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ORIGINAL PAPER

Xylem and soil CO_2 fluxes in a *Quercus pyrenaica* Willd. coppice: root respiration increases with clonal size

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Abstract

• ***Key message*** **Xylem and soil CO_2 fluxes in coppiced oak forests increase with clonal size, suggesting larger expenditures of energy for root respiration. An imbalance between root demand and shoot production of carbohydrates may contribute to the degradation of abandoned coppices.**

• ***Context*** Our understanding of root respiration is limited, particularly in root-resprouting species with many stems and a large system of interconnected roots resulting from long-term coppicing.

• ***Aims*** We tested the hypothesis that clone size influences the internal flux of CO_2 dissolved in xylem sap (F_T) from roots into the stem and soil CO_2 efflux (F_S) as indicators of root respiration. We predicted that large clones would exhibit higher F_T per stem and F_S than small clones due to larger root system per stem in large clones.

• ***Methods*** Genetic analyses were performed to elucidate clonal grouping. F_T was measured continuously for 100 days in 16 similar-sized stems of *Quercus pyrenaica* belonging to two large and two small clones. F_S was measured in 20 clones of varying size.

• ***Results*** F_T per stem and F_S were higher in large clones. F_T was 2 % of the root-respired CO_2 that diffused through soil to the atmosphere.

• ***Conclusions*** Relative to other studies, the contribution of F_T to root respiration was very low, pointing to large differences depending on species or site. Higher stem F_T and F_S in large clones compared with small clones suggest greater carbon consumption by roots in large clones, pointing to a root/shoot biomass and physiological imbalance resulting from long-term coppicing that would partially explain the degradation of currently abandoned stands of *Q. pyrenaica*.

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Contribution of the co-authors Roberto Salomón, María Valbuena-Carabaña, and Jesús Rodríguez-Calcerrada: designing the experiment, running data analysis, and writing the paper
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1 Introduction

Resprouting woody species are distributed worldwide among tropical, temperate, and Mediterranean forests. Resprouting is the dominant regeneration strategy in areas of low productivity or frequent disturbances (Del Tredici 2001; Bond and Midgley 2001; Bellingham and Sparrow 2009), such as Mediterranean landscapes where natural and anthropogenic perturbations have historically shaped vegetation and modes of ecosystem regeneration (see references in Valbuena-Carabaña et al. 2010). Although resprouting constitutes an important mechanism of natural ecosystem regeneration (Dietze and Clark 2008), in the general frame of plant ecology, the study of vegetative regeneration has been frequently disregarded in favor of sexual recruitment (Del Tredici 2001; Bond and Midgley 2001; Dietze and Clark 2008). In ecophysiological studies, trees have been traditionally considered as discrete individuals regenerating from seeds. Nevertheless, recent studies challenge this assumption as the processes of root grafting and resprouting can result in trees with multiple stems that share resources and function physiologically as a unit (Fraser et al. 2006; Tarroux et al. 2010; Baret and DesRochers 2011). Technical difficulties in the study of belowground structures have limited our understanding of root system dynamics, their relationships with aboveground structures, and their implications for ecosystem regeneration in clonal resprouting species.

Root respiration (R_R) (the list of acronyms is presented in Table 1) is an important component of the carbon budget of trees (Ryan et al. 2004) and contributes to soil CO_2 efflux (F_S) (Hanson et al. 2000; Rey et al. 2002; Tang and Baldocchi 2005), which constitutes the largest contributor to total ecosystem respiration (Reichstein et al. 2002; Guidolotti et al. 2013). R_R can be fueled by either recently assimilated (Högberg et al. 2001) or stored (Aubrey et al. 2012) carbon. The non-structural carbohydrate pool is particularly important for root growth and maintenance in resprouting species. In fact, non-structural carbohydrate pools stored in root systems of resprouters are directly related to resprouting ability and initial growth (Drake et al. 2009; Zhu et al. 2012) and have been shown to be much larger in resprouters than in non-sprouting species (Bond and Midgley 2001 and references therein). A greater amount of parenchyma and more carbohydrate storage may lead to higher maintenance respiration costs in root systems of vegetatively regenerated trees relative to sexually originated ones. In coppiced stands, periodic removal of all or part of aboveground biomass—while underground biomass remains preserved—initiates an imbalance in root/shoot biomass that may persist over time (DesRochers and Lieffers 2001; Landhäusser and Lieffers 2002; Corcuera et al. 2006; Bravo et al. 2008; Drake et al. 2009). Since fine-root shedding likely occurs after coppicing in relation to lower transpiration needs, persistence of coarse roots would contribute to a greater extent to this disequilibrium. Assuming clonal size as a proxy of clonal age (Steinger et al. 1996; Wesche et al. 2005), older and larger clones subjected to more coppicing events would be constrained by a larger imbalance. For instance, the root biomass left after a stem is felled in a clonal tree can be partially maintained by connected stems for several years, as has been shown in *Populus tremuloides* (DesRochers and Lieffers 2001; Jelínková et al. 2009). Therefore, an increasing disequilibrium may arise over time if the foliage that develops after repeated coppicing barely compensates for the disproportionately large amount of carbon consumed by the intact coarse root system (Iwasa and Kubo 1997; Landhäusser and Lieffers 2002). The general stagnation in growth observed worldwide in many overaged coppices after abandonment could be related to the high demand for carbohydrates by the root system, which draws resources from aboveground tree parts that would otherwise be used for stem growth and seed yield. This might be the case of *Quercus pyrenaica* Willd., a marcescent, root-resprouting species distributed in siliceous sub-Mediterranean mountain ranges over southwestern

Table 1 Acronym, unit, and description of respiration rates (R) and CO_2 and water fluxes (F)

Respiration rates and CO_2 and water fluxes	Units	Acronym
Root respiration	mmol day^{-1} $stem^{-1}$	R_R
Root-respired CO_2 through xylem	mmol day^{-1} $stem^{-1}$	F_T
Sap flux	L day^{-1} $stem^{-1}$	F_{H2O}
Dissolved CO_2 at stem base	mmol L^{-1}	$[CO_2^*]_{base}$
Soil CO_2 efflux	μmol s^{-1} m^{-2}	F_S
Soil CO_2 efflux at 16 °C	μmol s^{-1} m^{-2}	F_{S16}
Heterotrophic respiration in soil at 16 °C	μmol s^{-1} m^{-2}	R_{H16}
Litter respiration at 16 °C	μmol s^{-1} m^{-2}	R_{L16}
Root-respired CO_2 through soil at 16 °C	mmol day^{-1} $stem^{-1}$	$F_{S16\text{-}root}$

France, the Iberian Peninsula, and northern Morocco. Woodlands of *Q. pyrenaica* have been historically subject to short turns (7–15 years) of coppicing to obtain charcoal, firewood, and woody pastures. Currently, most of these coppices are abandoned due to the general rural exodus and the transition away from wood as a primary energy source that has occurred since the middle of the twentieth century. Repeated coppicing of these woodlands likely produced large clonal assemblies within stands (Valbuena-Carabaña and Gil 2013). The resulting large root-to-shoot ratio due to heavy coppicing may cause an unbalanced respiratory demand for carbohydrates in the root system which could contribute to the current degraded state of many *Q. pyrenaica* stands as evidenced by low productivity, high mortality rates, stem top drying, and scarce acorn yield (Cañellas et al. 2004; Bravo et al. 2008; Salomón et al. 2013).

Measuring R_R presents a number of difficulties. It has been demonstrated that CO_2 originating from respiration of woody tissues can diffuse radially to the atmosphere and/or dissolve in sap and move upwards in the transpiration stream (e.g., Aubrey and Teskey 2009; Cerasoli et al. 2009; Bloemen et al. 2014). Thus, the internal flux of CO_2 through xylem (F_T) may be responsible for large inconsistencies found in rates of woody tissue respiration, classically estimated by measurements of radial CO_2 flux (Teskey and McGuire 2002). It was observed that a substantial portion of locally respired CO_2 in woody tissues was transported upward instead of diffusing to the atmosphere, e.g., 35 % for tropical trees (Angert et al. 2012); 45–55, 14, and 15 % for sycamore, sweetgum, and beech trees, respectively (McGuire and Teskey 2004; Teskey and McGuire 2007); and 11 % for rimu trees (Bowman et al. 2005). Moreover, Teskey and McGuire (2007) suggested that a large portion of CO_2 dissolved in tree xylem could originate in the root system and that large among-tree variability in $[CO_2]$ at the base of the stem might be explained by root size. Using different techniques such as carbon isotopes, xylem $[CO_2]$ monitoring, and stem girdling, it has been demonstrated that the autotrophic (root) component of belowground respiration is underestimated by F_S measurements by up to 50 %, because root-respired CO_2 dissolves in xylem sap and moves upward to the stem and leaves (Aubrey and Teskey 2009; Grossiord et al. 2012; Bloemen et al. 2014). Aubrey and Teskey (2009) estimated that, in a *Populus deltoides* plantation, the amount of root-respired CO_2 transported aboveground via the xylem stream was twice that diffusing to the soil atmosphere. The authors calculated that only a small portion (7.8 %) of F_T at the base of the stem resulted from root uptake of CO_2 dissolved in soil water. Soil $[CO_2]$ is much lower than xylem $[CO_2]$ at the base of the stem (Teskey and McGuire 2007; Aubrey and Teskey 2009), suggesting that most movement of CO_2 is in the direction from root to soil along the concentration gradient. Therefore, under the same soil structure and environmental conditions, differences in the relative contribution of soil water CO_2 to F_T among trees are likely negligible. Thus, measurements of F_T at the base of the stem could be used as a proportional indicator of R_R activity (Teskey and McGuire 2007; Aubrey and Teskey 2009) for comparison among different stems of the same species under the same conditions. This recently developed approach avoids the pitfalls of classic measurements of radial CO_2 fluxes from roots, which are methodologically complicated by root inaccessibility and perturbations, or measurements of F_S, which are controversial due to the difficulty of discriminating between heterotrophic and autotrophic respiration and their high spatial variability (Hanson et al. 2000).

Recently, the effect of clonal clump characteristics on stem growth of *Q. pyrenaica* was evaluated: stems belonging to large-biomass clones (characterized by large spatial extent and high number of within-clone stems and stumps) exhibited slower growth than those belonging to small-biomass clones (Salomón et al. 2013). Reduced stem growth in large clones might be conditioned by high R_R, which consumes resources otherwise available for stem growth, flower production, and fruiting. To address this hypothesis, genetic and physiological approaches were integrated in this experiment: (i) genetic analyses were used to assess clonal membership since this species does not form clearly visible clonal assemblies (Valbuena-Carabaña and Gil 2013), and (ii) continuous measurements of F_T were used as a proportional index to compare R_R in dominant similar-sized *Q. pyrenaica* stems belonging to clones of differing size. Additionally, F_S was measured to estimate F_T contribution to R_R across different clonal sizes. We predicted that large clones would have higher F_T per stem and F_S than small clones due to respiration of a root system disproportionate to stem size.

2 Materials and methods

2.1 Experimental site

The study was performed in an experimental plot located in the Matas de Valsaín public woodland, in the Guadarrama mountain range located in central Spain. This monospecific *Q. pyrenaica* forest was historically managed as a coppice since at least the twelfth century (Manuel Valdés and Rojo y Alboreca 1993) and abandoned after 1970. The 1-ha study plot represents a one-storied stand of *Q. pyrenaica* (781 stems ha^{-1}) with 45-year-old stems. Site annual rainfall is 885 mm, average temperature is 10.5 °C, and soil type is humic cambisol. Climatic conditions were monitored by a weather station located 2.1 km away from the plot. Soil water content was recorded using a dielectric aquameter sensor (ECH_2O; Decagon Devices, Inc.) inserted at 15 cm in depth.

2.2 Clonal assignment

Prior to the start of physiological measurements, the clonal membership of all stems in the plot was elucidated by performing genetic analyses in a hierarchical manner by gradually increasing sampling density. At the end, 541 stems were analyzed to delimit clonal clumps. The total genomic DNA was isolated from dry leaves collected in 2009 using an Invitek kit (Invisorb Spin Plant Mini Kit). Genetic analyses were made using seven nuclear microsatellite markers (*Qp*ZAG9, *Qp*ZAG36, *Qp*ZAG110, MSQ4, MSQ13, *Qr*ZAG11, and *Qr*ZAG39, developed by Dow et al. 1995, Steinkellner et al. 1997, and Kampfer et al. 2004) scored by PCR in a thermal cycler (GeneAmp® PCR System 9700; Applied Biosystems, Foster City, CA, USA) following conditions described in Valbuena-Carabaña et al. (2007). Electrophoresis and scoring of fragments were performed on a Li-Cor 4300 automated DNA sequencer (Li-Cor Biosciences, Lincoln, NE, USA) using Saga GT software (Li-Cor Biosciences, Lincoln, NE, USA) with commercial (SequaMark™; Invitrogen, Carlsbad, CA, USA) and specific internal standards. Multilocus genotypes were used to assign clonal membership of stems using GeneClone software (Arnaud-Haond and Belkhir 2007), based on the probability of occurrence of a given genotype at the observed frequency in the sample (which depends on the allele frequencies) and on the likelihood that all identical replicates were actually clones (which also depends on the sample sizes). The probabilities of the clonal stems to have a sexual origin were extremely low (P_{sex} ranging from $2.31 \cdot 10^{-19}$ to $1.08 \cdot 10^{-205}$); thus, clonal assignment was performed with high statistical confidence. Clonal groups were defined as polygons whose stems had identical genotypes, including non-genotyped stems located among them.

Four clones of contrasting surface extension and stem number were selected and classified as large or small clones (two of each size class). Four even-sized and even-aged stems within each clone were selected for measurement, for a total of 16 stems. Figure 1 shows the spatial distribution of selected clones and the location of measured stems. Stem and clonal features are shown in Tables 2 and 3, respectively. Clonal and stem growth was obtained from Salomón et al. (2013) wherein two wood cores per stem were used to measure the average normal section increment during the last 10-year growing period.

2.3 Flux of root-derived CO_2 through xylem (F_T)

To calculate F_T, measurements of sap flux and dissolved CO_2 in xylem sap were needed. Sap flux density (L cm^{-2} sapwood s^{-1}) was measured using constant heat thermal dissipation probes operated similar to those originally designed by Granier (1985). Pairs of thermocouples were inserted at 20 mm deep into each stem at breast height and separated at 100 mm vertically. Sap flux density was calculated from the temperature difference between the upper and lower thermocouple. Zero flow was calculated daily from the maximum temperature difference between 0400 and 0600 hours. Two pairs of probes were placed on each stem on opposite sides. Data from the two pairs were averaged to account for circumferential non-uniformity of sap flow. Calibration parameters developed by Sun et al. (2011) were applied to improve the accuracy of the measurements according to the ring porous xylem anatomy of the *Q. pyrenaica* stems. Sap flux (F_{H2O}, L day^{-1}) for each stem was calculated by multiplying 15-min average sap flux density integrated over 24 h by sapwood area. Sapwood area was determined from the color change between heartwood and sapwood of stem cores.

Xylem [CO_2] was measured by inserting a solid-state non-dispersive infrared (NDIR) CO_2 sensor (model GMM221; Vaisala, Helsinki, Finland) into the stem 10 cm aboveground level. Holes of 40 mm in length and 25 mm in diameter were drilled to place the sensor, which measures gaseous [CO_2] (%) in equilibrium with CO_2 dissolved in xylem sap ([CO_2^*]). NDIR sensors were isolated from the external atmosphere with rubber sealant. Gas concentration measured at the base of the stem was converted to dissolved CO_2 ([CO_2^*]$_{base}$, mmol L^{-1}) by applying Henry's law (McGuire and Teskey 2002). For this calculation, xylem temperature and sap pH were measured. A type T thermocouple was inserted in the xylem 50 mm away from the CO_2 sensor to measure temperature. pH of sap expressed from twigs was determined using a portable pH meter (model 25+; Crison, Barcelona, Spain) and a pH electrode (model 52 07; Crison, Barcelona, Spain). The scarce published data regarding sap pH indicates that pH remains relatively constant within a diel period but can fluctuate during the growing season, which would affect calculations of internal [CO_2^*] (Aubrey et al. 2011; Erda et al. 2014). Therefore, we measured pH every 20–25 days at peak sap flow, when F_T reaches maximum values, and interpolated daily pH values between measurements. For detailed information on F_T measurement methodology, see Aubrey and Teskey (2009). Xylem temperature, xylem [CO_2], and sap flux density were measured every minute, averaged every 15 min, and recorded with a data logger (model CR23X; Campbell Scientific, Barcelona, Spain). Data recorded at 15-min intervals were scaled to daily total F_{H2O} and daily average [CO_2^*]$_{base}$. F_T (mmol day^{-1}) was integrated over 24 h as the product of 15-min averages of F_{H2O} and [CO_2^*]$_{base}$. Data recorded for individual stems were averaged per clone. Measurements were conducted in 2012 and lasted from day of year (DOY) 101 until DOY 200.

2.4 Soil CO_2 efflux measurements (F_S)

Ten large clones (≥9 stems and 30 m^2) and ten small clones (≤5 stems and 15 m^2) (Fig. 1) were selected for measurements of F_S on DOY 198 of 2014. In each clone, three soil collars

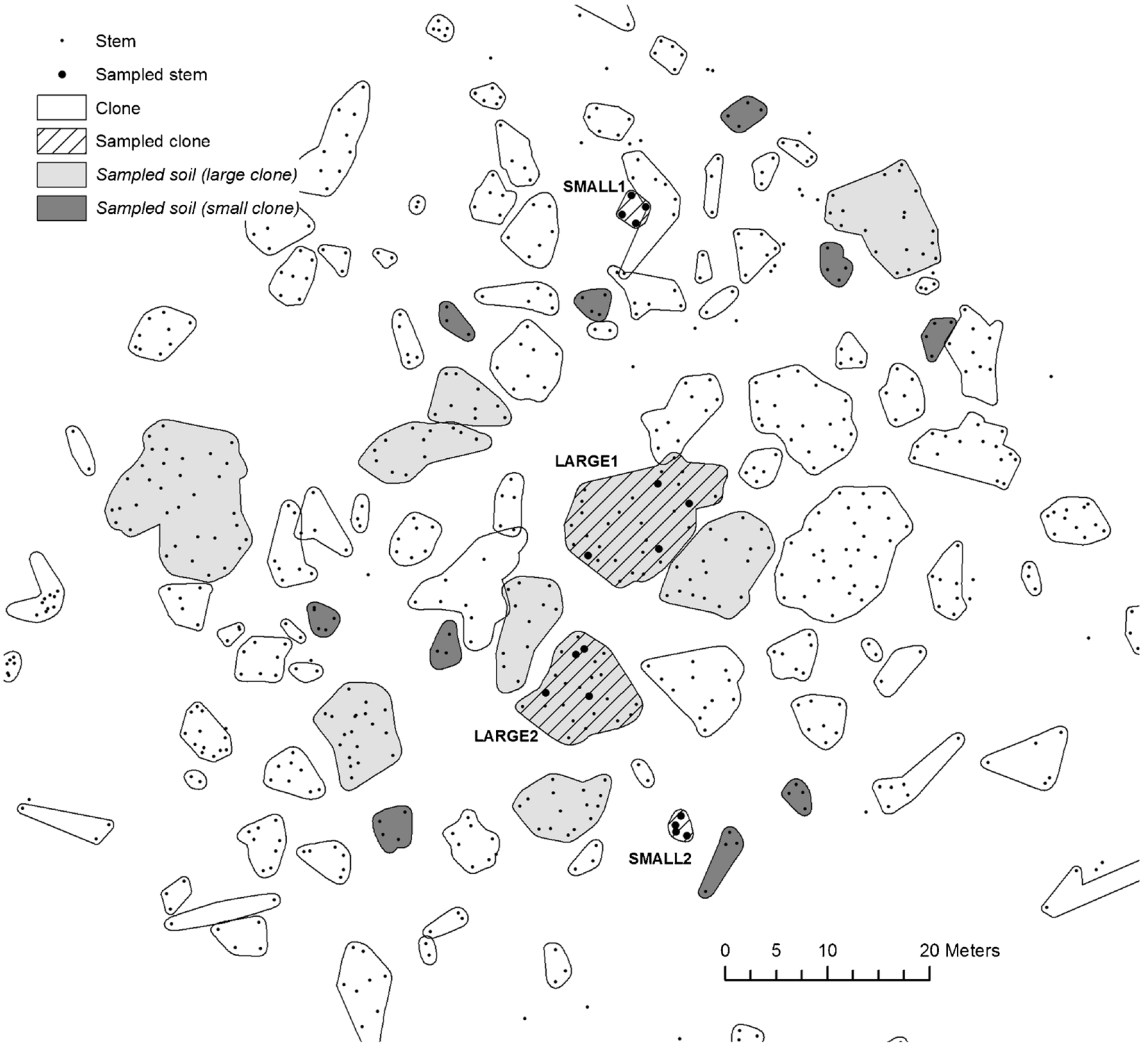

Fig. 1 Map of clones and stems in an experimental plot in a coppiced *Quercus pyrenaica* stand at Matas de Valsaín, Spain. Sampled stems were measured continuously from DOY 101 to 200 of 2012 for sap flux, temperature, and xylem [CO_2]; sampled soil was measured on DOY 198 of 2014 for soil CO_2 efflux

made of PVC, 10 cm in diameter and 4.5 cm in length, were inserted 1.5 cm into the soil at random locations 1 week before measurements. Additionally, F_S was measured in six collars located in an excavated (in autumn 2013) and refilled (in spring 2014) soil area where roots and herbaceous ground cover had been removed to eliminate the autotrophic (i.e., root) respiratory component of F_S. These measurements of root-free soil CO_2 efflux were assumed to be equal to belowground heterotrophic respiration. F_S was measured with a portable infrared gas analyzer (LI-6400; Li-Cor, Inc., Lincoln, NE, USA) and a soil chamber (LI-6400-09). Measurements of gas exchange between soil and chamber were made in closed configuration by attaching the chamber to each collar, reducing CO_2 concentration inside the chamber, and then letting it increase to an upper concentration limit. These limits varied depending on efflux rate and ambient CO_2 concentration. Measurements were made at ambient [CO_2] and humidity across three consecutive cycles per collar. Each collar was sampled twice, at early morning and afternoon, to calculate the temperature coefficient (Q_{10}) of efflux, as in Zaragoza-Castells et al. (2007). From mean Q_{10}, F_S was estimated at 16 °C, the mean soil temperature during F_T monitoring (F_{S16}, μmol m^{-2} s^{-1}). We calculated this estimate based on the relationship between soil temperature and air temperature (R^2=0.7345, p<0.001) and the average air temperature from DOY 146 to 200 of 2012, when substantial transpiration occurred (Fig. 2), to provide a means to compare the single-day measurements of F_S with season-long measurements of F_T. F_{S16} was partitioned into autotrophic and heterotrophic components as in Rey et al. (2002). The contribution of soil organic matter decomposition by heterotrophs (belowground heterotrophic respiration, R_{H16}) to F_{S16} was obtained from the ratio root-free F_{S16}/root F_{S16}. The contribution of aboveground litter decomposition (R_{L16}) was assumed to be 50 % of the remaining F_{S16}–R_{H16}, based on measurements in a Mediterranean coppice of *Quercus cerris* (Rey et al. 2002).

Table 2 Characteristics of instrumented stems in two large and two small clones in a coppiced *Quercus pyrenaica* stand at the beginning of the 2012 growing season

Clone	Stem	Dbh (cm)	Ring number at bh
LARGE1	1	19.35	47
	2	21.40	46
	3	20.05	46
	4	22.75	42
LARGE2	5	21.70	47
	6	21.35	46
	7	18.95	48
	8	20.25	46
SMALL1	9	22.30	45
	10	20.75	43
	11	19.90	43
	12	22.75	46
SMALL2	13	21.90	44
	14	19.20	47
	15	20.45	43
	16	21.55	46

Dbh diameter at breast height

The portion of root-respired CO_2 that diffused through soil to the atmosphere ($F_{S16\text{-root}}$) was calculated as

$$F_{S_{16}\text{-root}} = F_{S_{16}} - R_{H_{16}} - R_{L_{16}}$$

Additionally, to express F_T and $F_{S16\text{-root}}$ in the same units (mmol CO_2 day^{-1} $stem^{-1}$) for comparison, $F_{S16\text{-root}}$ (on surface area basis) was multiplied by the clonal extension (m^2) and divided by the number of stems within the clone (Table 3).

2.5 Data analysis

Since F_{H2O} (and F_T) was negligible before budburst, only data from DOY 146 to 200 (Fig. 2) was used to calculate daily average of $[CO_2^*]_{base}$ and daily accumulated F_{H2O} and F_T per stem. Within-clone variation in $[CO_2^*]_{base}$, F_{H2O}, and F_T was examined by performing multiple comparisons of daily values (Tukey's test). Differences between the two clone sizes in F_{H2O}, $[CO_2^*]_{base}$, and F_T were compared with hierarchical mixed models performed in R using the *lme* function in the *nlme* library (Pinheiro et al. 2014). Clone size (n=2) was treated as a fixed factor, whereas individual clone (n=4) was treated as a random factor. Logarithmic transformation was made to satisfy Shapiro-Wilk normality tests. To assess variability in F_{S16} (on area basis) and $F_{S16\text{-root}}$ (per stem) with clonal size (as a qualitative variable, n=2) and clonal extension (as a quantitative variable, m^2), mixed models were performed and clone was treated as a random factor (n=20). Additionally, to assess relationships between clonal extension and clonal average F_{H2O}, $[CO_2^*]_{base}$, F_T, F_{S16}, and $F_{S16\text{-root}}$, we performed linear regressions to provide R^2 as an estimate of the variance explained by the model and compare magnitudes between root-respired CO_2 transported in the xylem (F_T) and diffused through soil to the atmosphere ($F_{S16\text{-root}}$). All values presented in the text are mean (SE).

3 Results

The average temperature during the experiment was 14.7 (0.7)°C. Temperature reached its minimum of −1.9 °C on DOY 108 and its maximum of 35.2 °C on DOY 178. Total rainfall from DOY 101 to 200 was 157 mm, of which 90 % occurred before DOY 143 (Fig. 2(a)). Soil water content ranged from 17.5 % on DOY 175 (sensor installation) to 9.2 % on DOY 200. Average sap pH to calculate $[CO_2^*]_{base}$ was 6.43 (0.03). Budburst took place ca. between DOY 137 and 145, when a substantial increase in F_{H2O} was registered (Fig. 2(b)). STEM9 had visual symptoms of wilting, remarkably low levels of F_{H2O} and high $[CO_2^*]_{base}$ (Fig. 3). F_{H2O} in STEM9 was 4.1 times lower than the lower quartile of the data distribution, and so, according to the standard boxplot rule, STEM9 was considered an outlier and excluded from statistical analyses to avoid bias in clone SMALL1.

In general terms, before budburst, daily mean $[CO_2^*]_{base}$ was high and positively correlated to daily mean temperature (R^2=0.688, p<0.001) and showed no relationship with daily

Table 3 Features of two large and two small clones in a coppiced *Quercus pyrenaica* stand at the beginning of the 2012 growing season

Clone	Surface extension (m^2)	Number of stems	Mean stem diameter (cm)	Min-max stem diameter (cm)	Mean stem growth[a] (cm^2 $year^{-1}$)	P_{sex}[b]
LARGE1	130	30	16.9 (0.6)	9.1–22.8	2.92 (0.26)	$1.08 \cdot 10^{-205}$
LARGE2	82	24	17.1 (0.6)	12.8–21.7	3.14 (0.38)	$1.02 \cdot 10^{-158}$
SMALL1	10	4	21.4 (0.7)	19.9–22.8	4.27 (0.50)	$2.31 \cdot 10^{-19}$
SMALL2	6	4	20.8 (0.6)	19.2–21.9	6.25 (1.24)	$6.17 \cdot 10^{-21}$

[a] Mean stem normal section increment in the previous 10 years

[b] The probability of the stems bearing the same genetic variables to have been originated by sexual events

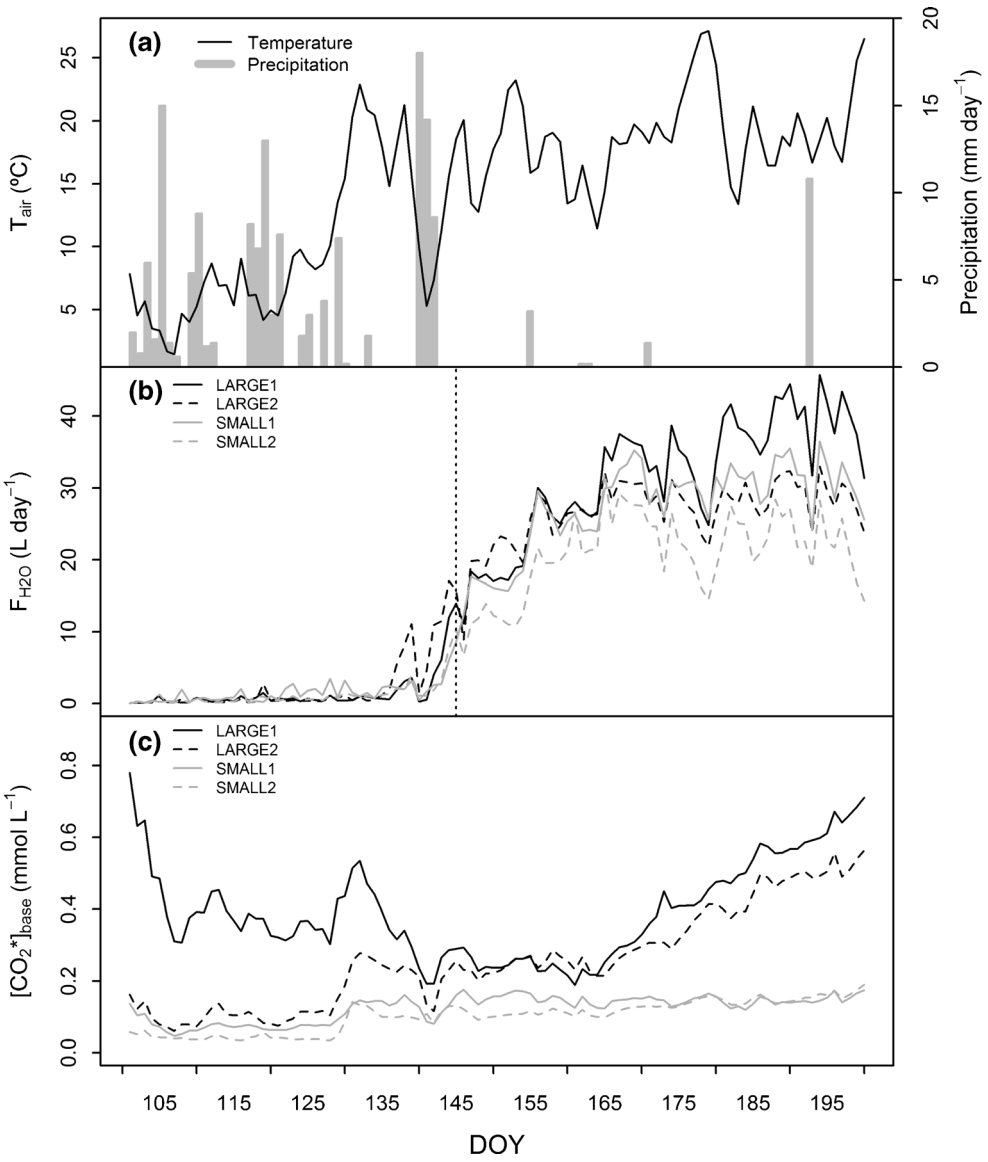

Fig. 2 Seasonal patterns of daily mean air temperature and daily total precipitation (*a*), daily total sap flux (F_{H2O}) (*b*), and mean dissolved [CO_2^*] in sap solution at stem base ([CO_2^*]$_{base}$) (*c*) in a coppiced *Quercus pyrenaica* stand measured during the growing season in 2012 in two large and two small clones. Daily total sap flux and [CO_2^*]$_{base}$ are the mean of four sampled stems per clone (three stems in clone SMALL1). *Vertical dashed line* on DOY 145 shows the first day on which substantial F_{H2O} was observed. Beginning on this day, daily F_{H2O} and [CO_2^*]$_{base}$ were considered for comparison among clonal sizes

F_{H2O} (p=0.420). After budburst, [CO_2^*]$_{base}$ relationships with both temperature (R^2=0.397, p=0.004) and F_{H2O} (R^2=0.428, p=0.002) were positive. Overall daily mean (from DOY 146 to 200) per-stem [CO_2^*]$_{base}$, F_{H2O}, and F_T were 0.27 (0.07)mmol CO_2 L^{-1}, 26.66 (2.02)L day^{-1}, and 8.21 (2.89)mmol CO_2 day^{-1}, respectively. F_{H2O} was not affected by clonal size (p=0.308) (Table 4, Fig. 2(b)). However, stems of large clones had higher [CO_2^*]$_{base}$ than stems of small clones at α=0.10 significance level (p=0.084) (Table 4, Fig. 2(c)). Stems belonging to large clones transported larger amounts of CO_2 dissolved in the sap than stems of small clones; there were significant differences in F_T among clonal sizes at α=0.10 (p=0.095) (Table 4). F_{H2O} made only a small contribution to the differences in F_T among clonal sizes, which were affected to a greater extent by [CO_2^*]$_{base}$ (see Tukey's test similarities between [CO_2^*]$_{base}$ and F_T, Fig. 3). Differences in [CO_2^*]$_{base}$ and F_T were found not only among clones, but also among stems within the same clone (Fig. 3). Linear regressions between clonal extension and clonal average F_{H2O}, [CO_2^*]$_{base}$, and F_T lead to the same conclusions: [CO_2^*]$_{base}$ and F_T were directly related to clonal extension (R^2=0.849, p=0.079, and R^2=0.838, p=0.085, respectively), whereas F_{H2O} was not (p=0.219) (Fig. 4).

Mean F_{S16} and its Q_{10} were 6.91 (0.31)μmol m^{-2} s^{-1} and 1.44, respectively. Given that the heterotrophic component of belowground respiration (R_{H16}) measured in the root-free soil was 4.42 (0.75)μmol m^{-2} s^{-1}, average $F_{S16\text{-root}}$ estimated per stem was 333.47 (53.69)mmol day^{-1}. F_{S16} and $F_{S16\text{-root}}$ were positively related to clonal extension (p=0.027 and p=0.023, respectively, n=20) (Table 4, Fig. 4). However, when clonal size as a qualitative variable was considered, F_{S16} and $F_{S16\text{-root}}$ were not significantly different among large and small clones (p=0.312 and p=0.247, respectively). Since F_S was not measured in SMALL1 and SMALL2 (which were root trenched or harvested prior to F_S measurements), the comparison between

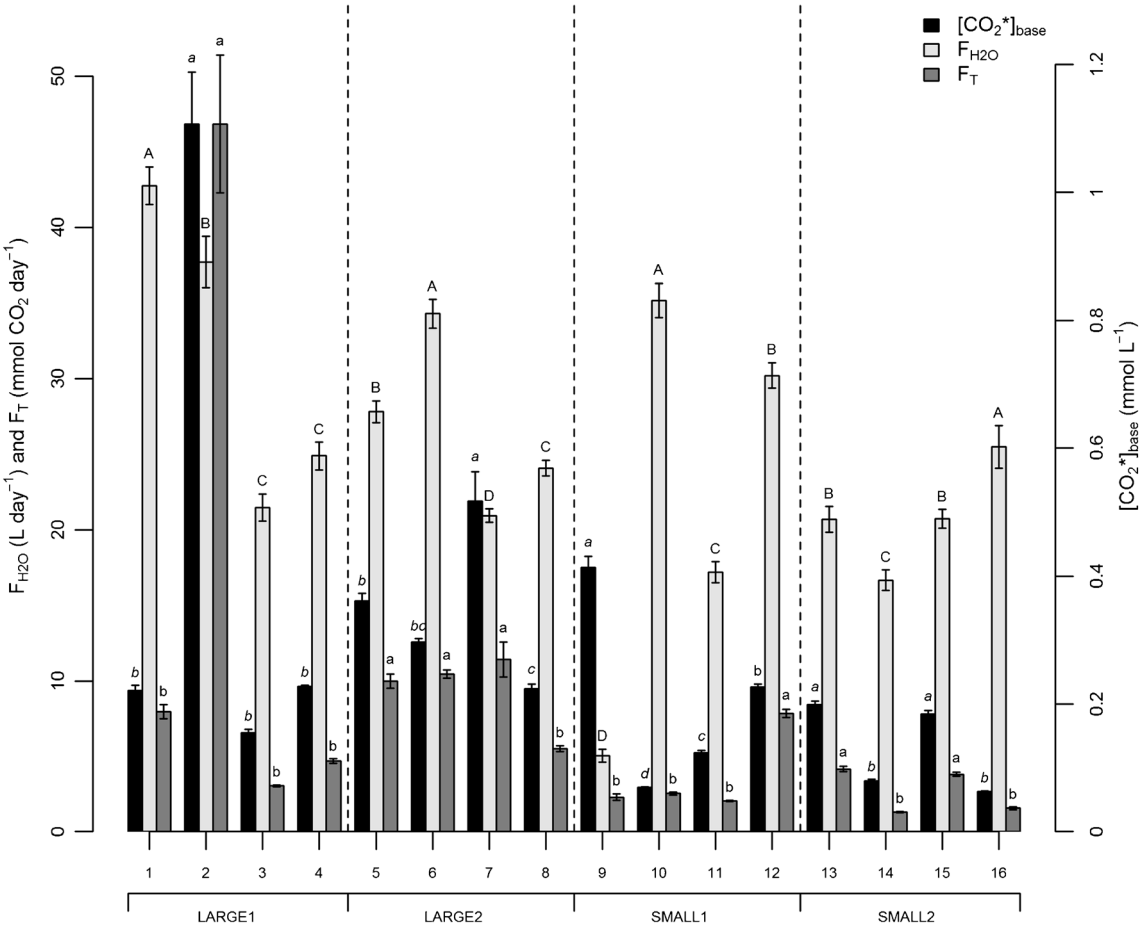

Fig. 3 Mean daily sap flux (F_{H2O}), CO_2 flux through xylem (F_T), and dissolved CO_2 concentration at stem base ($[CO_2^*]_{base}$) of 16 stems belonging to two large and two small clones in a coppiced *Quercus pyrenaica* stand measured during the growing season of 2012. Since F_{H2O} and F_T were insubstantial before and during budburst (DOY 101–145), data from DOY 146 to 200 were used to calculate the means. *Different letters* indicate significant differences within clone for $[CO_2^*]_{base}$ (*italic*), F_{H2O} (*capital*), and F_T (*lowercase*) (Tukey's test at $p<0.05$)

$F_{S16\text{-}root}$ and F_T was assessed from the relationships of $F_{S16\text{-}root}$ and F_T with clonal extension (Fig. 4(c, e)). The average ratio (%) between F_T and $F_{S16\text{-}root}$ per stem over the sampled range of clonal extension (from 6 to 155 m^2) was 1.8 %. Similarly, the $F_T/F_{S16\text{-}root}$ ratio of the mean measured values was 2.5 %; i.e., root-respired CO_2 flux through xylem was ca. 2 % of the root-respired CO_2 that diffused to the atmosphere through soil (Table 5).

Mean stem diameter increment of large clones was lower compared to that of small clones (2.92 and 3.14 cm^2 $year^{-1}$ for large clones and 4.27 and 6.25 cm^2 $year^{-1}$ for small clones; Table 3). Relationships between individual stem growth and stem F_{H2O}, $[CO_2^*]_{base}$, and F_T were not significant (data not shown).

4 Discussion

Two methodologies were combined to understand root carbon flux in a clonal-sprouting species and its possible importance to stand degradation. First, genetic analyses provided information on the origin of the stems, and second, F_T and F_S were measured to compare root respiration among clonal sizes. This integrative approach provided experimental support for our hypotheses: different clonal sizes exhibited different F_T and $F_{S16\text{-}root}$, indicating that larger clones have higher respiration per stem than smaller clones. Moreover, in contrast to other studies, the proportion of root-respired CO_2 transported through xylem (F_T) was low compared to root-respired CO_2 diffused through soil in our experimental system (2 %), suggesting that there will be large differences in the F_T contribution to root respiration depending on species and/or site.

4.1 F_T as a proportional index of root respiration

Measurements of F_S are often used to elucidate R_R. However, unambiguous discrimination of autotrophic ($F_{S\text{-}root}$) and heterotrophic (R_H) components of F_S, together with F_T measurements, is necessary to accurately estimate R_R ($R_R=F_{S\text{-}root}+F_T$) (Hanson et al. 2000; Aubrey and Teskey 2009). Considering the same species under the same plot conditions, we assumed

Table 4 ANOVA tables for mixed models to compare daily average of sap flux (F_{H2O}, L day^{-1}), dissolved [CO_2^*] at the base of the stem ([CO_2^*]$_{base}$, mmol L^{-1}), internal flux of CO_2 dissolved in xylem sap (F_T, mmol $CO_2 day^{-1}$), of *Q. pyrenaica* stems among large and small clones (class variable); and modeled soil CO_2 efflux (F_{S16}, μmol m^{-2} s^{-1}) and root respiration that diffused through soil to the atmosphere ($F_{S16\text{-root}}$, mmol $stem^{-1}$ day^{-1}) among clonal extension (quantitative variable, m^2)

	numDF	*DenDF*	*F* value	*p* value
F_{H2O}				
Intercept	1	11	174.1781	*<0.0001***
Clonal size	1	2	1.8351	0.3083
[CO_2^*]$_{base}$				
Intercept	1	11	112.5734	*<0.0001***
Clonal size	1	2	10.4784	*0.0836*•
F_T				
Intercept	1	11	74.5154	*<0.0001***
Clonal size	1	2	9.1012	*0.0945*•
F_{S16}				
Intercept	1	35	2363.8111	*<0.0001***
Clonal extension	1	18	5.7967	*0.0270**
$F_{S16\text{-root}}$				
Intercept	1	35	1438.6130	*<0.0001***
Clonal extension	1	18	6.1742	*0.0230**

[CO_2^*]$_{base}$, F_T, F_{S16}, and $F_{S16\text{-root}}$ were log transformed to satisfy Shapiro-Wilk normality tests. Italic type indicates statistical significance ($p<0.10$)

$p<0.0001$ **; $p<0.05$ *; $p<0.1$•, significance codes

that values of F_T were proportional to total R_R. In a poplar plantation, Aubrey and Teskey (2009) estimated that F_T was approximately 66 % of total R_R. In a study on *Quercus robur*, at high transpiration rates, the relative contribution of the autotrophic component of belowground respiration increased from 25 to 45.4 % when F_T was considered (Bloemen et al. 2014). From those data, it can be inferred that 45 % [i.e., (45.4–25)/45.4] of R_R was transported in the xylem stream. However, over a 5-day period, which included periods of lower transpiration, the calculated F_T portion of R_R decreased to 18 % [(32.8–27)/32.8]. Similarly, carbon isotope techniques applied in a *Eucalyptus* plantation showed that there was a reduction in the $F_{S\text{-root}}$ component of F_S during the day, which was attributed to diversion of respired CO_2 to F_T, estimated to be 17 % of R_R on a daily basis and 24 % at high transpiration rates (Grossiord et al. 2012). Compared to these observations in temperate and tropical trees, the magnitude of F_T relative to $F_{S\text{-root}}$ in *Q. pyrenaica* was much lower (2 %).

The estimated R_{H16} contribution to F_{S16} was 64 % (i.e., 4.42 to 6.91 μmol m^{-2} s^{-1}), slightly higher than the 55 and 59 % reported for a *Q. cerris* coppice and a *Quercus douglasii* parkland in the dry season (Rey et al. 2002; Tang and Baldocchi 2005). Assuming a similar contribution of R_L and $F_{S\text{-root}}$ to F_S as in the *Q. cerris* Mediterranean coppice, the proportion of root-respired CO_2 transported through xylem (F_T) relative to the root-respired CO_2 that diffused through soil to the atmosphere ($F_{S\text{-root}}$) was 2 %. We suspect that this upscaling underestimates the magnitude of F_T compared to $F_{S16\text{-root}}$ because of the drier conditions in 2012 (when F_T was measured) than in 2014 (when F_S was measured), since F_S increases with soil water availability in drought-prone regions (Reichstein et al. 2002; Rey et al. 2002; Tang and Baldocchi 2005; Guidolotti et al. 2013). In any case, F_T accounted for a low proportion of R_R in *Q. pyrenaica*. This observation highlights large variability of F_T among species. In *Q. pyrenaica*, measured values of [CO_2^*] in sap solution, ranging from 0.06 to 0.93 mmol L^{-1} (Fig. 3), were lower than those previously reported for several species, applying this methodology (Table 1 in Teskey et al. 2008). Nevertheless, this is the first time that [CO_2^*] was measured in the Mediterranean region during summer drought (note that the study of Cerasoli et al. (2009) was performed during autumn). Summer drought in Mediterranean climates restrains respiratory processes at the ecosystem (Reichstein et al. 2002; Guidolotti et al. 2013; Rambal et al. 2014), soil (Rey et al. 2002; Tang and Baldocchi 2005), stem, and foliage levels (Maseyk et al. 2008; Rodríguez-Calcerrada et al. 2014) and may be the cause of the comparatively low [CO_2^*] and F_T measured in this study. Moreover, lower root water content caused by limited soil water availability would enhance $F_{S\text{-root}}$ to the detriment of F_T due to reduced resistance to radial CO_2 diffusion (Steppe et al. 2007). Accordingly, [CO_2^*] as low as 0.3 mmol L^{-1} was observed for *Q. robur* in the only [CO_2^*]-drought experiment existent to date after 9 days without watering, with a sharp increase in [CO_2^*] once watering resumed (Saveyn et al. 2007), as was seen in this species after first autumn rain.

4.2 Relationship between F_T, F_S, and clonal structure

Differences in F_T and [CO_2^*]$_{base}$ between clonal sizes were significant at $\alpha=0.10$. Considering the low sample size for F_T (two clones per clonal size), the $\alpha=0.10$ significance level was considered reasonable to detect actual differences. In this regard, it is worth noting that monitoring 16 stems simultaneously over 100 days represents the largest investment in the study of F_T to date. Soil CO_2 effluxes (F_{S16} and $F_{S16\text{-root}}$) showed similar relationships with clonal structure. Clonal extension and F_{S16} and $F_{S16\text{-root}}$ were positively related (Table 4, Fig. 4), despite no differences among large and small clones when clonal size was considered as a qualitative variable ($n=2$), likely due to the large range of extension of the ten large clones. Therefore, larger clones showed higher [CO_2^*]$_{base}$, F_T, F_{S16}, and $F_{S16\text{-root}}$ than smaller clones (Table 4, Fig. 4), which suggests higher consumption of carbohydrates for root metabolism with increasing clonal size. Although F_T and stem growth were not related in this study, likely due to the reduced

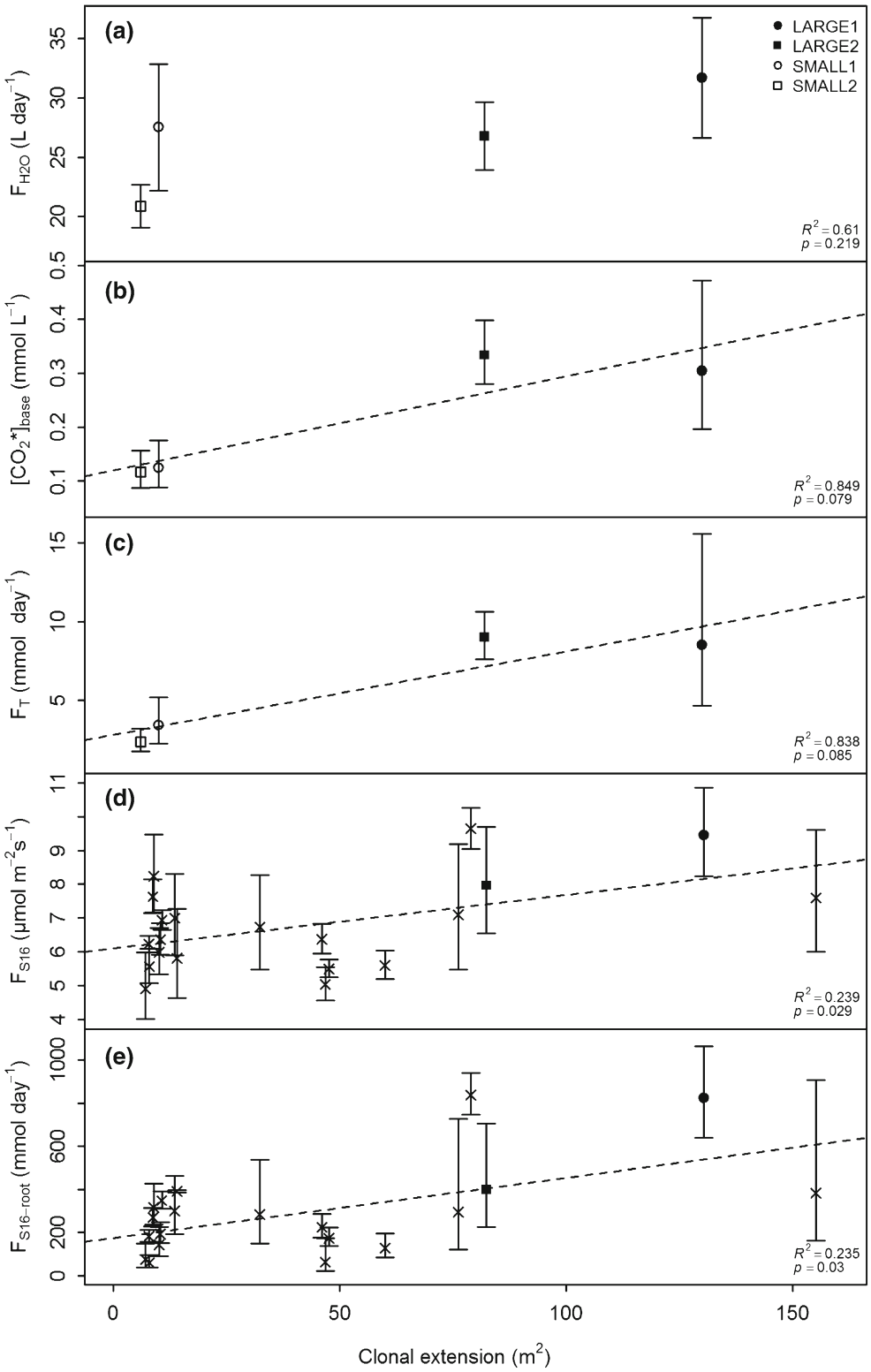

Fig. 4 Linear regressions between clonal extension and clonal average sap flux (F_{H2O}, L day^{-1}) (*a*), [CO_2] dissolved at stem base ([CO_2^*]$_{base}$, mmol CO_2 L^{-1}) (*b*), and CO_2 flux through the xylem (F_T, mmol CO_2 day^{-1}) (*c*) in two large and two small *Quercus pyrenaica* clones measured during the growing season of 2012. Soil CO_2 efflux on surface basis (F_{S16}, µmol m^{-2} s^{-1}) (*d*) and root-respired CO_2 diffused through soil to the atmosphere per stem ($F_{S16\text{-root}}$, mmol CO_2 day^{-1}) (*e*) in 20 *Q. pyrenaica* clones measured during the growing season of 2014. [CO_2^*]$_{base}$, F_T, F_{S16}, and $F_{S16\text{-root}}$ were log transformed to satisfy Shapiro-Wilk normality tests. Statistics (R^2 and *p* values) apply to transformed regressions. Back-transformed values were used to display (*geometric*) means, SE intervals, and regression lines. Only significant ($p<0.1$) regression *lines* are depicted

sample size, higher root respiratory costs per stem in larger clones could partially explain lower stem diameter growth relative to stems of smaller clones reported in temperate and Mediterranean coppices (Tanentzap et al. 2012; Salomón et al. 2013). Similarly in this study, despite even-sized and even-aged sampled stems, mean stem diameter growth increment over the last 10-year period was lower for stems in larger clones compared with smaller clones [4.44 (0.31) and 5.26 (0.72) cm^2 year^{-1}, respectively]. This difference in growth might be explained by higher initial growth of stems in larger

Table 5 Fluxes of root-respired CO_2 through xylem (F_T, mmol CO_2 $stem^{-1}$ day^{-1}) and root-respired CO_2 diffused through soil to the atmosphere ($F_{S16\text{-}root}$, mmol CO_2 $stem^{-1}$ day^{-1}). To compare magnitudes between fluxes, the ratio $F_T/F_{S16\text{-}root}$ is shown on a percentage basis. $F_{S16\text{-}root}$ was estimated at 16 °C for comparison

	F_T	$F_{S16\text{-}root}$	$F_T/F_{S16\text{-}root}$ (%)
Average fluxes among clones[a]	8.21 (2.89)	333.47 (53.69)	2.46
Average fluxes within clone LARGE1[a]	15.63 (10.45)	881.85 (231.99)	1.77
Average fluxes within clone LARGE2[a]	9.35 (1.31)	511.92 (192.42)	1.83
Average fluxes over the sampled range of clonal extension[b]	7.08 (0.90)	399.53 (53.96)	1.77

[a] Measured values ($F_{S16\text{-}root}$ was not measured in clones SMALL1 and SMALL2)

[b] Fluxes predicted from F_T and $F_{S16\text{-}root}$ relationships with clonal extension (Fig. 4 (c, e))

clones (Zhu et al. 2012) followed by a greater rate of decline after 15–20 years (Corcuera et al. 2006) compared with stems in smaller clones. The root-to-shoot biomass (R/S) ratio is higher in clonal tree species compared to non-sprouters (Bond and Midgley 2001 and references therein). For instance, in oak species, a review of R/S measurements reported median values of 0.3 in temperate forests (Table 2 in Mokany et al. 2006), whereas R/S of resprouting *Quercus coccifera* and *Quercus aquifolioides* showed average values of 3.5 and 2.2, respectively (Cañellas and San Miguel 2000; Zhu et al. 2012). The ratio of aerial to belowground biomass can approach unity along broad ranges of stem size in *Quercus ilex* (e.g., Rambal et al. 2014). However, as clonal extension increases in *Q. ilex*, the relationship deviates from an isometric one due to the development of massive root systems (Canadell and Roda 1991). Assuming that clonal extension increases with age (Steinger et al. 1996; Wesche et al. 2005), the larger F_T, larger F_S, lower stem growth, and higher R/S ratio in large clones reinforce the hypothesis that age-related growth decline is caused partly by increased carbon allocation to storage in roots in old trees (see Ryan et al. 2004; Genet et al. 2010; Sala et al. 2012). In the particular case of old abandoned coppices, a disproportionately large energetic demand to maintain living parenchyma in the root system could become a burden because the means of carbohydrate production has been repeatedly removed (Landhäusser and Lieffers 2002). The strong root sink for carbohydrates may reduce not only aboveground growth but also flowering and fruiting, and the large carbohydrate demand would increase with increasing clonal age and size (Iwasa and Kubo 1997) in clones subjected to more coppicing events. Therefore, this physiological imbalance between root demand and shoot production should be considered as a potential cause of the degraded state of abandoned coppiced stands in Mediterranean regions, such as *Q. pyrenaica* stands in the Iberian Peninsula (Corcuera et al. 2006; Bravo et al. 2008).

It is worth noting that the hypothetical carbon disequilibrium holds at both stem and clone scales. When the average F_T of the four stems sampled in large clones was extrapolated to the rest of their stems, accumulated F_T increased to 469 and 224 mmol $CO_2 day^{-1}$ in LARGE1 and LARGE2, respectively, whereas in the small clones, the F_T sum was 17 and 11 mmol $CO_2 day^{-1}$. Since the stem density within the clone (stem number/clonal extension) decreased with clonal extension ($p=0.003$), differences in $F_{S16\text{-}root}$ between large and small clones increased when $F_{S16\text{-}root}$ was expressed on a stem basis (multiplying by the clonal surface and dividing by the stem number, data not shown), suggesting higher root biomass supported per stem. However, we must be cautious with these extrapolations because high intra-clonal variability was found in F_T and $F_{S16\text{-}root}$ (Fig. 4(c, e)), suggesting an irregular distribution of living root biomass within clones, and because stem diameter was variable in the large clones. In this regard, high intra-clonal variability in F_T, mainly caused by differences in $[CO_2^*]_{base}$ (Fig. 3), may be explained by the particular interconnection of roots to stems within the clone. Stems closely connected to a large amount of live root biomass might exhibit higher $[CO_2^*]$ at the base of the stem than those with fewer connections to live root biomass. It has been seen in *P. tremuloides* that stump roots with connections to living stems could remain alive and functional for 70 years (DesRochers and Lieffers 2001). A similar phenomenon was observed at our experimental site, in which one excavated clone composed of eight stems and more than 50 old stumps was interconnected by more than 200 living root grafts and parental roots (R. Salomón, personal observation). This observation highlights the structural complexity that might develop in root systems of resprouting coppiced forests. For instance, this particular stand has been subjected to coppicing since at least the twelfth century (Manuel Valdés and Rojo y Alboreca 1993) and clonal extension exceeds 100 m^2 in some cases (Fig. 1).

The carbon imbalance suggested by our results has silvicultural implications. Management of oak coppices is one of the largest problems currently facing Mediterranean silviculture (Cañellas et al. 2004; Montes et al. 2004). The increasing interest in implementing new uses for these abandoned stands justifies the urgent need to study them. These systems of root-resprouting clonal species present unique challenges to the application of ecophysiological and silvicultural studies. The

main problems result from a lack of knowledge about what constitutes a functional unit in these ecosystems, and the technical difficulties of surveying the belowground attributes of individuals. Thinning has been advised for conversion of abandoned oak coppices into high forest, as it can enhance growth of residual trees, improve stand structure, and reduce excessive stand density (Montes et al. 2004; Bravo et al. 2008; Rodríguez-Calcerrada et al. 2011). However, thinning multi-stemmed trees could also intensify structural and physiological imbalances between shoots and roots. The reduction of stem density would result in a decrease of carbon input, whereas, for an unknown amount of time, root biomass would remain unaffected and continue to consume resources in respiratory processes. Similarly, a thinning experiment with jack pine (*Pinus banksiana*) revealed that the positive growth response of living residual trees connected by grafts to the root systems of removed trees was lower than that of non-grafted trees within the plot, suggesting that living residual trees were supporting roots and stumps of removed trees at the cost of their own growth (Tarroux et al. 2010). In the same way, since stems in coppiced stands are connected by the parental root system, their response to thinning could differ from that expected in classic silviculture in non-clonal stands, which could explain the limited success of thinning in Mediterranean *Quercus* stands to date (Valbuena-Carabaña and Gil 2013).

5 Conclusions

This work highlights the importance of clonal structure in research on root respiration and tree carbon budgets of resprouting species. The low and constant magnitude of F_T compared to $F_{S16\text{-root}}$ (2 %) emphasizes the large variability of the contribution of F_T to R_R depending on species or site. On the other hand, in a formerly coppiced stand of *Q. pyrenaica*, large clones showed higher F_T and $F_{S16\text{-root}}$ per stem than small clones (Table 4, Fig. 4), pointing to a direct relationship between clonal extension and the carbohydrate demands of roots. These results suggest an increasing physiological imbalance between the shoot and the root system with increasing clonal size. The genetic analyses we performed allowed identification of individual clones and provided more specific information about factors causing constrained stem growth and the general observed decay of abandoned coppiced oak stands in Mediterranean environments. High intra-clonal variability implies that in some clonal species, belowground attributes cannot be estimated from allometric relationships with aboveground features, such as stem diameter. Clonal structure after centuries of coppicing and the subsequent root biomass distribution within clones are commonly overlooked factors, although they may have important effects on respiratory carbon losses, physiological performance of individual stems, and stand dynamics.

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