Stem CO_2 efflux in six co-occurring tree species: underlying factors and ecological implications

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Running title: stem carbon loss in six deciduous trees

ABSTRACT

Stem respiration plays a role in species coexistence and forest dynamics. Here we examined the intra- and inter-specific variability of stem CO_2 efflux (E) in dominant and suppressed trees of six deciduous species in a mixed forest stand: *Fagus sylvatica* L., *Quercus petraea* [Matt.] Liebl, *Quercus pyrenaica* Willd., *Prunus avium* L., *Sorbus aucuparia* L. and *Crataegus monogyna* Jacq. We conducted measurements in late autumn. Within species, dominants had higher E per unit stem surface area (E_s) mainly because sapwood depth was higher than in suppressed trees. Across species, however, differences in E_s corresponded with differences in the proportion of living parenchyma in sapwood and concentration of nonstructural carbohydrates (NSC). Across species, E_s was strongly and NSC marginally positively related with an index of drought tolerance, suggesting that slow growth of drought-tolerant trees is related to higher NSC concentration and E_s. We conclude that, during the leafless period, E is indicative of maintenance respiration and is related with some ecological characteristics of the species, such as drought resistance; that sapwood depth is the main factor explaining variability in E_s within species; and that the proportion of NSC in the sapwood is the main factor behind variability in E_s among species.

Key-words: forest succession; shade tolerance, tree canopy class, carbon balance, xylem live cell content, tree survival.

Abbreviations: E, stem CO_2 efflux; E_{s5}, E per unit surface area at 5 ºC; E_{v5}, E per unit sapwood volume at 5 ºC; E_{p5}, E per unit living sapwood volume at 5 ºC; E_{m5}, E per unit mass of sapwood at 5 ºC; E_{n5}, E per unit mass of nitrogen at 5 ºC; N, nitrogen; NSC, nonstructural carbohydrates; Q_{10}, the proportional change in E per 10 ºC change in temperature; R, stem respiration; SS, soluble sugars.

INTRODUCTION

The living cells of the stem provide energy and carbon skeletons through respiration that help to maintain different tree functions. Consumption of assimilated carbon in stem respiration (*R*) makes it an important component of tree and ecosystem carbon budgets (e.g. Litton *et al*. 2007). However, despite the likely importance of *R* in tree carbon balance and survival, there is still little information on the causes of variability in *R* within and among species and its ecological implications.

Part of the variability in *R* per unit surface area among trees resides in the volumes of cambium, phloem (Pruyn *et al*. 2002a; Pruyn *et al*. 2005) and sapwood (Ryan 1990; Ryan *et al*. 1995; Edwards & Hanson 1996; Pruyn *et al*. 2005; Yang *et al*. 2012a). For a given volume of sapwood, *R* may be related to the proportion of living parenchyma (Ryan 1990; Ryan *et al*. 1995; Ceschia *et al*. 2002; Gruber *et al*. 2009); while at a finer scale, it may depend on the concentration of nitrogen (N) – which affects the cell respiratory potential (Cannell & Thornley 2000; Vose & Ryan 2002; Bosc *et al*. 2003; Pruyn *et al*. 2005; Reich *et al*. 2008) – and nonstructural carbohydrates (NSC) – which affects the cell respiratory rate (Pruyn *et al*. 2005; Wang *et al*. 2006; Wertin & Teskey 2008; Maier *et al*. 2010; Maunoury-Danger *et al*. 2010). Therefore, the weighted rate of *R* per unit surface area depends on the amount of living cells and their activity, which is related to the concentration of N and NSC. The inverse relationships between some of the above traits could lead to some unexpected results. For example, high sapwood depth may not necessarily translate into high *R* per unit surface area because of the trade-off between sapwood depth and proportion of parenchyma in sapwood (Spicer & Holbrook 2007). Similarly, high parenchyma volume may not lead to high *R*, because of the trend for respiratory potential of living cells to decline with increasing parenchyma volume (Pruyn *et al*. 2003; Pruyn *et al*. 2005; Spicer & Holbrook 2007).

Anatomical and biochemical features can vary among trees occupying different positions in the forest canopy and thus affect *R*. Suppressed trees can have less sapwood (Whitehead & Jarvis 1981; Deckmyn *et al*. 2006; Renninger *et al*. 2007) and thus lower *R* than dominants. In support of this, Guidolotti *et al*. (2013) found higher CO_2 efflux per unit stem surface area in dominant than co-dominant *Fagus sylvatica* trees. It is also possible that the balance between the supply and use of NSC (Deckmyn *et al*. 2006) as well as the uptake and allocation of N (Couto-Vázquez & González-Prieto 2010) differ between trees of different crown classes, thus affecting NSC and N concentrations, and rates of *R* of dominant, co-dominant and suppressed trees.

Similarly to variation within species, *R* can differ between species in relation to their functional and ecological characteristics, but this is something which has been barely studied. Fast growing species, with abundant foliage (Körner 1991), can have higher sapwood depth but lower sapwood N concentration than less competitive species (Martin *et al*. 1998). Drought-adapted species can be highly resistant to xylem cavitation and have high sapwood density (Hacke *et al*. 2001); they can also store large amounts of NSC in xylem parenchyma cells to resprout (Palacio *et al*. 2007) or restore water transport capacity after hydraulic failure (Zwieniecki & Holbrook 2009). Shade tolerance can also be related to the amount of carbon losses occurring through respiration. In the same way as shade-tolerant species tend to show lower leaf respiration (Bazzaz 1979; Veneklaas & Poorter 1998; Walters & Reich 1999; Valladares & Niinemets 2008), low stem respiration (*R*) may contribute to persistence of shade tolerant trees under low light conditions imposed by the canopy.

Despite a likely role of *R* in tree adaptation/acclimation to environmental conditions, no empirical evidence has been found between *R* and species ecological requirements. Here we investigated the intra- and inter-specific variability of *R* by measuring radial CO_2 efflux (*E*) from intact stems of six tree species of different ecological characteristics growing together in a mixed sub-Mediterranean forest. Eight trees were selected from each species, half occupying a dominant position in the canopy and half a suppressed position. Measurements were made at the end of autumn in leafless trees to minimize the effects of stem growth and transpiration on *E*, and so using this variable as a proxy of maintenance *R* (see McGuire *et al.* 2007). We hypothesized that i), within and across species, bigger trees having deeper crowns and sapwood depth would have higher *E* per unit surface area than smaller ones; ii) *E* per unit mass or volume of sapwood would be related to the proportion of parenchyma and concentration of N and NSC in sapwood; and iii) *E* per unit sapwood parenchyma would decline as trees had higher amounts of parenchyma. Finally, we expected that *E* would be related with some ecological requirements of the species, such as shade- and drought-tolerance, in support of the role of *R* in plant fitness.

MATERIAL AND METHODS

Study area and species

The study was conducted in central Spain, at the "El Hayedo de Montejo" forest (3º30'W, 41º07' N; altitude: 1240-1575 m a.s.l.). This forest was managed for centuries as a wood pasture and used to keep livestock until 1961 (Pardo et al. 2004). When grazing was banned, recruits from the multi-centennial trees composing the savannah-like woodland rapidly established and generated the current uneven-aged structure and mixed-species composition of the forest. The soil is relatively deep and classified as Luvisol Orthodystric (FAO 2006), and dominant lithology is granitic micaceous gneiss. The climate is Mediterranean, with the highest temperature and lowest precipitation occurring during summer months. Meteorological data collected in the forest since 1994 to 2013 indicate mean monthly temperatures range from 2.3 ºC in January to 18.9 ºC in July; mean annual precipitation is 901 mm, of which only 147 mm occur from June through September.

The species compared in the study were: *Quercus petraea* [Matt.] Liebl (sessile oak), *Quercus pyrenaica* Willd. (melojo oak), *Fagus sylvatica* L. (beech), *Prunus avium* L. (wild cherry), *Sorbus aucuparia* L. (rowan). and *Crataegus monogyna* Jacq. (common hawthorn). All are broadleaved winter deciduous tree species. *F. sylvatica*, *Q. petraea*, *P. avium* and *S. aucuparia* are mostly found in temperate forests, but are rare in sub-Mediterranean forests such as the one of study, where these species are at or close to their southernmost distribution limit. On the contrary, *Q. pyrenaica* and *C. monogyna* are typical of sub-Mediterranean regions with a moderate drought period. Overall, the three Fagaceae species (*F. sylvatica*, *Q. petraea* and *Q. pyrenaica*) form the late stage of forest succession, whereas the three Rosaceae species (*P. avium*, *S. aucuparia* and *C. monogyna*) appear in earlier successional stages or sparsely in edges and gaps of mature forests. Further, all species vary in ecological traits such as growth rate, shade and drought tolerance. Based on our observations in the study plot from 2006 to 2012 and data from Gil *et al*. (2010), the ranking of growth rate is: *F. sylvatica* > *P. avium* > *Q. petraea* > *S. aucuparia* > *C. monogyna* > *Q. pyrenaica*. Based on quantitative indexes from Niinemets & Valladares (2006) and data from Rodríguez-Calcerrada *et al*. (2010), the ranking of shade tolerance is *F. sylvatica* > *P. avium* > *S. aucuparia* = *Q. petraea* > *Q. pyrenaica* > *C. monogyna*; while species ranking in drought tolerance is roughly inverse: *Q. pyrenaica* > *C. monogyna* > *Q. petraea* > *P. avium* > *F. sylvatica* > *S. aucuparia.* All species resprout after perturbation, *Q. pyrenaica* being the species that sprouts more profusely.

Study plot and sampling

The study plot was located in a mixed forest stand. All species were found within 1ha of similar slope (≈ 10%) and orientation (Fig. 1). The canopy was around 16 m height and was formed by *Q. petraea*, *Q. pyrenaica*, *F. sylvatica* and less so by *P. avium*, with *S. aucuparia* and *C. monogyna* only reaching the canopy around gaps.

For each species we selected four dominant (D) and four suppressed (S) trees. Dominants had most of the crown exposed to full sunlight while suppressed trees had the crown completely overtopped (Davies *et al.*, 2008). We selected trees with diameter greater than 5 cm at breast height for the study but did not include ancient multi-centennial trees. We measured stem CO_2 efflux (E) and morphological and biochemical features of inner bark and sapwood at breast height. E was measured five times from dawn to midday during three late autumn days (3rd, 9th and 10th December 2012). Anatomical and biochemical properties were examined from stem cores extracted at breast height within the three days following the last measurement of E. Sampling for biochemistry alternated among trees from different species and canopy positions to minimize any effect of sampling time. Tree age was also estimated by counting growth rings in fine-sanded cores extracted at the base of the stem.

Stem CO_2 efflux measurements

Stem CO_2 efflux (E) was measured with a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) coupled to a soil respiration chamber, using the protocol described in Xu *et al.* (2000). The method consists in measuring the increase in air CO_2 concentration in the closed system formed by the measuring device and an adapter previously fixed to the stem. The PVC adapter houses the soil chamber and was fixed to the stem with silicon sealant and wire, which was placed around the stem upon thick rubber strips to avoid any damage to trees with thin bark. The rise in CO_2 concentration coming from the stem was recorded in three successive cycles. Each cycle started with the machine pumping the air of the system through soda lime to reduce the CO_2 concentration and finished when the CO_2 flowing through the bark reached an upper CO_2 concentration limit. The increase in CO_2 concentration was recorded over 2 minutes, at the most, and then used to compute the rate of E per unit stem surface area (E_s) at ambient air CO_2 concentration. The upper limit of measurement cycles was typically 2 to 10 ppm units higher than the ambient CO_2, which varied between 380 and 395 ppm during the day. The calculation of E_s requires additional corrections for the volume of the adapter and the curvature of the stem (Xu *et al*. 2000). Measurements were made at ambient air temperature and relative humidity.

Climatic variables

Air temperature and relative humidity were measured at 1.5 m height in the center of the plot with a HOBO microstation data logger H21-002 (Onset Computer Corporation, MA, USA). Stem temperature was measured with type T (copper – constantan) thermocouples inserted 2 cm into the sapwood. Air and stem temperatures were measured every minute and stored every five minutes in two data loggers (CR23X and CR10X Campbell Scientific Ltd. UK).

Soil moisture measured with a TDR (Trime-FM, IMKO, Germany) at five points on the plot at 10, 30, 50 and 70 cm depths, was 22.1 ± 0.7 %, providing evidence that drought had ended by the beginning of the experiment.

Morphological and anatomical variables

We measured the thickness of inner bark and sapwood and the density of sapwood for all trees in the study. Increment cores were taken at 1.5 m height with a 5 mm increment borer, taking care to reach the pith of the stem. The separation of sapwood and heartwood was marked by a clear change in color for the two *Quercus* species, *P. avium* and *S. aucuparia*, but not for *F. sylvatica* and *C. monogyna*, for which it was necessary to stain the cores. We used Lugol's Iodine reagent (IKI), which reacts with starch of living parenchyma and stains the sapwood with a deep blue-black color. Sapwood density was computed as the ratio of oven-dried weight to water-saturated volume (measured as displaced water in a precision balance) of 1-cm deep samples. The amount of sapwood was also expressed relative to total diameter (Whitehead & Jarvis 1981).

Increment cores were then stored in FAA (formalin–acetic acid–70% ethanol in proportions 10:5:90) to estimate the proportion of living parenchyma cells in the sapwood. Tangential and cross sections 15–20 µm thick were cut using a sliding microtome, stained with an aqueous solution of 1% safranine and 1% alcian blue, mounted for optical microscopy, and photographed with a digital camera (Canon EOS 600D with a LM microscopy adapter, MicroTech Lab; Supporting Information Fig. S1). Tangential sections were obtained from the early wood of the last five growth rings. Three randomly located images per section of 1 mm^2 size were processed and the proportion of tangential surface occupied by rays was manually measured with ImageJ (http://rsb.info.nih.gov/ij/). The mean value of the 15 images was used to calculate the proportion of ray parenchyma for each tree. Axial parenchyma was quantified in five cross sections per tree. Two randomly located images per section of 0.2 mm^2 size were analyzed in the earlywood and two in the latewood. The mean value of 20 images per tree was used to calculate the proportion of surface area occupied by axial parenchyma. The total volume of parenchyma in each sample was determined as the sum of radial and axial parenchyma.

Biochemical measurements

We measured the concentration of nitrogen (N) and total nonstructural carbohydrates (NSC) in lyophilized samples from the inner bark and sapwood. Analyses of N were made with an Elemental Combustion System CHNS-O 4010 analyzer (Costech Instruments, Italy/USA). Analyses of NSC were made separately for soluble sugars (SS) and starch as described by Haissig and Dickson (1979) and Hansen and Møller (1975). SS were extracted in a 12:5:3 (by volume) solution of methanol, chloroform and water, incubated with anthrone reagent, and measured at 625 nm wavelength in a spectrophotometer within the next 30 min (UV–1700 PharmaSpec, UV-Visible Spectrophotometer, Shimadzu, Japan). Starch contained in the residue left by the extraction was transformed into glucose by amyloglucosidase activity, and glucose was oxidized with the peroxidase-glucose oxidase complex. Glucose was measured at

450 nm within 30 min of further reaction with dianisidine. Additional details on the protocols can be found in Oleksyn *et al*. (2000) and Karolewski *et al*. (2013).

Data analyses

Limitations in radial transport of CO_2 can cause E at a given time to reflect the metabolic activity of living tissues at previous temperatures, rather than the concurrent stem temperature (Ryan *et al*. 1995; Acosta *et al*. 2008). Thus, we examined the relationship between E_s and stem temperature registered at the same time, and at 30, 60, 90 and 120 minutes earlier, and used the relationship with the highest coefficient of determination (r^2) to compute the Q_{10} of E and the value of E per unit surface area at a constant temperature of 5 ºC (E_{s5}; µmol CO_2 m^{-2} s^{-1}) for each tree (Ryan *et al*. 1995; Acosta *et al*. 2008).

From the slope (k) of the relationship between stem temperature and the natural logarithm of E_s, we calculated the Q_{10} of E as:

$$Q_{10} = e^{10k}.$$

Using the value of Q_{10} we calculated E_{s5} as:

$$E_{s5} = E_{s0} Q_{10}^{\left(\frac{5-0}{10}\right)}.$$

In this equation, E_{s0} is E_s at 0 ºC, and is obtained from the intercept of the relationship between stem temperature and the natural logarithm of E_s (Atkin *et al*. 2005).

E was expressed per unit of sapwood volume (E_{v5}; µmol CO_2 m^{-3} s^{-1}) as:

$$E_{v5} = E_{s5} \frac{S}{V}$$

where S and V are the surface area and volume of sapwood, respectively. Thus,

$$E_{v5} = E_{s5} \frac{2\pi r_{h+s} height}{\pi({r_{h+s}}^2 - {r_h}^2)\, height} = E_{s5} \frac{2 r_{h+s}}{({r_{h+s}}^2 - {r_h}^2)}$$

where r_h and r_{h+s} denote the radius of heartwood and heartwood plus sapwood, respectively.

We expressed E on the basis of living sapwood volume (E_{p5}; μmol CO_2 m^{-3} s^{-1}), by dividing E_{v5} by the summed proportion of ray and axial parenchyma in the sapwood. Finally, based on sapwood density and N concentration, we estimated E per mass of sapwood (E_{m5}; nmol CO_2 kg^{-1} s^{-1}) and mass of N (E_{n5}; nmol CO_2 g^{-1} s^{-1}).

Means of all variables were compared between species and canopy positions by analysis of variance (ANOVA). We first included tree age as a covariate, but it was never significant at $P < 0.05$ and was removed from the final models. Whenever the main effect of species was significant, a post-hoc Tukey HSD test was applied to delineate significant differences among species. The interaction term between species and canopy position in ANOVA models was never significant at $P < 0.05$, so differences between species were analyzed by pooling together dominant and suppressed trees. Variables were transformed when necessary to meet assumptions of parametric analysis. The two groups formed by the three Rosaceae and three Fagaceae species were also compared by ANOVA, with species as the experimental unit. The relationships among variables were studied within and across species separately through regression analysis. We used the software Statistica 8.0 (StatSoft Inc. Tulsa, OK, USA).

RESULTS

Dendrometric characteristics

The number of growth rings at the stem base was used as a proxy of tree age. All trees had a similar number of rings, 37 on average, and differences among species or canopy positions were not significant at $P < 0.05$ (Fig. 2). On the contrary, stem diameter, tree height and crown height differed significantly among species and canopy positions (all $P < 0.01$). Suppressed trees had lower values in all these metrics than dominants, although differences were small for some species, such as *C. monogyna* (Fig. 2). Moreover, in general, *F. sylvatica*, *P. avium* and *Q. petraea* were bigger than *Q. pyrenaica*, *S. aucuparia* and *C. monogyna* (Fig. 2). A strong relationship existed between the quantitative index of shade-tolerance and the height of the living crown, with more shade-tolerant species having deeper crowns (n = 6, $r^2 = 0.94$, $P < 0.01$).

Morphological and anatomical characteristics

Sapwood density was higher in *C. monogyna* (0.55 ± 0.01 g cm^{-3}) than *P. avium* (0.48 ± 0.01 g cm^{-3}; $P = 0.049$; Fig. 3), and similar intermediate densities were found in the other species. The depth of inner bark and sapwood also varied significantly among species (both $P < 0.001$). Sapwood depth was the highest in *F. sylvatica*, intermediate in *C. monogyna* and lower in the other species (Fig. 3).

Sapwood density was similar between canopy positions but sapwood and inner bark depth were higher in dominant than suppressed trees (both $P < 0.01$; Fig. 3). Relative to stem diameter, however, the sapwood occupied a larger proportion in suppressed trees than dominants (69.1 ± 4.4% *vs* 58.1 ± 4.1%, respectively; $P = 0.027$). A marginally significant relationship between the quantitative index of shade-tolerance and sapwood depth pointed to more shade-tolerant species having deeper sapwood (n = 6, $r^2 = 0.56$, $P = 0.085$).

Regarding anatomical characteristics, *F. sylvatica*, *P. avium*, *C. monogyna* and *S. aucuparia* had diffuse-porous wood and the two *Quercus* species ring-porous wood. The proportion of parenchyma in the sapwood differed markedly among species (Fig. 3). The proportion of axial parenchyma was approximately 15% in the two *Quercus*, nil in *P. avium*, and lower than 4% in the rest of species. Both ray and total parenchyma were highest in *Q. pyrenaica* (33 ± 2% and 48 ± 2% for ray and total parenchyma, respectively), lowest in *S. aucuparia* (12 ± 1% and 14 ± 1%) and intermediate in the other species (Fig. 3; Supporting Information Fig. S1). A marginally significant positive relationship was observed between the drought-tolerance index and the proportion of xylem parenchyma (n = 6, $r^2 = 0.56$, $P = 0.087$; Supporting Information Fig. S2). Ray and total parenchyma were significantly lower in the sapwood of Rosaceae than of Fagaceae species (both $P < 0.05$). Regarding canopy position, dominant trees had slightly higher proportion of ray ($P < 0.001$) and total ($P = 0.015$) parenchyma than suppressed trees.

Biochemical characteristics

Averaged across species, N concentration was 0.41 ± 0.06 % in inner bark and 0.33 ± 0.03 % in sapwood. For either inner bark or sapwood, N concentration differed significantly among species but not between canopy positions, at $P < 0.05$. In sapwood, *Q. pyrenaica* showed higher values of N concentration than the Rosaceae species, while *F. sylvatica* and *Q. petraea* had intermediate values (Fig. 4; both $P < 0.001$). Comparing the two botanical families, we observed that the Fagaceae had significantly higher concentrations of N in inner bark and sapwood than the Rosaceae at $P < 0.05$. However, N concentrations in parenchyma cells tended to be higher in the Rosaceae species (8.4 ± 0.8 mg N cm^{-3} parenchyma) than in the Fagaceae (5.7 ± 0.8 mg N cm^{-3} parenchyma; $P = 0.056$).

Similarly to N concentration, NSC concentration in inner bark (7.8 ± 0.4 %) was higher than in sapwood (5.1 ± 1.0 %), and in both stem parts, it did not differ significantly between dominant and suppressed trees at $P < 0.05$, but it did differ among species ($P < 0.01$). In sapwood, NSC, and in general starch and SS, were all higher in the two *Quercus* than in the rest of species, *F. sylvatica* and *S. aucuparia* having particularly low concentrations of starch (Fig. 4). A marginally significant positive relationship was observed between drought-tolerance index and concentrations ($n = 6$, $r^2 = 0.61$, $P = 0.067$) and pools ($n = 6$, $r^2 = 0.55$, $P = 0.091$) of NSC in sapwood (Supporting Information Fig. S2).

The proportion of parenchyma in the sapwood was positively related with its concentrations of N and NSC across species (Fig. 5). The strength and significance of relationships were similar using total, ray or axial parenchyma as explanatory variables, suggesting a similar storage role of both axial and ray parenchyma cells.

Sensitivity of stem CO_2 efflux to temperature

There was a variable response of E to temperature among trees. 67% of trees showed a moderate positive relationship between E_s and temperature ($r^2 > 0.50$), but the remaining trees showed a weaker response, or even a strong negative relationship (two trees with $r^2 > 0.80$). Accordingly, the Q_{10} was very variable; it was not significantly different between canopy positions but varied significantly among species ($P < 0.01$; Fig. 6). *P. avium* showed the highest value of Q_{10}, and *F. sylvatica* and *S. aucuparia* the lowest.

Stem CO_2 efflux at a comparable temperature of 5 ºC

Making use of the relationship between temperature and E_s, we estimated E_s at a comparable temperature of 5 ºC. This value was reliable even for trees for which E_s was poorly predicted by temperature, because the temperature of 5 ºC was in the measurement range of all trees (from -1 to 10 ºC). Actually, there were consistent differences among species and canopy positions when either the E_s modeled from temperature or that observed at near 5 ºC (5.1 ± 0.2 ºC) were compared, both values differing by just 0.7%.

E was significantly different among species irrespective of how it was expressed. The highest coefficient of variation was observed for E_{s5} (70%) and the lowest for E_{n5} (55%). Species ranking was similar for E_{s5}, E_{v5}, and E_{m5}, indicating species differences in sapwood depth or density did not explain variability in E_{s5}. These rankings were headed by *Q. pyrenaica*, with *Q. petraea*, *C. monogyna* and *P. avium* having intermediate values, and *F. sylvatica* and *S. aucuparia* the lowest values (Fig. 6). Species ranked in a different order when we expressed E per unit of sapwood parenchyma volume (E_{p5}) and N mass (E_{n5}). The higher values of E_{p5} were found in *P. avium*, followed by *S. aucuparia* and *C. monogyna*, and then the Fagaceae species of which *F. sylvatica* had the lowest values (Fig. 6). Actually, the parenchyma of Rosaceae species exhibited significantly higher E rates than that of Fagaceae ($P < 0.01$). When we took into account the concentration of N in sapwood, the difference in *E* (E_{n5}) between families was no longer significant, suggesting that parenchyma cells of Rosaceae respired more mainly because they contained more N (Fig. 6).

Regression analyses across species indicated that sapwood depth was not related with E_{s5} (Fig. 7; Table 1). On the contrary, the proportion of parenchyma was related with E_{s5}, species with higher proportion of xylem parenchyma having higher E_{s5} (Fig. 7; Table 1). Sapwood parenchyma volume (resulting from multiplying sapwood depth by parenchyma proportion, over 1 m^2 of stem surface area) was negatively correlated with E_{p5} ($r^2 = 0.80$, $P = 0.015$; Fig. 8), but not with any other CO_2 efflux variable. The concentration of NSC was strongly related with the rate of E_{s5} (Fig. 7; Table 1). Similarly, species quantified as more drought tolerant showed significantly higher *E* per unit surface area (E_{s5}; Fig. 7; Table 1), sapwood volume (E_{v5}; $r^2 = 0.74$, $P = 0.028$; Table 1) and sapwood mass (E_{m5}; $r^2 = 0.65$, $P = 0.051$; Table 1). Finally, species quantified as more shade tolerant exhibited lower E_{n5} ($r^2 = 0.71$, $P = 0.034$; Table 1).

Regarding canopy position, *E* was significantly higher in dominant than suppressed trees when it was expressed per unit stem surface area ($P < 0.01$), but not when it was expressed per unit sapwood volume, mass, parenchyma volume or N mass (Fig. 6). The correlation between variables within species is shown in Supporting Information Table S1. It is worth noting significant correlations across species did not hold for all species, and that some significant relationships within species did not occur across species.

Finally, we noted that sapwood density was not significantly related with E_{s5} across or within any species ($P > 0.05$) and that inner bark depth was only marginally significantly related with E_{s5} for *Q. pyrenaica* ($n = 8$, $r^2 = 0.49$, $P = 0.053$) but not across species (data not shown). In summary, the availability of respiratory substrates in sapwood (mostly NSC; accounting for sapwood depth, parenchyma proportion, density and NSC concentration), and the activity of sapwood parenchyma per unit N (E_n5) govern E_s5 across trees of different species and canopy positions (see Principal Component and Classification Analysis in Supporting Information Fig. S4).

DISCUSSION

Differences in *E* between tree species

In seasonal climates, stem CO_2 efflux (*E*) peaks during the growing season, often coinciding with high respiratory energy requirements for lignification of new cells, and is lower in late autumn and winter, when temperatures are low and growth has ceased (Ceschia *et al.* 2002; Gaumont-Guay *et al.* 2006; Rodríguez-Calcerrada *et al.* 2014). But *E* is not negligible during the "winter-dormant" season. Here, the rate of stem CO_2 efflux per unit surface area at 5 ºC (E_{s5}) in late autumn varied from 0.28 μmol m^{-2} s^{-1} in *F. sylvatica* to 0.75 μmol m^{-2} s^{-1} in *Q. pyrenaica*, which agrees with literature values from angiosperms estimated at a comparable temperature (e.g. Levy & Jarvis 1998; Cernusak *et al.* 2006; Zach *et al.* 2010; Yang *et al.* 2012a, b; Guidolotti *et al.* 2013; Rodríguez-Calcerrada *et al.* 2014). Since stem diameter increment had long ceased at the time of *E* measurements (Aranda *et al. unpublished data*), we suggest that these rates most likely reflected the energy costs associated with maintenance of living cells under cold conditions – such as maintenance of ion gradients and synthesis of molecules involved in stability of membranes at freezing temperatures (Cavender-Bares 2005; Pagter *et al.* 2008) – and possibly "waste" respiration (Cannell. & Thornley 2000). Indeed, without the confounding effects of stem photosynthesis and sap flow on CO_2 fluxes, E_{s5} is a close surrogate of stem respiration (McGuire *et al.* 2007) that will depend on the amount of living cells and their respiratory potential and actual activity. Here, the proportion of parenchyma and NSC in sapwood governed the variability of E_{s5} across species; *Q. pyrenaica* had the highest proportion of parenchyma and the highest rate of E_{s5}, whereas *S. aucuparia* had the lowest proportion of parenchyma and the lowest rate of E_{s5} (Fig. 7). Interestingly, the volume of parenchyma in sapwood was not a reliable predictor of E_{s5} across species, because the respiratory activity of parenchyma cells decreased with and compensated for high parenchyma volume (Fig. 8).

Some trade-offs between the factors that compound stem respiration could reflect an evolutionary trend towards maintaining stem respiration within certain limits according to the growth environment, and be advantageous for survival. First, parenchyma cells tend to show low activity when the amount of parenchyma is important (Pruyn *et al*. 2003; Pruyn *et al*. 2005; Spicer & Holbrook 2007; Fig. 8), which could attenuate carbon losses from trees requiring a large depth of sapwood. For example, *E* per unit of sapwood parenchyma volume and N mass were the lowest in *F. sylvatica*, so that, even if sapwood was deep and contained a considerable proportion of parenchyma in this species, rates of E_{s5} were eventually moderate and similar to rates of Rosaceae species with half as much parenchyma and depth of sapwood (i.e. *P. avium* and *S. aucuparia*). In other words, abundant foliage of *F. sylvatica* is supplied with needed water through sapwood, but the stem does not incur in severe carbon losses, because the respiratory activity of living cells is low. A similar trade-off was found between the two families studied. The parenchyma of Rosaceae respired twice as much as that of Fagaceae, although E_{s5} was similar between families because Rosaceae had half as much parenchyma. The main reason for high parenchyma respiration in the Rosaceae species studied was the high N observed in this tissue, something which could be related to the presence of Rosaceae species in fertile soils of gaps and forest edges where more N is available than in the forest interior (Hamberg *et al*. 2009; Esen *et al*. 2012). Finally, trade-offs between respiratory components can also discriminate between functional groups of differing water-transport capacity. Sapwood tends to be thicker in diffuse- than ring-porous species (Martin *et al*. 1998; Gebauer *et al*. 2008), but sapwood-based *R* tends to be lower in diffuse- than ring-porous species (Edwards & Hanson 1996; Spicer & Holbrook 2007). In our study, diffuse- and ring-porous trees did not show clear differences in E_{s5} or E_{v5}. Diffuse-porous *F. sylvatica* and *S. aucuparia* had lower E_{v5} than the ring-porous *Q. pyrenaica*, but this pattern did not hold for the other species (Fig. 7).

It is remarkable that approximately half of the sapwood of *Q. pyrenaica* was formed by living axial and multiseriate ray parenchyma cells. Other studies have shown high proportions of parenchyma in sapwood and E_s in oaks compared to other co-occurring trees (Panshin *et al.* 1964; Yang *et al.* 2012a). Maintaining such a high fraction of parenchyma with considerable respiratory activity (see E_{p5} and E_{n5} as compared to the other Fagaceae in Fig. 6), incurs a high penalty in terms of carbon loss (see E_{s5} and E_{v5} in Fig. 6), but it must provide some fundamental advantages to the tree. The parenchyma is involved in nutrient storage (Pruyn *et al.* 2005), radial water transport (James *et al.* 2003), and possibly conduit refilling and tolerance of hydraulic failure (Zwieniecki & Holbrook 2009). All these functions are particularly important for Mediterranean species (such as *Q. pyrenaica*) prone to suffer cavitation and other perturbations, including wildfires (Gebauer *et al.* 2008).

Another interesting finding was that, although *Q. pyrenaica* has a large fraction of woody biomass, small crown, and the shortest leaf life span of all species, it had the highest proportion of living stem cells and E_{s5} without any symptom of NSC depletion. These apparently contradictory characteristics are reconciled by the slow growth, little expenditure in sexual regeneration (Salomón *et al.* 2013), and high rates of leaf net photosynthesis during summer (Gallego *et al.* 1994; Rodríguez-Calcerrada *et al.* 2008). Reduced growth seems to be a pivotal factor explaining the positive correlation of drought-tolerance index with NSC concentration and E_{s5} across species. Imposed by climatic constraints, drought-tolerant species have adapted to grow little (Mooney 1981). The residual carbon not consumed during the growing season needs to be stored in woody tissues in these deciduous trees. Accordingly, storage capacity and NSC reserves in stem sapwood tended to be higher in more drought-tolerant species. Xylem parenchyma may help to tolerate drought in different ways (see above), but it enhances respiration and adds to growth limitations already imposed by climatic factors.

There was also a significant relationship between E_{n5} and shade tolerance (Table 1), suggesting that xylem parenchyma of more shade tolerant species has lower respiratory activity per unit N than that of less shade tolerant ones. However, this did not translate into lower CO_2 emitted through bark (i.e. no significant relationship was found between E_{s5} and shade tolerance), suggesting that high biomass allocation to foliage (Niinemets 1998; this study) and sapwood volume (Martin *et al.* 1998; this study) in shade-tolerants offset its potentially low cellular respiratory activity.

Differences in E_{s5} among species did not correspond with differences in inner bark depth. The inner bark is much thinner than the sapwood, but it may have 2-5 times higher N and NSC concentration (Pruyn *et al.* 2005 in coniferous trees; Fig. 3), and 2-11 times higher respiratory potential than the more active outer sapwood, thus largely contributing to E (Stockfors & Linder 1998; Pruyn *et al.* 2002a; Bowman *et al.* 2005; Pruyn *et al.* 2005). We suggest that, during the growing season, cambium and phloem could contribute to E more than during the winter dormant season (Edwards & Hanson 1996).

Differences in *E* between tree canopy positions

Sapwood depth was the main factor behind the variability in E_{s5} within species. Sapwood depth increases up to species-specific limits imposed by constraints to respiration of inner xylem parenchyma cells as trees grow (Pruyn *et al.* 2003), such as reduced oxygenation and increased proportion of woody relative to photosynthetic tissues (see Spicer & Holbrook 2007). Foliage area determines parenchyma respiration and sapwood area (Walters & Reich 1999), and vice versa (Martin *et al.* 1998). Here, from the relationships of stem diameter *vs* sapwood, no asymptotic value of sapwood depth seemed to be attained. Sapwood depth increased linearly with stem diameter so that larger dominant trees had thicker sapwood and higher E_{s5} than suppressed ones. The significant difference between canopy positions in E_{s5} but not in E_{v5}, E_{m5}, E_{p5} or E_{n5} supports this reasoning and suggests that parenchyma of both dominant and suppressed trees respired at a similar rate. In support of these results Pruyn *et al.* (2003) found that respiratory potential of sapwood was similar in dominant and suppressed trees of *Tsuga heterophylla*, but Guidolotti *et al.* (2013) found similar E_s in dominants and co-dominants of *Fagus sylvatica* (of unknown diameter) during the dormant season. In the study of Pruyn *et al.* (2003), age was a confounding factor in the comparison of crown classes because suppressed trees were younger than dominants. Here the sampled trees were recruits that established almost simultaneously after the abandonment of a wood pasture, and differences between crown classes were due to differences in competition and growth rate rather than age. One corollary of these results is that sapwood volume is an appropriate variable to upscale E to the forest level in monospecific stands (Ryan 1990), at least during the period of low cambium and phloem activity.

Sensitivity of E to temperature: the use of E as a surrogate of R

Values of Q_{10} (1.7 on average) were similar to those reported for other species – ranging from 1.3 to 2.6 in Ryan *et al.* (1995), Ceschia *et al.* (2002), Yang *et al.* (2012a) and Guidolotti *et al.* (2013), to cite some examples. Surprisingly, however, we found low Q_{10} and predictability of E_s by temperature for some trees. The literature reveals variable coupling of temperature and E_s, ranging from nil (e.g. Edwards & McLaughlin 1978; Zach *et al.* 2010) to strong (e.g. Zach *et al.* 2010; Rodríguez-Calcerrada *et al.* 2014). In our case we expected a strong positive relationship between E_s and temperature because the study was made when growth and sap flow, two factors potentially affecting E_s (Ryan *et al.* 1995; McGuire *et al.* 2007; Yang *et al.* 2012b), were negligible. Low temperature and E_s for some trees likely contributed to a poor relationship between E_s and temperature, but it is also possible that across the range of temperatures measured in the sapwood (from -1 to 10 ºC), the solubility of CO_2 in the xylem sap experienced important changes and altered E_s. Temperature-induced changes of CO_2 solubility in xylem sap have already been said to affect Q_{10} when sap is flowing (Ryan *et al.* 1995; McGuire *et al.* 2007). In winter leafless trees, internal storage of CO_2 could vary around freezing temperatures and affect E_s depending on sap freezing point or pH (Bowman *et al.* 2005; see also Saveyn *et al.* 2008). Less likely, corticular photosynthesis in trees with more light-permeable bark might reduce E_s with increasing temperatures and weaken relationships of temperature and E_s (Saveyn et al. 2008; Bloemen et al. 2013).

Concluding statements

Stem CO_2 efflux measured in leafless deciduous trees during the dormant season is a good surrogate of maintenance respiration. The concentration of NSC and to lower extent the proportion and respiratory rate of parenchyma cells in sapwood determine variation of CO_2 efflux across species, whereas sapwood depth is the main factor behind variability of CO_2 efflux between dominant and suppressed trees within species. The relationships between drought-tolerance index and xylem parenchyma proportion and stem CO_2 efflux suggest a drought-adaptive role of parenchyma in deciduous trees. The benefits of storing NSC in parenchyma cells for tolerating drought and related perturbations such as fires come at the cost of high carbon respiratory losses, which can add to other factors limiting tree growth in Mediterranean species, such as low leaf area, leaf- to sapwood-area ratio and shoot to root ratio.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Images of stem cuttings showing axial and ray parenchyma of six tree species.

Figure S2. Relationship of species drought tolerance indexes with stem anatomical, biochemical and respiratory variables.

Figure S3. Principal Component and Classification Analysis with factors potentially affecting stem CO_2 efflux across trees of different species and canopy positions.

Table S1. Relationship of anatomical and biochemical variables with stem CO_2 efflux within species.

Tables

Table 1. Coefficient of correlation of anatomical, biochemical and ecological characteristics with stem CO_2 efflux expressed per unit of stem surface area (E_{s5}), sapwood volume (E_{v5}), sapwood mass (E_{m5}), sapwood parenchyma volume (E_{p5}) and sapwood nitrogen mass (E_{n5}) across species (n =6). [NSC] = concentration of nonstructural carbohydrates; [N] = concentration of nitrogen. Highlighted in bold are correlations significant at $P < 0.05$. Quantitative indexes of shade- and drought-tolerance are taken from Niinemets and Valladares (2006) and Rodríguez-Calcerrada *et al*. (2010), with positive values indicating increasing tolerance to shade and drought.

	Sapwood depth	**Sapwood parenchyma proportion**	**Sapwood [NSC]**	**Sapwood [N]**	**Shade-tolerance**	**Drought-tolerance**
E_{s5}	-0.27	**0.81**	**0.90**	0.74	-0.48	**0.96**
E_{v5}	-0.47	0.63	0.78	0.50	-0.70	**0.96**
E_{m5}	-0.50	0.61	0.80	0.50	-0.67	**0.94**
E_{p5}	-0.72	-0.65	-0.27	-0.69	-0.50	-0.05
E_{n5}	-0.70	-0.02	0.26	-0.17	**-0.84**	0.62

Figure legends

Fig. 1. Study plot showing trees with diameter at breast height (DBH) higher than 5 cm and the 48 sampled trees. Species are identified by different symbols and abbreviations: *Fagus sylvatica* (*Fsy*), *Quercus petraea* (*Qpt*), *Quercus pyrenaica* (*Qpy*), *Prunus avium* (*Pav*), *Sorbus aucuparia* (*Sau*) and *Crataegus monogyna* (*Cmo*).

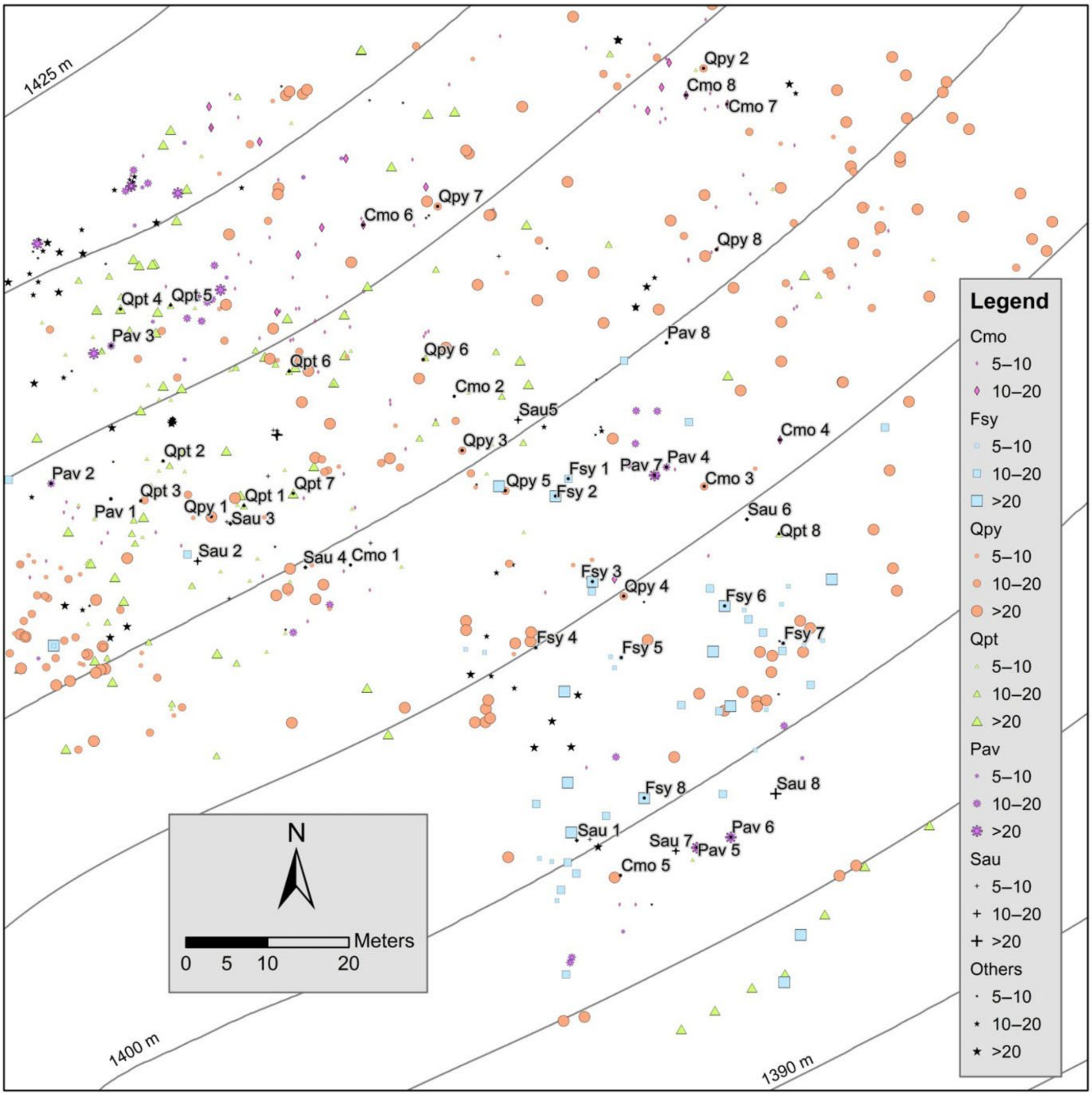

Fig. 2. Dendrometric characteristics of dominant (D) and suppressed (S) trees of six species: *Fagus sylvatica* (*Fsy*), *Quercus petraea* (*Qpt*), *Quercus pyrenaica* (*Qpy*), *Prunus avium* (*Pav*), *Sorbus aucuparia* (*Sau*) and *Crataegus monogyna* (*Cmo*). DBH = stem diameter at breast height. Different letters separate significantly different means among species based on post-hoc Tukey HSD tests at $P < 0.05$.

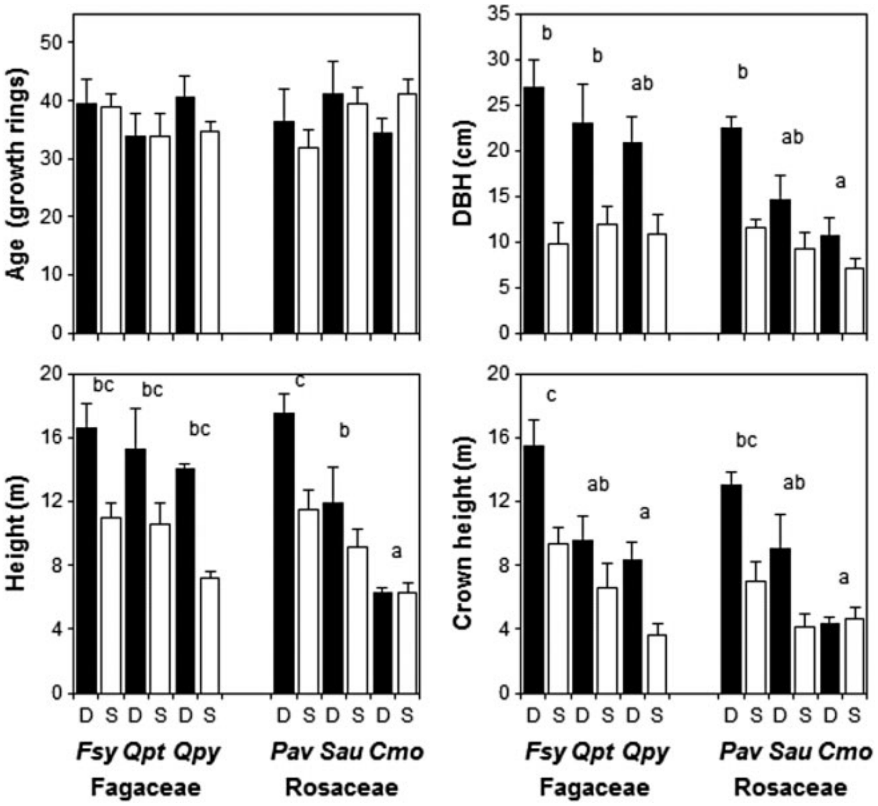

Fig. 3. Morphological and anatomical characteristics of dominant (D) and suppressed (S) trees of six species: *Fagus sylvatica* (*Fsy*), *Quercus petraea* (*Qpt*), *Quercus pyrenaica* (*Qpy*), *Prunus avium* (*Pav*), *Sorbus aucuparia* (*Sau*) and *Crataegus monogyna* (*Cmo*). Different letters separate significantly different means among species based on post-hoc Tukey HSD tests at $P < 0.05$. In the case of parenchyma, letters allude to differences in the summed proportion of axial (lower bars) and ray (upper bars) cells.

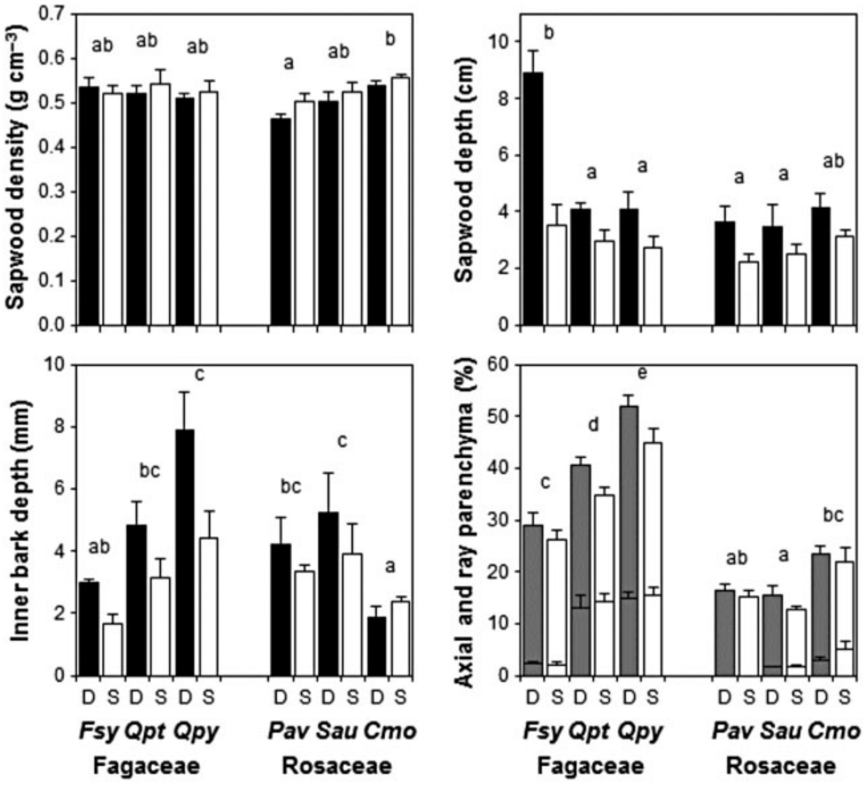

Fig. 4. Biochemical characteristics of dominant (D) and suppressed (S) trees of six species: *Fagus sylvatica* (*Fsy*), *Quercus petraea* (*Qpt*), *Quercus pyrenaica* (*Qpy*), *Prunus avium* (*Pav*), *Sorbus aucuparia* (*Sau*) and *Crataegus monogyna* (*Cmo*). N = nitrogen and SS = soluble sugars. Different letters separate significantly different means among species based on post-hoc Tukey HSD tests at $P < 0.05$. In the case of nonstructural carbohydrates, letters allude to differences in the summed proportion of starch (lower bars) and soluble sugars (upper bars).

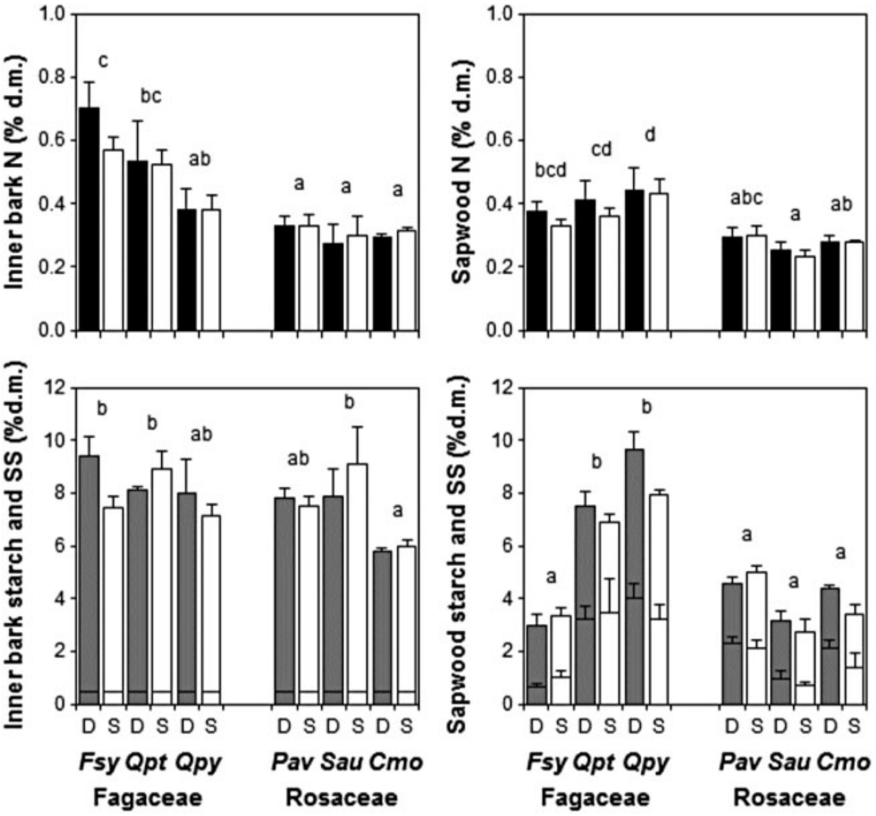

Fig. 5. Relationship between the proportion of living parenchyma and the concentration of nonstructural carbohydrates (NSC) and nitrogen (N) in sapwood for trees of six species: *Fagus sylvatica* (squares), *Quercus petraea* (triangles), *Quercus pyrenaica* (circles), *Prunus avium* (asterisks), *Sorbus aucuparia* (plus symbols) and *Crataegus monogyna* (diamonds). Symbols correspond to individual trees, and to species means (±2SE) in the insets, where the strength and significance of the linear regressions between variables is indicated.

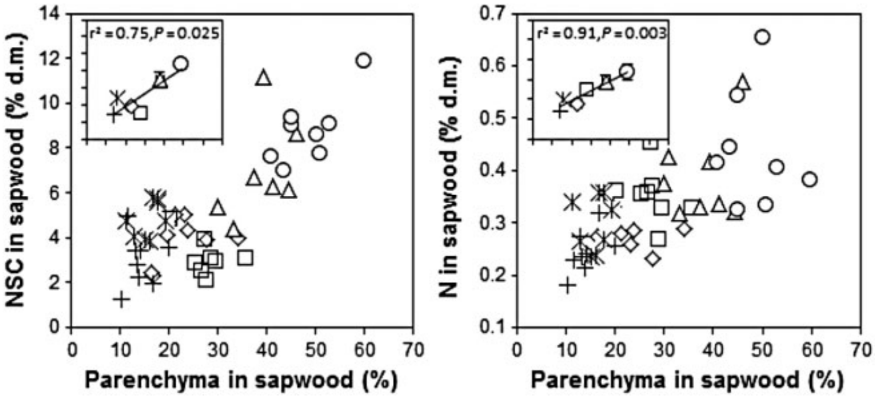

Fig. 6. Mean (±SE) of stem CO_2 efflux sensitivity to temperature (Q_{10}) and stem CO_2 efflux at 5 ºC expressed per unit of stem surface area (E_{s5}), sapwood volume (E_{v5}), sapwood mass (E_{m5}), sapwood parenchyma volume (E_{p5}) and sapwood nitrogen mass (E_{n5}) in dominant (D) and suppressed (S) trees of six species: *Fagus sylvatica* (*Fsy*), *Quercus petraea* (*Qpt*), *Quercus pyrenaica* (*Qpy*), *Prunus avium* (*Pav*), *Sorbus aucuparia* (*Sau*) and *Crataegus monogyna* (*Cmo*). Different letters separate significantly different means among species based on post-hoc Tukey HSD tests at $P < 0.05$ after.

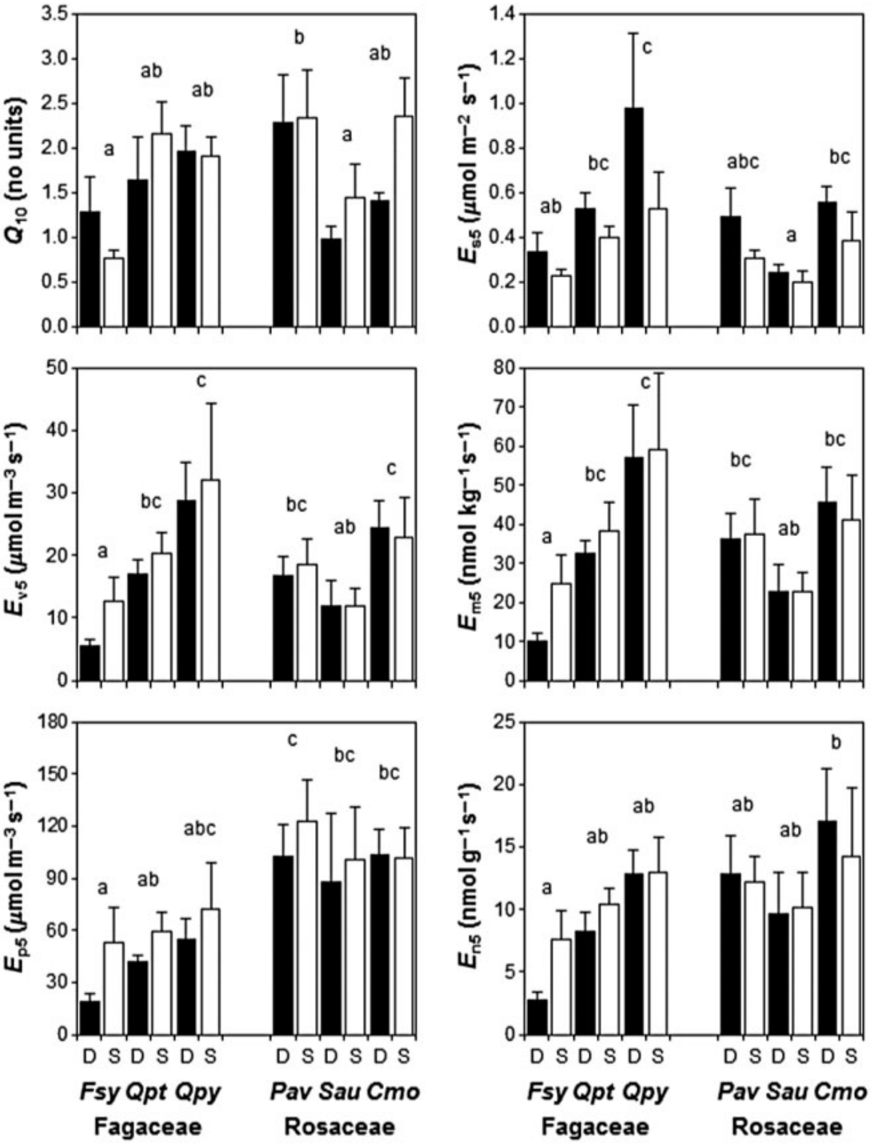

Fig. 7. Relationship of stem CO_2 efflux per unit surface area at 5 ºC (E_{s5}) with sapwood depth, the proportion of living parenchyma, the concentration of nonstructural carbohydrates (NSC) and an index of drought tolerance for trees of six species: *Fagus sylvatica* (*Fsy*), *Quercus petraea* (*Qpt*), *Quercus pyrenaica* (*Qpy*), *Prunus avium* (*Pav*), *Sorbus aucuparia* (*Sau*) and *Crataegus monogyna* (*Cmo*). Values are species means (±2SE). The strength and significance of relationships were indicated by the coefficient of determination and *P*-value of linear regression analyses within the panels. The drought-tolerance index is based on the review of Niinemets and Valladares (2006) where tolerance ranges from 0 to 5 (maximal tolerance). Based on Rodríguez-Calcerrada *et al*. (2010) tolerance for *Q. pyrenaica* was quantified as 4.0, similar to other Mediterranean broad-leaved oaks (e.g. *Q. pubescens*; 4.1 ± 0.25; Niinemets and Valladares 2006).

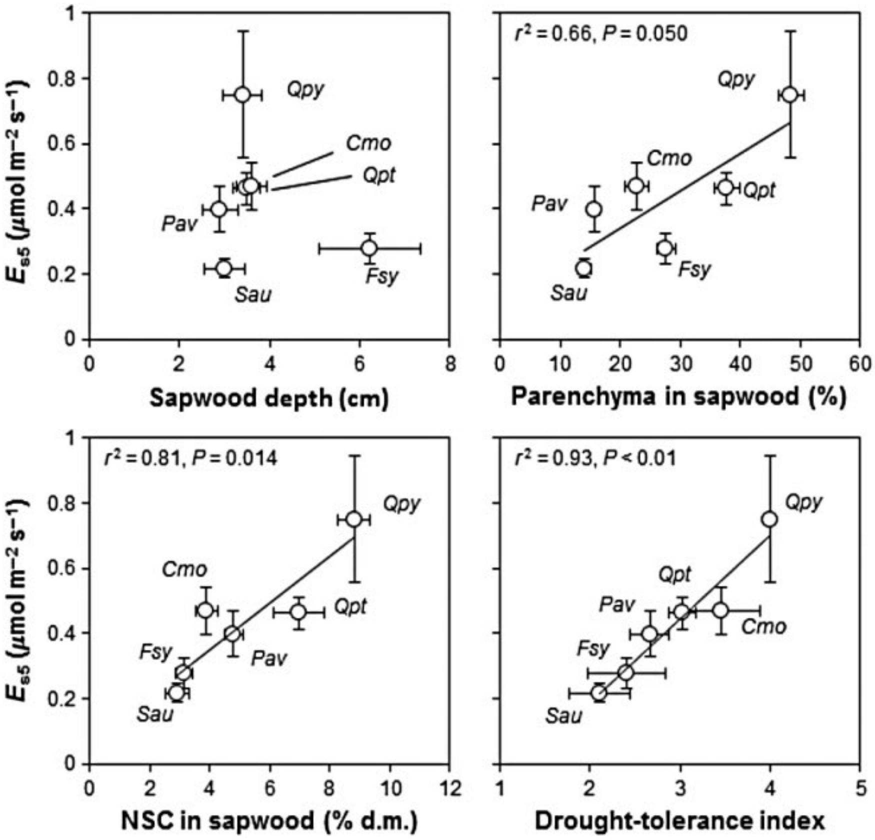

Fig. 8. Relationship between sapwood parenchyma volume (calculated as the product of sapwood depth by parenchyma proportion, beneath 1 m^2 of stem surface area) and stem CO_2 efflux at 5 ºC per unit of sapwood parenchyma volume (E_{p5}) in trees of six species: *Fagus sylvatica* (squares), *Quercus petraea* (triangles), *Quercus pyrenaica* (circles), *Prunus avium* (asterisks), *Sorbus aucuparia* (plus symbols) and *Crataegus monogyna* (diamonds). Symbols correspond to individual trees, and to species means (±2SE) in the insets, where the strength and significance of the linear regressions between variables is indicated.