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

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Total word count:

Abstract: 247

Manuscript: 5977 (without figures)

Figures: 4

Tables: 3

31 Appendices: 1

32

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.

ABSTRACT

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

- 60 **Keywords:** drought, sap flux, *Quercus*, species interactions, water use
 61 efficiency
- 62 **Abbreviations**
- 63 F_D : Sap flux density ($\text{L dm}^{-2} \text{ h}^{-1}$)
- 64 $F_{D \text{ Mean}}$: Mean daily sap flux density ($\text{L dm}^{-2} \text{ h}^{-1}$)
- 65 $F_{D \text{ Mean-S}}$: Mean daily sap flux density standardized by potential evapotranspiration ($\text{L dm}^{-2} \text{ h}^{-1}$
 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

1. INTRODUCTION

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

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

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

2. MATERIALS AND METHODS

2.1 Site description

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

2.2 Drought intensity

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

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

2.3 Tree transpiration

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

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

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

$$F_D = \frac{\Delta T(0)}{\Delta T(u)} \quad (1)$$

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

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

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

2.4 Foliar carbon isotope composition

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

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

2.5 Data analysis

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

3. RESULTS

3.1 Model simulation of drought intensity

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

3.2 Tree transpiration

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

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

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

4. DISCUSSION

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

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

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

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

4.2 Impact of species interactions on the response to drought

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

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

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

4.3 Leaf physiological adjustments in pure vs. mixed conditions

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

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.

407 **Acknowledgments**

408 We thank Eleftheria Dalmaris for her technical assistance with the sapflow sensor installation
409 and leaf sampling, Andrea Coppi and Matteo Feducci for their technical assistance with
410 sapflow measurements, the four tree climbers, Ciencia Sartia, Claudio Santelli, Giovanni Lotti
411 and Franscesco Ierimonti, who collected the leaf samples, and Christian Hossann for the leaf
412 carbon isotope composition analyses. We are grateful to Pierre Montpied for his statistical
413 support. We would like to acknowledge the three anonymous reviewers who greatly
414 contributed to the improvement of a previous version of this manuscript. This work was
415 conducted in the framework of the ARBRE Laboratory of Excellence project (ANR-12-
416 LABXARBRE-01) supported by the French National Research Agency. The research leading
417 to these results has received funding from the European Union Seventh Framework
418 Programme (FP7/2007-2013) under grant agreement n° 265171. CG was supported by a
419 FunDivEUROPE grant from the INRA Nancy.

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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 index (*PAI*, m² m⁻²) with standard errors (*N* = 5), basal and sapwood area (m² ha⁻¹), and species' contribution to basal area (%) for each study plot. 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)	<i>PAI</i> (m ² m ⁻²)	Basal area (m ² ha ⁻¹)	Sapwood area (m ² 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

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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 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

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

	Sampled trees	DBH range (m)	Equation	R ²
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

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Figure Legends:

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

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

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

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

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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.

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