m , Φ_{PSII} and g_s are included in Appendix S1. When necessary,
 233 data were transformed (logarithm, square root) to meet normality and homoscedasticity
 234 assumptions.

235 A generalised linear mixed model was implemented for final survival (i.e. survival
 236 at the end of the experiment) using a binomial distribution and logit link function. The
 237 model was only applied to seedlings from the dry treatment because there was no
 238 mortality in the well-watered treatment. This model included Species as a fixed-effect
 239 factor, and Block and Family nested within Species as random-effect factors. The model
 240 equation for survival was:

$$241 \quad Y_{ijklp} = \mu + S_i + F(S)_{ik} + B_l + M_p + E_{ijklp}$$

242 where the description of the terms is consistent with that of equation (1). Analyses were
 243 performed with the package lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al.,
 244 2017) packages in R 3.4.2 (R Core Team 2015). Degrees of Freedom (df), chi-square

245 values (χ^2) and significance P values were obtained from likelihood-ratio tests comparing
246 reduced models to the full model using function “anova”.

247 We also tested differences between species in acorn mass using an ANOVA. To
248 test for associations between traits within treatments, we performed linear regressions at
249 the level of the individual and maternal family. Three linear regressions were performed
250 for each pair of traits, one for *Q. ilex*, one for *Q. suber* and one for both species together.
251 The trait g_s was log-transformed for these tests. Relationships between survival and traits
252 were examined by performing linear regressions using family means. To test for
253 associations across treatments, g_s , Ψ_{PD} and Φ_{PSII} were log-transformed (see Savage et al.
254 2009 for a similar approach). These analyses were performed in STATISTICA 10.0
255 (StatSoft, Inc., Tulsa, OK, USA). Sigmaplot 12.5 (Systat Software Inc., San Jose, CA,
256 USA) was used to plot figures.

257

258 **Results**

259 In the well-watered treatment, volumetric soil water content (VWC) ranged between 20
260 and 30% at 20, 30 and 40-cm depth, and between 25 and 40 % at 60 cm (Fig. S1). In the
261 dry treatment, VWC decreased from 20-30 % to 5-10 % after two months in the top 20
262 cm of soil (Fig. S1). Reductions of VWC were also evident at 30, 40 and 60-cm soil depth
263 but to a lesser extent than those at 20-cm depth (Fig. S1). The experiment finished with
264 first rains of autumn (60 days after the start of the differential watering treatments). The
265 average temperature during this period was $T = 22.9^\circ\text{C}$ (maximum $T = 39.2^\circ\text{C}$ and
266 minimum $T = 7.8^\circ\text{C}$). There were differences between species in acorn dry mass ($P <$
267 0.001). On average, acorns of *Q. ilex* had a higher mass (0.872 ± 0.022 g) than those of
268 *Q. suber* (0.699 ± 0.019 g). The effect of acorn mass was significant for basal diameter,
269 stomatal conductance and final survival (Tables 1-4).

270 There were differences between treatments in absolute and relative growth rates
271 (AGR and RGR), during the period after the start of watering treatments, and in
272 aboveground growth (height and number of leaves) at the end of the experiment (Table
273 1). However, treatment effects on above-ground growth traits were only evident for *Q.*
274 *suber* not *Q. ilex*, with the exception of RGR (Fig. 2) (see also species-by-treatment
275 interactions below). As expected, plants growing in the dry treatment had lower AGR,
276 RGR, accumulated height growth and number of leaves (Fig. 2). Predawn leaf water
277 potential (Ψ_{PD}) differed between treatments in both species and plants growing in the dry
278 environment exhibited lower values (Table 1, Fig. 2). Differences between treatments in
279 root ratio and root:shoot ratio were only evident for *Q. suber*, such that seedlings growing
280 in the dry environment had higher root and root:shoot ratios (Table 1, Fig. 3). Leaf ratio
281 did not differ between treatments and stem ratio only differed between treatments for *Q.*
282 *suber* (Table 1, Fig. 3). The watering treatment affected leaf physiological traits (Tables
283 2 & 3): seedlings had lower F_v/F_m , Φ_{PSII} and g_s in the dry treatment compared to the well-
284 watered treatment for both species (Fig. 4). The differences between treatments in F_v/F_m
285 and g_s increased at the end of the experiment (significant interaction watering treatment
286 \times date of measurement), as expected since the summer drought increased the difference
287 between the treatments in VWC over time (Tables 2 & 3, Fig. 4).

288 There were also differences between species, or species-by-treatment interaction,
289 for most traits. In the well-watered treatment, *Q. suber* had higher AGR, total height,
290 number of leaves, stem ratio and stem biomass but a lower leaf ratio than *Q. ilex*, which
291 typically has larger thicker leaves (Table 1, Table S2, Fig. 3, Fig. S2), implying that the
292 two species use different strategies in allocating resources to leaf traits. In the dry
293 treatment, *Q. suber* had higher predawn leaf water potential (Ψ_{PD}), root ratio and
294 root:shoot ratio than *Q. ilex* (Table 1, Fig. 2). In the dry treatment, both species had similar

295 growth rates (AGR, RGR), aboveground growth (height, number of leaves and diameter)
296 and aboveground, leaf, root and stem biomass. *Q. suber* had higher F_v/F_m , and g_s in the
297 dry treatment in the latter half (from day 35) of the experiment (Tables 2 & 3, Fig. 4).
298 Differences between species in the well-watered treatment for these traits were small or
299 absent (Fig. 4). *Q. suber* seedlings had greater survival than *Q. ilex* at the end of the
300 experiment (Table 4, Fig. 5).

301 Linear regressions did not show an association between acorn mass and survival
302 using family means, indicating that differences in survival between species could not be
303 explained by differences in acorn mass (Fig. S3). Root biomass was positively associated
304 with survival (Fig. S3). Acorn mass was not associated with root ratio but it was
305 associated with root biomass (Fig. S3), which could explain the positive effect of acorn
306 mass on survival at the individual level (Table 4).

307 In the dry treatment, traits related to the water status of the plants such as Ψ_{PD} ,
308 F_v/F_m and g_s were associated with root ratio and leaf ratio across species (Fig. 6).
309 Seedlings with a higher root ratio and lower leaf ratio had higher Ψ_{PD} , F_v/F_m and g_s (Fig.
310 6, Fig. S4). *R*-squared values were moderate, particularly for associations of leaf traits /
311 survival with leaf ratio / root ratio (Fig. 6, Fig. S4). Similar associations were present
312 between Ψ_{PD} , F_v/F_m and g_s with root:shoot ratio or root biomass (data not shown). Plants
313 that had higher root ratio and Ψ_{PD} maintained higher g_s in the dry treatment (Fig. 6, Fig.
314 S5). However, when species were analysed separately, linear regressions between root
315 ratio and Ψ_{PD} , F_v/F_m and g_s were not significant indicating that associations were caused
316 by species differences in these traits (Fig. 6, Fig. S4). Higher investment in roots was also
317 associated with greater survival after two months of drought (Fig. 6). In the well-watered
318 treatment, RGR was positively associated with g_s and Φ_{PSII} using family means (Fig. S6).

319 Both g_s and Φ_{PSII} were associated across treatments with predawn water potential (Ψ_{PD})
320 in negative log-linear relationships (Fig. S7).

321

322 **Discussion**

323 Contrary to our hypothesis, *Q. suber* was better able to survive the summer drought than
324 *Q. ilex* in our experiment (Fig. 5). Nearly 55 % of the *Q. suber* seedlings remained alive
325 after a two-month period without water: this represents almost 20 % higher survival than
326 *Q. ilex* over the same period. At the end of the dry period, the surviving seedlings of *Q.*
327 *suber* had strikingly higher water potential, F_v/F_m and g_s than those of *Q. ilex* (Figs. 2 &
328 4). The most striking difference between the species was that *Q. suber* had a higher root
329 ratio than *Q. ilex* (Fig. 3), and this trait was positively associated with Ψ_{PD} , F_v/F_m , g_s and
330 survival across species (Fig. 6). Taken together, our results suggest that higher relative
331 investment in roots by *Q. suber* allowed this species maintain better physiological status
332 and overall condition than *Q. ilex*, increasing its probability of survival under dry
333 conditions in a sandy acidic substrate.

334 Numerous studies have reported that bigger acorns lead to increased seedling
335 growth, which can improve seedling performance by providing the resources to grow
336 deep and extensive root systems, giving access to water from deep within the soil profile
337 (Stock et al. 1990, Tripathi and Khan 1990, Osunkoja et al. 1994, Gómez 2004, Ramírez-
338 Valiente et al. 2009; Rodríguez-Calcerrada et al. 2011). In fact, strong associations have
339 been reported between acorn mass and survival in *Q. ilex* and *Q. suber* (Gómez 2004,
340 Ramírez-Valiente et al. 2009). In our experiment, high acorn mass was positively
341 associated with the probability of survival under dry conditions at the individual level
342 probably because of an increased capacity for root growth (Table 4, Fig. S3), but this did
343 not explain the species differences in survival (Fig. S3).

344 On the other hand, although the total and aboveground biomass, height and
345 number of leaves in the dry treatment were similar in the two species, *Q. ilex* had higher
346 relative investment in leaves, i.e. leaf ratio. Higher leaf ratio might be caused by a larger
347 leaf lamina area, thicker leaves or both. The interpretation of our results would differ
348 depending on the main mechanism underlying the difference in leaf ratio. If leaf area is
349 the main factor mediating differences in leaf ratio between the two species, then the lower
350 survival and poor water status of *Q. ilex* seedlings could, at least in part, be the result of
351 depletion of soil moisture due to their higher whole-plant transpiration (Poorter 1990,
352 Villar et al. 2017). We did not measure specific leaf area (ratio between leaf area and leaf
353 mass) in our study, but the facts that *Q. ilex* usually has thicker leaves than *Q. suber* (e.g.
354 Cavender-Bares et al. 2005, Peguero-Pina et al. 2017) and that root ratio was better
355 correlated with survival and water status of seedlings than leaf ratio (see Fig. S4) suggest
356 that whole-plant transpiration area have had a low impact on the differences in the
357 performance between *Q. ilex* and *Q. suber*.

358 Root ratio, which represents the investment in roots relative to the total biomass,
359 seemed to underpin the different probability of survival under dry conditions between
360 these two evergreen oak species. Mediterranean oak species have tended to favour
361 belowground growth over aboveground growth in studies where water is limiting (Villar-
362 Salvador et al. 2004, Cubera et al. 2009, Corcobado et al. 2014). In our study, *Q. suber*
363 exhibited higher root ratio than *Q. ilex* after two months of drought, similar to the findings
364 reported by Quero et al. (2008) and González-Rodríguez et al. (2011). A higher root ratio
365 in oaks is produced by increases in both lateral and tap roots allowing a larger volume
366 and depth of soil to be explored (Chiatante et al. 2005, Chirino et al. 2008). The size and
367 length of the root system have also been found to predict predawn water potential and
368 survival under seasonal drought (Bellot et al. 2002, Grossnickle 2005, Padilla and

369 Pugnaire 2007, Chirino et al. 2008, Villar-Salvador et al. 2012). Although high relative
370 investment in roots is a characteristic of most species from the genus *Quercus*, there can
371 be species-specific differences such as those reported by Espelta et al. (2005) when
372 comparing *Q. ilex* and *Q. cerrioides*. We did not measure root length, but the fact that
373 root ratio was positively correlated with leaf water potential, F_v/F_m and stomatal
374 conductance across species suggests that high root biomass allocation allowed seedlings
375 of *Q. suber* to increase water uptake, probably because their deeper roots reached soil
376 with higher water content (Otieno et al. 2006). Consistent with these findings, three-year-
377 old seedlings of *Q. suber* exhibited higher survival than *Q. ilex* seedlings of the same age
378 growing in a fluvisol (González-Rodríguez et al. 2011). In another study, adult trees of
379 *Q. suber* had higher predawn leaf water potentials than those of *Q. ilex* under summer
380 drought in an open woodland where both species coexist (Mediavilla and Escudero 2003).
381 However, the opposite result has been also recorded on several occasions in both adult
382 trees and seedlings, i.e. *Q. ilex* exhibited higher predawn leaf water potentials than *Q.*
383 *suber* when growing under low water availability (Acherar and Rambal 1992, David et al.
384 2007, Vaz et al. 2010). In addition, seedlings of *Q. ilex* typically have a lower water
385 potential at the turgor loss point (π_{tlp}) than those of *Q. suber*, indicating higher drought
386 tolerance including when grown in common environmental conditions (Salleo and Lo
387 Gullo 1990, Sala and Tenhunen 1994, Tognetti et al. 1996, Nardini et al. 1996, Villar-
388 Salvador et al. 2004, Pardos et al. 2005, Otieno et al. 2006, Castro-Díez and Navarro
389 2007). Only Corcuera et al. (2002) reported similar values of π_{tlp} for both species.

390 Taken together, these previous findings suggest that *Q. ilex* seedlings better
391 tolerate low soil water potentials than *Q. suber* seedlings probably because of their better
392 ability to accumulate solutes and lower their osmotic potential under dry conditions (a
393 drought-tolerance strategy). In contrast, our results suggest that *Q. suber* seedlings

394 increased root biomass in response to low soil water potentials whereas *Q. ilex* did not (a
395 drought avoidance strategy), which is consistent with a previous study (González-
396 Rodríguez et al. 2011). Evolutionary trade-offs between drought tolerance and avoidance
397 strategies have been reported for other oaks from seasonally-dry ecosystems (Ramírez-
398 Valiente and Cavender-Bares 2017, Cavender-Bares and Ramírez-Valiente 2017). The
399 key question is; what are the conditions which favour one strategy over the other in terms
400 of fitness? The fact that *Q. suber*, which is usually found in more mesic areas than *Q. ilex*,
401 survived better under dry conditions opens a debate on whether rainfall and the severity
402 and length of the dry season are the main selective forces shaping the distribution ranges
403 of *Q. ilex* and *Q. suber*. Several studies have shown that *Q. suber* is more sensitive to cold
404 temperatures than *Q. ilex*. For example, Cavender-Bares et al. (2005) found that *Q. suber*
405 was less resistant to xylem embolisms than *Q. ilex* under freezing conditions. Aranda et
406 al. (2005) found that PSII efficiency of *Q. suber* seedlings was sensitive to cold
407 temperatures, but PSII is not usually sensitive to cold in *Q. ilex* (Nardini et al. 2000,
408 Corcuera et al. 2005, Camarero et al. 2012). *Q. suber* is also considered a strictly
409 calcifugous species and is absent from basic soils, whereas *Q. ilex* tolerates a wider range
410 of soil types (Valdecantos et al. 2006). For example, Serrasolses et al. (2009) showed that
411 *Q. suber* seedlings, established in siliceous sandstone soils under high annual
412 precipitation, had higher growth rates than seedlings growing in calcareous soils and low
413 annual precipitation regimes. In contrast, Sardans et al. (2006) did not find any effect of
414 soil type (siliceous or calcareous) on seedling growth and biomass allocation patterns in
415 *Q. ilex*. These differences in physiological tolerance and habitat preference highlight the
416 need to explore the effect on the performance of *Q. ilex* and *Q. suber* of interactions
417 among multiple abiotic factors including drought, soil chemical properties, soil

418 granulometry and temperature to ascertain their relative roles in shaping the distributions
419 of these evergreen oaks.

420 In conclusion, our results revealed that *Q. suber* seedlings survived better than *Q.*
421 *ilex* during a two-month summer drought period without any precipitation. Surviving
422 seedlings of *Q. suber* also had strikingly higher water potential, F_v/F_m and g_s than those
423 of *Q. ilex*. The relative investment in roots was strongly linked to survival, water potential,
424 F_v/F_m and stomatal conductance across species, suggesting that the higher probability of
425 survival and the better water status of seedlings of *Q. suber* was probably explained by a
426 drought avoidance strategy that allowed them to maintain higher water content
427 throughout the dry season in a sandy soil. Comparative studies of these two species
428 regarding complementary drought tolerant traits, such as osmotic adjustment and xylem
429 vulnerability to cavitation and their relationship with fitness at different ontogenetic
430 stages, are needed to provide a definitive perspective on the drought resistance
431 mechanisms of these two oaks and explain the main factors driving their distribution and
432 coexistence patterns.

433

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441

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694 **Figure legends**

695 Fig. 1. Distribution ranges of *Quercus ilex* (green) and *Q. suber* (purple). Overlapping
696 areas between ranges of both species are shown in brown. Modified from EUFORGEN
697 database (www.euforgen.org/species) and Caudullo et al. (2017). The open circle
698 indicates the area where the maternal families for the common garden were sampled. The
699 black triangle indicates where the field experiment was established.

700 Fig. 2. LS-means (\pm SE) of (a) absolute growth rate (AGR), (b) relative growth rate
701 (RGR), (c) height, (d) number of leaves, (e) basal diameter and (f) predawn water
702 potential (Ψ_{PD}) for *Q. suber* (triangles) and *Q. ilex* (circles) under well-watered (W) and
703 dry (D) treatments. Asterisks (*) indicate differences between treatments for each species.
704 Letters indicate differences between species within treatments.

705 Fig. 3. LS-means (\pm SE) of (a) root ratio, (b) root:shoot ratio, (c) leaf ratio and (d) stem
706 ratio for *Q. suber* (triangles) and *Q. ilex* (circles) under well-watered (W) and dry (D)
707 treatments. Asterisks (*) indicate differences between treatments for each species. Letters
708 indicate differences between species within treatments.

709 Fig. 4. Least-squared-means (\pm SE) of (a) potential quantum yield of PS II in dark adapted
710 leaves (F_v/F_m), (b) effective quantum yield of PS II in leaves receiving full solar
711 irradiance (Φ_{PSII}) and (c) stomatal conductance (g_s) for *Q. suber* (triangles) and *Q. ilex*
712 (circles) under dry (red) and well-watered (blue) treatments on three occasions during the
713 summer drought vs watering treatments (t = 50, 35 and 50 days). Asterisks (*) indicate
714 differences between treatments for each species. Letters indicate differences between
715 species within treatments.

716 Fig. 5. Means (\pm SE) of survival measured in *Q. suber* (triangles) and *Q. ilex* (circles) on
717 three occasions during the summer drought vs watering treatments. Only the dry (red)
718 treatment results are shown since all seedlings survived in the well-watered treatment.

719 Letters indicate differences between species within treatments at the end of the
720 experiment.

721 Fig. 6. Relationships between root ratio and (a) predawn leaf water potential (Ψ_{PD}), (b)
722 F_v/F_m and (c) stomatal conductance (log-transformed, g_s) in the dry treatment measured
723 50 days after the start of watering treatments. Results of regression analyses conducted
724 for each species separately are shown with subscripts (*i* - *Quercus ilex*, *s* - *Quercus suber*)
725 in linear fits (R^2) and significant levels. (d) The relationship between root ratio and
726 survival in the dry treatment at the end of the experiment using family means (\pm SE).
727 *Quercus ilex*: circles, *Quercus suber*: triangles. Linear fit (R^2) and significant level are
728 shown when excluding one of the *Q. suber* families, which was an extreme outlier for
729 some traits including survival and Φ_{PSII} (see also Fig. S6).

Table 1. Results of mixed models for Absolute Growth Rate (AGR), Relative Growth Rate (RGR), height, number of leaves (N leaves), diameter, pre-dawn water potential (Ψ_{PD}), root ratio, root:shoot ratio, leaf ratio and stem ratio. Chi-squares (χ^2) and *P*-values are shown. Fixed factors: Species, Treatment. Random factors: Family(Species), Block. Covariate: Seed mass.

		AGR	RGR	Height	N leaves	Diameter	Ψ_{PD}	Root ratio	Root:shoot	Leaf ratio	Stem ratio
Species	χ^2	5.61	3.22	9.99	8.65	0.12	38.64	11.02	12.53	32.93	0.71
	<i>P</i>	0.004	0.073	0.002	0.003	0.7241	<0.001	0.001	<0.001	<0.001	0.400
Treatment	χ^2	38.46	60.67	9.10	31.01	1.52	172.75	18.51	10.97	1.83	9.92
	<i>P</i>	<0.001	<0.001	0.003	<0.001	0.217	<0.001	<0.001	0.001	0.176	0.002
Species \times Treatment	χ^2	8.82	4.33	5.99	5.71	0.47	49.24	7.76	5.92	0.32	6.22
	<i>P</i>	0.003	0.038	0.014	0.017	0.492	<0.001	0.005	0.015	0.571	0.013
Block	χ^2	0	0.00	0.01	0	0.14	0	0	0	0	0
	<i>P</i>	-	0.975	0.908	-	0.710	-	-	-	-	-
Family(Species)	χ^2	0	0	0.01	0.01	3.04	0	4.01	2.31	0.11	0
	<i>P</i>	-	-	0.944	0.910	0.081	-	0.045	0.059	0.740	-
Treatment \times Family(Species)	χ^2	0	0	3.17	0.02	0.00	0	0	0	0	0
	<i>P</i>	-	-	0.075	0.898	0.966	-	-	-	-	-
Seed mass	<i>F</i>	3.44	1.37	0.18	1.32	6.47	1.07	1.98	1.80	1.85	0.50
	<i>P</i>	0.063	0.241	0.674	0.250	0.011	0.302	0.159	0.180	173	0.478

Table 2. Results of mixed models for F_v/F_m and effective quantum yield of PS II (Φ_{PSII}). Degrees of freedom (df), Chi-squares (χ^2) and P -values are shown.

Fixed factors: Species, Treatment, Date. Random factors: Family(Species), Block. Covariate: Seed mass.

	df	χ^2	P -value
F_v/F_m			
Species	1	33.56	< 0.001
Treatment	1	167.1	< 0.001
Date	2	150.5	< 0.001
Species \times Treatment	1	26.99	< 0.001
Species \times Date	2	31.10	0.001
Treatment \times Date	2	71.91	< 0.001
Species \times Treatment \times Date	2	19.44	< 0.001
Block	1	2.76	0.097
Family(Species)	1	0.39	0.532
Treatment \times Family(species)	1	0	-
Date \times Family(Species)	1	0	-
Seed mass	1	3.70	0.054
Φ_{PSII}			
Species	1	20.87	< 0.001
Treatment	1	466.6	< 0.001
Date	2	30.80	< 0.001
Species \times Treatment	1	0.33	0.564
Species \times Date	2	2.59	0.274
Treatment \times Date	2	3.45	0.178
Species \times Treatment \times Date	2	0.90	0.639
Block	1	10.85	< 0.001
Family(Species)	1	0	-

Treatment × Family(species)	1	0	-
Date × Family(Species)	1	0	-
Seed mass	1	0.23	0.631

Table 3. Results of the mixed model for area-based stomatal conductance (g_s). Degrees of freedom (df), Chi-squares (χ^2) and P -values are shown. Fixed factors: Species, Treatment, Date, Daytime. Random factors: Family(Species), Block. Covariate: Seed mass.

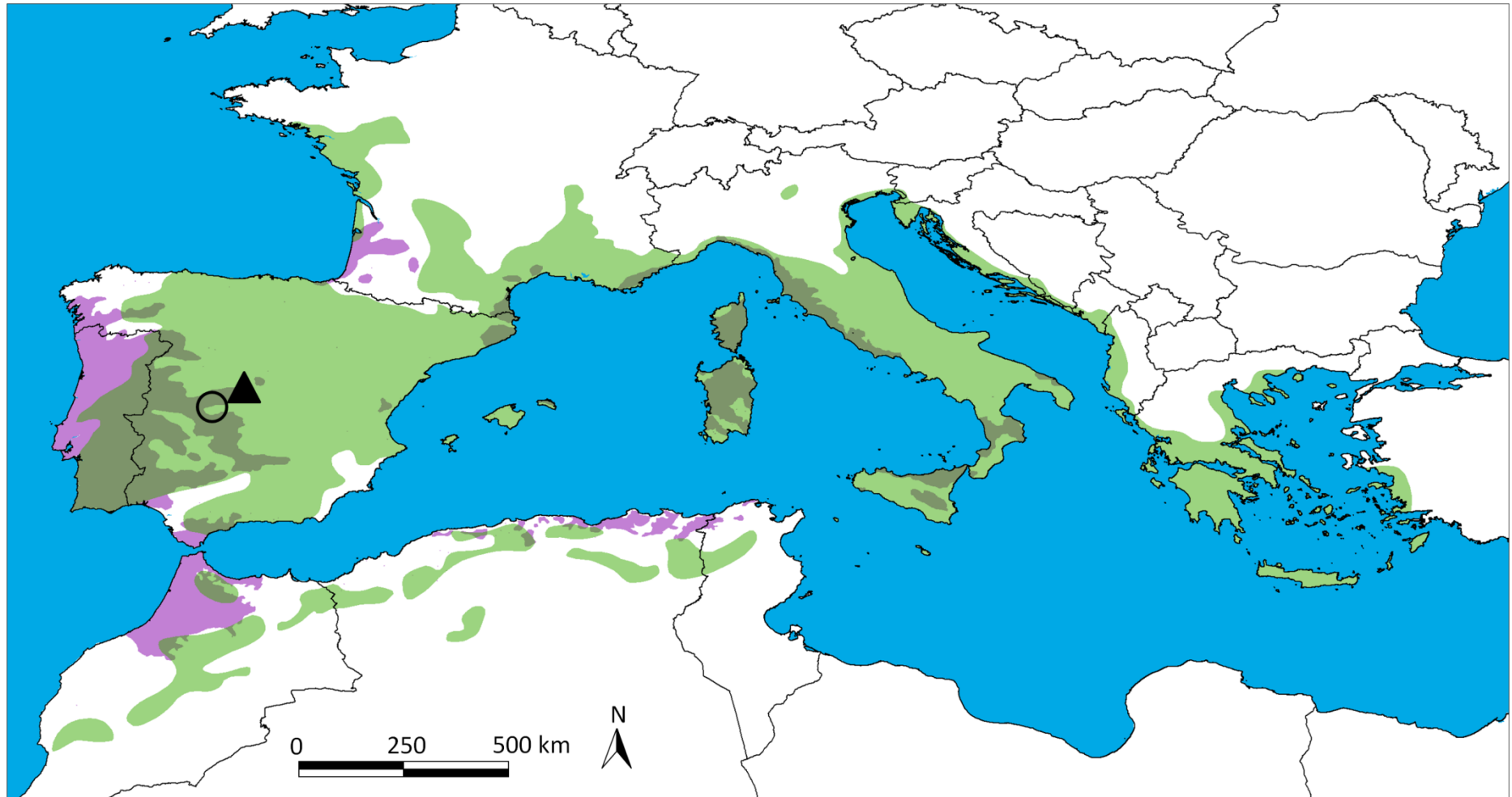
	df	χ^2	P -value
Species	1	22.51	< 0.001
Treatment	1	501.3	< 0.001
Date	2	42.27	< 0.001
Daytime	1	78.57	< 0.001
Species \times Treatment	1	28.61	< 0.001
Species \times Date	2	41.18	< 0.001
Species \times Daytime	1	3.68	0.055
Treatment \times Date	2	44.10	< 0.001
Treatment \times Daytime	1	66.26	< 0.001
Species \times Treatment \times Date	2	33.86	< 0.001
Species \times Treatment \times Daytime	1	1.57	0.211
Block	1	15.60	< 0.001
Family(Species)	1	4.49	0.034
Treatment \times Family(Species)	1	0.21	0.650
Date \times Family(Species)	2	0	-
Daytime \times Family(Species)	1	0	-
Seed mass	1	9.93	0.002

Table 4. Results of the generalized linear mixed model for final survival (t = 50 days). Degrees of freedom (df), Chi-squares (χ^2) and *P*-values are shown. Fixed factor: Species Random factors: Family(Species), Block. Covariate: Seed mass.

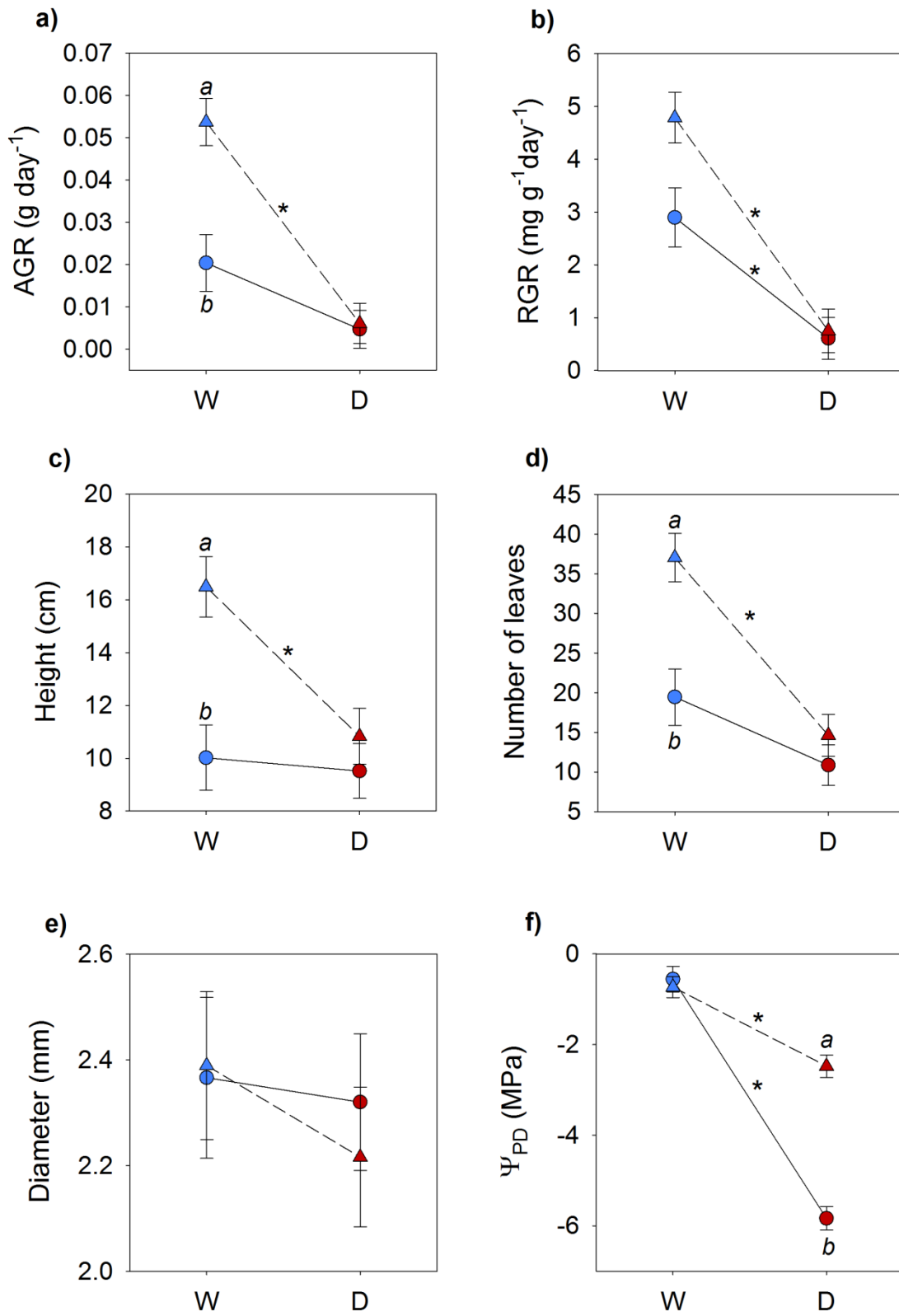
	df	χ^2	<i>P</i> -value
Species	1	5.78	0.016
Block	1	8.32	0.004
Family(Species)	1	0.00	-
Seed mass	1	6.68	0.010

Figures

Fig. 1



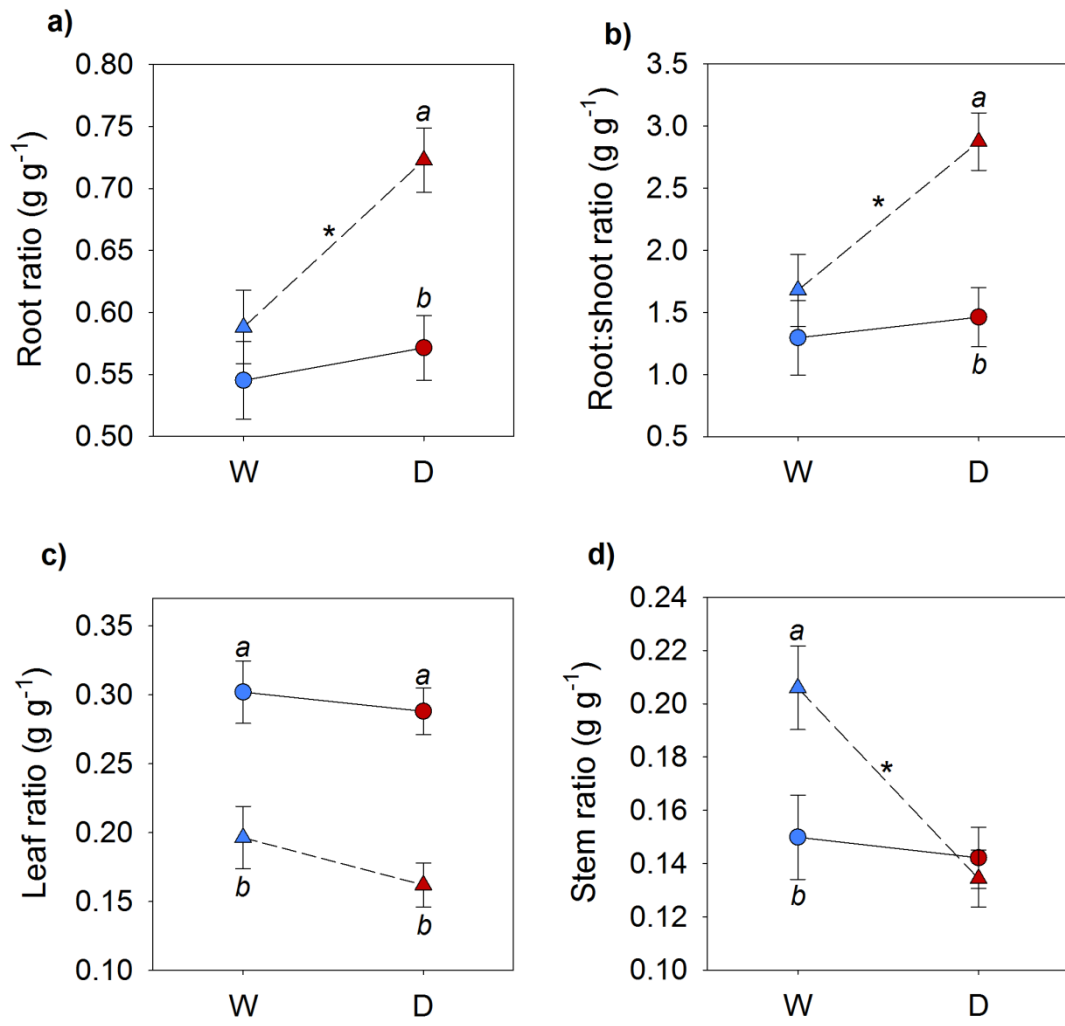
1 Fig. 2.



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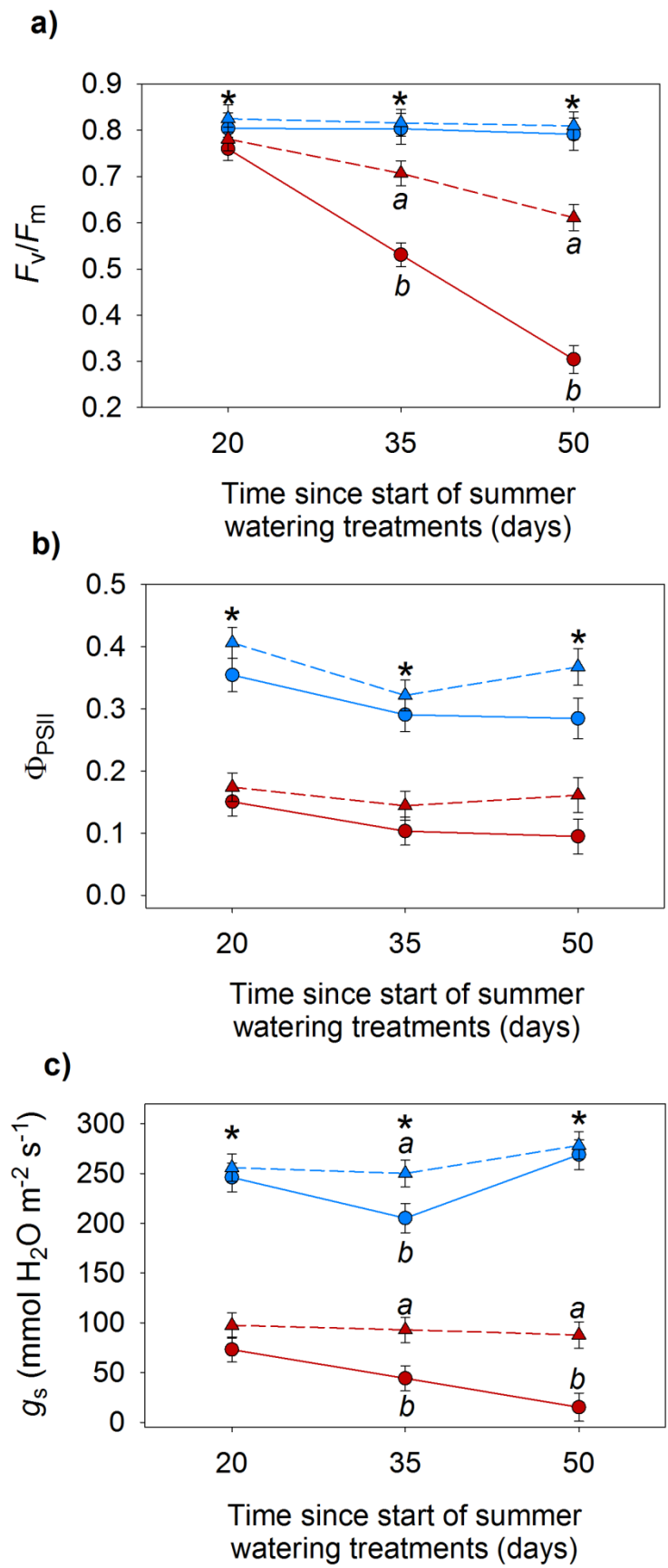
4 Fig. 3.



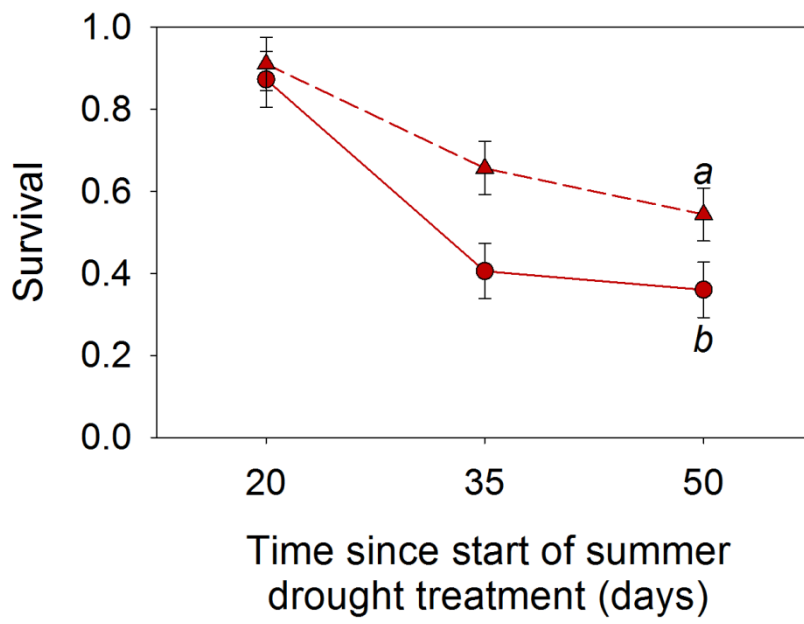
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7 Fig. 4.



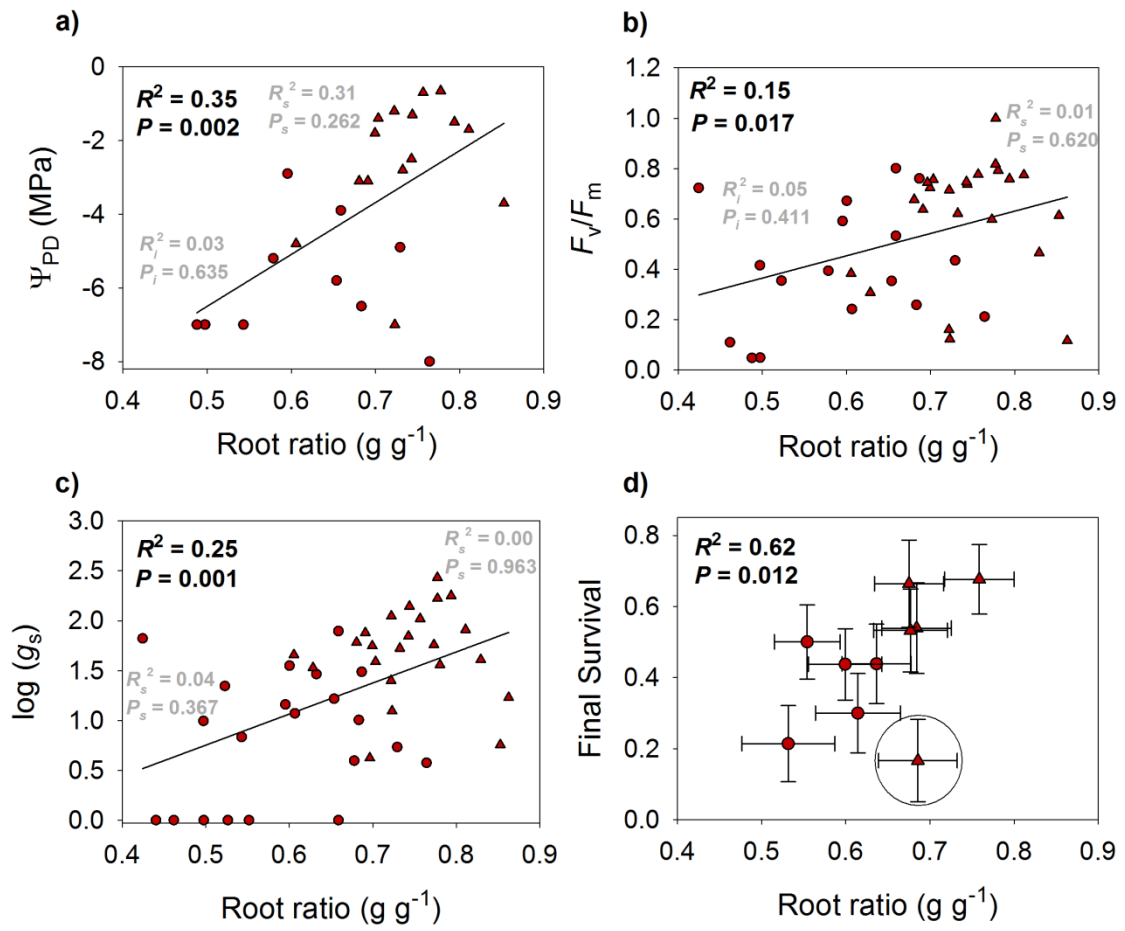
9 Fig. 5.



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12 Fig. 6.



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