

1 **Contrasting functional strategies following severe drought in two**
2 **Mediterranean oaks with different leaf habit: *Quercus faginea* and**
3 ***Quercus ilex* subsp. *rotundifolia***

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20 photoprotection, photosynthesis, vulnerability segmentation, water stress.

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22 **Running head:** Responses to drought in two Mediterranean oaks

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24

25 **ABSTRACT**

26

27 Nowadays, evergreen sclerophyllous and winter-deciduous malacophyllous oaks with
28 different paleogeographical origins coexist under Mediterranean-type climates, such as
29 the mixed forests of the evergreen *Quercus ilex* subsp. *rotundifolia* and the winter-
30 deciduous *Quercus faginea*. Both Mediterranean oaks constitute two examples of
31 contrasting leaf habit, so it would be expected different functional strategies to cope with
32 summer drought. In this study, we analysed photosynthetic, photochemical and hydraulic
33 traits of different organs for *Q. faginea* and *Q. ilex* subsp. *rotundifolia* under well-watered
34 conditions and subjected to very severe drought. The coordinated response between
35 photosynthetic and hydraulic traits explained the higher photosynthetic capacity of *Q.*
36 *faginea* under well-watered conditions, which compensated its shorter leaf life span at the
37 expense of a higher water consumption. The progressive imposition of water stress
38 evidenced that both types of Mediterranean oaks displayed different functional strategies
39 to cope with water limitations. Specifically, the decrease in mesophyll conductance (g_m)
40 associated to edaphic drought seems to be the main factor explaining the differences
41 found in the dynamics of net CO₂ assimilation (A_N) throughout the drought period. The
42 sharp decline in photosynthetic traits of *Q. faginea* was coupled with a strong decrease in
43 shoot hydraulic conductance (K_{shoot}) in response to drought. This fact probably avoided
44 extensive xylem embolism in the stems (i.e. “vulnerability segmentation”), which enabled
45 new leaf development after drought period in *Q. faginea*. By contrast, leaves of *Q. ilex*
46 subsp. *rotundifolia* showed effective photoprotective mechanisms and high resistance to
47 drought-induced cavitation, which would be related with the longer leaf life span of the
48 evergreen Mediterranean oaks. The co-occurrence of both types of Mediterranean oaks
49 could be related to edaphic conditions that ensure the maintenance of soil water potential

50 above critical values for *Q. faginea*, which can be severely affected by soil degradation
51 and climate change.

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53

54 **Introduction**

55

56 Two types of oaks (*Quercus* L.; Fagaceae) currently coexist in locations under genuine
57 Mediterranean-type climates, i.e. the well-known evergreen sclerophyllous type and the
58 sparsely accepted winter-deciduous malacophyllous type (see Gil-Pelegrín et al. 2017 and
59 references therein). Several authors have reported such co-occurrence (Tognetti et al.
60 1998, Nardini et al. 1999, Montserrat-Martí et al. 2009) and considered as a peculiarity
61 of the Mediterranean Biome (Baldocchi et al. 2010). The evergreen sclerophyllous oaks
62 have been considered keystone Mediterranean tree species that would have their origin in
63 pre-Mediterranean lineages of the Palaeotropical Geoflora (Valiente-Banuet et al. 2006,
64 Denk et al. 2017, Jiang et al. 2019) and display morphological traits traditionally
65 associated to Mediterranean-type climates with dry and hot summers (Walter 1985,
66 Traiser et al. 2005). Nevertheless, most of the winter-deciduous Mediterranean oak
67 species, in spite of having an Arcto-Tertiary origin (Grímsson et al 2005), seem to be able
68 to cope with the extreme summer aridity period in a similar way to their evergreen
69 relatives (Radoglou 1996, Scarascia-Mugnozza et al. 2000), and some of them inhabit
70 under extremely dry Mediterranean sub-types (Dufour-Dror and Ertas 2004).

71 The mixed forests of the evergreen sclerophyllous *Quercus ilex* subsp. *rotundifolia*
72 and winter-deciduous *Quercus faginea* located in the inland areas of the Iberian Peninsula
73 would be a good example of this situation (Montserrat-Martí et al. 2009, Forner et al.
74 2018a). These areas are characterized by the occurrence of two potential stress periods
75 throughout the year, the cold winter and the dry and hot summer (Mediavilla and
76 Escudero 2004, Montserrat-Martí et al. 2009). Leaf shedding during the autumn allows
77 *Q. faginea* to evade the negative effect of low temperatures experienced by the leaves of
78 *Q. ilex* subsp. *rotundifolia* (Corcuera et al. 2005a, 2005b). However, the incidence of a

79 dry period during summer severely affects the performance and growth of the two co-
80 occurring species (Corcuera et al. 2004a, 2004b). In this context, it is crucial for
81 Mediterranean tree species to develop different mechanisms and strategies to survive
82 under situations of water deficit (Baldocchi et al. 2010, Forner et al. 2018b).

83 Stomatal closure under drought conditions regulates water consumption at the expense
84 of a reduction in photosynthesis, which has been extensively reported for both types of
85 Mediterranean oaks (Acherar and Rambal 1992, Mediavilla and Escudero 2003, 2004,
86 Peguero-Pina et al. 2008a, 2009, 2018). Besides stomatal limitations, the decrease of the
87 diffusion of CO₂ from the sub-stomatal cavities to the sites of carboxylation (i.e. a reduced
88 mesophyll conductance, g_m) is also one of the key factors limiting photosynthesis under
89 water stress (Flexas et al. 2012, Niinemets and Keenan 2014). However, to the extent of
90 our knowledge, only a few studies have dealt with this topic in Mediterranean oaks (see
91 Peguero-Pina et al. 2017a and references therein). An increase of limitations to
92 photosynthesis in response to drought implies that light incident on the leaf surface may
93 exceed the amount that can be used for photosynthesis. Under this situation, different
94 mechanisms allow the protection of the photosynthetic apparatus, such as the non-
95 photochemical quenching (NPQ) of chlorophyll fluorescence and the de-epoxidation of
96 the xanthophyll cycle, which have been described for some evergreen and deciduous oaks
97 in response to moderate or severe drought conditions (see García-Plazaola et al. 2017 and
98 references therein).

99 Another efficient strategy to survive under water deficit is the development of xylem
100 with a high resistance to water stress-induced embolism, as the failure of the water
101 transport system is a primary cause of plant mortality during drought (Martin-StPaul et
102 al. 2017, Rodriguez-Dominguez et al. 2018). This seems to be the case in both types of
103 Mediterranean oaks, which show very negative values of water potential inducing 50%

104 and 88 loss of hydraulic conductivity in the stems (PLC₅₀ and PLC₈₈, respectively), as
105 reviewed by Gil-Pelegrín et al. (2017). Thus, the combination of an early stomatal closure
106 together with a high resistance of the stem to embolism (i.e. a wide hydraulic safety
107 margin, Martin-StPaul et al. 2017) in Mediterranean oaks would constitute a key factor
108 for increasing the survival under Mediterranean severe drought conditions (Vilagrosa et
109 al. 2010).

110 Besides stem vulnerability, plant tolerance to drought would be constrained by the
111 capacity of other plant organs to withstand xylem embolism (Skelton et al. 2018). This
112 phenomenon is known as “vulnerability segmentation” (Hochberg et al. 2017), and could
113 create hydraulic fuses within the plant in order to confine the drought-induced cavitation
114 to the most expendable parts of the plant (e.g. leaves) and further protect the more costly
115 tissues (e.g. stems or roots) (Creek et al. 2018, Rodriguez-Dominguez et al. 2018).
116 However, contrastingly to stem xylem embolism, only a few studies have dealt with the
117 dynamic of leaf or shoot hydraulic conductance under water stress conditions in
118 Mediterranean oaks (Nardini and Tyree 1999, Nardini et al. 2012, Scoffoni et al. 2012,
119 Peguero-Pina et al. 2015, Skelton et al. 2018). Furthermore, leaf hydraulic conductance
120 is also considered a key factor for net CO₂ assimilation (Scoffoni et al. 2012), possibly
121 through its covariation with stomatal and mesophyll conductance (Flexas et al. 2013,
122 Wang et al. 2018).

123 In particular, regarding *Q. ilex* subsp. *rotundifolia*, a considerable number of studies
124 have dealt with its physiological performance under drought stress from different
125 viewpoints. Thus, a drop in water potential increased the diffusive and non-diffusive
126 limitations to net CO₂ assimilation in this species (Limousin et al. 2010, Gallé et al. 2011,
127 Zhou et al. 2014, Peguero-Pina et al. 2018), which responded through photoprotective
128 mechanisms for dissipating the excess of light energy (Peguero-Pina et al. 2009, Sancho-

129 Knapik et al. 2018). Moreover, the reported curves of stem xylem cavitation seem to
130 indicate a high resistance to drought-induced cavitation (e.g. PLC₅₀ ca. -6.5 MPa
131 according to Peguero-Pina et al. 2014). Nevertheless, the tolerance of other tissues (i.e.
132 leaves or shoots) of *Q. ilex* subsp. *rotundifolia* to drought-induced cavitation has not been
133 studied yet. A high resistance would be also expected, as recently found by Rodriguez-
134 Dominguez et al. (2018) in *Olea europaea*, another very resilient Mediterranean
135 evergreen tree that is able to resist high level of water stress. On contrast, concerning *Q.*
136 *faginea*, several studies have reported an early stomatal closure in response to water
137 scarcity, even more marked than that found for *Q. ilex* subsp. *rotundifolia* (Acherar and
138 Rambal 1992, Mediavilla and Escudero 2003, 2004, Forner et al. 2018a). However, as far
139 as we know, no studies have dealt with the dynamic of mesophyll conductance, the role
140 of non-stomatal limitations to photosynthesis and the existence of efficient
141 photoprotective mechanisms when this species is subjected to severe water shortage.
142 Regarding hydraulic traits, although Esteso-Martínez et al. (2006) reported a relatively
143 low sensitivity of the stem xylem of seedlings of *Q. faginea* to drought-induced cavitation
144 (PLC₅₀ = -3.9 MPa), there is a lack of studies about the sensitivity of leaves or shoots to
145 drought-induced cavitation in *Q. faginea*.

146 Therefore, it could be expected that both species show different functional strategies
147 to cope with water limitations that go beyond they constitute two examples of contrasting
148 leaf habit (Escudero et al. 2017). Very few comparative ecophysiological studies have
149 analysed simultaneously the response to intense water deficit of photosynthetic,
150 photochemical and hydraulic traits of different organs involving co-occurring
151 Mediterranean oaks with contrasting leaf habits (Ramírez-Valiente et al. 2020). In this
152 sense, the specific objectives of this study are: (i) to analyse the existence of a differential
153 response to very severe drought in photosynthetic, photochemical and hydraulic traits of

154 *Q. faginea* and *Q. ilex* subsp. *rotundifolia*, and (ii) elucidate the possible existence of a
155 coordination among these traits for both species to withstand water shortage.

156

157 **Materials and methods**

158

159 *Plant material and experimental conditions*

160

161 This study was carried out with plants of *Quercus faginea* Lam. and *Quercus ilex* subsp.
162 *rotundifolia* from the same provenance (“Alcarria-Serranía de Cuenca” provenance,
163 40°19’N, 2°15’W, 950 m above sea level, Spain). Acorns from both species were sown
164 and cultivated in 2009 under the same conditions with a mixture of 80% compost
165 (Neuhaus Humin Substrat N6; Klasman-Deilmann GmbH, Geeste, Germany) and 20%
166 perlite in 0.5 l containers inside a greenhouse. After the first growth cycle, seedlings were
167 transplanted to 25 l containers filled with the same mixture described above and cultivated
168 outdoors in a common garden at CITA de Aragón (41°39’N, 0°52’W, Zaragoza, Spain)
169 under Mediterranean conditions (mean annual temperature 15.4 °C, total annual
170 precipitation 298 mm). A slow-release fertilizer (15:9:12 N:P:K, Osmocote Plus, Sierra
171 Chemical, Milpitas, CA, USA) was added twice a year to the top 10-cm layer of substrate
172 (3 g l⁻¹ growth substrate). All plants were grown under the same environmental
173 conditions, drip-irrigated to field capacity every 2 days and pruned when necessary until
174 just before the beginning of the vegetative period of 2018.

175 Two weeks before the onset of the experiment, five potted plants per species (10-years-
176 old) were placed under a polyethylene roof (200 µm thickness) with open sides that
177 allowed passing 90% of photosynthetic photon flux density (PPFD) (~1800 µmol photons
178 m⁻² s⁻¹ at midday, over the course of the experiment). We used this cover to avoid re-

179 watering by unwanted precipitation events throughout the drought period. Irrigation was
180 stopped on 11 July 2018 and all measurements started on 12 July 2018 under well-watered
181 conditions in the five plants per species (thereafter “stressed plants”). During the
182 following days, all measurements were simultaneously performed in the same five
183 stressed plants per species every 2-4 days with increasing levels of drought: 12, 16, 19,
184 23, 25, 27, 30 July and 1 August 2018 for *Q. faginea*; 12, 16, 20, 24, 28 July and 1 August
185 2018 for *Q. ilex* subsp. *rotundifolia*. Drought stress was imposed until 2 August 2018,
186 when the percentage of green leaves was almost negligible for both species (11% for *Q.*
187 *faginea* and 12 % for *Q. ilex* subsp. *rotundifolia*, Figure 1). Subsequently, stressed plants
188 were irrigated again to field capacity after the last measurement under drought conditions.

189 Plants of *Q. faginea* burst its buds again at the end of the summer; we took advantage
190 of this event to examine the hydraulic capacity of the new stems (thereafter “second
191 growth”) and those that experienced the drought period (thereafter “first growth”) in the
192 stressed plants. For comparative purposes, we also measured the hydraulic capacity of the
193 first and second growth in another set of five plants of *Q. faginea* that were grown under
194 the same environmental conditions than stressed plants although drip-irrigated to field
195 capacity every 2 days throughout the summer (thereafter “control plants”).

196

197 *Water potential measurements*

198

199 Leaf water potential was measured at predawn and midday (Ψ_{PD} and Ψ_{MD} , respectively,
200 MPa) throughout the drought period in one shoot of each one of the five studied stressed
201 plants per species (with leaves still attached to the shoots) with a Scholander pressure
202 chamber, according to the methodology described by Turner (1988).

203

204 *Percentage of leaf damage using visual scoring and spectral reflectance*

205

206 Percentage of green, yellow and brown leaves was estimated by means of visual scoring
207 (VS) throughout the drought period for each one of the five studied stressed plants per
208 species. In order to test that colour changes were associated to changes in leaf chlorophyll
209 concentration, we made spectral reflectance measurements between 9 and 10 h (solar
210 time) in one green, yellow and brown fully-developed current-year attached leaf for each
211 one of the five studied stressed plants per species throughout the drought period. To do
212 this, we used a visible/near-infrared spectroradiometer USB-2000 (Ocean Optics,
213 Dunedin, USA) connected into one end of a bifurcated fiber optic cable, which were also
214 connected into the other end to a tungsten halogen light source LS-1-LL (Ocean Optics,
215 Dunedin, USA). Leaf reflectance was calculated by dividing leaf spectral radiance by the
216 radiance of a white standard (Spectralon, Labsphere, North Sutton, NH, USA).
217 Integration time was 200 ms. The normalized difference vegetation index (NDVI) was
218 used for the estimation of changes in leaf chlorophyll concentration and calculated as
219 $(R_{750} - R_{705}) / (R_{750} + R_{705})$, where R_{750} and R_{705} represent, respectively, the reflectance at
220 750 and 705 nm (Richardson and Berlyn 2002).

221

222 *Leaf gas exchange and chlorophyll fluorescence measurements*

223

224 Chlorophyll (Chl) fluorescence parameters were measured firstly at predawn on fully
225 developed current-year attached leaves and cortical bark chlorenchyma of each one of the
226 five studied stressed plants of *Q. faginea* and *Q. ilex* subsp. *rotundifolia*. Measurements
227 were performed with a FMS II modulated fluorometer (Hansatech Instruments, Norfolk,
228 UK) on one green, yellow and brown leaf per studied stressed plant, and the weighted

229 mean was calculated for each plant according to the percentage of each leaf type (Figure
230 1). Initial Chl fluorescence in the dark-adapted state (F_0) was measured only with the
231 modulated light (0.6 kHz, PPFD below $0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$), which avoids electron transport
232 through photosystem II (PSII). Maximal Chl fluorescence in the dark-adapted state (F_M)
233 was measured by closing reaction centres with a 0.8-s pulse of $6000 \mu\text{mol m}^{-2} \text{s}^{-1}$ of white
234 light (20 kHz). F_0 was measured in presence of far-red light that fully oxidizes the PSII
235 acceptor side (Belkhodja et al. 1998). The dark-adapted, maximum quantum efficiency
236 of PSII was calculated as F_V/F_M (Abadía et al. 1999), being $F_V = F_M - F_0$, both for leaves
237 and cortical bark chlorenchyma (Peguero-Pina et al. 2008b).

238 Afterwards, simultaneous gas-exchange and chlorophyll fluorescence measurements
239 were carried out in the same leaves between 9 and 10 h (solar time) with the portable
240 photosystem system CIRAS-2 (PP-Systems, Amesbury, MA, USA) fitted with an
241 automatic universal leaf cuvette (PLC6-U, PP-Systems) and the FMS II portable
242 fluorometer mentioned above. All measurements were conducted under controlled
243 conditions for CO_2 concentration surrounding the leaf ($C_a = 400 \mu\text{mol mol}^{-1}$), leaf
244 temperature (25°C), vapour pressure deficit (1.25 kPa) and saturating PPFD ($1500 \mu\text{mol}$
245 $\text{m}^{-2} \text{s}^{-1}$). After steady-state in terms of gas exchange was reached (typically ca. 10 minutes
246 after leaf clamping), net photosynthesis (A_N , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s ,
247 $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) and Chl fluorescence at steady-state photosynthesis (F_S) were
248 recorded. The maximum Chl fluorescence in the light-adapted state (F'_M) was
249 subsequently determined with a 0.8-s pulse of $6000 \mu\text{mol m}^{-2} \text{s}^{-1}$ of white light. Finally,
250 the minimum Chl fluorescence in the light-adapted state (F'_0) was determined after
251 switching off the actinic light and in presence of far-red light ($7 \mu\text{mol m}^{-2} \text{s}^{-1}$).

252 The actual (Φ_{PSII}) and intrinsic (Φ_{exc}) PSII efficiency were calculated according to
253 Genty et al. (1989) as $(F'_M - F_S)/F'_M$ and $(F'_M - F'_0)/F'_M$, respectively. Non-photochemical

254 quenching (NPQ) was calculated as $(F_M/F'_M) - 1$ (Bilger and Björkman 1990). The
 255 fraction of light absorbed that is dissipated in the PSII antenna $(1 - \Phi_{exc})$ was also
 256 estimated (Morales et al. 1998). Photosynthetic electron transport rate (J_F) was
 257 determined according to Krall and Edwards (1992). The measured fluxes were corrected
 258 by quantifying the leakage of CO₂ in and out of the cuvette with a photosynthetically
 259 inactive leaf obtained by heating (Flexas et al. 2007a).

260 Mesophyll conductance (g_m) and maximum velocity of carboxylation (V_{cmax}) were
 261 estimated according to the variable J method of Harley et al. (1992) (eq. 1) and the one-
 262 point method of De Kauwe et al. (2016) (eq. 2), respectively, as follows:

$$g_m = \frac{A_N}{C_i - \frac{\Gamma^*(J_F + 8(A_N + R_L))}{J_F - 4(A_N + R_L)}} \quad (1)$$

$$V_{cmax} = \frac{A_N}{\left(\frac{C_i - \Gamma^*}{C_i + K_m}\right) - 0.015} \quad (2)$$

263 where A_N and the substomatal CO₂ concentration (C_i) were taken from the gas-exchange
 264 measurements at saturating light. The chloroplastic CO₂ compensation point in the
 265 absence of mitochondrial respiration (Γ^*) and the respiration rate in the light (R_L) were
 266 estimated as described in Flexas et al. (2007b), whereas K_m (the Michaelis-Menten
 267 constant) was estimated as described in De Kauwe et al. (2016).

268 The relative controls on A_N were separated into their functional components according
 269 to the proposal by Jones (1985) as implemented and comprehensively described by Grassi
 270 and Magnani (2005). This methodology allows comparing relative changes in limitations
 271 to net CO₂ assimilation into different components related to g_s (stomatal limitations, l_s),

272 g_m (mesophyll limitations, l_m) and leaf biochemistry (biochemical limitations, l_b). l_s , l_m
273 and l_b , with value between zero and one ($l_s + l_m + l_b = 1$), were calculated as:

$$l_s = \frac{g_{tot}/g_s \cdot \delta A_N/\delta C_c}{g_{tot} + \delta A_N/\delta C_c} \quad (3)$$

$$l_m = \frac{g_{tot}/g_m \cdot \delta A_N/\delta C_c}{g_{tot} + \delta A_N/\delta C_c} \quad (4)$$

$$l_b = \frac{g_{tot}}{g_{tot} + \delta A_N/\delta C_c} \quad (5)$$

274 where g_s is the stomatal conductance to CO₂, g_m is the mesophyll conductance according
275 to Harley et al. (1992) (eq. 1), and g_{tot} is the total conductance to CO₂ from leaf surface
276 to carboxylation sites in the chloroplasts ($1/g_{tot} = 1/g_s + 1/g_m$). The values of g_m obtained
277 were used to calculate the chloroplastic CO₂ concentration (C_c) using the equation $C_c =$
278 $C_i - A_N/g_m$. $\delta A_N/\delta C_c$ was calculated for *Q. faginea* and *Q. ilex* subsp. *rotundifolia* as the
279 slope of the relationship between C_c and A_N obtained for each species, considering a C_c
280 range of 50–100 $\mu\text{mol mol}^{-1}$ (Peguero-Pina et al. 2017b).

281

282 *Native xylem embolism*

283

284 Native xylem embolism was determined throughout the drought period in current-year
285 twigs of *Q. faginea* and *Q. ilex* subsp. *rotundifolia* collected from branches of each one
286 of the five studied stressed plants per species where Ψ_{MD} had previously been measured.
287 We used the same experimental procedure that is described in Peguero-Pina et al. (2018).
288 Twigs were cut under water in the experimental field and immediately transported to the

289 laboratory while maintaining them under water and covered with a plastic bag. Once
290 there, stem segments of the twigs were recut under water (50 mm long), and both ends
291 were shaved with a razor blade. Segments were placed in a tubing immersed in distilled
292 water to avoid desiccation and to maintain a near constant temperature, and connected to
293 a digital mass flowmeter Liqui-Flow (Bronkhorst High-Tech, Ruurlo, Netherlands). We
294 perfused distilled, degassed and filtered (0.22 μm) water containing 0.005 %
295 (volume/volume) Micropur (Katadyn Products, Wallisellen, Switzerland) to prevent
296 microbial growth. The hydraulic conductivity (K_{stem} , $\text{kg m s}^{-1} \text{MPa}^{-1}$) was measured at a
297 pressure of 0.005 MPa and after removing air embolisms at a pressure of 0.15 MPa for
298 60–90 s. Native embolism was calculated as the ratio between K_{stem} values obtained
299 before and after removing embolism.

300

301 *Shoot hydraulic conductance (K_{shoot})*

302

303 Shoot hydraulic conductance (K_{shoot} , $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) was measured for *Q. faginea*
304 and *Q. ilex* subsp. *rotundifolia* throughout the drought period following the methodology
305 detailed in Brodribb et al. (2005). One sun-exposed branch of each one of the five studied
306 stressed plants per species was sampled at 07:00–08:00 h (solar time), minimizing the
307 possibility for midday reduction in leaf hydraulic conductance (K_{leaf}) (Brodribb and
308 Holbrook 2004). Branches were enclosed in sealed plastic bags to impede water loss, and
309 stored in complete darkness during at least 1 h to achieve complete stomatal closure. This
310 procedure ensures that all leaves from the same branch reached the same water potential,
311 which is assumed to be that of the leaves before rehydration (Ψ_0). Afterwards, one shoot
312 per branch was cut under water to avoid air entry and allowed to take up water for 30 to

313 60 seconds (t). The water potential after rehydration was subsequently measured (Ψ_f).

314 The leaf hydraulic conductance was calculated as:

$$K_{shoot} = \frac{C \cdot \ln\left(\frac{\Psi_0}{\Psi_f}\right)}{t} \quad (6)$$

315 where C , ($\text{mol MPa}^{-1} \text{ m}^{-2}$) is the shoot capacitance for each species, calculated as the

316 initial slope of the P-V relationships, normalized by the leaf area (Brodribb et al. 2005).

317 P-V relationships for *Q. faginea* and *Q. ilex* subsp. *rotundifolia* were determined in six

318 leaves per species following the free-transpiration method (Vilagrosa et al. 2003).

319

320 *Nitrogen, phosphorous and potassium concentration in green, yellow and brown leaves*

321

322 The macro-nutrients N, P and K were selected to document nutrient resorption prior to

323 abscission in response to leaf damage for *Q. faginea* and *Q. ilex* subsp. *rotundifolia*. N, P

324 and K concentration were analysed for each one of the studied stressed plants in i/ green

325 leaves at the beginning of the experiment, and ii/ green, yellow and brown leaves at the

326 end of the drought period. N was quantified using an Organic Elemental Analyzer (Flash

327 EA 112, Thermo Fisher Scientific Inc., MA, USA). P and K were quantified using an

328 inductively coupled plasma optical emission (ICP-OES) spectrometer (Varian 725 ES,

329 Varian Inc., CA, USA).

330

331 *Stem hydraulic conductivity in Q. faginea after the drought period*

332

333 As explained above, we examined the hydraulic capacity of the new stems (“second

334 growth”) and the possible hydraulic recovery of the stems that experienced the drought

335 period (“first growth”) in the five stressed plants of *Q. faginea*. Moreover, for
336 comparative purposes, we also measured the hydraulic capacity of the first and second
337 growth in another set of five control plants of *Q. faginea* that were well irrigated
338 throughout the summer. To do this, K_{stem} was determined two months after the end of
339 experiment (1-2 October 2018) in one stem segment of each one of the five control and
340 the five stressed plants of *Q. faginea* before and after removing air embolisms, and native
341 embolism was then calculated as described above and expressed as percentage loss of
342 conductivity (PLC, %). Leaf-specific conductivity (LSC, $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was calculated
343 as the ratio between K_{stem} before removing air embolism and total leaf surface area
344 supplied. Leaf area was measured by digitalizing the leaves and using the ImageJ image
345 analysis software (<http://rsb.info.nih.gov/nih-image/>).

346

347 *Statistical analysis*

348

349 Data are expressed as means \pm standard error of the mean. Student’s t-tests were used to
350 determine the point during the drought period when the change for each measured
351 parameter was statistically significant compared to well-watered conditions. PLC₅₀ values
352 were calculated for stems and shoots by adjusting pairs of data (Ψ_{MD} vs. percentage of
353 maximum K_{stem} and Ψ_{MD} vs. percentage of maximum K_{shoot} , respectively) by using a
354 sigmoidal function according to Pammenter and Van derWilligen (1998). One-way
355 ANOVAs were performed to identify the effect of the type of leaf (green, yellow and
356 brown) on NDVI and nutrient composition for stressed plants of both species. Multiple
357 comparisons were carried out among types of leaf for NDVI and nutrient composition
358 using post hoc Tukey’s Honest Significant Difference test. Student’s t-tests were used to
359 compare the values of K_{stem} , PLC, LSC and Total Leaf Area between control and stressed

360 plants both for the first and the second growth of *Q. faginea* two months after the drought
361 period. All statistical analyses were performed in the R software environment (version
362 4.0.0, R development Core Team, 2020).

363

364 **Results**

365

366 We found a differential response to changes in water potential between *Q. faginea* and *Q.*
367 *ilex* subsp. *rotundifolia* in terms of leaf damage (Figure 1). Thus, *Q. faginea* showed a
368 gradual decrease in the percentage of green leaves for a Ψ_{PD} range between ca. -2 MPa
369 and -4 MPa, as the percentage of yellow and brown leaves gradually increased (Figure
370 1A). The percentage of green leaves experienced a drastic reduction (15 %) when Ψ_{PD}
371 was below -4.5 MPa in comparison with the values under well-watered conditions (100
372 %) and the percentage of brown leaves showed its maximum value (64 %) at the end of
373 the drought period ($\Psi_{PD} = -5.6$ MPa) (Figure 1A). By contrast, *Q. ilex* subsp. *rotundifolia*
374 maintained all the leaves green at this stage ($\Psi_{PD} = -5.2$ MPa), and only experienced a
375 sharp increase in the percentage of brown leaves (88 %) at the end of the drought period
376 ($\Psi_{PD} = -8$ MPa) (Figure 1B). It should be noted that *Q. ilex* subsp. *rotundifolia* never
377 showed yellow leaves throughout the drought period (Figure 1B).

378 Leaf damage in *Q. faginea* was subsequently followed by a decrease in F_V/F_M in
379 yellow and, especially, brown leaves with respect to green leaves (Figure 2A). Thus, at
380 the end of the drought period, when the percentage of green leaves was almost negligible,
381 F_V/F_M in yellow and brown leaves were ca. 0.5 and 0.1, respectively (Figure 2A).
382 Nonetheless, F_V/F_M in stems of *Q. faginea* remained high throughout the drought period
383 (Figure 2B). By contrast, *Q. ilex* subsp. *rotundifolia* did not show this pattern, as F_V/F_M
384 showed a decrease in green leaves and, albeit delayed, also in stems, reaching very low

385 values at the end of the drought period (ca. 0.2 and 0.3, respectively) (Figures 2C and
386 2D).

387 The values of the normalized difference vegetation index (NDVI) of green leaves were
388 much higher than those measured for yellow or brown leaves for drought stressed plants
389 of both species ($P < 0.05$, Figure S1), suggesting that leaf discoloration was associated to
390 a decrease in chlorophyll concentration. NDVI did not show statistically significant
391 differences ($P > 0.05$) throughout the drought period within each leaf type (i.e. green,
392 yellow or brown) both for drought stressed plants of *Q. faginea* and *Q. ilex* subsp.
393 *rotundifolia* (data not shown). The drop in NDVI associated to changes in leaf colour was
394 accompanied in *Q. faginea* by a decrease in N, P, and K concentrations, especially for
395 brown leaves (Figure S2). By contrast, *Q. ilex* subsp. *rotundifolia* did not experience
396 changes in N/P/K content between green and brown leaves (Figure S2). No differences
397 in N/P/K concentrations were found between green leaves under well-watered conditions
398 at the beginning of the drought period (Gr_i) and the leaves that remained green at the
399 end of the drought period (Gr_f) for stressed plants of *Q. faginea* and *Q. ilex* subsp.
400 *rotundifolia* (Figure S2).

401 The values of net photosynthesis and stomatal conductance (A_N and g_s) for *Q. faginea*
402 were 1.4 and 1.6-fold higher, respectively, than those recorded for *Q. ilex* subsp.
403 *rotundifolia* at the beginning of the experiment (i.e. when Ψ_{PD} was about -0.2 MPa)
404 (Figures 3A and 3B). The intrinsic water use efficiency ($iWUE = A_N/g_s$) did not show
405 statistically significant differences ($P > 0.05$) between for both species at this stage
406 (Figure 3C). Both A_N and g_s decreased in *Q. faginea* and *Q. ilex* subsp. *rotundifolia* when
407 Ψ_{PD} became more negative, reaching negative values for A_N and almost negligible values
408 for g_s at the end of the drought period (Figure 3). The main difference in the response to
409 changes in water potential between the two species was found when water stress was

410 moderate (i.e. when Ψ_{PD} ranged between ca. -1 and -2 MPa). At this stage, *Q. faginea*
411 experienced a sharp decrease in A_N (Figure 3B), even higher than that found for g_s (Figure
412 3A), with a concomitant reduction in iWUE when Ψ_{PD} reached -1.5 MPa (Figure 3C). By
413 contrast, *Q. ilex* subsp. *rotundifolia* showed a more gradual decrease in A_N and g_s ,
414 displaying higher values than *Q. faginea* at this stage (Figures 3A and 3B). Moreover, the
415 drops in A_N and g_s in *Q. ilex* subsp. *rotundifolia* when Ψ_{PD} became more negative were
416 coordinated, such that iWUE remained fairly constant for this species throughout the
417 drought period (Figure 3C). This pattern was also observed for mesophyll conductance
418 (g_m) and maximum velocity of carboxylation (V_{cmax}), with higher values for *Q. faginea* at
419 the beginning of the experiment but a sharp decrease than *Q. ilex* subsp. *rotundifolia*
420 throughout the drought period (Figure 4). The analysis of the partitioning of
421 photosynthetic limitations revealed that A_N was mainly limited by mesophyll conductance
422 (l_m) for *Q. ilex* subsp. *rotundifolia*, with a gradual increase throughout the drought period
423 (Figure 5B). Stomatal (l_s) and biochemical (l_b) limitations for this species slightly
424 decreased and remained fairly constant, respectively, during the drought period (Figure
425 5A and 5C). Regarding *Q. faginea*, A_N was mainly limited by l_m and l_b at the beginning
426 of the experiment (Figure 5). However, when Ψ_{PD} became more negative, l_b strongly
427 decreased at the expense of a sharp increase in l_m , whereas l_s only experienced a slight
428 reduction (Figure 5).

429 The rest of parameters derived from Chl fluorescence measurements also showed
430 outstanding differences between the two species. The actual PSII efficiency (Φ_{PSII}) was
431 much higher in *Q. ilex* subsp. *rotundifolia* than in *Q. faginea* at the beginning of the
432 drought period (i.e. when Ψ_{PD} was about -0.2 MPa) (Figure 6A). However, contrary to *Q.*
433 *faginea*, Φ_{PSII} in *Q. ilex* subsp. *rotundifolia* experienced a strong decrease when Ψ_{PD}
434 diminished, showing lower values than *Q. faginea* even when stress was moderate (i.e.

435 when Ψ_{PD} was ca. -1 MPa) (Figure 6A). The fraction of light absorbed that is dissipated
436 in the PSII antenna ($1 - \Phi_{exc}$) and non-photochemical quenching (NPQ) were much
437 lower for *Q. ilex* subsp. *rotundifolia* at the beginning of the drought period, while both
438 parameters experienced a sharp increase in this species when water stress was moderate
439 (Figures 6B and 6C). By contrast, the increase in Φ_{exc} and NPQ throughout the drought
440 period was more gradual in *Q. faginea* (Figures 6B and 6C).

441 The increasing levels of maximum daily drought stress (estimated through the
442 measurement of Ψ_{MD}) induced a progressive loss of hydraulic conductivity in *Q. faginea*
443 and *Q. ilex* subsp. *rotundifolia*, both in shoots (K_{shoot}) and stems (K_{stem}) (Figure 7).
444 Regarding K_{stem} , *Q. ilex* subsp. *rotundifolia* reached values of native xylem embolism ca.
445 50% at water potential values similar than *Q. faginea* (ca. -5.9 and -5.5 MPa, respectively,
446 Figure 7B). By contrast, the water potential inducing the 50% loss of hydraulic
447 conductivity in the shoots occurred at water potential values much more negative in *Q.*
448 *ilex* subsp. *rotundifolia* than in *Q. faginea* (ca. -6.8 and -4.9 MPa, respectively, Figure
449 7A).

450 Two months after the end of the experiment, the values of the percentage loss of
451 conductivity (PLC) in the stems that were subjected to the drought period (first growth)
452 of stressed plants of *Q. faginea* were much higher than that for the first growth of control
453 plants (Figure 8B), which implied a strong reduction in K_{stem} (Figure 8A). PLC in the
454 second growth was almost negligible for both control and stressed plants (Figure 8B). In
455 spite of this, K_{stem} in the second growth of stressed plants was much lower than that for
456 control plants (Figure 8A), which can be explained by the strong decrease in the
457 conductive xylem area (data not shown). Leaf-specific conductivity (LSC) did not show
458 differences ($P > 0.05$) between control and stressed plants for the first growth (Figure 8C)
459 due to the strong reduction in supported leaf area experienced by stressed plants (Figure

460 8D), which compensated the differences in K_{stem} (Figure 8A). The differences in LSC
461 between control and stressed plant for the second growth (Figure 8C) reflected the
462 differences in K_{stem} (Figure 8A), as supported leaf area did not show differences ($P > 0.05$)
463 between both types of plants for the second growth (Figure 8D).

464

465 **Discussion**

466

467 *Segregation between Q. faginea and Q. ilex subsp. rotundifolia in the absence of water*
468 *stress*

469

470 This study has evidenced that *Q. faginea* displayed higher values of A_N , g_s and g_m than *Q.*
471 *ilex* subsp. *rotundifolia* under well-watered conditions at the beginning of the experiment
472 (first point of the graphs in Figures 3 and 4), with similar values than those previously
473 reported for *Q. faginea* (Peguero-Pina et al. 2016) and for *Q. ilex* subsp. *rotundifolia*
474 (Peguero-Pina et al. 2017b, 2018). Regarding this, some comparative studies already
475 stated the lower g_s and A_N in *Q. ilex* subsp. *rotundifolia* when compared with *Q. faginea*
476 in the absence of water stress (Mediavilla and Escudero 2003, 2004, Juárez-López et al.
477 2008, Forner et al. 2018a). Our results also show that, under well-watered conditions at
478 the beginning of the experiment, K_{shoot} was much higher for *Q. faginea* than for *Q. ilex*
479 subsp. *rotundifolia* (45.4 ± 3.9 and 27.2 ± 1.4 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$, respectively) (data not
480 shown). Taken together, these facts reflect the existence of a coordinated response
481 between gas exchange and leaf hydraulic when these species are compared under well-
482 watered conditions, as the evergreen *Q. ilex* subsp. *rotundifolia* showed a similar
483 percentage of decrease for these parameters with respect to the winter-deciduous *Q.*
484 *faginea* (i.e. 34%, 39%, 45% and 40% for A_N , g_s , g_m and K_{shoot} , respectively). Previous

485 studies have established that the link between photosynthesis and leaf hydraulic arises
486 from g_s (Brodribb et al. 2007, Scoffoni et al. 2016), as water loss and CO₂ uptake share a
487 common pathway through stomata (Boyer 2015). Moreover, several studies have also
488 pointed out the existence of a coordination of g_m and leaf hydraulic (Flexas et al. 2013,
489 Xiong et al. 2017, 2018, Lu et al. 2019), as recently reviewed by Xiong and Nadal (2020).
490 These authors suggested that water movement in mesophyll tissues also share a common
491 pathway with CO₂ transport through the gas phase that would connect g_m and the outside-
492 xylem component of leaf hydraulic conductance (K_{ox}). Overall, this coordination would
493 explain the increased ability for carbon assimilation found in *Q. faginea* when compared
494 with *Q. ilex* subsp. *rotundifolia* in the absence of water stress, albeit at the expense of a
495 higher water consumption under the atmospheric evaporative demand experienced by this
496 species during summer (Peguero-Pina et al. 2016).

497

498 *The progressive imposition of water stress induced a differential physiological response*
499 *between Q. faginea and Q. ilex subsp. rotundifolia*

500

501 We confirmed that winter-deciduous Mediterranean oaks such as *Q. faginea* showed a
502 higher stomatal sensitivity to soil water deficit than evergreen Mediterranean oaks such
503 as *Q. ilex* subsp. *rotundifolia* (Figure 3B), as previously reported by Acherar and Rambal
504 (1992) and Mediavilla and Escudero (2003). This phenomenon minimized water losses
505 in *Q. faginea* at the expense of an extreme reduction in A_N , and may explain the slightly
506 higher rates of A_N in *Q. ilex* subsp. *rotundifolia* at low water potentials (Figure 3) (Forner
507 et al. 2018a). However, unlike previous studies, this is the first comparative research
508 between two species representing the two types of Mediterranean oaks dealing with the

509 role of non-stomatal factors constraining photosynthetic activity under drought
510 conditions.

511 According to our results, the differential response of g_m to edaphic drought seems to
512 be the main factor explaining the differences found between *Q. faginea* and *Q. ilex* subsp.
513 *rotundifolia* in the dynamics of net CO₂ assimilation when Ψ_{PD} became more negative
514 (Figure 4). Thus, we have demonstrated for the first time the important role of g_m in
515 determining the photosynthetic response of *Q. faginea* to water stress, with increasing
516 relative importance under progressive drought (Figure 5). Specifically, when water stress
517 was moderate, the decrease in g_m in *Q. faginea* (ca. 95%) were more pronounced than
518 those estimated for *Q. ilex* subsp. *rotundifolia* (ca. 51%) (Figure 4), which could explain
519 the additional decrease in A_N (ca. 96%) with respect to g_s (ca. 80%) experienced by *Q.*
520 *faginea* (Figure 3). Similar observations were obtained in other winter-deciduous oaks,
521 such as *Q. pubescens* (Zhou et al. 2014), *Q. robur* (Grassi and Magnani 2005) and *Q.*
522 *petraea* (Cano et al. 2013). The underlying mechanisms to the response of g_m to drought
523 are yet to be elucidated, but they may be associated to changes in aquaporin conductance
524 and/or carbonic anhydrase expression (Cano et al. 2013). In parallel to g_m , we also
525 observed a sharp decrease in V_{cmax} for *Q. faginea* under progressive water deficit, which
526 strongly limited the fixation of CO₂ into sugars (Figure 5). By contrast, *Q. ilex* subsp.
527 *rotundifolia*, regardless of the predominant role of mesophyll limitations throughout the
528 drought period (Figure5), showed a higher degree of co-regulation in the response of A_N ,
529 g_s , g_m and V_{cmax} to progressive drought, which allowed this species to maintain fairly
530 constant values of iWUE throughout the drought period (Figures 3 and 4).

531 Besides photosynthetic traits, the ability of plants to supply water to transpiring leaves
532 can be strongly limited under drought conditions, as it happens to both species here
533 studied (Figure 7). In spite of this common trend, *Q. faginea* experienced an early

534 decrease of K_{shoot} in response to drought, with a PLC_{50} value less negative than that
535 estimated for *Q. ilex* subsp. *rotundifolia* (ca. -4.9 and -6.8 MPa, respectively, Figure 7A).
536 This differential response in leaf hydraulic resembled the one observed in photosynthetic
537 traits. Recently, Xiong and Nadal (2020) stated that only a few studies have examined the
538 coordination of photosynthetic and leaf hydraulic traits upon water stress, especially
539 considering g_m . Thus, Wang et al. (2018) proposed that the drought-induced decrease in
540 leaf hydraulic conductance could act as a potential trigger for declines in g_s and g_m in
541 *Oryza sativa*. By contrast, Theroux-Rancourt et al. (2014) reported a delayed response of
542 g_m with respect to g_s and leaf hydraulic conductance in poplar clones exposed to soil
543 drying. Therefore, the interdependence between photosynthetic and hydraulic traits could
544 be species-dependant (Xiong and Nadal 2020). In our case, we observed an early response
545 in g_s and g_m with respect to K_{shoot} for both species, but especially pronounced in *Q. ilex*
546 subsp. *rotundifolia*. This situation agrees with the hypothesis of stomata as a safety valve
547 to prevent hydraulic failure under moderate drought conditions (Hochberg et al. 2017,
548 Flexas et al. 2018).

549 In contrast with K_{shoot} , both species showed a similar evolution of K_{stem} with increasing
550 levels of drought stress (Figure 7). In other words, PLC_{50} in shoots was lower than in
551 stems of *Q. faginea* (ca. -4.9 and -5.5 MPa, respectively), which is consistent with the
552 “vulnerability segmentation” hypothesis (Hochberg et al. 2017). Skelton et al. (2018) also
553 found that leaves were more vulnerable than stems in *Q. douglasii*, a winter-deciduous
554 Mediterranean oak that experiences leaf shedding under severe drought associated with
555 leaf embolism and avoidance of extensive xylem embolism (i.e. “vulnerability
556 segmentation”). In our study, a similar behaviour was described for *Q. faginea*, as the
557 percentage of green leaves experienced a drastic reduction when Ψ_{PD} was ca. -4.5 MPa
558 (Figure 1A), as reported by Hinckley et al. (1983) for the winter-deciduous Mediterranean

559 oak *Q. pubescens*. This Ψ_{PD} value corresponded to a Ψ_{MD} value ca. -5.7 MPa during the
560 drought period (data not shown), which would imply a reduction of K_{shoot} below 20% of
561 its initial value (Figure 7A) while K_{stem} was reduced only to about 50% of the initial value
562 (Figure 7B). Peguero-Pina et al. (2015) suggested that “vulnerability segmentation” in
563 the winter-deciduous Mediterranean *Q. subpyrenaica* explained the premature leaf
564 withering during intense summer drought as a way to reduce water losses by transpiration,
565 ensuring bud survival and subsequent new leaf development. This last phenomenon was
566 observed in *Q. faginea* several weeks after the end of the drought period, probably
567 because the embolism threshold for irreversible drought damage (88% loss of stem
568 conductivity, Urli et al. 2013) was not reached (Figure 7B), as also indicated the lack of
569 F_V/F_M decrease in the stems of this species (Figure 2B). The new bud bursting in
570 previously stressed plants of *Q. faginea* was not associated to xylem refilling in the stems
571 that experienced water stress (i.e. first growth), but to the formation of new xylem without
572 embolism (i.e. second growth) after drought period (Figure 8B). In line with this, Creek
573 et al. (2018) also found that three arid zone Australian species with “vulnerability
574 segmentation” did not refill xylem embolisms, and hydraulic recovery was dependent on
575 new plant growth after drought. However, the hydraulic capacity of the second growth of
576 *Q. faginea* was much lower than that for control plants (Figure 2A) for an equivalent
577 supported leaf area (Figure 2D), which implied a strong reduction of LSC in the new
578 xylem of previously stressed plants (Figure 2C). Another negative consequence of
579 premature withering in *Q. faginea* could be a decrease in nutrient use efficiency
580 (Montserrat-Martí et al. 2009), as resorption of N, P and K was only partial in yellow and
581 brown leaves (Figure S2).

582 By contrast, PLC_{50} in shoots of *Q. ilex* subsp. *rotundifolia* was even higher than in
583 stems (ca. -6.8 and -5.9 MPa, respectively). Indeed, Skelton et al. (2018) did not find

584 evidences of “vulnerability segmentation” for the evergreen Mediterranean oaks of
585 California, and even *Q. sadleriana* showed the opposite trend, in line with the results here
586 obtained for *Q. ilex* subsp. *rotundifolia*. In this way, *Q. ilex* subsp. *rotundifolia* maintained
587 all the leaves green throughout almost all the drought period (Figure 1B), which pointed
588 out the high resistance of their leaves to drought-induced cavitation. Peguero-Pina et al.
589 (2015) suggested that this strategy could be related with the longer leaf life span of the
590 evergreen species and the ability of *Q. ilex* subsp. *rotundifolia* for maintaining its
591 photosynthetic capacity for more than one vegetative period (Corcuera et al. 2005a).
592 Therefore, this species ensures the keeping of foliage by avoiding irreversible damage
593 even under intense drought conditions (i.e. when Ψ_{PD} was ca. -5 MPa).

594 To do this, besides leaf hydraulic resistance, Mediterranean evergreen oaks have
595 developed a great capacity for protecting the photosynthetic apparatus through the
596 thermal dissipation of the excess of energy in response to water scarcity (Peguero-Pina et
597 al. 2009, García-Plazaola et al. 2017, Sancho-Knapik et al. 2018). Thus, we have shown
598 that *Q. ilex* subsp. *rotundifolia* showed an early strong decrease in Φ_{PSII} in response to
599 drought (Figure 6A), downregulating the photosynthetic electron transport when CO_2
600 assimilation is severely limited. Furthermore, this species also showed a sharp increase
601 in the fraction of light absorbed that is dissipated in the PSII antenna ($1 - \Phi_{exc}$) and in the
602 non-photochemical quenching (NPQ) in response to drought (Figures 6B and 6C,
603 respectively). However, although these mechanisms were also present in *Q. faginea*, our
604 results indicated a limited capacity to cope with water stress in terms of photoprotection
605 when compared with *Q. ilex* subsp. *rotundifolia* (Figure 6).

606

607 *Ecophysiological implications*

608

609 Both *Q. faginea* and *Q. ilex* subsp. *rotundifolia* are well-known representatives of the two
610 co-occurring types of Mediterranean oaks, with different leaf type, paleogeographical
611 origin and contrasting strategies to cope with water limitations. Winter-deciduous
612 Mediterranean oaks have been considered components of the so-called “sub-
613 Mediterranean” vegetation (Sánchez de Dios et al. 2009), inhabiting transitional areas
614 where the Mediterranean summer aridity is partially mitigated by a higher annual
615 precipitation and a lower mean temperature (Río and Penas, 2006, Martín-Gómez et al.
616 2017). Their co-existence with the evergreen congeneric species in many areas of the
617 western Mediterranean basin is a complex matter that has been examined in several
618 ecophysiological studies (e.g. Nardini et al. 1999, Montserrat-Martí et al. 2009, Peguero-
619 Pina et al. 2015), but further research is needed to fully understand it.

620 On one hand, the leaf life span of the winter-deciduous Mediterranean oaks limits the
621 photosynthetic activity to a shorter period, which would explain the coordination between
622 hydraulic and photosynthetic traits in order to maximize the ability for carbon
623 assimilation in *Q. faginea* under favourable conditions. On the other hand, the great water
624 consumption required under the high atmospheric evaporative demand experienced by
625 this species during summer (Peguero-Pina et al. 2016) could induce an additional drop in
626 soil water availability that could negatively affect leaf hydraulics, gas exchange
627 (Mediavilla and Escudero 2003, 2004) and, ultimately, can induce a premature leaf
628 withering. This fact may definitively shorten the vegetative period and explain the large
629 dependence of *Q. faginea* on edaphic conditions that ensure the maintenance soil water
630 potential values above critical values for this species (Esteso-Martínez et al. 2006). Thus,
631 when soil water was depleted, leaves of *Q. ilex* subsp. *rotundifolia* exhibited a more
632 conservative water-use strategy and a higher resistance to drought-induced cavitation than
633 *Q. faginea* (Figures 3C and 6B, respectively). This conservative leaf strategy of the

634 evergreen Mediterranean oaks allows them to assimilate carbon throughout a longer time
635 period during summer and other periods of the year such as the early spring or late
636 autumn, as reported by Corcuera et al. (2005a) for *Q. ilex* subsp. *rotundifolia*. Therefore,
637 the co-occurrence of both types of Mediterranean oaks in these transitional areas of the
638 western Mediterranean basin could be associated to local edaphic conditions that result
639 in a lower water storage capacity as a consequence of the soil degradation associated to
640 the human management Corcuera et al. (2005a, 2005b). This situation would be
641 exacerbated in the context of climate change, which predicts an increase in temperature
642 and aridity in several regions, especially in the Mediterranean basin (Klausmeyer and
643 Shaw 2009, Ruiz-Labourdette et al. 2012). For this reason, there are some prediction
644 models that indicate a notable reduction in the potential distribution range of winter-
645 deciduous Mediterranean oaks (e.g. *Q. faginea*) and their substitution by evergreen
646 Mediterranean oaks such as *Q. ilex* (Sanchez de Dios et al. 2009).

647

648 **Conclusions**

649

650 We have evidenced in the present study that *Q. faginea* and *Q. ilex* subsp. *rotundifolia*
651 show a differential response in terms of photosynthetic, photochemical and hydraulic
652 traits, both in the absence of water stress and under intense drought. The contrasting leaf
653 life span may condition the different functional strategies shown by different organs (i.e.
654 leaves, shoots and stems) of these species, with important consequences for the co-
655 occurrence of both types of Mediterranean oaks, which could be dependent on local
656 conditions related to soil water availability.

657 Thus, the shorter leaf life span of the winter deciduous *Q. faginea* was compensated
658 through an increased ability for carbon assimilation by means of enhanced g_m , V_{cmax} and

659 K_{shoot} , although at the expense of a higher water consumption under favourable
660 conditions. However, this advantage in terms of carbon gain was rapidly suppressed, even
661 when soil water deficit was moderate, due to the sharp decline in g_m , the most limiting
662 factor for photosynthetic activity in *Q. faginea*. This fact was coupled with an early
663 increase in leaf embolism and the lack of effective photoprotective mechanisms, which
664 could trigger the drastic reduction of green leaves in response to drought. As a
665 counterpart, stems of *Q. faginea* were protected from extensive xylem embolism, which
666 allowed new leaf development after drought period. This fact could be crucial for the
667 survival and growth of this species, as well as partially offsetting the loss of
668 competitiveness with co-occurring evergreen Mediterranean oaks with longer leaf life
669 span. This was the case of *Q. ilex* subsp. *rotundifolia*, which showed a conservative leaf
670 strategy, characterized by a high resistance to drought-induced cavitation and effective
671 photoprotective mechanisms, which kept their leaves functional even under intense
672 drought conditions for more than one vegetative period. This conservative strategy would
673 confer a competitive advantage with respect to winter-deciduous Mediterranean oaks
674 when soil water availability is restricted.

675

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677

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683

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950 **Figure legends**

951

952 Figure 1. Time course of the percentage of green, yellow and brown leaves with predawn
953 water potential (Ψ_{PD}) for *Q. faginea* (A) and *Q. ilex* subsp. *rotundifolia* (B) during the
954 drought period. Data are mean \pm SE of each date when stressed plants were measured
955 (see Materials and Methods for details). Asterisks indicate the point of the drought period
956 when the decrease of the percentage of green leaves was statistically significant (pairwise
957 *t*-test, $P < 0.05$) compared to well-watered conditions (first point of the graph).

958

959 Figure 2. Time course of the maximum potential PSII efficiency (F_V/F_M) with predawn
960 water potential (Ψ_{PD}) for *Q. faginea* and *Q. ilex* subsp. *rotundifolia* leaves (A and C,
961 respectively) and stems (B and D, respectively) during the drought period. Data are mean
962 \pm SE of each date when stressed plants were measured (see Materials and Methods for
963 details). Asterisks indicate the point of the drought period when the decrease was
964 statistically significant (pairwise *t*-test, $P < 0.05$) compared to well-watered conditions
965 (first point of the graph).

966

967 Figure 3. Time course of (A) net photosynthesis (A_N), (B) stomatal conductance (g_s) and
968 (C) intrinsic water use efficiency with predawn water potential (Ψ_{PD}) for *Q. faginea*
969 (white symbols) and *Q. ilex* subsp. *rotundifolia* (grey symbols) leaves during the drought
970 period. Data are mean \pm SE of each date when stressed plants were measured (see
971 Materials and Methods for details). Measurements were performed on green, yellow and
972 brown leaves when present, and the weighted mean was calculated for each plant
973 according to the percentage of each leaf type (Figure 1). Data were adjusted for a better
974 visualization and interpretation of the results. Asterisks indicate the point of the drought

975 period when the decrease was statistically significant (pairwise t -test, $P < 0.05$) compared
976 to well-watered conditions (first point of the graph). Triangles represent unreliable iWUE
977 values due to the combination of negative A_N and extremely low g_s at the end of the
978 drought period.

979

980 Figure 4. Time course of (A) mesophyll conductance (g_m) and (B) maximum velocity of
981 carboxylation (V_{cmax}) with predawn water potential (Ψ_{PD}) for *Q. faginea* (white symbols)
982 and *Q. ilex* subsp. *rotundifolia* (grey symbols) leaves during the drought period. Data are
983 mean \pm SE of each date when stressed plants were measured (see Materials and Methods
984 for details). Measurements were performed on green, yellow and brown leaves when
985 present, and the weighted mean was calculated for each plant according to the percentage
986 of each leaf type (Figure 1). Data were adjusted for a better visualization and
987 interpretation of the results. Asterisks indicate the point of the drought period when the
988 decrease was statistically significant (pairwise t -test, $P < 0.05$) compared to well-watered
989 conditions (first point of the graph).

990

991 Figure 5. Time course of relative (A) stomatal (l_s), (B) mesophyll (l_m) and (C) biochemical
992 (l_b) photosynthesis limitations with predawn water potential (Ψ_{PD}) for *Q. faginea* (white
993 symbols) and *Q. ilex* subsp. *rotundifolia* (grey symbols) leaves during the drought period.
994 Data are mean \pm SE of each date when stressed plants were measured (see Materials and
995 Methods for details). Measurements were performed on green, yellow and brown leaves
996 when present, and the weighted mean was calculated for each plant according to the
997 percentage of each leaf type (Figure 1). Asterisks indicate the point of the drought period
998 when the change was statistically significant (pairwise t -test, $P < 0.05$) compared to well-
999 watered conditions (first point of the graph).

1000

1001 Figure 6. Time course of (A) actual PSII efficiency (Φ_{PSII}), (B) the fraction of light
1002 absorbed that is dissipated in the PSII antenna ($1 - \Phi_{\text{exc}}$) and (C) non-photochemical
1003 quenching (NPQ) with predawn water potential (Ψ_{PD}) for *Q. faginea* (white symbols) and
1004 *Q. ilex* subsp. *rotundifolia* (grey symbols) leaves during the drought period. Data are
1005 mean \pm SE of each date when stressed plants were measured (see Materials and Methods
1006 for details). Measurements were performed on green, yellow and brown leaves when
1007 present, and the weighted mean was calculated for each plant according to the percentage
1008 of each leaf type (Figure 1). Data were adjusted for a better visualization and
1009 interpretation of the results. Asterisks indicate the point of the drought period when the
1010 change was statistically significant (pairwise *t*-test, $P < 0.05$) compared to well-watered
1011 conditions (first point of the graph).

1012

1013 Figure 7. Relationship between midday water potential (Ψ_{MD}) and the percentage of (A)
1014 shoot hydraulic conductance (K_{shoot}) and (B) stem hydraulic conductivity (K_{stem}) for *Q.*
1015 *faginea* (white symbols) and *Q. ilex* subsp. *rotundifolia* (grey symbols) leaves during the
1016 drought period. Data are mean \pm SE of each date when stressed plants were measured
1017 (see Materials and Methods for details). Asterisks indicate the point of the drought period
1018 when the decrease was statistically significant (pairwise *t*-test, $P < 0.05$) compared to
1019 well-watered conditions (first point of the graph). Vertical dashed lines indicate the water
1020 potential inducing 50% loss of hydraulic conductivity (PLC₅₀) in shoots and stems.

1021

1022 Figure 8. (A) Stem hydraulic conductivity (K_{stem}), (B) percentage loss of stem hydraulic
1023 conductivity (PLC), (C) leaf-specific conductivity (LSC) and (D) supported leaf area for
1024 the first and the second growth in control (white bars) and stressed (grey bars) plants of

1025 *Q. faginea* two months after the end of the drought period. Data are mean \pm SE. Asterisks
1026 indicate statistically significant differences between control and stressed plants ($P < 0.05$).
1027