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1 **Interspecific competition influences the response of oak transpiration to**
2 **increasing drought stress in a mixed Mediterranean forest**

3

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33 **Highlights (max 85 characters including spaces per line):**

- 34 • The influence of species interaction on oak transpiration was studied in a
35 Mediterranean forest.
- 36 • Oak species differed in the response to drought in terms of transpiration and water-use
37 efficiency.
- 38 • The presence of sessile oak in mixtures increased drought effect on turkey oak.
- 39 • Managing for mixed stands might increase the detrimental effect of drought on species
40 functioning.

41 **ABSTRACT**

42 Increasingly severe droughts are expected to negatively impact forest functioning in the
43 future, especially in the Mediterranean region. Favouring mixed species stands has been
44 advocated as a compromise between wood production and biodiversity conservation, but
45 whether such management practices would allow forest ecosystems to acclimate to future
46 climate conditions remains to be addressed. We tested whether the transpiration of *Quercus*
47 *cerris* (Qc) and *Quercus petraea* (Qp) during droughts differ when they grow in pure or mixed
48 forests. We measured sap flux density (F_D) and leaf carbon isotope composition ($\delta^{13}C$), as a
49 proxy for intrinsic water use efficiency (WUE_{int}), in pure and mixed Qc and/or Qp forest plots
50 in Italy during the summer 2012. Qc and Qp showed a drop in F_D of 41% and 52%
51 respectively during the summer drought independently of the mixtures, but with different
52 seasonal trends. Qp transpiration response to soil drought did not differ between pure and
53 mixed plots. In contrast, Qc transpiration was reduced by 56% in mixed plots at the maximum
54 of the drought and only by 31% in the pure plot. Furthermore, we observed higher WUE_{int} for
55 Qc in the mixed plot and no change for Qp, supporting the higher water stress intensity in the
56 mixed plot for Qc. Our study illustrated the negative impact of Qp on Qc when these species
57 compete for water resources. We suggest that managing for mixed stands in the Mediterranean
58 region might, in some cases, increase the detrimental effect of drought on species functioning.
59

- 60 **Keywords:** drought, sap flux, *Quercus*, species interactions, water use
61 efficiency
- Abbreviations**
- 62 F_D : Sap flux density ($\text{L dm}^{-2} \text{ h}^{-1}$)
- 63 $F_{D \text{ Mean}}$: Mean daily sap flux density ($\text{L dm}^{-2} \text{ h}^{-1}$)
- 64 $F_{D \text{ Mean-S}}$: Mean daily sap flux density standardized by potential evapotranspiration ($\text{L dm}^{-2} \text{ h}^{-1}$
65 mm^{-1})
- 66 PET : Daily potential evapotranspiration (mm)
- 67 Qc : *Quercus cerris* L.
- 68 Qp : *Quercus petraea* (Matt) Liebl.
- 69 REW : Relative extractable water (unitless)
- 70 VPD : Vapour pressure deficit (kPa)
- 71 WUE_{int} : Intrinsic water use efficiency ($\mu\text{mol}^{-1} \text{ mol}^{-1}$)
- 72 $\delta^{13}\text{C}$: Leaf carbon isotope composition (‰)
- 73

74 1. INTRODUCTION

75 Both fundamental and applied questions are emerging about potential changes in the
76 functioning of European forest ecosystems in regions subjected to severe climatic changes.
77 Scenarios predict a global increase in the frequency and intensity of atmospheric and soil
78 drought during the growing season throughout Europe (IPCC, 2007) and whether today's
79 forest ecosystems will acclimate and survive in the upcoming decades remains to be seen. The
80 Mediterranean zone will be one of the regions most affected during the next decades, with a
81 considerable reduction in rainfall during the growing season accompanied by more frequent
82 and more intense heat-waves (Giorgi 2006; Somot *et al.* 2008). Ecological stability and
83 productivity of Mediterranean forests is particularly sensitive to climatic conditions
84 (Scarascia-Mugnozza *et al.* 2000). In this context, forest management practices adapted to
85 future climatic change must be implemented (Fabbio *et al.* 2003). Specifically, these practices
86 should be able to counteract the likely decrease in productivity and increase of catastrophic
87 events such as fires and pests outbreaks so that acceptable levels of goods and services can
88 continue to be provided.

89 Mixed forests have been advocated as a potential compromise between high wood production
90 and biodiversity conservation (Kelty 2006) and seem to be an interesting management option
91 to help forest ecosystems acclimate to future climatic conditions. The two mechanisms
92 underlying the positive effects of biodiversity on ecosystem services in mixed forests are:
93 complementarity of resource use arising from facilitation and/or niche stratification between
94 species with different functional traits, and selection effects explained by a higher probability
95 of including one or several highly productive species in more diverse stands (Loreau &
96 Hector 2001). Positive effects of biodiversity on productivity in mixed Mediterranean forests
97 have already been demonstrated (i.e. Vilà *et al.* 2007); however, species interactions in mixed
98 forests may also strongly influence other ecosystem functions, particularly related to water
99 use under soil water deficit conditions, though studies to date are lacking for these
100 ecosystems.

101 The influence of tree species diversity on ecosystem transpiration has already been observed
102 (Forrester *et al.* 2010; Gebauer *et al.* 2012; Grossiord *et al.* 2013; Kunert *et al.* 2012). In
103 tropical and temperate tree plantations, higher water use seems to be triggered by a
104 complementary behaviour between the interacting species through differential canopy and/or
105 root stratification in mixed and pure stands (Forrester *et al.* 2010; Kunert *et al.* 2012).

106 Conversely, in a mature temperate forest and a boreal tree plantation, higher transpiration of

107 mixed-species stands was due to the presence of a species with particularly high water use per
108 canopy projection area (Gebauer *et al.* 2012; Grossiord *et al.* 2013). Including species with
109 high transpiration rates in mixed species stands subjected to periods of reduced precipitation
110 may therefore exhaust soil water reserves earlier during drought as compared to pure stands.
111 In addition, soil water limiting conditions may have negative consequences on species
112 survival, ecosystem productivity and ecosystem resistance to soil drought.

113 This paper presents a field study on the impact of soil water deficit on the tree-level
114 transpiration of two oak species (*Quercus cerris* L., turkey oak, and *Quercus petraea* (Matt
115 Liebl., sessile oak) growing in pure or mixed conditions in Tuscany in central Italy. We tested
116 whether the transpiration response of these species to severe soil water depletion would differ
117 in pure and mixed plots. We also analyzed whether these differences were related to differing
118 intrinsic water use efficiencies. We hypothesized that mixing the two oak species would lead
119 to a better utilization of available resources during a summer drought and that, consequently,
120 transpiration rates would decrease less than in the pure plots. Indeed, these two oak species
121 are known to have different water use strategies (Tognetti *et al.* 1996) and to differ in their
122 rooting systems (Nardini 1999). Turkey oak is considered to be highly opportunistic for water
123 uptake and keeps stomata open during summer drought periods, whereas sessile oak has a
124 more conservative strategy of controlling transpiration earlier during drought.

125

126 2. MATERIALS AND METHODS

127 2.1 Site description

128 The study was conducted in a thermophilous deciduous forest situated in south-central
129 Tuscany in the natural forest of Alto Merse, Italy (43.08°N, 11.19°E). One pure plot of turkey
130 oak (*Quercus cerris* L., Qc), one of sessile oak (*Quercus petraea* (Matt) Liebl., Qp) and one
131 mixed plot with both oaks (Qc/Qp) were selected. The 30 m × 30 m plots were no more than 2
132 km from one another and are included in the exploratory platform of the FP7-
133 FunDivEUROPE project (“Functional Significance of Forest Biodiversity in Europe”)
134 (Baeten *et al.* 2013). They are situated on Cambisol soils (World Reference Base for Soil
135 Resources, FAO classification) above quartzite and anagenite bedrock and were selected to
136 maximize soil type homogeneity and reduce as much as possible differences in diameter
137 distribution, basal area, tree height, tree density, or canopy structure. The plots were
138 composed of 50- to 60-year-old trees at altitudes ranging from 250 to 450 m a.s.l.. The past
139 management of all plots is coppice with standards and the last cut was about 20 years ago.
140 Mean annual rainfall in the region is 950 mm, occurring mainly during the winter, and mean
141 annual temperature is around 13.5°C. Detailed plot characteristics are presented in Table 1.

142

143 2.2 Drought intensity

144 In order to characterize the drought intensity experienced by the study trees, we estimated the
145 daily relative extractable water in the soil over the whole root zone (*REW*, unitless) in each
146 plot over 2012. *REW* is defined as the ratio between available soil water and maximum
147 extractable water. It varies between 1.0 (field capacity) and 0.0 (permanent wilting point).
148 When *REW* is higher than 0.4, soil water conditions are non-limiting for transpiration of a
149 large range of tree species and stomatal conductance mainly depends on air vapour pressure
150 deficit, irradiance and forest phenology (Granier *et al.* 1999). When *REW* is below 0.4, the
151 resulting soil water shortage induces a drop in stomatal conductance and thus in leaf gas
152 exchange, causing most tree species to cease radial growth (Granier *et al.* 1999). In order to
153 simulate *REW* at a daily time-scale, we ran the BILJOU water balance model (Granier *et al.*
154 1999) with measurements of daily climate conditions (rainfall, global radiation, air
155 temperature and humidity, wind speed) from a nearby meteorological station in Pentolina
156 (43.12°N, 11.10°E, 414 m a.s.l.), 1 km from the study plots. This generic model calculates the
157 different water fluxes at daily time-scale in the ecosystem: tree transpiration, understory

158 evapotranspiration, rainfall interception and drainage. These fluxes are dependent on plant
159 area index and evaporative demand, i.e. the potential evapotranspiration, calculated using the
160 Penman equation (*PET*, mm). The difference between rainfall and evapotranspiration fluxes
161 refills the soil reservoir, whereas excess of water is drained out. All the functions implemented
162 in the model were calibrated using various field experiments in different forests, climate
163 conditions, and soil types. In order to parameterize the model to our study site, the plant area
164 index (*PAI*, m² m⁻²) of each selected plot was measured in September 2012. We used a LAI
165 2000 Plant Canopy Analyzer (PCA, LI-COR, Lincoln, NE, USA) to measure *PAI* at five
166 locations in each plot and then calculated plot average (Table 1). Maximum extractable water
167 (i.e. the water holding capacity) in this soil type was assumed to be 140 mm (Badeau V, pers.
168 comm.). Sensitivity tests using lower (100 mm) and higher (160 mm) water holding capacities
169 did not change the seasonal patterns of *REW* (data not shown).

170

171 **2.3 Tree transpiration**

172 We followed the thermal dissipation method described by Granier (1985) to measure sap flux
173 density in the three selected plots at four periods in the 2012 growing season (P1 = June, P2 =
174 July, P3 = August, and P4 = September). In the pure plots, five trees were equipped with
175 sapflow sensors; in the mixed plot, five trees per species were equipped with sapflow sensors,
176 for a total of 20 trees (Table 2). This number of replicates was considered representative of the
177 response of single tree species to soil drought and it is currently used in the literature on tree-
178 level transpiration research. All sapflow sensors were installed on the North faced side of trees
179 in order to reduce the effect of direct sunshine that can be responsible of thermal gradients that
180 would alter measurements. Each sensor was supplied with a power of 0.2 W.

181 Studied trees were randomly selected among the dominant and co-dominant tree classes
182 (canopy stage) for each species in each plot. These classes were defined according to the
183 diameter of the trees in each plot and an index of light interception (Dawkins index) that was
184 visually assigned for each tree. Together, the dominant and co-dominant classes represented
185 86%, 93% and 80% of the sapwood area in the Qc, Qp and Qc/Qp plots, respectively.
186 Measurements were restricted to trees of the dominant and co-dominant classes in order to
187 minimize other confounding factors on transpiration such as sun exposure. Because of sensor
188 failure, one tree in the Qc pure plot had to be removed from the analyses (Table 2). Due to
189 technical constraints (power supply system), the sap flux density measurements were
190 restricted to 4 or 5 consecutive days in each period (Fig. 1).

191 The two 20-mm-long probes of the sapflow sensors (UP-GmbH, Cottbus, Germany) were
192 installed under the bark and the cambium at around 1.3 m above soil level with a 10-cm
193 vertical spacing between the probes. Data were collected every ten seconds with a CR1000 or
194 a CR800 data logger (Campbell Scientific Inc, Loughborough, Leicestershire, UK) and thirty-
195 minute averages were recorded. The volumetric sap flux density (F_D , L dm⁻² h⁻¹) was
196 calculated according to Granier (1985):

197

198 , (1)

199

200 where $\Delta T(0)$ is the maximum temperature difference between the two temperature probes
201 during the night and $\Delta T(u)$ is the current temperature difference. Although this equation was
202 not originally validated for Q_c and Q_p directly, literature shows validation of this method for
203 a large number of species with great physiological differences (e.g. Braun and Schmid 1999;
204 Mc Culloh *et al.* 2007; Liu *et al.* 2008).

205 Allometric equations to calculate tree sapwood area and thus sapwood thickness were
206 established for both species by extracting wood cores from the North aspects of the stems on
207 21 and 48 Q_c and Q_p trees selected right outside the study plots with a Pressler increment
208 borer (Suunto, Finland) in July 2012 (Table 3). Sapflow sensors integrate sap flux density
209 over a 20-mm-long section of sapwood. As only a small proportion of sap flux density beyond
210 20 mm-depth has been observed in *Quercus* species (Cermak *et al.* 1992), we did not apply
211 any radial distribution corrections for F_D . For two Q_p trees, the sapwood length was smaller
212 than 20 mm and we applied the correction proposed by Clearwater *et al.* (1999).

213 As sapflow sensors signals were stable during nights among the 4 investigated periods, we
214 concluded that there was no substantial occurrence of night-time transpiration. We then
215 calculated mean daily sap flux density ($F_{D \text{ Mean}}$, L dm⁻² h⁻¹) and mean daily sap flux density
216 standardized by PET ($F_{D \text{ Mean-S}}$, L dm⁻² h⁻¹ mm⁻¹) for each tree.

217

218 **2.4 Foliar carbon isotope composition**

219 To estimate leaf intrinsic water use efficiency (WUE_{int}), 20 fully expanded sunlit leaves were
220 sampled once from each of the selected trees by tree climbers at the beginning of July 2012.
221 The samples were oven dried at 60°C for 48 h at INRA Nancy and then finely ground. About

222 1.0 mg of the powdered material from each sample was placed into tin capsules for analysis of
223 leaf carbon isotope composition ($\delta^{13}\text{C}$, ‰). Analyses were performed by the Technical
224 Platform of Functional Ecology (OC 081) at the INRA Forest Ecology and Ecophysiology
225 Unit with an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany; \pm
226 0.2 ‰). Isotopic measurements are reported in the delta notation (δ , ‰) relative to the Vienna
227 Pee Dee Belemnite (VPDB) standard. $\delta^{13}\text{C}$ is positively related to WUE_{int} (Farquhar *et al.*
228 1982) and provides information on WUE_{int} integrated over the leaf life-span.

229

230 **2.5 Data analysis**

231 The analyses were performed with the statistical software R 2.14.1 (R Development Core
232 Team, 2011). For the pure plots, mixed linear models were used to determine the fixed effects
233 of *REW*, *PET*, species, and their interactions on $F_{D\text{ Mean}}$. The day of measurement and tree
234 identity were included in these tests as a repeated measure and a random effect, respectively.
235 Tests were conducted separately for the first three measurement periods and for all four
236 periods together in order to test the impact of the decreasing soil water content (first three
237 periods) separately from the recovering period (fourth period). For all plots, mixed linear
238 models were used 1) to determine the effect of mixture, measurement period, species, and
239 their interactions on $F_{D\text{ Mean-s}}$, with “days” as a repeated measure and “tree” as a random effect,
240 and 2) to analyze the effect of mixture and species on $\delta^{13}\text{C}$ values, with “tree” as random
241 effect. Finally, contrasts (Tukey test) were used to test for relevant differences between fixed
242 effects when the overall models were significant.

243

244 3. RESULTS

245 3.1 Model simulation of drought intensity

246 On June 1st, i.e. just before the first measurement period (P1), soil water content was close to
247 field capacity (Fig. 1). During P1, *REW* was well above 0.4 for all plots, suggesting that
248 transpiration was not yet limited by soil water (Fig. 1). From P1 to P3, *REW* decreased
249 progressively in all three plots (Fig. 1a). During P2, *REW* was already lower than 0.4 in all
250 plots. P3 revealed even lower *REW* values in all three plots; furthermore, *REW* was still
251 decreasing during this period. Over the whole study period, *REW* reached minimum value in
252 all plots on August 23rd, 2012, i.e. between P3 and P4. *REW* was lower than 0.4 for a
253 minimum period of 45 days in the mixed plot and a maximum period of 65 days in the Qc
254 pure plot. Finally, rain events between August 23rd and September 9th 2012 resulted in a sharp
255 increase in *REW*. During the last period (P4), *REW* was higher than 0.4 in all plots. Radiation,
256 *VPD*, and *PET* were high during P1 and P2 and then decreased in P3 and P4 (Fig. 1b).

257

258 3.2 Tree transpiration

259 As the drought intensified during the summer months, $F_{D \text{ Mean}}$ of both species and in all plots
260 progressively decreased to a minimum during P3. Thereafter, $F_{D \text{ Mean}}$ increased from P3 to P4
261 for both species and in all plots (Fig. 2).

262 In the pure plots, the mixed-effect models revealed significant species ($P = 0.001$), *PET* and
263 *REW* effects ($P < 0.001$) on $F_{D \text{ Mean}}$ when considering the four periods individually or
264 combined. For all plots and all periods combined, we observed significant species ($P = 0.012$)
265 and measurement period ($P < 0.001$) effects on $F_{D \text{ Mean-s}}$, but no effect of the mixture ($P =$
266 0.081). The interactions between mixture and measurement period or species and
267 measurement period were significant ($P < 0.001$) (Fig. 3), whereas the interaction between
268 mixture and species was not ($P = 0.068$). Qc always had significantly higher $F_{D \text{ Mean-s}}$ values
269 than Qp in all plots.

270 The Tukey contrast tests revealed that $F_{D \text{ Mean-s}}$ values did not differ from P1 to P2 in the Qc
271 pure plot (Fig. 3), whereas values in P3 were significantly lower (Fig. 3). Conversely, in the
272 Qp pure plot, $F_{D \text{ Mean-s}}$ was already lower in P2 than in P1 (Fig. 3), and no further decrease in
273 $F_{D \text{ Mean-s}}$ was observed between P2 and P3.

274 After the rain events at the end of the summer (Fig. 1), $F_{D \text{ Mean-s}}$ values strongly increased
275 during P4 for both Qc and Qp in pure plots. The values reached higher levels than during P1
276 for Qc and similar levels for Qp (Fig. 3).

277 The Tukey contrast tests also revealed that the two oak species showed different trends in $F_{D \text{ Mean-s}}$
278 values between pure and mixed conditions. For any given period, Qp did not show any
279 significant differences in $F_{D \text{ Mean-s}}$ values between pure and mixed plots. In contrast, for any
280 given period, significant differences were observed for Qc (Fig. 3). Furthermore, for Qc, the
281 difference in $F_{D \text{ Mean-s}}$ values between pure and mixed plots increased from P1 to P2, then
282 remained similar during P2 and P3, resulting in very low values in $F_{D \text{ Mean-s}}$ in the mixed plot
283 in P3 (Fig. 3).

284

285 **3.3 Foliar carbon isotope composition**

286 For Qc, mean tree leaf $\delta^{13}\text{C}$ was $-26.1 \text{ ‰} (\pm 0.2 \text{ ‰})$ in the mixed plot and $-27.2 \text{ ‰} (\pm 0.4 \text{ ‰})$
287 in the pure one. For Qp, mean tree leaf $\delta^{13}\text{C}$ was $-27.5 \text{ ‰} (\pm 0.4 \text{ ‰})$ in the mixed plot and
288 $-27.4 \text{ ‰} (\pm 0.3 \text{ ‰})$ in the pure one. Overall, there was no species ($P = 0.063$) nor mixture ($P =$
289 0.051) effect and no interaction between species and mixture ($P = 0.203$) on $\delta^{13}\text{C}$. However,
290 when considering each species separately, a significantly higher $\delta^{13}\text{C}$ value was observed for
291 Qc in the mixed plot compared to the pure one ($P = 0.038$) (Fig. 4). For Qp, no significant
292 differences were found between plots ($P = 0.991$) (Fig. 4).

293 The absence of any difference in $\delta^{13}\text{C}$ between plots for Qp was consistent with the absence of
294 any significant percentage change in $F_{D \text{ Mean-s}}$ values between the periods P1 and P3, whereas
295 higher $\delta^{13}\text{C}$ values in the mixed plot for Qc were consistent with a clear reduction in $F_{D \text{ Mean-s}}$
296 values (Fig. 4).

297 4. DISCUSSION

298 4.1 Response to drought of the two oak species in pure conditions

299 Before any soil water deficit occurred (P1), daily sap flux density of Qc was higher than that
300 of Qp in the pure plots (Fig. 2 and 3); this is consistent with previous measurements
301 conducted by Tognetti *et al.* (1996) on the same species in Italy.

302 Both oak species displayed a marked decrease in *PET* standardized sap flux density ($F_{D \text{ Mean-s}}$)
303 throughout the summer (from P1 to P3) in the pure plots (Fig. 2 and 3), but with different
304 intensities and timing. The decrease in $F_{D \text{ Mean-s}}$ for Qc occurred between P2 and P3 whereas it
305 occurred between P1 and P2 for Qp (Fig. 2 and 3). The pattern observed for Qc, a “drought
306 tolerant” species sensu Kramer (1983), was consistent with observations by Tognetti *et al.*
307 (2007) who concluded that this species has a non-conservative water use strategy. Indeed, Qc
308 transpiration regulation occurred late during the dry period. This strategy allows the tree to
309 avoid limiting its carbon assimilation during medium soil water deficit periods, as has
310 previously been observed for other *Quercus* species (Epron & Dreyer 1993). Conversely, the
311 pattern observed for Qp corresponded to a “water conservative” strategy, which is consistent
312 with previous studies conducted on this species (Bréda *et al.* 1993). Physiological and
313 morphological mechanisms potentially supporting this strategy include rapid stomatal
314 regulation when soil water deficit increases and developing extensive lateral and deep rooting
315 systems (Kramer 1983). It is noteworthy that these different water use strategies actually led
316 to a similar relative decrease in transpiration from P1 to P3 for these species in our study (Fig.
317 4). A differential influence of atmospheric conditions (radiation, *VPD*) on species
318 transpiration is not a likely explanation for this pattern since we were able to show that these
319 conditions impacted transpiration similarly for the two species; furthermore, we used *PET*
320 standardized daily sap flux density means in our calculations. Our results indicate that under
321 similar environmental conditions, contrasted water use strategies may not necessarily confer
322 any clear advantage to a given species in terms of transpiration response to drought.

323 Nevertheless, the rainy episodes at the end of August (between P3 and P4) were clearly more
324 beneficial in terms of transpiration for Qc than for Qp (Fig. 3); this can be explained by the
325 shallower rooting system in Qc (Nardini 1999) than for Qp. As evaporative demand (*VPD* and
326 *ETP*) was very low during P4 in comparison to P1, the lower transpiration rates of Qc during
327 P1 could be explained by stricter stomatal control against atmospheric drought at the
328 beginning of the summer than at the end of the drought.

329

330 4.2 Impact of species interactions on the response to drought

331 In this study, we revealed the differential impact of competition for water resources between
332 the two species on their response to soil water deficit. Indeed, while tree transpiration rates for
333 Qp did not differ between the mixed and the pure plot, Qc had much lower transpiration rates
334 in the mixed plot (Fig. 3). Furthermore, Qc response to drought was timed differently between
335 pure and mixed conditions (Fig. 3): $F_{D\text{ Mean-s}}$ of Qc decreased much earlier during the summer
336 in the mixed plot than in the pure plot, even though the decrease in REW was slightly lower in
337 the mixture (Fig. 1). We have thus demonstrated a clearly detrimental effect of interspecific
338 competition for water resources on Qc transpiration under soil drought in a Mediterranean
339 forest.

340 Several morphological and/or physiological adaptations may explain the observed patterns.
341 Sap flow measurements were conducted on dominant and co-dominant trees in each plot so
342 differences in crown exposure to atmospheric conditions can be excluded as a potential cause
343 for the patterns we found for $F_{D\text{ Mean-s}}$. However, a different vertical distribution of the Qc root
344 system in pure and mixed plots could lead to a different mean depth of water extraction, thus
345 explaining the patterns observed for Qc. The response of stomatal conductance is assumed to
346 be linked to the soil water deficit experienced by the majority of the roots, even if water is
347 being extracted from deeper depths (Irvine *et al.* 1998). We can therefore assume that the Qc
348 roots are mostly restricted to the upper soil horizons in the mixed plot. We were unable yet to
349 directly evaluate the mean rooting depth of the sampled trees or to estimate it through indirect
350 approaches such as soil water labelling experiments (e.g. Zapater *et al.* 2011). Contrary to Qc,
351 Qp typically develops a deep root system when competing with other species, thus allowing
352 the tree to maintain high levels of transpiration during soil water deficit periods (Bréda *et al.*
353 1993; Leuschner *et al.* 2001; Zapater *et al.* 2013). We therefore hypothesize that in mixed
354 plots, where the limiting soil water conditions occur every year, Qp roots consistently
355 outcompeted Qc roots in the deeper and wetter soil horizons, which explained the absence of
356 a negative response in terms of transpiration of Qp although mixed with a higher water
357 consumer such as Qc (Fig. 3). Interspecific competition thus resulted in a negative impact on
358 Qc transpiration as compared to the pure plot situation. For Qp, on the other hand, usable
359 water resources were neither reduced nor improved when the species was mixed with Qc. Our
360 results thus show a negative interaction between the two oak species for soil water use in this
361 Mediterranean ecosystem and, contrary to our initial hypothesis, do not support any

362 complementarity effect in Qp/Qc mixture. This result contrasts with previous studies
363 conducted on tropical or temperate forest ecosystems (Forrester *et al.* 2010; Kunert *et al.*
364 2012). It should be noted that, even though the plots were selected in order to minimize
365 differences in soil characteristics, some minor differences in soil depth, texture and porosity
366 might also partly explain the observed differences in transpiration between pure and mixed
367 plots. Furthermore, differences in transpiration of Qc between the pure and mixed plots might
368 be partly due to differences in tree density and diameter (Table 2). Some studies indeed
369 reported a positive relationship between tree sap flux density and tree size (Mc Jannet *et al.*
370 2007; Jung *et al.* 2011) although some works also found negative relationships (Meinzer *et al.*
371 2001). However, those differences would contribute to explain the distinct seasonal patterns
372 we observed for Qc in the pure vs. mixed plot only if they significantly affected their root
373 depth. A clear relationship between tree size and root depth in mature Mediterranean forests
374 has not yet been demonstrated.

375

376 **4.3 Leaf physiological adjustments in pure vs. mixed conditions**

377 Differences between Qc and Qp in the response of tree-level transpiration to mixture
378 conditions were accompanied by different leaf-level physiological adjustments. Indeed, while
379 no difference in $\delta^{13}\text{C}$ was found between pure and mixed trees for Qp, higher $\delta^{13}\text{C}$ values (and
380 thus WUE_{int}) were found for Qc in the mixed plot (Fig. 4). Higher $\delta^{13}\text{C}$ values for Qc is
381 consistent with the large body of literature showing higher $\delta^{13}\text{C}$ and WUE_{int} under higher
382 drought conditions (e.g., Galle *et al.* 2010; Zhang & Marshall 1994). Enhanced $\delta^{13}\text{C}$ and
383 WUE_{int} for Qc in the mixed plot was consistent with reduced transpiration and supported our
384 hypothesis of reduced water resource availability in the mixed plot for this species. It should
385 be noted that our $\delta^{13}\text{C}$ values were obtained on leaves sampled in July 2012, i.e. before any
386 intensive soil water deficit occurred (Fig. 1). As Qc is a deciduous species, these data
387 therefore might reflect a strong, long-term physiological acclimation of Qc trees to the
388 different soil water deficit conditions encountered every year in the different plots. For Qp,
389 the similar $\delta^{13}\text{C}$ values between plots was also consistent with transpiration data and further
390 indicated that this species neither benefited nor suffered from the interactions with Qc in
391 terms of water use.

392

393 In conclusion, under severe soil water deficit conditions, we highlighted an interesting
394 differential response pattern in terms of transpiration and water use efficiency between
395 *Quercus cerris* (Qc) and *Quercus petraea* (Qp) when they are growing in pure plots or when
396 they are competing for water resources in mixed species conditions. Qp sap flux density was
397 neither negatively affected nor favoured by species interactions as would have been expected
398 had complementarity in water resource use occurred. On the contrary, Qc was negatively
399 impacted in its water use when growing in mixed conditions with Qp, an oak species that is
400 known to be able to extract water from deep soil layers. Whether carbon fluxes and
401 productivity of Qc were also negatively influenced still remains to be evaluated. Our results
402 suggest that management practices in the Mediterranean region that favour mixing of Qp and
403 Qc could be detrimental for Qc in terms of water use and survival in the expected context of
404 more frequent and more severe droughts. Nevertheless, whether interactions between these
405 oak species and other broadleaf or coniferous species would be beneficial for them, or at least
406 would have no negative effects, remains to be addressed.

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521 **Table 1:** Elevation (m), tree density (ha^{-1}), mean tree height (m) and tree diameter at breast height (DBH, m) with standard errors, plant area
 522 index (PAI , $\text{m}^2 \text{m}^{-2}$) with standard errors ($N = 5$), basal and sapwood area ($\text{m}^2 \text{ha}^{-1}$), and species' contribution to basal area (%) for each study plot.
 523 Qc and Qp stand for turkey and sessile oak, respectively.

Plot	Species combination	Elevation (m)	Tree density (ha^{-1})	Mean tree height (m)	Mean DBH (m)	PAI ($\text{m}^2 \text{m}^{-2}$)	Basal area ($\text{m}^2 \text{ha}^{-1}$)	Sapwood area ($\text{m}^2 \text{ha}^{-1}$)	Species contribution to basal area (%)	
									Qc	Qp
1	Qc	254	933	16.4 ± 0.7	0.22 ± 0.01	3.5 ± 0.1	28.0	19.9	100	
2	Qp	421	867	17.5 ± 0.5	0.20 ± 0.01	4.0 ± 0.1	27.7	12.3		100
3	Qc / Qp	436	689	15.1 ± 0.6	0.17 ± 0.01	2.9 ± 0.2	27.2	7.9	45	55

524

525 **Table 2:** Number of trees, mean tree height (m), mean tree diameter at breast height (m), and sapwood area (cm²) for trees equipped with sapflow
526 sensors.

Number of species in mixture	Species	Number of trees	Mean tree height (m)	Mean tree diameter (m)	Sapwood area (cm ²)
1	Qc	4	23.9 ± 1.6	0.40 ± 0.03	802 ± 109
1	Qp	5	22.5 ± 0.6	0.30 ± 0.02	210 ± 19
2	Qc	5	20.3 ± 0.2	0.25 ± 0.03	380 ± 66
2	Qp	5	20.2 ± 0.3	0.27 ± 0.02	171 ± 23

527

528

529

530 **Table 3:** Number of trees sampled, diameter range of the sampled trees at breast height (m), and allometric equations between sapwood area (SA,
531 m²) and tree diameter at breast height (DBH, m) with coefficient of determination (R²) established for turkey and sessile oak (Qc and Qp
532 respectively).

	<i>Sampled trees</i>	<i>DBH range (m)</i>	<i>Equation</i>	<i>R²</i>
Qc	21	0.15 - 0.47	SA = 1.843 × (DBH ^{1.6351})	0.90
Qp	48	0.16 – 0.52	SA = 0.3653 × (DBH ^{1.8483})	0.94

533

534 **Figure Legends:**

535 **Figure 1:** Seasonal pattern of (a) modelled relative soil extractable water (*REW*) for each plot,
536 daily sum of precipitation (*P*, mm), (b) daily sum of global radiation (*R_G*, MJ m⁻²), daily
537 evapotranspiration (*PET*, mm) and daily mean vapour pressure deficit (*VPD*, kPa) during the
538 summer 2012 (01/04/2012 to 31/09/2012). Periods indicated in grey represent the periods of
539 sap flux density measurements (P1, P2, P3 and P4). The dashed grey line stand for the
540 threshold of 0.4 of *REW* under which the resulting soil water shortage induces a drop in
541 stomatal conductance and thus in leaf gas exchange.

542 **Figure 2:** Daily variations in potential evapotranspiration (*PET*, mm h⁻¹) and mean sap flux
543 density (*F_D*, L dm⁻² h⁻¹) of sampled turkey and sessile oak trees (*Q_C* and *Q_p* respectively) in
544 pure and mixed plot with standard mean errors for four sunny days, during the four
545 measurement periods.

546 **Figure 3:** Mean of the daily mean sap flux densities standardized for potential
547 evapotranspiration (*F_{D Mean-s}*, L dm⁻² h⁻¹ mm⁻¹) for each measurement period and for each study
548 plot for turkey and sessile oak (*Q_C* and *Q_p* respectively). Letters denote significant differences
549 between plots and periods revealed by contrast tests for each species after mixed effect
550 models had been applied.

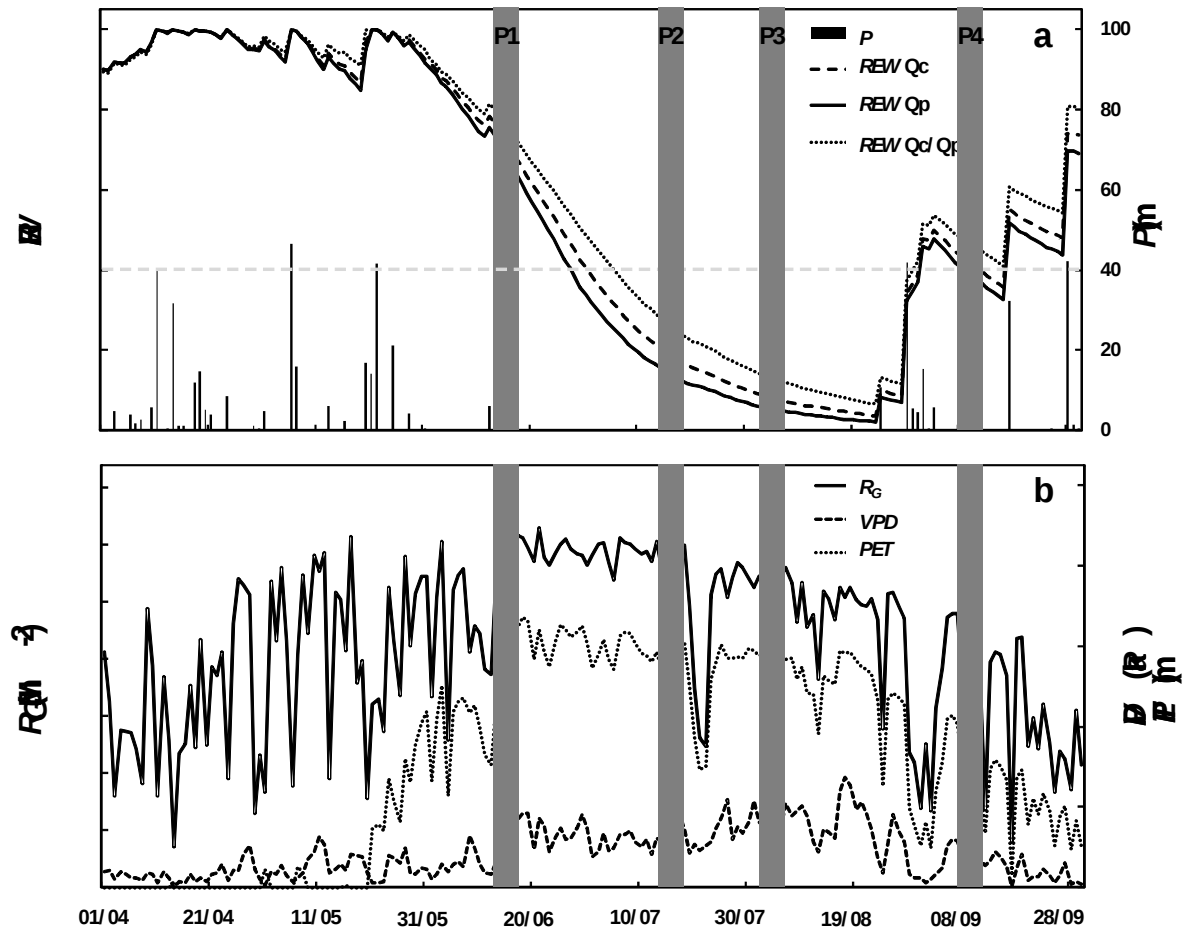
551 **Figure 4:** Mean leaf carbon isotope composition ($\delta^{13}\text{C}$, ‰) in July 2012 for turkey (black
552 symbols, *Q_C*) and sessile (white symbols, *Q_p*) oak as a function of the percentage of decrease
553 in mean daily sap flux density between P1 and P3 (*F_{D Mean-s}* % *P1-P3*, %) for each plot and each
554 species. Bars are standard errors of the mean. Arrows indicate the change in $\delta^{13}\text{C}$ and *F_{D Mean-s}*
555 % *P1-P3* from pure to mixed plots for each species.

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Figure 1



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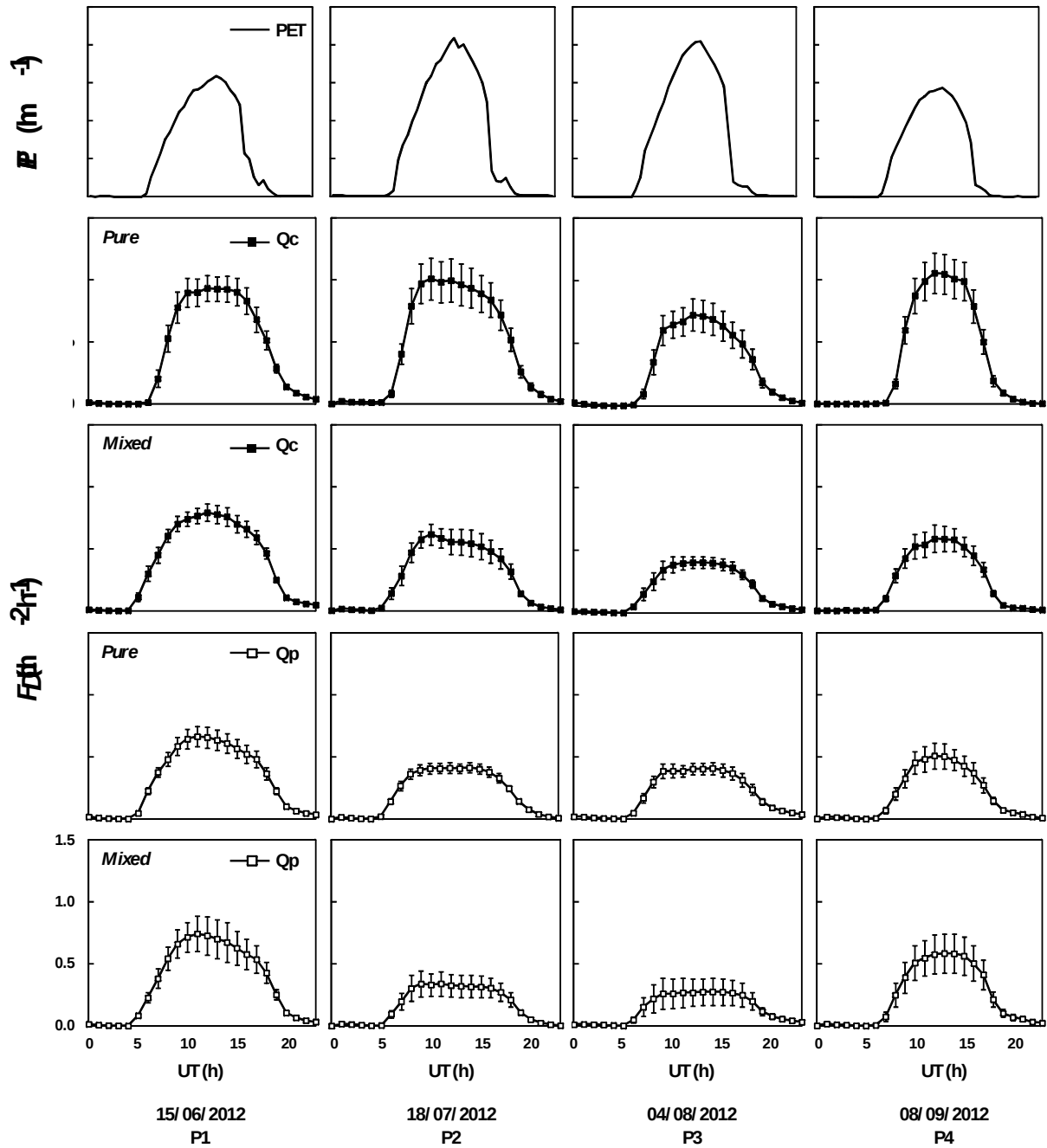
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Figure 2



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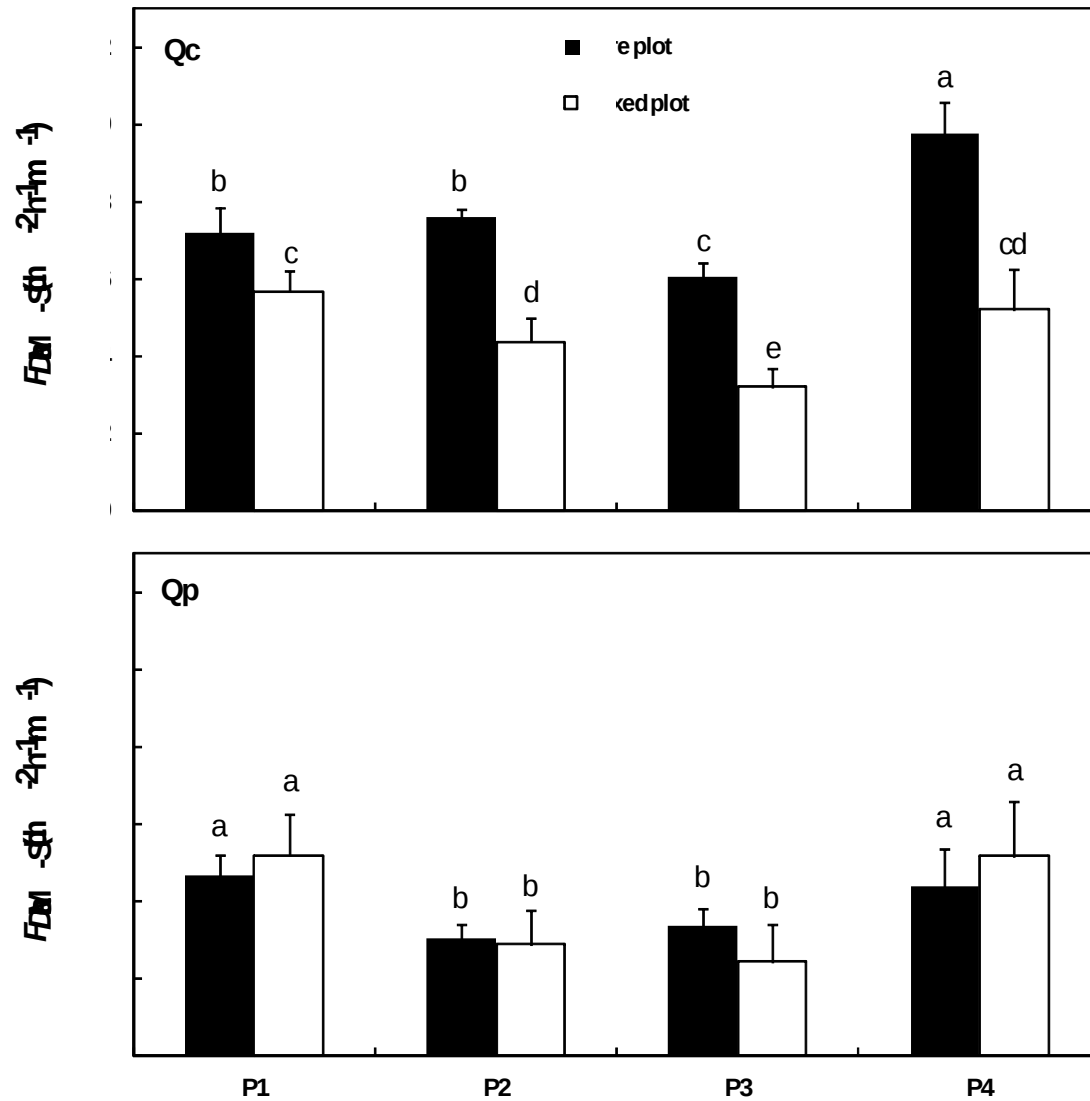
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Figure 3

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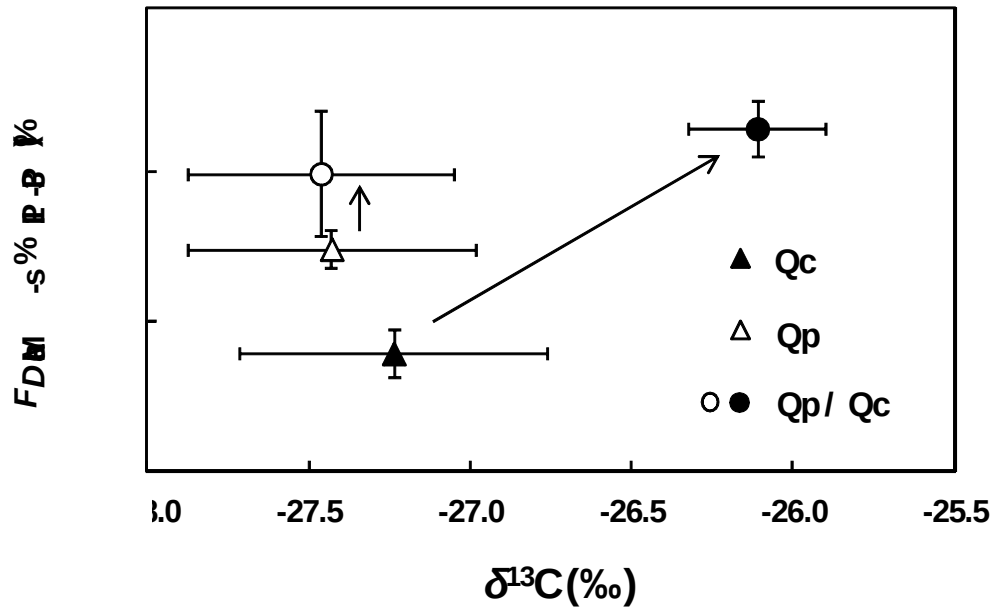
Figure

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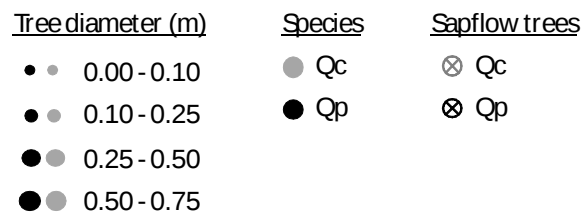
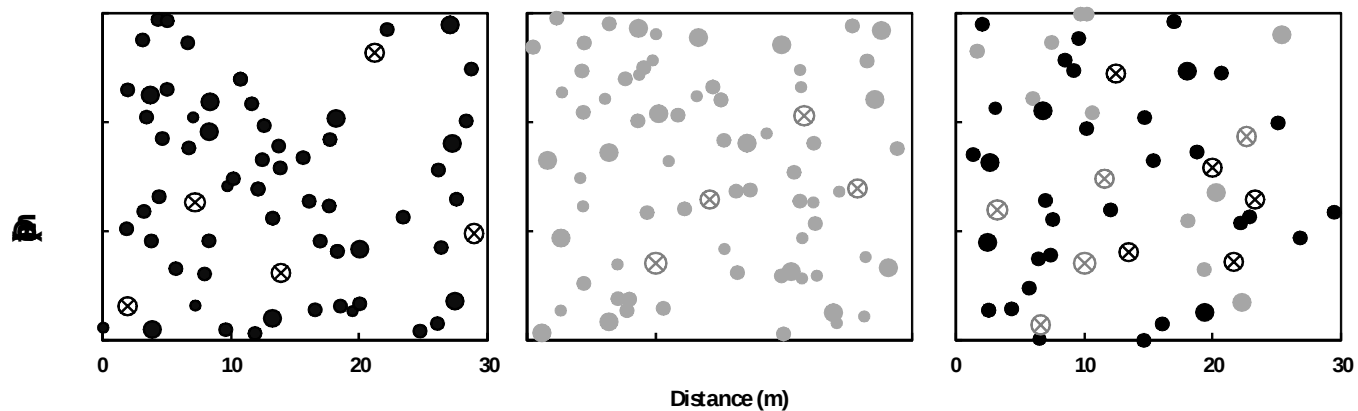


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577

578 **Appendix 1:** Maps of the selected plots with tree diameter class distribution (m). Grey circles stand for turkey oak (Qc) and black circles stand
 579 for sessile oak (Qp). Circles that contain a cross are selected trees for sap flux density measurements.

580



581