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Influence of species interactions on transpiration of Mediterranean tree species during a summer drought

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Abstract Recent research has shown that interactions between species with different functional traits can promote forest ecosystem processes. In the context of climate change, understanding whether species interactions in mixed-species ecosystems can improve the adaptation of these ecosystems to extreme climatic events is crucial to developing new management strategies. In this study, we investigated the impact of species interactions on the sap flux density of three Mediterranean tree species (*Quercus faginea*, *Pinus nigra* and *Pinus sylvestris*) during a summer drought. Measurements of foliar carbon isotopic composition (δ^{13}C) were also conducted on the same trees. The decline in transpiration during drought was the greatest for *P. sylvestris* and the least pronounced for *Q. faginea*. For *P. nigra* and *Q. faginea,* the decrease in transpiration as the drought progressed was lower when these species where interacting with another tree species, particularly with *P. sylvestris*. In contrast, the decrease for *P. sylvestris* was higher when this species was interacting with another species. Differing drought effects were consistent with the δ^{13}C values. We showed that the identity of the species present in the direct neighbourhood of a given tree can differentially influence water availability and water-use of these three co-existing Mediterranean tree species during a summer drought. Our findings suggest that species interactions play an important role in modulating the response of tree species to drought. Favouring tree species diversity in this region does not seem to be systematically beneficial in terms of soil water availability and water-use for all the interacting species.

Keywords Complementarity · Competition · Facilitation · Species interaction · Drought · Transpiration · δ^{13}C

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Introduction

Climate models forecast a decrease in annual mean precipitation in large areas in the northern hemisphere in the future, accompanied by an increase in air temperatures (IPCC 2013). As a result, the evaporative demand over these regions will increase and drought episodes will not only become more frequent, but will also increase in severity and duration. Such expected conditions may lead to gradually increasing tree mortality rates and rapid forest die-off events during the next century (Allen et al. 2010).

Tree species in drought-prone forest ecosystems have a wide range of water-use strategies involving leaf- or plant-level structural and/or physiological adaptations (e.g. Kramer 1983). The major physiological adjustment trees undergo to preserve leaf water status, and thus leaf—and even whole tree—functionality during drought, is related to stomatal aperture. Leaf stomatal conductance is driven by atmospheric water pressure deficit and soil water supply. By closing their stomata during drought, trees reduce leaf transpiration and therefore water-use, but in the mean time, CO_2 uptake and assimilation rates are restricted.

Differences in structural characteristics among tree species contribute to the considerable variability in their responses to water depletion in drought-exposed forests. Among these features, the vertical development of rooting systems plays a large role in tree functional response to drought stress (Burgess 2006). Some species develop a deep-rooting system which allows them to maintain high rates of transpiration by using water from deep soil layers as superficial layers dry out (e.g. Zapater et al. 2013). Other species mainly rely on a superficial rooting system, and they suffer sooner from soil water exhaustion as drought conditions worsen. However, a shallow rooting pattern can also sometimes translate into a competitive advantage since the species can absorb water from summer rainy episodes more efficiently (Xu and Li 2006).

These different strategies have been widely studied, but less is known about how the interactions among species with contrasting water-use strategies during drought stress affect their physiological responses to water depletion in natural forest ecosystems. Since the early nineteenth century, ecologists have been trying to understand how ecosystem processes are influenced by community assembly and species interactions. In many cases, studies have shown that species interactions can be beneficial for ecosystem functions and services involving the forest carbon and water cycles (Pretzsch et al. 2013a, b; Zhang et al. 2012), even though a negative influence of species interactions can also be found (e.g. Grossiord et al. 2014a). Different mechanisms of species interactions have been suggested to explain these positive effects: Facilitation among species whereby one species will benefit from the presence of another species can take place in mixed-species ecosystems and have an overall positive effect on ecosystem functioning (Loreau et al. 2001). Furthermore, complementarity among co-occurring species can lead to a more effective use of available resources and may also be a driver of higher performance in mixed-species ecosystems (Loreau et al. 2001). This mechanism occurs when functional traits enable plant species in the community to exploit resources unavailable to others or to use the same resource at a different place or time. This resource partitioning in mixed-species communities can take place both aboveground and belowground. Indeed, species interactions optimize the aboveground stratification of the canopy and improve overall light interception (Kelty 2006; Pretzsch 2014), resulting in higher gross and net ecosystem carbon uptake rates (Forrester et al. 2010). Inversely, belowground complementarity can impact the accessibility of different water reservoirs and may impact plant and ecosystem water uptake (Schmid and Kazda 2001).

Among various biotic and abiotic factors, positive species interactions (i.e. facilitation and/or complementarity) have been suggested to contribute to the higher transpiration in mixed forests as compared to mono-specific ones (Forrester et al. 2010; Kunert et al. 2012). However, other studies also demonstrated no impact of species diversity on tree- or stand-level transpiration (Gebauer et al. 2012, 2013), or contrasting results according to species identity (Grossiord et al. 2014b). The discrepancy between these results can partially be explained by differences in climatic conditions during measurements periods: Studies were conducted under dry soil conditions tended to show higher transpiration in mixed forests in comparison to mono-specific ones (Forrester et al. 2010; Kunert et al. 2012), whereas the others that were conducted under wet soil conditions showed no effect of species diversity (Gebauer et al. 2012, 2013). Consequently, one could expect that in ecosystems frequently subjected to extreme soil water conditions (i.e. drought), like Mediterranean forests, tree species interactions, are likely to have a positive effect on water availability and/or accessibility. The underlying mechanisms that could drive such an effect could be both facilitation processes such as hydraulic lift (Zapater et al. 2011) and/or complementarity processes such as the differentiation in belowground rooting patterns among species (e.g. Schmid and Kazda 2001) that would lead to a better sharing of the available soil water among tree species.

Here, we assessed tree species identity in the local neighbourhood of three co-existing Mediterranean tree species known to present important differences in functional traits related to drought tolerance (rooting strategy, stomatal regulation under water shortage, phenology…): *Quercus faginea* a drought-adapted, broadleaved, deciduous/semi-evergreen, oak species with a deep-rooting system, *Pinus nigra* a drought-adapted, Mediterranean, evergreen, conifer species and *Pinus sylvestris* an evergreen conifer tree species with a shallow rooting system and a rather sensitive stomatal response to soil drought. We tested whether interactions among these species would influence their transpiration response to a summer drought. We also tested whether species interactions would influence the carbon isotope composition ($\delta^{13}C$) of leaf or needle samples. Indeed, differences in $\delta^{13}C$ of organic material are related to changes in forest soil moisture and/or air humidity (Saurer et al. 2004). We hypothesized that

(1) community assembly of tree species with differing functional characteristics in this Mediterranean forest ecosystem would affect soil water availability and (2) processes of complementarity and/or facilitation would develop when species compete with non-conspecific neighbours. This would be reflected by a lesser decline in transpiration of target trees and by lower $\delta^{13}C$ values of target trees in interaction with non-conspecific neighbours as compared to target trees in interaction with conspecific ones.

Materials and methods

Study site

The study was conducted in a continental Mediterranean mixed forest situated in the Alto Tajo natural park (Guadalajara, Castilla La Mancha, 40.66°N, 02.27°W) in central Spain. A total of ten 30 m-by-30 m plots including monocultures and mixtures of Portuguese oak (*Q. faginea* Lam.), black pine (*Pinus nigra* J.F. Arnold) and Scots pine (*Pinus sylvestris* L.) were selected in the natural park. Three monocultures (one for each species) and seven two-species mixtures were included, with characteristics as described in Table 1. The selected stands are all within 20 km of each other and are included in the exploratory platform of the FP7-FunDiv EUROPE project (www.fundiveurope.eu). Plots were selected to reduce soil type heterogeneity and differences in diameter distribution, basal area, tree height, tree density and canopy structure (Table 1; for more information, see also Baeten et al. 2013). Understory vegetation in the selected plots is mainly composed of shrub species (*Arctostaphylos uva-ursi*, *Buxus sempervirens* and *Genista Scorpius*) and juveniles of the dominant tree species. The ten selected plots are situated on calcic cambisol soils (Food and Agriculture

Organization of the United Nations classification) on a cracked limestone bedrock. Soil depth is very shallow in the selected plots and varied between 20 and 40 cm, but roots may extend down to several metres through the cracked bedrock (Peñuelas and Filella 2003). Mean annual rainfall in this region is 499 mm and mean annual temperature is 11 °C. Plot altitudes range from 980 to 1,300 m a.s.l.

Our objective was to characterize two types of species interactions: conspecific or non-conspecific ones. Selected trees of a given species should be in interaction with trees of one of the two other species or with conspecific neighbours (i.e. three neighbourhood levels). To select the trees, we retained in each plot only dominant or co-dominant trees (crown illumination index strictly higher than four, Clark and Clark 1992) with a diameter at breast height of more than 0.10 m that were located at least 5 m from the edge of the plots. We chose dominant or co-dominant trees to avoid any confounding effects caused by differing light acquisition levels. We then characterized species interactions within the immediate neighbourhood around each tree. The neighbourhood of a given tree was defined as all the other trees located within a 5 m radius of its trunk (Fig. 1). This radius was chosen because (1) the majority of the lateral roots of a given tree are supposed to be largely confined within its crown width projection (Schenk and Jackson 2002) and (2) the mean radius of the crown projection of the trees in the selected plots was 1.9 m. The root systems of trees distant by 3.8 m are thus supposed to be competing for resources. We arbitrarily extended this radius to 5 m to ensure other potential interactions.

The size of the interacting trees within an immediate neighbourhood and their distance to the target tree have a strong effect on the water availability of a given tree during a drought event. To take these effects into account, we calculated the distant-dependent Hegyi competition index (CI) (Hegyi 1974) within the 5 m radius of a tree. This

Table 1 Species combination, elevation (m), tree density (ha^{-1}), leaf area index (LAI, m^2 m^{-2}), mean tree diameter at breast height (DBH, cm), and mean tree height (m), with standard errors of the mean (SE), for the 10 plots selected in this study

Plot	Species combination	Elevation (m)	Tree density (ha^{-1})	LAI (m^2 m^{-2})		DBH (cm)		Tree height (m)	
				Mean	SE	Mean	SE	Mean	SE
1	QF	1,187	889	1.6	0.0	11.4	0.9	6.4	0.3
2	PN	980	1,244	1.9	0.3	17.1	1.1	11.2	0.3
3	PS	1,310	789	2.3	0.1	24.4	1.4	12.8	0.3
4	PS–QF	1,224	1,689	1.9	0.1	11.6	0.9	7.4	0.3
5	PS–QF	1,325	644	1.9	0.5	19.2	1.5	8.5	0.4
6	PN–QF	1,010	1,289	1.9	0.4	14.1	1.3	5.1	0.2
7	PN–QF	1,228	422	1.3	0.1	24.0	1.7	10.1	0.6
8	PN–QF	1,207	1,589	1.8	0.1	15.7	2.0	7.3	0.2
9	PN–PS	1,311	778	2.3	0.4	18.9	1.1	10.3	0.3
10	PN–PS	1,304	811	2.6	0.3	23.1	1.7	12.8	0.5

PN, *Pinus nigra*; PS, *Pinus sylvestris*; QF, *Quercus faginea*

Fig. 1 Schematic representation characterizing the local neighbourhood for selected and excluded trees in a given stand. The local neighbourhood of a given selected tree was used to characterize the identity of the interacting species and to calculate the Hegyi competition index
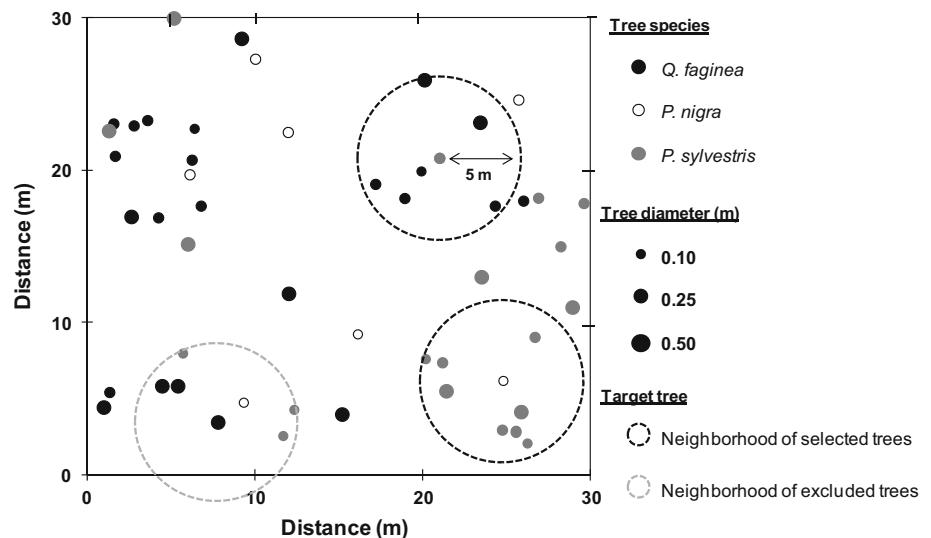
index uses the ratio of the basal area of the competitor trees and the subject tree weighted by the distance between the subject tree and the competitors. We also characterized the species identity of the trees in the neighbourhood of each tree.

Finally, we randomly selected six trees per species for each of the three species interaction levels (i.e. interaction with conspecific neighbours and with each one of the two other tree species) that covered the full gradient of CI.

Soil water balance

In order to characterize the drought intensity potentially encountered by the selected trees, we calculated the relative extractable water (REW, unitless) in the soil of each plot daily from January 2013 to November 2013 (Fig. 2). REW varies between 1.0 (field capacity) and 0.0 (permanent wilting point). When REW remains between 1.0 and 0.4, tree transpiration depends only on air humidity, irradiance and leaf area index (LAI) (Granier et al. 1999). When REW falls below the threshold of 0.4, water shortage induces a drop in stomatal conductance and leaf gas exchange for most tree species (Granier et al. 1999).

In order to calculate REW, we used the generic model for forest water balance "BILJOU" (Granier et al. 1999). The model calculates the different water fluxes in the ecosystem at a daily time scale: tree transpiration, understory evapotranspiration, rainfall interception and drainage. These fluxes are mainly dependent on LAI and evaporative demand, i.e. potential evapotranspiration (PET). The difference between rainfall and evapotranspiration fluxes refills the soil reservoir, whereas the excess of water is drained out. All the functions in the "BILJOU" model were calibrated through various field experiments in

different forests, climates and soil types (Granier et al. 1999). This model was applied by using above-canopy measurements of rainfall, global radiation, air temperature and humidity, and wind speed, which were recorded at a nearby meteorological station (40.66°N, 02.27°W, 1,000 m a.s.l.) no more than 15 km from any of the study plots. These data were also used to calculate daily PET following the Penman formula (Fig. 2). In order to parameterize the model, in July 2013, we used an LAI-2000 leaf area metre (LI-COR, Nebraska, USA) to measure the LAI at five locations within each plot and then averaged the five values. Mean plot LAI was $1.9 \text{ m}^2 \text{ m}^{-2} \pm 0.3$. Maximum extractable soil water (i.e. the soil water-holding capacity) of calcic cambisols is low so we set the reference value at 80 mm. However, to address uncertainties concerning the water-holding capacity in the different plots, two additional simulations per plot were performed with higher (100 mm) and lower (60 mm) water-holding capacities (REW_{max} and REW_{min}, respectively). REW_{mean} is the modelled value for 80 mm of extractable water (Fig. 2).

Sap flux density

We used the thermal dissipation method (Granier 1987) to measure sap flux density (F_D, L dm^{-2} h^{-1}) over the 2013 summer with five distinct collection periods (Fig. 2). The five periods were selected to characterize tree transpiration before drought and to assess the effect of decreasing REW on tree transpiration once drought conditions had appeared (Table 2). Due to technical constraints (power supply), sap flux measurements were restricted to three to five consecutive days in each measurement period (Fig. 2). Climate within each selected periods was rather homogenous, with warm and sunny conditions. The five periods were selected

Fig. 2 Seasonal pattern of modelled relative extractable water and climatic conditions for the summer 2013. **a** maximum, minimum and mean modelled relative soil extractable water averaged for all plots (REW$_{max}$, REW$_{min}$ and REW$_{mean,}$ respectively) and daily cumulative precipitation (P, mm), **b** daily cumulative global radiation (R_G, MJ m^{-2}) and daily potential evapotranspiration (PET, mm), and **c** daily mean vapour pressure deficit (VPD, kPa) and daily mean atmospheric temperature (T_A, °C). Periods indicated in *grey* represent the five sap flux measurement periods (P1, P2, P3, P4 and P5 in chronological order). The *horizontal arrow* indicates the period when late frosts occurred in May 2013. The *dashed grey line* shows the REW threshold of 0.4 under, which the resulting soil water shortage induces a drop in stomatal conductance and thus in leaf gas exchange
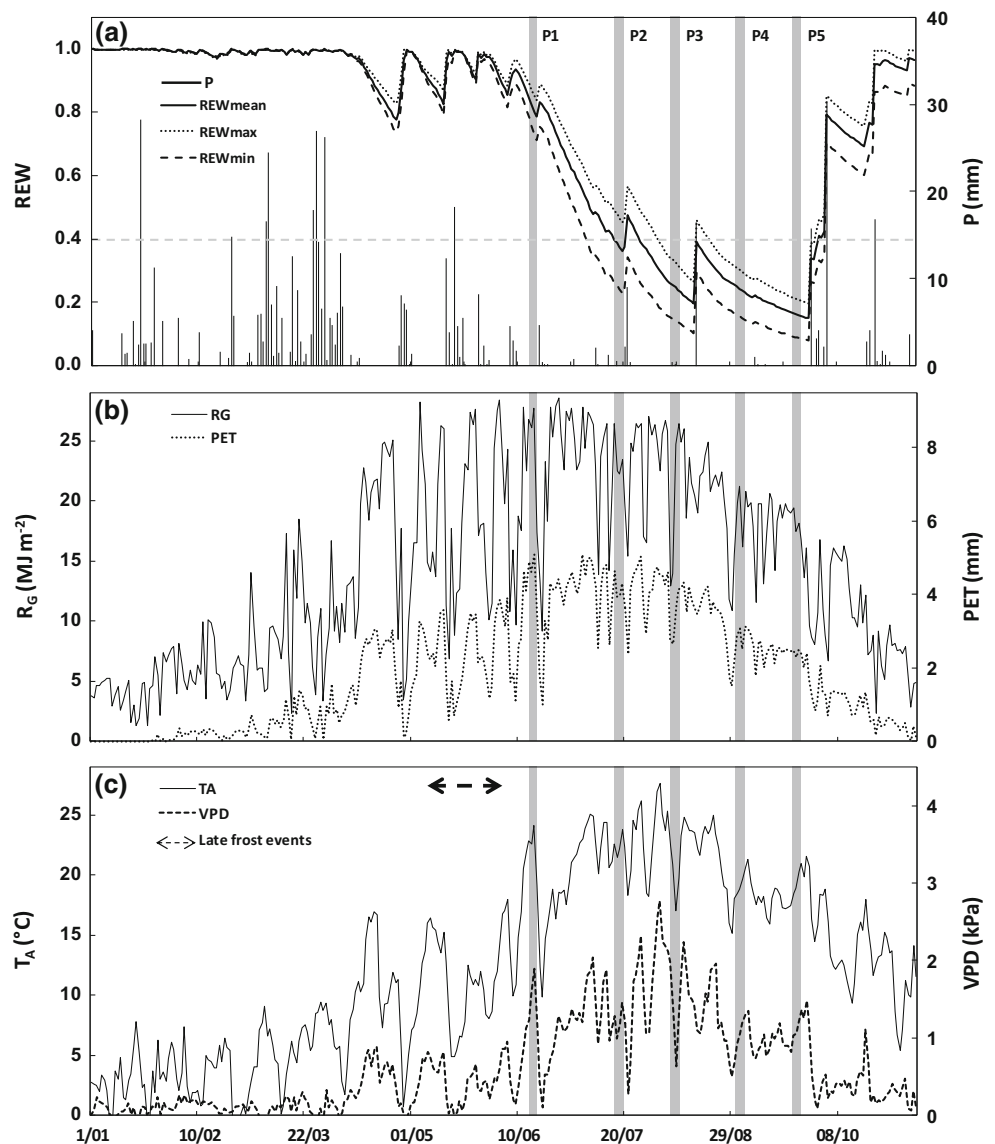
Table 2 Mean daily relative extractable water modelled using a water balance model (Granier et al. 1999) during the five measurement periods (P1, P2, P3, P4 and P5) for a water-holding capacity of 60 mm (REW$_{min}$), 80 mm (REW$_{mean}$) and 100 mm (REW$_{max}$) with standard errors of the mean (SE)

Period	N	REW$_{min}$		REW$_{mean}$		REW$_{max}$	
		Mean	SE	Mean	SE	Mean	SE
P1	3	0.76[a]	0.02	0.82[a]	0.01	0.89[a]	0.01
P2	4	0.26[b]	0.01	0.39[b]	0.01	0.49[b]	0.01
P3	3	0.14[c]	0.01	0.25[c]	0.01	0.33[c]	0.01
P4	5	0.16[c]	0.01	0.24[c]	0.01	0.34[c]	0.01
P5	4	0.09[d]	0.01	0.17[d]	0.01	0.22[d]	0.01

N are the total number of days for each period of measurement

Letters denote significant differences among periods for a given REW

according to weather forecast and spaced by 16 to a maximum of 31 days (P1, P2, P3, P4 and P5) (Fig. 2). Finally, because of sensor failures, data for a few trees had to be removed from the final analyses (Table 3).

The two 20-mm-long probes on the sap flux sensors (UP-GmbH, Cottbus, Germany) were installed under the bark and the cambium at ca. 1.3 m aboveground level with a 10-cm vertical spacing between the probes. All sap flux sensors were installed on the North facing side of the tree to reduce the risk of direct sunlight causing thermal gradients and distorting the measurements. Each sensor was supplied with 0.2 W of constant power. Data were collected every 10 s with a CR800 or CR1000 data logger (Campbell Scientific Inc, Leicestershire, UK), and 30 min averages were recorded. F_D was calculated following the equation proposed by Granier (1987).

Table 3 Number of trees analysed for sapflow measurements (N), mean diameter at breast height (DBH, cm), mean tree height (m) and mean competition index (CI) for each species and each level of species interactions tested with standard errors of the mean (SE)

Type of interaction	Pinus nigra							Pinus sylvestris							Quercus faginea						
	N	DBH (cm)		Tree height (m)		CI		N	DBH (cm)		Tree height (m)		CI		N	DBH (cm)		Tree height (m)		CI	
		Mean	SE	Mean	SE	Mean	SE		Mean	SE	Mean	SE	Mean	SE		Mean	SE	Mean	SE	Mean	SE
PN–PN	5	26.9	3.3	14.3	0.8	2.5	1.0														
PN–PS	6	35.5	3.0	16.1	0.9	1.9	0.7	5	27.4	2.0	12.3	0.8	2.1	1.5							
PN–QF	5	32.4	3.9	12.6	0.3	1.4	0.3								6	20.8	3.4	9.9	1.1	2.5	1.4
PS–PS								4	32.2	4.7	15.6	1.3	2.4	1.0							
PS–QF								5	26.6	2.4	13.7	0.3	2.4	0.7	5	20.6	0.5	12.1	0.5	2.2	0.7
QF–QF															6	16.8	0.9	7.7	0.4	1.6	0.5

PN, *Pinus nigra*; PS, *Pinus sylvestris*; QF, *Quercus faginea*

As Granier-type probes can only measure F_D along a radial cylinder 20 mm in width, if the sapwood is thicker than the sensor length, part of the total tree sap flux is not measured. As we were unable to find any allometric equations between tree diameter and sapwood thickness for *Q. faginea* in the literature, we established an equation to calculate sapwood thickness (ST, mm) and sapwood area (SA, cm^2) for this species. To do so, we used a Pressler increment borer (Suunto, FI) to extract wood cores from the North side of the stems of 21 *Q. faginea* trees (diameter at breast height (DBH) between 10 and 36 cm) selected outside the study plots in July 2013. The sapwood–heartwood transition was determined with visual colouration differences (Rybnicek et al. 2006; Herrero de Aza et al. 2011). We then related SA to tree DBH through the following equation ($R^2 = 0.92$):

$$SA = 0.64 \times DBH^{1.81} \tag{1}$$

For both pine species, we used equations from the literature to calculate SA of each tree (Poyatos et al. 2005). This assumption may lead to some slight overestimation of *SA*, but as our objective was to compare trees within a given tree species, these slight errors had no significant consequences on our results.

Based on SA, we calculated ST of all trees. For five of the *Q. faginea* trees, ST was <20 mm, so we applied the correction proposed by Clearwater et al. (1999). For the remaining *Q. faginea* trees, we did not apply corrections for changes in F_D radial patterns since it has been shown that F_D decreases to zero after the first 20 mm for *Quercus* species (Cermak et al. 1992). For the pines, ST was always above 20 mm, so we applied corrections for radial changes in F_D based on the modal distribution provided by Nadezhdina et al. (2002):

$$\text{Total } F_D = F_D\text{m}*\left[1 + \frac{\frac{2}{3}*\pi*(ST - 20)^2}{\frac{2}{3}*\pi*ST^2}\right] \tag{2}$$

where Total F_D represents the sap flux density along the whole sapwood, F_Dm represents the sap flux density measured with the sensor, and ST represents the sapwood thickness of the tree.

During the five measurement periods, sap flux sensor signals were stable throughout the night and from night to night, showing that there was no occurrence of night-time transpiration. This was consistent with the fact that the vapour pressure deficit (VPD) at night during these periods was very low (between 0.0 and 0.1 kPa).

Based on F_D values, we calculated the mean daily sap flux density (F_{Dmean}) for each tree and each measurement day. We then defined the maximum value of mean daily sap flux density (F_{Dmax}) for each tree over the whole measurement campaign (i.e. all five periods) as the value of

F_{Dmean} on the day with the highest amount of global radiation (i.e. DOY 165). We also calculated the daily relative mean sap flux density ($F_{D\%}$) for each tree as the ratio of F_{Dmean} to F_{Dmax} in order to compare the decline in transpiration among trees along the drought period.

Foliar analyses

For each selected tree, we used 18-m-long pruning shears to sample 20–30 fully expanded sunlit leaves (*Q. faginea*) and three to five 30–40-cm-long branches supporting fully mature, sunlit needles in June 2013. For conifers, current- and previous-year needles were sampled and bulked. The samples were oven-dried at 65 °C for 48 h at INRA Nancy and finely ground (CB2200, Sodemi, St-Ouen l'Aumône, FR). About 1.0 mg of the powdered material from each tree was weighed out (MX5, Mettler Toledo, Viroflay, FR) and placed into tin capsules (Elemental Microanalysis Limited, Devon, UK) for carbon isotope composition ($\delta^{13}C$, ‰) analysis at the Technical Platform of Functional Ecology (OC 081) at the INRA Forest Ecology and Eco-physiology Unit; an EA-GC/IRMS (Delta S, Finnigan MAT, Bremen, Germany; ±0.2 ‰) was used for the analyses. Isotopic measurements are reported in the delta notation (δ, ‰) according to the Vienna Pee Dee Belemnite (VPDB) standard.

Data analyses

To test the effect of the period of measurements (P1–P5) on REW_{min}, REW_{mean} and REW_{max}, one-way ANOVAs were used followed by Tukey-type post hoc tests to determine differences among periods. To determine differences among species in their response to drought, we used mixed linear models where the fixed effects of *species*, *period* and their interaction were tested on F_{Dmean} with "tree" as a random factor. Prior to these tests, we employed Bartlett's tests to confirm homogeneity of variance. To determine the influence of the identity of species in the local neighbourhood (*neighbourhood*), the competition intensity (CI), the size of the trees (DBH) and the drought (*period*) on the decrease in sap flux density for each species, we used mixed linear models where the fixed effects of *neighbourhood*, CI, DBH and *period* were tested on $F_{D\%}$ with "tree" as a random factor. The interactions between *neighbourhood* and *period* and between CI and *period* were also included in the model to determine whether the effects of species interactions and competition intensity changed during the drought. Finally, we used mixed linear models to analyse the effect of *neighbourhood*, DBH and CI on $\delta^{13}C$ values of each species, with "tree" as a random factor. All analyses were performed with the statistical software R 2.14.1 (R Development Core Team 2011). For the mixed models, we used the package *lme4* (Bates et al. 2013). Tukey-type post hoc tests were used to reveal significant differences when a given factor was significant by using the package *multcomp* (Hothorn et al. 2008).

Results

Seasonal variations in climate and soil water

At the beginning of June, i.e. at the first measurement period (P1), REW was high in all the study plots, indicating that soil water was close to field capacity and thus not yet limiting transpiration. From P1 to P5, REW significantly decreased ($P < 0.001$) (Fig. 2, Table 2). At P2, REW was close to 0.4, possibly indicating a first effect of restricted soil water availability on tree transpiration (Granier et al. 1999). REW then continued to drop and remained below 0.4 during P3, P4 and P5. Between P2 and P3, and between P3 and P4, light rain events occurred that induced slight increases in REW (Fig. 2). Over the whole study period, REW reached its lowest value on September 26, 2013, i.e. just after the end of P5. REW was lower than 0.4 for a minimum of 78 days and a maximum of 87 days depending on the water-holding capacity. Finally, rain events at the end of September, 2013 resulted in a sharp increase in REW in all the plots (Fig. 2). Radiation, VPD and PET were high during P1 and P2 and then decreased steadily throughout the summer (Fig. 2). Late frosts were observed in May 2013 that induced late leaf establishments in several broadleaf species in the region, including *Q. faginea* (Valladares unpubl.).

Sap flux density

We observed no significant species effect on F_{Dmean} ($P = 0.202$) but a significant effect of the period of measurement ($P < 0.001$) and of the interaction between species and the period of measurement ($P < 0.001$), indicating that the three tree species differently responded to the drought (Fig. 3). Before the start of the drought (P1), *P. nigra* had the highest F_{Dmean} values and *Q. faginea* the lowest (Fig. 3). As the drought progressed, F_{Dmean} for the two conifers decreased steadily to the minimum values in September (P5), while *Q. faginea* values decreased more slowly (Fig. 3).

For *P. nigra*, we found a significant effect of the identity of species in the immediate neighbourhood and the period of measurements on $F_{D\%}$, ($P < 0.001$), but no effect of CI and of DBH (Table 4). The mixed model also revealed significant interactions between the level of species interaction and the period ($P < 0.001$) as well as between CI and the period ($P = 0.001$) (Table 4). $F_{D\%}$ of *P. nigra*
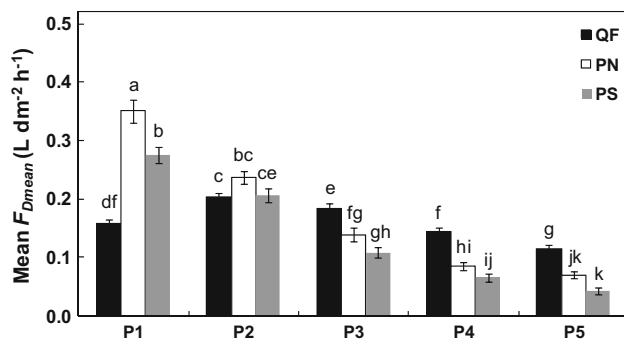
Fig. 3 Mean of the daily mean sap flux density (Mean F_{Dmean}, L dm^{-2} h^{-1}) for *Quercus faginea* (QF), *Pinus nigra* (PN) and *Pinus sylvestris* (PS) for each period of measurements (*P1*, *P2*, *P3*, *P4* and *P5*). *Vertical bars* represent the standard error of the mean for each period of measurement. *Letters* denote significant differences in daily mean sap flux density among all species and periods

trees decreased from P1 to P5 with the less intense decrease for trees interacting with *P. sylvestris* trees (Fig. 4).

For *P. sylvestris*, we found a significant effect of the identity of species in the immediate neighbourhood ($P = 0.025$) and of the period of measurement ($P < 0.001$) on $F_{D\%}$, but no effect of CI and of DBH (Table 4). The interactions between the level of species interaction and the period as well as between CI and the period were also significant ($P < 0.001$, Table 4). $F_{D\%}$ of *P. sylvestris* trees decreased from P1 to P5 with a less intense decrease for trees interacting with conspecific neighbours (Fig. 4).

For *Q. faginea*, we observed a significant effect of the identity of species in the immediate neighbourhood and of the period of measurement ($P < 0.001$) on $F_{D\%}$, but no effect of CI and of DBH (Table 4). Furthermore, the interactions between the level of species interaction and the period ($P < 0.001$) as well as between CI and the period ($P = 0.005$) were also significant (Table 4). $F_{D\%}$ of *Q. faginea* trees increased from P1 to P2 and then slightly decreased until P5 (Fig. 4). Trees interacting *P. sylvestris*

trees were able to maintain high $F_{D\%}$ values throughout the summer, while trees interacting with conspecific neighbours showed the highest decrease in $F_{D\%}$ (Fig. 4).

Foliar isotope composition

Mean species foliar carbon isotope composition ($\delta^{13}C$) varied between -25.4 ‰ for *Q. faginea* and -26.4 ‰ for *P. nigra*. Tests revealed a significant effect of the identity of species in the immediate neighbourhood on $\delta^{13}C$ for *P. nigra* ($P = 0.009$) with more negative values for the trees interacting with *Q. faginea* or conspecific neighbours (Fig. 5). A significant effect of the type of species interaction was also found for *Q. faginea* ($P = 0.001$) with more negative values for trees interacting with *P. nigra* or *P. sylvestris* (Fig. 5). For *P. sylvestris*, no effect of the type of species interaction could be found on $\delta^{13}C$ ($P = 0.136$). None of the three species showed a significant effect of CI on $\delta^{13}C$ ($P > 0.05$). There was a significant effect of DBH on $\delta^{13}C$ for *Q. faginea* ($P = 0.045$) and *P. sylvestris* ($P = 0.031$), but not for *P. nigra* ($P = 0.226$).
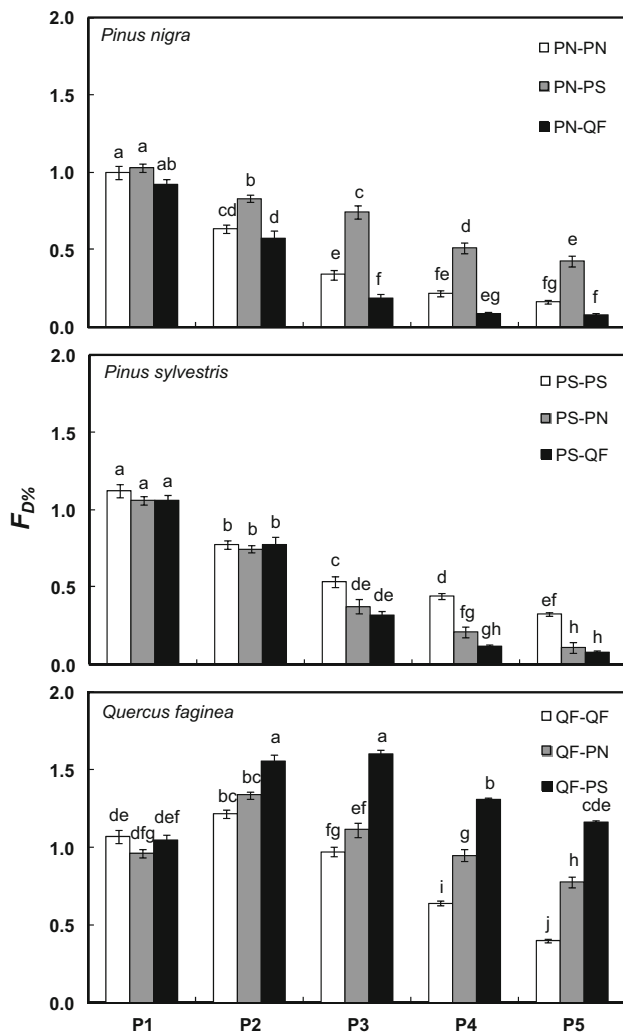
Discussion

Transpiration under non-limiting soil water conditions

Before any drought occurred, we found higher rates of sap flux density for the two coniferous species than for *Q. faginea* (Fig. 3). This result contrasts with previous studies, which showed that Mediterranean broadleaves generally have higher rates of sap flux density than do conifers (Carnicer et al. 2013). The discrepancy between these previous results and ours might be explained by the fact that needles of conifer species are fully transpiring early in spring, while leaves of ring-porous species such as *Q. faginea* are established later in the season when new xylem tissues are built.

Table 4 Degrees of freedom (*df*), *F* and *P* values from the mixed linear models used to test the fixed effects of the level of species interaction (*Neighbourhood*), the Hegyi competition index (CI), the period of measurement (*Period*), the diameter at breast height (DBH) and the interactions between *Neighbourhood* and *Period* and between CI and *Period*, on mean daily relative sap flux density ($F_{D\%}$) for *Pinus nigra*, *Pinus sylvestris* and *Quercus faginea*
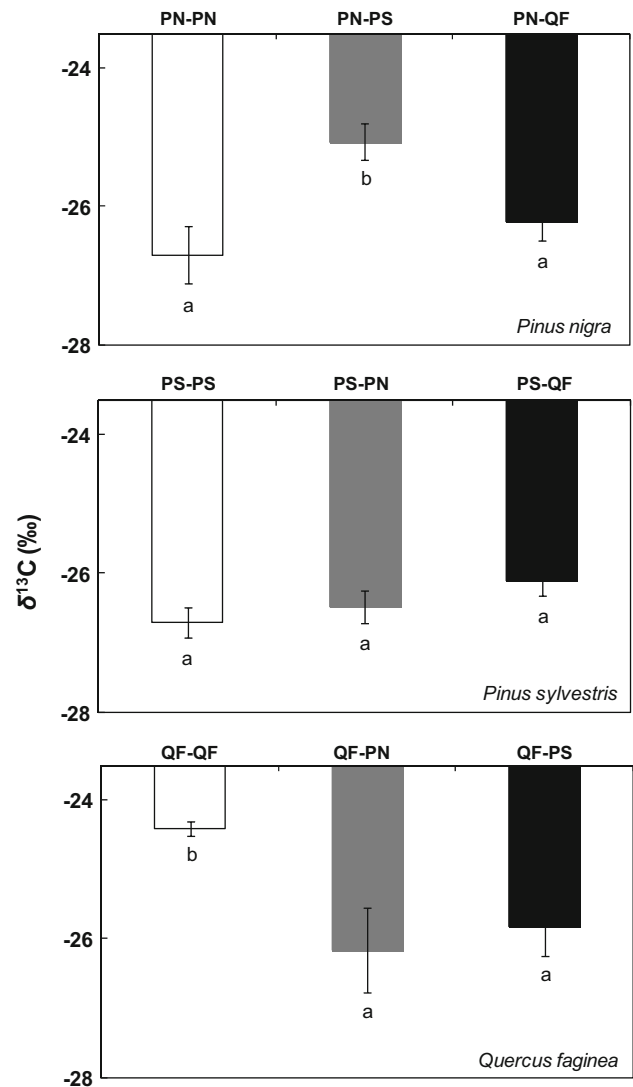
	Pinus nigra			*Pinus sylvestris*			*Quercus faginea*		
	df	*F* value	*P* value	*df*	*F* value	*P* value	*df*	*F* value	*P* value
Neighbourhood	2	16.5	<0.001***	2	5.6	0.025*	2	14.5	<0.001***
CI	1	0.2	0.671	1	0.5	0.990	1	1.5	0.246
Period	4	460.2	<0.001***	4	652.1	<0.001***	4	227.9	<0.001***
DBH	1	0.8	0.388	1	2.2	0.178	1	0.2	0.642
Neighbourhood × period	8	12.3	<0.001***	8	8.5	<0.001***	8	36.2	<0.001***
CI × *period*	4	4.5	0.001**	4	15.9	<0.001***	4	7.7	0.005**

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Fig. 4 Mean of the daily mean relative sap flux density ($F_{D\%}$) for each measurement period (*P1*, *P2*, *P3*, *P4* and *P5*) and each level of species interaction for *Quercus faginea* (QF), *Pinus nigra* (PN) and *Pinus sylvestris* (PS). *Vertical bars* represent the standard error of the mean for each period of measurements. For a given species, *letters* denote significant differences in $F_{D\%}$ among all periods and mixture levels

Fig. 5 Species mean carbon isotope composition (δ^{13}C, ‰) of target trees of *Pinus nigra*, *Pinus sylvestris* and *Quercus faginea* when interacting with conspecific (PN–PN, PS–PS, QF–QF) or non-conspecific (PN–PS and PN–QF for *Pinus nigra*; PS–PN and PS–QF for *Pinus sylvestris*; QF–PN and QP–PS for *Quercus faginea*) neighbours. *Vertical bars* represent the standard error of the mean for each neighbourhood. For a given species, *small letters* denote significant differences in δ^{13}C among mixture levels

Another explanation for higher rates of sap flux density for the two coniferous species before the drought might be the delayed establishment of the leaves of *Q. faginea* at our study site due to unusually late frosts in May 2013 (Fig. 2). The leaves on *Q. faginea* were still completing their development during the first measurement period, so maximum sap flux density rates were only reached during the second measurement period (P2, Fig. 3).

Influence of drought on transpiration

Both *P. nigra* and *P. sylvestris* showed a strong decrease in transpiration from P1 on, whereas the decrease for *Q. faginea* was less severe and started after P2 (Fig. 3).

Drought negatively affected the transpiration of all three species, but at clearly different levels (Fig. 3). When pooling all the trees of a given species, the mean decrease in sap flux density ranged from 30 % for *Q. faginea* to 85 % for *P. sylvestris*. Poyatos et al. (2005) also found a stronger decrease in transpiration for *Pinus* than for *Quercus* trees during a drought stress. This result reflects the higher sensitivity of coniferous species to drought (Carnicer et al. 2013) and is explained by differences among species in their ecophysiological strategies to cope with water deficit. Indeed, *Q. faginea* can be characterized as a drought-adapted species, thanks to its deep-rooting

system (Corcuera et al. 2004; Montserrat-Marti et al. 2009) which access higher water resources during droughts and allow the tree to maintain stomata open over longer periods (Cochard et al. 1996). Regulation of water loss for *Quercus* species starts only after a very low value of water potential has been crossed (typically −2.0 MPa; Bréda et al. 1993). Leaf and soil water potential values were not measured here, but our results suggest that such a threshold value was reached late during the summer, probably after P3. Inversely, pines, which have a shallower rooting system than oaks, close their stomata early on during periods of limited water availability in order to avoid very negative leaf water potential values and thus potential risks of vessel cavitation (Irvine et al. 1998). However, it must be noted that the two pine species also present important differences in their strategy to deal with drought stress. Indeed, *P. sylvestris* is less susceptible to xylem embolism than is *P. nigra* (Choat et al. 2012). Furthermore, *P. sylvestris* relies mostly on superficial soil layers to extract water (Kalliokoski et al. 2008), while *P. nigra* seems better adapted to drought-prone climates (Peñuelas and Filella 2003).

Functional and ecological interpretation

Our main findings were: (1) the strong effect of tree species identity around the target trees on their response to drought and (2) the considerable differences in these effects among species. Our results strongly support the hypothesis that local tree species interactions influence the response of these Mediterranean tree species transpiration to drought, but to different extents. Throughout the summer and during the course of the drought, *Q. faginea* trees that were surrounded by *Pinus* species exhibited a much lower decline in $F_{D\%}$ than *Q. faginea* trees surrounded by conspecific competitors (Fig. 4). Such a response seems to indicate that *Q. faginea* trees experienced a reduced competition for available soil water when surrounded by *Pinus* trees than when surrounded by conspecific trees. Similarly, the interaction with another *Pinus* species resulted in a less pronounced decline in $F_{D\%}$ of *P. nigra* trees during the drought than when interacting with conspecific neighbours (Fig. 4). However, *P. nigra* trees that were in interaction with *Q. faginea* exhibited the same decline in $F_{D\%}$ as in the mono-specific neighbourhoods (Fig. 4). In contrast, the presence of *P. nigra* or *Q. faginea* trees in the neighbourhood of *P. sylvestris* individuals resulted in a higher decline in $F_{D\%}$ with progressing drought as compared to trees mainly surrounded by conspecific competitors (Fig. 4). The differences in the response of *P. sylvestris* transpiration to drought probably indicate lower water availability for the trees in interaction with another species.

The transpiration response observed in the different neighbourhoods suggests the following conclusions: (1)

Community assembly in this Mediterranean ecosystem with coexisting species differing in functional characteristics strongly affects soil water content. (2) Interactions among species can have contrasting consequences for water availability levels depending on the species considered. We develop below our interpretation of the functional and ecological response of each species to these interactions.

For *P. nigra* and *Q. faginea*, the results suggest that when these species are interacting with *P. sylvestris*, soil water depletion is reduced throughout a drought period (Fig. 4). We assume that complementarity mechanisms for soil water uptake could partially explain this pattern. Species interactions may improve the availability and/or access to water resources during the dry period of *P. nigra* and *Q. faginea* when competing with *P. sylvestris*. Furthermore, the interaction between *P. nigra* and *Q. faginea* seemed also to increase the water accessibility of *Q. faginea* at the outmost levels of drought stress (P4 and P5; Fig. 4). Comparable positive species interactions were previously observed in tree plantations (Forrester et al. 2010; Kunert et al. 2012) where complementary water-use led to less competition for soil water resources among the interacting species and to higher overall transpiration rates. This interpretation is consistent with $\delta^{13}C$ values of these species in the different neighbourhoods (Fig. 5). Reduced water availability usually parallels increased (i.e. less negative) $\delta^{13}C$ values in organic material (Saurer et al. 2004). As *P. nigra* exhibited more negative $\delta^{13}C$ values when interacting with *P. sylvestris,* and *Q. faginea* had the most negative values when interacting with the two *Pinus* species (Fig. 5), this indicates greater soil water availability in these neighbourhoods for these two species. As leaf samples were taken before drought started to affect tree transpiration, these results reflect a strong, long-term physiological acclimation of these trees to the different soil water deficit conditions encountered every year in the different neighbourhoods.

For *P. sylvestris*, the lower $F_{D\%}$ rates in the neighbourhoods where *P. nigra* or *Q. faginea* are present (Fig. 4) could be explained by the fact that this species relies mostly on water from shallow soil horizons (Irvine et al. 1998) and, in our case, was interacting with species with higher transpiration rates (Fig. 3). Therefore, the water available to a given *P. sylvestris* tree may have dropped faster in these neighbourhoods than in situations where a tree was competing with the less "water-hungry" individuals of its own species. The fact that any significant effect of species interactions was found on $\delta^{13}C$ (Fig. 5) could suggest that this negative effect of species interactions remains rather small. The differences in local water availability between the neighbourhoods with the two other species and the one with *P. sylvestris* trees solely were

probably not great enough to cause any notable leaf-level physiological acclimation in *P. sylvestris*.

Finally, it must be noted that even though we only selected dominant and co-dominant trees, differences in the aboveground vertical stratification of the canopy might occur in the different plots. As the canopy architecture of the three study species is highly contrasted (Poorter et al. 2012), differences in light interception and in vapour pressure deficit in the different neighbourhoods could have also contributed to the variability in transpiration rates we observed.

Conclusion

Our study brings new insights into the management of mixed Mediterranean forests. We have shown that managing these forests as mixed-species stands would only alleviate the effect of water stress during summer droughts for the deepest-rooted species like *Q. faginea* and *P. nigra*. When such deep-rooted species interact with shallower-rooted species, they seem to benefit from a reduction in competition for water resources in the deep soil layers which dry out more slowly than superficial ones. In contrast, mixing a shallow-rooted conifer species such as *P. sylvestris* with deeper-rooted species may actually be detrimental to its water acquisition and use. Our results also provide worrisome evidence that mixing *Pinus* and *Quercus* species in Mediterranean forests could accelerate the ongoing conversion from mixed conifer-broadleaved forests to pure broadleaved forests in this region under future drier climatic conditions (Carnicer et al. 2014).

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