Response of fine root respiration to variations in biotic and abiotic factors in a mixed Mediterranean forest affected by drought-induced secondary succession

Eva Pereira Blanco
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Tutores del Proyecto:
Francisco Lloret Maya
Jorge Curiel Yuste
Josep Barba

CREAF

UAB
Universitat Autònoma de Barcelona

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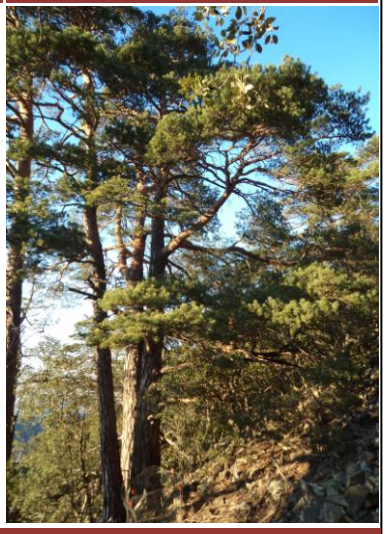

Màster de Ecologia Terrestre i Gestió de la Biodiversitat
Especialitat en Ecologia Terrestre
Dpt. Biologia Animal, Biologia Vegetal i Ecologia, Facultat de Biociències, UAB

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Author Contributions:

Francisco Lloret Maya conceived and designed the study, and revised the paper.

Jorge Curiel Yuste conceived and designed the study, and revised the paper.

Josep Barba conceived and designed the study, developed the methodology, performed research and revised the paper.

Rafael Poyatos collected and treated sap flow and environmental data, and offered methodology support.

Eva Pereira designed the study, developed the methodology, performed research, treated-analyzed data and wrote the paper.

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Abstract

Understanding the factors controlling fine root respiration (FRR) at different temporal scales will help to improve our knowledge about the spatial and temporal variability of SR and to improve future predictions of CO_2 effluxes to the atmosphere. Here we present a comparative study of how FRR respond to variability in soil temperature and moisture in two widely spread species, Scots pines (*Pinus sylvestris* L.) and Holm-oaks (HO; *Quercus ilex* L.). Those two species show contrasting water use strategies during the extreme summer-drought conditions that characterize the Mediterranean climate. The study was carried out on a mixed Mediterranean forest where Scots pines affected by drought induced die-back are slowly being replaced by the more drought resistant HO. FRR was measured in spring and early fall 2013 in excised roots freshly removed from the soil and collected under HO and under Scots pines at three different health stages: dead (D), defoliated (DP) and non-defoliated (NDP). Variations in soil temperature, soil water content and daily mean assimilation per tree were also recorded to evaluate FRR sensibility to abiotic and biotic environmental variations. Our results show that values of FRR were substantially lower under HO (1.26 ± 0.16 µg CO_2 /g_{root}·min) than under living pines (1.89 ± 0.19 µg CO_2 /g_{root}·min) which disagrees with the similar rates of soil respiration (SR) previously observed under both canopies and suggest that FRR contribution to total SR varies under different tree species. The similarity of FRR rates under HO and DP furthermore confirms other previous studies suggesting a recent Holm-oak root colonization of the gaps under dead trees. A linear mixed effect model approach indicated that seasonal variations in FRR were best explained by soil temperature ($p<0.05$) while soil moisture was not exerting any direct control over FRR, despite the low soil moisture values during the summer sampling. Plant assimilation rates were positively related to FRR explaining part of the observed variability ($p<0.01$). However the positive relations of FRR with plant assimilation occurred mainly during spring, when both soil moisture and plant assimilation rates were higher. Our results finally suggest that plants might be able to maintain relatively high rates of FRR during the sub-optimal abiotic and biotic summer conditions probably thanks to their capacity to re-mobilize carbon reserves and their capacity to passively move water from moister layers to upper layers with lower water potentials (where the FR were collected) by hydraulic lift.

Abbreviations

A CO_2 assimilation
C Carbon
D Dead pines
DBH diameter at breast high
DP defoliated pines
FRR Fine root respiration
GPP Gross primary production
HO Holm-oaks
NDP non-defoliated pines
RH Relative humidity
SR Soil respiration
ST Soil temperature
SWC Soil water content
T Transpiration water loss
WUE Water use efficiency

Introduction

Soil respiration (SR), the product of the autotrophic and heterotrophic aerobic respiration in soils, is the principal terrestrial source of CO_2 to the atmosphere only after gross CO2 flux from oceans (Schlesinger & Andrews, 2000). Recent works point to root respiration as an important process underlying SR variability (Hopkins *et al.*, 2013; Gonzalez-Meler & Taneva, 2005; Vargas & Allen, 2008) and identify photosynthesis as key factor ultimately needed to understand spatial and temporal variability of SR (Kuzyakov & Gavrichkova, 2010; Bahn *et al.*, 2009). Particularly root respiration alone can account for more than a half of soil CO_2 efflux in forest ecosystems (Hanson *et al.*, 2000; Högber *et al.*, 2001; Fahey *et al.*, 2005), eventually representing the majority of soil CO_2 respired during periods of high productivity (Subke *et al.*, 2006; Gomez-Casanovas *et al.*, 2012). Fine roots (<0.5 cm Ø) responsible of water and nutrient uptake are metabolically more active and more dynamics than coarse roots which have a structural and storage role (Pregitzer *et al.*, 1998). Therefore fine roots exhibit higher specific respiration rates and faster turnover (Vogt, 1991) representing a major loss of CO_2 from plants. Approximately 52% of gross diary assimilation is respired back to the atmosphere by roots, with fine roots alone accounting for 22-32% of the total autotrophic respiration (Janssens *et al.*, 2002, Ruehr & Buchmann, 2009). Understanding the factors controlling fine root respiration (FRR) at different temporal scales will help to improve our knowledge about the spatial and temporal variability of SR and to improve future predictions of CO_2 effluxes to the atmosphere.

Soil temperature increase FRR and has been classically considered the most determinant factor influencing it (Atkin *et al.*, 2000; Atkin *et al.*, 2005) although other studies point to soil moisture as an important abiotic driver of FRR variability (Bryla *et al.*, 2001, Burton & Pregitzer, 2003). Atkin and Tjoelker (2003) assign variations in temperature sensitivity of respiration to either limitations of substrate availability under high temperatures or limitations of enzyme catalytic activity under low temperatures. During the last years growing evidences have demonstrated that root respiration was not exclusively controlled by soil temperature and moisture but instead there is a strong and dynamic linkage between canopy assimilation rates and root respiration in trees (Horwath *et al.*, 1994; Högber *et al.*, 2001; Trueman & Gonzalez-Meler, 2005). However there are many difficulties in the study of the coupling between carbon assimilation and root respiration in trees. Firstly, many studies that focused on the temperature effect on root respiration have been developed in temperate or boreal areas where water availability is not a limiting factor and high temperature periods are often correlated with elevated radiation and peaks of gross primary production (GPP) (Burton & Pregitzer, 2003; Subke et al., 2006). Thus, in these ecosystems, responses to temperature could have been masking the photoassimilation effect on root respiration. In contrast, Mediterranean ecosystems where the highest summer temperatures are accompanied by severe water droughts and where some species can maintain their assimilation capacity under such conditions, offer the possibility to discern between the effect on root respiration of photosynthetic activity and soil temperature and moisture. Secondly, the time lag between canopy assimilation and root respiration in mature trees is still discussed due to the uncertainties associated with gas diffusivity delay through the soil or to the existence of artefacts in isotopic methods (Drake *et al.*, 2008; Irvine *et al.*, 2008; Kuzyakov & Gavrichkova 2010). Finally root respiration may be determined by the phenological patterns of carbon (C)

allocation coupled to photosynthetic activity. The fuelling of C for fine root respiration may come from recent photosynthates (Högber *et al.*, 2001; Steinmann *et al.*, 2004; Kuzyakov & Gavrichkova, 2010) or from stored C (Bahn et al., 2006; Bahn *et al.*, 2009). Carbon reserves would support FRR when C allocation to roots is restricted, as it happens during the growing season (Lynch *et al.*, 2013), when GPP is low as it under stressful conditions like drought, high vapour pressure deficit (VPD) or extreme temperatures (Czimczik *et al.*, 2006; Schuur&Trumbore, 2006). Hence coupling between C assimilation and root respiration largely depends on stored C pools that buffer the variability of current photosynthate supply.

Successional processes in ecosystems which imply species replacements due to exposure to different kind of stressful conditions (i.e. high competition pressure combined with extreme environmental events as drought) could entail important implications in FRR through changes in plant species composition and possible species-specific patterns of photosyntathe-root respiration coupling and carbon allocation processes. However, at our knowledge no previous studies have been done to study how a successional process may affect FRR rates. Under this context, the current and widespread drought induced-mortality events (Allen et al., 2010) that often entail succession processes (Royer et al., 2011) provide an exceptional experimental frame to evaluate the effects die-off at FRR level and elucidate the main ecological drivers of FRR at different time scales.

Here, we studied seasonal variations in excised FRR rates in a Scots pine (*Pinus sylvestris* L.) and Holm-oak (*Quercus ilex* L.) Mediterranean mixed forest at Prades Mountains (northeastern Spain). Scots pine exhibits a widely distribution range, occupying about one third of Northern hemisphere (Critchfield & Little, 1966) and presents its southernmost limit in the Mediterranean basin, concretely in the Iberian peninsula(Jalas & Suominen, 1976). In this region *P. sylvestris* is often restricted to mountain areas and persists in isolated locations facing with ecological conditions very different from those present in the main distribution area (Ceballos & Ruiz de la Torre, 1971). In contrast, Holm-oak is a very common tree in the western Mediterranean where finds its optimal conditions (Barbero *et al.*, 1992), showing their major populations in the Iberian peninsula (Blanco Castro et. al., 2005). The Scots pine population in Prades is affected by drought-induced dieback (Martínez-Vilalta & Piñol, 2002; Hereş *et al.*, 2011) and it is slowly being replaced by the more drought-adapted Holm-oak (Martínez-Vilalta *et al.*, 2012; Vilà-Cabrera *et al.*, 2012). A recent study of SR spatial patterns done in the same area (Barba *et al.*, 2013) shows an important effect of forest structure (i.e. tree identity and basal area) and proximity to drought-affected trees, with highest SR rates in sites close to dead pines and Holm oaks. There are several non-exclusive possible reasons explaining such high SR values under Holm-oaks and under areas available for colonization: high FRR rates, high fine root productivity or major fine root biomass, major root exudation processes and consequently elevated rhizomicrobial activity or finally high quantities of dead plant tissues and elevated bacterial activity associated.

We applied a root excision methodology "*in situ*" and developed a procedure using an open system (Licor 6400, Lincoln, US) to measure FRR rates directly excluding a great part of mycorrhizal respiration and a significant portion of rhizomicrobial respiration (Burton & Pregitzer, 2003).

Thus, the aims of this study were: (1) to study the seasonal variation in FRR associated to the different stages of drought-induced tree replacement, considering non-defoliated pines,

defoliated pines, dead pines and Holm-oaks; (2) to analyze their sensibility to environmental factors as soil temperature (ST) and water content (SWC); (3) to evaluate the seasonal coupling between FRR and tree assimilation in both coexisting species and in pines with different drought-induced stress grade.

Specifically, we aim to test the following hypothesis: (1) based on the results from Barba *et al.*, 2013, we predict highest FRR rates in roots collected under dead pines than under Holm-oaks as well as highest FRR under such both stages with respect to living pines. (2) we predict higher FRR seasonality in Pines than in Holm-oaks since pines are less adapted to dry conditions and their assimilation capacity will be drastically reduced during summer, as well as (3) FRR rates will respond differently to dry conditions in pines with different grade of affectation by drought, since more affected trees could be more reliant on carbon reserves (Galiano *et al.*, 2012) and have higher sensitivity to summer drought (Poyatos *et al.*, 2013).

Materials and Methods

Study area

The study site was carried out in a steep north facing hillside within the Titllar Valley at the Poblet Natural Reserve (Prades Mountains) located on NE Spain (41˚ 19' 58.05'' N, 1˚ 0' 52.26'' E, 1015 m asl). The climate is typically Mediterranean with a strong seasonality characterized by summer drought, with mean annual rainfall of 700 mm, peaking in spring and autumn, and minimal and maximal temperature of -2.5 and 28.7 ºC reached in January and July respectively (Ninyerola *et al.*, 2005). The substrate consists on a Palaeozoic base of schist that outcrops in a 44% of the soil surface. The soils are xerochreps with high stoniness and clay loam texture, and have a mean deep of ca 40 cm. Organic horizons cover most of the soil surface with variable thickness (Barba *et al.*, 2013).

Holm-oak (*Quercus ilex* L.) is the most extended species at Prades Mountains, constituting dense forest in lower areas, while Scots pine (*Pinus sylvestris* L.) appears on north hillsides and become dominant at altitudes over 800 m asl (Gutiérrez, 1989). Several drought events since 1990s have been specially affecting Scots pine population (Martínez-Vilalta & Piñol, 2002). Our study site is located on a mixed forest where Scots pine is gradually being replaced by the more resistant to drought Holm oak. As a result of this replacement process there is a mixture of healthy, defoliated and dead pines with Holm-oak growing in the understory. Pine stand mortality in Titllar valley is 12% (Vilà-Cabrera *et al.*, 2013) and the study is located in an especially high mortality area (>20%) where more than 50% of alive pines are seriously affected by defoliation (Jordi Martínez-Vilalta, unpublished data). Other species frequent are *Quercus cerrioides* Willk. & Costa and *Ilex aquifolium* L., and at lesser extent *Taxus baccata* L., *Amelanchier ovalis* Medik., *Prunus mahaleb* L., *Sorbus aria* (L.) Crantz, *Sorbus torminalis* (L.) Crantz and *Cistus laurifolius* L.. A detailed stand structure is summarized on Appendix 1, Table S1.

Root respiration

Roots were sampled on soil close to 28 individuals distributed in four tree categories representing different stages of the species replacement process: non-defoliated pines (NDP), defoliated pines (DP), dead pines (D) and Holm-oaks (HO). Individuals from the first three categories had similar diameter at breast high (DBH, mean±sd= 43.78 ± 14.19 cm; lm, t-

Student≈0, p>0.1), Holm-oaks were smaller (mean±sd=20.55±6.27 cm; lm, t-Student =8.59, p=0.0005). Defoliated pines were defined as those with 50% or less of green leaves relative to healthy canopies after visually identification (Galiano *et al.*, 2010). The study was performed in two seasons, in spring (22-24 of April) and early fall (30 September to October 2), before the first fall rain when conditions were still quite dry. All trees (seven per category) were sampled once per day in two consecutive days and the sampling time (morning-afternoon) was randomly chosen for each day and tree category.

Fine roots (<0.5 cm Ø) were removed from the upper soil layer (c.a. 20 cm deep). The protocol used was essentially the same as described by Burton and Pregitzer (2003). First order roots of each individual excluding dead pines were followed until fine roots were found. Under dead pines we collected the first living-tree roots founded independently of its identity. We minimized wounding and drying effects of root excision and transport by applying the minimal number of cuts as possible and keeping samples in a damp cloth. Adhering soil and organic debris were removed with a dry brush. Sample handling between excision and respiration measures took less than 15 minutes, being mostly 5-10 minutes, a time period much shorter than the four to six hours of constant respiration rate after excision described by some authors (Burton *et al.*, 2002; Bahn *et al.*, 2006; Burton & Pregitzer, 2003).

FRR was measured using an open system Licor 6400 coupled to the insect respiration chamber (6400-89 Insect respiration chamber, 25.31 cm^3) that was characterized by having two infrared gas analyzers (IRGAs) located in the sensor head, which measure CO_2 and H_2O concentrations in the air coming from the sample and the reference chambers. The initial incoming CO_2 concentration established has been demonstrated not have an effect over the measured respiration rates (Bouma *et al.*, 1997; Bryla *et al.*, 2001; Burton & Pregitzer, 2002). Therefore in order to reduce leak chance CO_2 concentration was set to atmospheric levels (400 ppm) instead of soil CO_2 concentrations (c.a. 500 to 2500 ppm at 5 cm depth; J. Barba & J.C. Yuste, unpublished data). Preventing root drying with an open system supposes a challenge since has a limited humidity control of incoming air from Li 6400 and it is not possible to raise its relative humidity (RH) over atmospheric RH. Root drying was avoided by lowering the air flux to 200 µmol/s and raising incoming-air RH over 80%. Such RH was reached by passing the air through water before arriving to the sample chamber (Figure 1). System modifications were made maintaining maximal measurement accuracy, i.e. shortening the circuit length and taking care of tube junctions to avoid leaks. The measurement precision reached with a flow rate of 200 µmol/s and a mean root weight between 1-2 g were smaller than 5% even for very low respiration rates (Appendix 2, “Configuration topics,” 2012). FRR was registered every minute for 15 minutes until the equilibrium state was reached, being the minimal time required to avoid overestimation due to the great quantities of CO_2 diffusing from roots freshly removed from soil (Appendix 1, FigureS1). Data processing includes (1) remove the dilution effect of water on CO_2 (Appendix 2, Eq. S1) since differences in air RH between reference and sample were influenced by air pass through the inserted circuit, (2) refer respiration data to root dry weight and (3) averaging both measures of FRR per tree in each season obtained on the two consecutive sampling days.

Mean daily carbon assimilation rate

Sap flow were measured and subsequently conversed to mean daily carbon assimilation through water use efficiency (WUE) values. WUE is defined as the ratio between CO_2 assimilation (A) and transpiration water loss (T) (Lambers *et al.*, 2008).

Photosynthesis was measured once per season between 10-13.00 am the day before root sampling in three trees per category (HO, DP and NDP). Water use efficiency at leaf level (WUE_L) was calculated from assimilation and transpiration rates measured on leaves unfolded the previous year (A_L, T_L) with a Licor 6400XT portable photosynthesis system and the standard chamber (2×3cm, 6400-08 Clear-Bottom Chamber). In the case of pine needles a subsequent estimation of leaf functional area was required. All measurements were done at ambient conditions of CO_2 (400 ppm), light (PAR=640-130 µmol/m^2 s), temperature (16-21˚C) and RH (48%).

Sap flow density was measured with handmade constant heat dissipation sensors (Granier, 1985) installed in 2010 (Poyatos *et al.*, 2013). Proves length was 2 and 1 cm for pines and Holm-oak respectively. Sensor pairs were separated 12 cm and covered with reflecting bubble wrap. For detailed description on sensor signal corrections by natural temperature gradients, sensor calibration, radial correction coefficients calculation and further knowledge on employed methods see Poyatos *et al.* (2013). Whole-tree sap flow (J_T) was calculated from sap flow densities corrected by radial coefficients δ_C and referred to sapwood area (a_S) (Equation 1). To obtain whole-tree daily mean sap flow ($J_{T,dm}$), J_T was firstly averaged per day including only the active photosynthetic hours with irradiance higher than 11.62 W/m^2; value calculated averaging sun and shade light compensation points of Scots pine (Fernández and Tapias-Martín, 2004) and Holm-oak (Valladares *et al.*, 2000) Iberian populations; and finally refereed to 24h in order to account for daily variations and counteract the possible underestimation of assimilation due to only have midday WUE values, especially in early fall.

$$J_T = \delta_c \times a_S \qquad \text{Equation 1}$$

Whole-tree daily mean assimilation rate ($A_{T,dm}$) defined as the mean quantity of CO_2 fixed by a tree in one day, was calculated from WUE_L and whole-tree daily mean sap flow ($J_{T,dm}$) as a measure of transpiration rate (Hu *et al.*, 2010; Rascher *et al.*, 2010) (Equation 2).

$$A_{T,dm} = WUE_L \times J_{T,dm} \qquad \text{Equation 2}$$

$A_{T,dm}$ were calculated for all trees since one week before sampling season (Appendix 1, Figure S2). Due to sensor failures in early fall there were no data from three Holm-oak, one DP and one NDP trees as well as various trees with missing assimilation data in certain days.

We accounted for a possible lag between assimilation and FRR, by averaging the values of the assimilation from two to six days, including the days of root sampling. The final assimilation variable was constituted by the mean assimilation of the 2 sampling days (Assim_m2) in spring and of the 6 days including the sampling days (Asim_m6) in early fall (Appendix 1, Table S2). Missing data in the assimilation variable were accounted by averaging assimilation of all trees in both seasons.

Ancillary data

Competition between trees was evaluated through Hegyi index (Hegyi, 1974) calculated for a 5 meter radio (Appendix 3, Eq.S3). Soil temperature (ST) was measured in the first layer of soil at roughly 10 cm deep, on the vicinity of each tree at the time of root extraction. Once per sampling season soil samples around each tree were collected with a soil core to a depth of 5, 10 or 15 cm. Soil water content (SWC) was measured gravimetrically, weighting the soil before and after drying at 105˚C for 24 hours and expressed as percentage of volumetric soil water content (SWCv) (Appendix 3, Eq.S4). Meteorological variables, soil moisture and sap flow were calculated as 15 min average of data that were registered every 30 seconds by a data acquisition system (CR1000 datalogger and AM16/32 multiplexers, Campbell Scientific Inc., Logan, UT, USA). SWCv was monitored in the upper 30 cm of soil using six frequency domain reflectometers (TDRs, CS616, Campbell Scientific Inc.) randomly distributed within the study area. Further detailed information about the system is specified in Poyatos *et al.* (2013).

Data analysis

Statistical analyses and data treatment were carried out with R Statistical Software 2.15.3 (R Development Core Team, 2013). Differences between environmental conditions among seasons were tested by a non parametric Kruskal-Wallys test. To test overall differences in all measured variables between treatments (season and tree category-NDP, DP, D, HO) and to account for repeated measures in time we used linear mixed effect models (*lme;* Pinheiro *et al.*, 2013) with tree as random factor. Constant air temperature and RH inside sampler chamber along tree categories and days in each season also were verified through an lme-model. DBH or Hegyi index comparisons along tree categories were done with general linear models (*lm*). To test linear correlations between continuous variables (FRR, ST, SWC and $A_{T,dm}$) we used the Pearson or Spearman-rank test. Analysis of FRR were applied to two data bases, one with data of all tree categories and the other with data from living trees (excluding dead pines) and with assimilation data. The best model explaining FRR was obtained by ANOVA comparison between lme-models by the method of maximal likelihood (ML) (Cayuela, 2012). Model assumptions were tested and fulfilled for all fitted models. Hegyi index (lm) and assimilation (lm, lme) were square root transformed and SWC (lme) was log transformed to achieve normal distribution. All variables managed with their units and symbols are summarized in the Appendix 1 Table S3.

Results

Ancillary data

Hegyi competition index was higher for Holm-oak (2.74 ± 1.67 sd) than for defoliated and non-defoliated pines (1.26 ± 0.83 sd; *lm*, t-Student≈-2.42, $p<0.05$), while dead pines show intermediate values (1.55 ± 0.68 sd; *lm*, t-Student=-1.75, $p=0.09$).

Daily temperature oscillations were similar for both seasons but average temperature was 10 ºC higher in early fall than spring (Figure 2, Table 1). SWC was relatively low in both seasons (<0.2 cm^3/cm^3) being extremely low during early fall (Table 1). Moreover both seasons exhibit significant daily vapour pressure deficit (VPD) oscillations, which were slightly more extremes in early fall (Kruskal-Wallis, Figure 2 & Table 1). Daytime ST measured under each tree was

consistent with mean air temperatures (Table 2). Values of SWC gravimetrically measured under each tree were much lower than the values obtained from the TDRs (Table 1 & Table 2).

No differences in air RH and temperature measuring conditions among tree classes were observed along each sampling season (lme, L.ratio≈0.54, p>0.1).

FRR under tree categories

ST and SWC were inversely correlated (Spearman, rho=-0.42, p=0.0017) and both exhibited significant differences between seasons but no between tree categories; only SWC showed slight tendency to lower values under dead pines (lme, t-Student=1.72, p=0.09) (Table 2). Within each season ST and SWC were not correlated (Spearman, p>0.1).

Total FRR was higher in early fall than spring (lme, L.Ratio=12.96, p=0.0003) and the same pattern was observed in both seasons (lme, L.Ratio=1.08, p=0.78) (Table 3 & Appendix 1, Figure S4): FRR was significantly higher under living pines (defoliated and non-defoliated) than under Holm-oak (lme, t-Student≈-3.02, p<0.01), while under dead pines FRR showed intermediate values between living pines and Holm-oaks (lme, t-Student≈-1.77, p≤0.1).

FRR response to ST was constant within each tree categories (Figure 3) exhibiting a positive correlation (Spearman, rho=0.42, p=0.002), although this relationship was not significant in any season when considering the overall pool of trees (Spearman, spring: rho=0.01, p-value=0.96; early fall: rho=0.21, p-value=0.31). Conversely there was no direct sensibility of FRR to SWC neither along seasons nor within tree categories (lme, t-Student=0.57/L.Ratio=0.18, p>0.1) (Figure 4).

The best lme-model explaining FRR variability only contained ST and tree category as explicative variables (AIC=89.85, p=0.01) (Appendix 1, Table S4). Model parameters and graphical description are showed in Table 4 (M1) and Figure 3.

FRR and daily mean assimilation

WUE in pines were strongly reduced from spring (8.05 ± 2.32) to early fall (4.41 ± 1.92 mmol CO_2/mol H_2O) while Holm-oak increased their WUE from 6.69 ± 3.73 to 10.35 ± 2.38 mmol CO_2/mol H_2O (lme, L.Ratio=8.49, p=0.01) being greater than for pines in early fall (lm, F=6.75, p=0.05). Both living pine categories shared similar assimilation behaviour along seasons, showing a steep decline from spring mean values of 255.43 ± 57.82 to early fall with 35.61 ± 18.15 g CO_2/day·tree (lme, L.Ratio=8.8, p<0.05). Conversely, Holm-oaks maintained constant assimilation rate in both seasons of roughly 99.49 ± 22.59 g·CO_2/day·tree, assimilating less than pines in spring (lme, t-Student=2.36, p=0.03) but slightly more in early fall (lme, t-Student=1.95, p=0.06) (Appendix 1, Table S6). Assimilation and SWC were positively correlated in spring (Spearman, rho=0.51, p<0.05) and not in early fall (Spearman, rho=-0.24, p=0.29) (Appendix 1, Figure S3)

The best lme-model explaining FRR variability includes tree category and the interaction between assimilation and ST as explicative factors (AIC=76.52, p<0.01) and neither Season nor SWC had enough explicative power in the model (lme, ML, p>0.1) (Appendix 1, Table S5). FRR response to assimilation depends on the temperature and was constant in all categories following the same pattern described previously (Table 4, M2 & Figure 5).

Discussion

Die-off impact on canopy

FRR under Holm-oaks was ca. 35% lower than under living pines while pine die-off tended to decrease FRR in 20% with respect to living pines (Table 3). This pattern was consistent in both sampling seasons (Table 3 & Appendix 1 Figure S4). Results partially differed from previous observations of SR made in spring 2010 (Barba *et al.*, 2013) where soil CO2 fluxes were highest under dead pines and Holm-oaks. It could be that a higher FR biomass Holm-oaks trees may have counteracted the observed differences in FRR between pines and Holm-oaks but not differences in FR biomass were observed by Barba et al. (2013). This disagreement, therefore, suggest that in general FRR under pines was contributing more to total SR than FRR under Holm-oak, and that the high SR observed in spring under Holm-oak and dead pines with respect to SR under living pines (Barba *et al.*, 2013) could be explained from the activity of other plant derived CO_2 sources (Kuzyakov & Gavrichkova, 2010), i.e mycorrhizal, rhizomicrobial and from microbial respiration of plant dead tissues.

The trend to decrease FRR from living to dead pines and Holm-oak (Table 3 & Appendix 1 Figure S4) was accompanied by increases in the Hegyi index. Considering the higher values of competition index for dead trees with respect to living ones and the similar FRR values between FR under dead pines and Holm-oaks, our results points to a recent Holm-oak root colonization under dead trees. Such recent Holm-oak roots colonization of dead pines gaps was also suggested by a current work in the area that shows a convergence of bacterial communities under dead pines and Holm-oaks (Curiel Yuste *et al.*, 2012). It is, therefore, likely that the observed aerial colonization of dead pine gaps was associated with a belowground colonization by FR of colonizers Holm-oaks, profiting from the lack of competition for resources under dead pine individuals.

Seasonal variation in FRR and sensibility to environmental factors and tree assimilation

FRR changed seasonally but unexpectedly the FRR values registered in the growing season (spring) when both biotic and abiotic conditions were more optimal for FRR, were 30% lower than in early fall when SWC was extremely low and pine assimilation were close to 0 (Table 3). For example, values of FRR during the growing season in another pine species range between 1.92-22.21 µg CO_2/g_{root}·min at ST from 5 to 20 ºC respectively in *Pinus resinosa* Ait. (Burton & Pregitzer, 2003) or between mean values of 15.93-25.92 µg CO_2/g_{root} in *Pinus taeda* L. (Drake *et al.*, 2008), being in all cases higher than the mean value of 1.55 ± 0.17 µg CO_2/g_{root} obtained for defoliated and non-defoliated individuals of *Pinus sylvestris* in spring at ST of 9 ºC. The low productivity that characterize the Mediterranean forests with respect to more productive temperate forest could partially explain the low values of FRR under pines obtained with respect to FRR of pines from temperate climatic zones. Data of excised FRR were neither found for Holm-oaks nor for other trees typically found in Mediterranean or semiarid ecosystems.

Soil temperature was the variable that better explained FRR when considering data from both seasons together (Table4). Soil temperature affected positively, and similarly, to FRR under all four ecotypes under study (Figure 3). However, the within-season variability in FRR were not explained by temperature, accordingly to preceding works pointing to temperature as an

important factor conditioning FRR but highlighting the importance of other drivers such as substrate availability (Pregitzer *et al.*, 2000; Atkin et al., 2000; Atkin & Tjoelker, 2003; Burton & Pregitzer, 2003; Atkin *et al.*, 2005). Moreover SWC did not seem to exert any direct control over the seasonal and/or the spatial variability of FRR (Figure 4). Experimental studies showed a marked decrease of FRR with low SWC (Bryla *et al.*, 2001, Burton & Pregitzer, 2003) and reductions of SWC during summer drought usually entail a significant reduction in SR attributed to some extent to reductions in root-rhizosphere respiration, at least in perennial species (Irvine *et al.*, 2005; Nikolova *et al.*, 2008; Ruehr *et al.*, 2012). Knowing the importance of water for the functioning of Mediterranean soils, which are deeply conditioned by summer drought conditions (Tang et al. 2005; Misson et al. 2006; Curiel Yuste et al. 2007), it is possible that the drought effect was reflected in a reduction in FR population/biomass (senescence of FR) as were previously evidenced in Holm-oaks (Claramunt Lopez, 1999), rather than in the FRR´s. A lower FR population during summer drought could have maintained the relatively high metabolic rates observed because trees are able to passively move water from tissues located in moister soil layers to tissues from layers with low water potential (upper layers) in a well-known process called hydraulic lifting (Caldwell *et al.*, 1998). However, this are only speculations and future studies should take into account both FR population dynamics and hydraulic lift to understand the relative role of FR in total SR.

Assimilation was an important factor determining FRR, significantly improving the AIC scores of the abiotic model and having a positive effect on FRR (Table 4). However we found a partial decoupling between FRR and assimilation during early fall when trees, and especially pines, presented extremely low assimilation rates (resulting from sap flow interruption at SWC values under 11%; see Poyatos *et al.*, 2013). Other SR studies on semiarid coniferous or mixed forests show high correlation of FRR with gross primary productivity (GPP) or photosynthetic active radiation (PAR) during the growing season which decreased during the dry period (Irvine *et al.*, 2005;Irvine *et al.*, 2008; Vargas *et al.*, 2010; Martin *et al.*, 2012). FRR-assimilation decoupling during drought indicates, therefore, that FRR during those periods was probably fuelled by stored carbon as observed in recent studies (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006; Lynch *et al.*, 2013).

Tables and Graphics

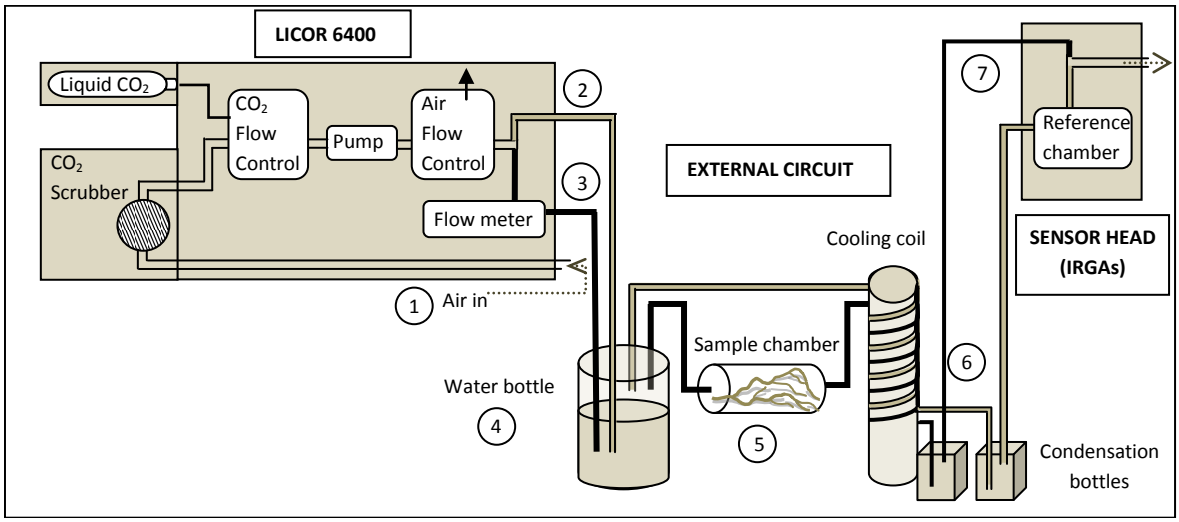

Figure 1. System developed to measure FRR with the Licor-6400. Water bottle was inserted in the original circuit in order to maintain high relative humidity in the sample chamber. Cooling coil and water condensation bottles were installed before the sensor head to avoid water condensation in the infrared gas analyzers sensors (IRGAs). (1) Air inflow to Licor-6400 where CO_2 and flow is regulated; (2, 3) Air outflow to reference (grey) and sample (black) tubes respectively; (4) Water bottle where air is water saturated; (5)the 6400-89 Insect respiration chamber (25.31 cm^3) used for root samplers; (6) Cooling air coil and water condensation bottles; (7) Sensor head containing reference and sample IRGAs.

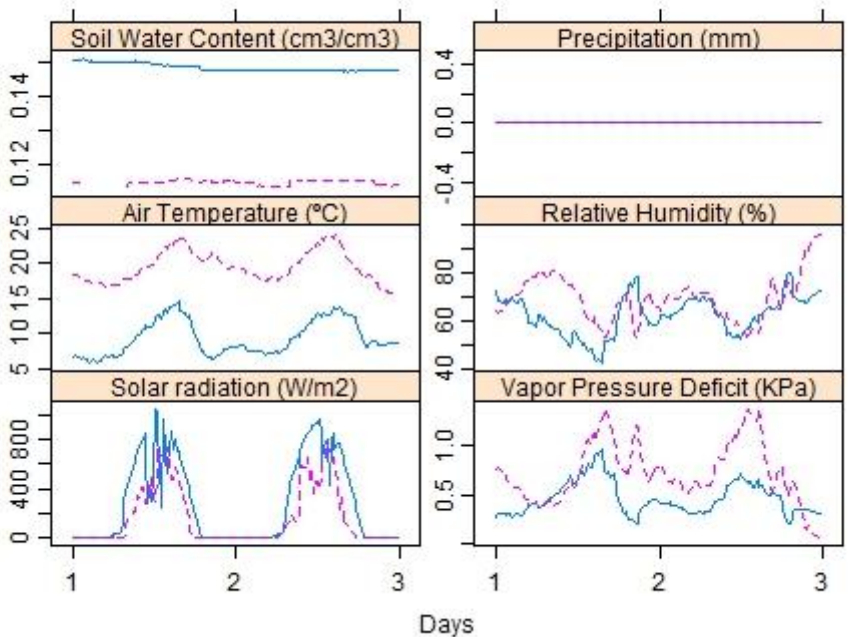

Figure 2. Daily evolution of environmental variables along fine root respiration sampling days in spring (continuous line) and early fall (dotted line).

468 *Table 1. Environmental conditions in both seasons (spring, early fall) during the period of FRR*
469 *measurements (April and October, 2013).*

	SPRING	EARLY FALL	p-val
SWC –Reflectometer (%)			
Mean	15 ± 8.4e-03	11 ± 4.61e-03	<0.0001
Max	15.09	11.60	
Min	14.70	11.35	
Global radiation (W/m2)			
Mean	284.15 ± 24.39	162.41 ± 16.9	<0.0001
Max	1050.00	766.10	
Air Temperature (˚C)			
Mean	9.33 ± 0.18	19.51 ± 0.16	<0.0001
Max	14.690	24.18	
Min	5.860	15.82	
Air relative humidity (%)			
Mean	62.19 ± 0.58	69.42 ± 0.68	<0.0001
Max	80.50	96.40	
Min	42.41	52.67	
Water vapor pressure deficit (KPa)			
Mean	0.46 ± 0.01	0.72 ± 0.02	<0.0001
Max	0.9627	1.3633	
Min	0.2072	0.0655	
Precipitation (mm^3)	0	0	

Data are absolute maxim and minimal values and means±sd for 2 days of every 30-seconds recording. Statistical differences between seasons were assessed by a non parametric Kruskall-Wallys test.

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474 *Table 2. Soil conditions in both seasons (spring, early fall) during the period of FRR*
475 *measurements (April and October, 2013) and its values for each tree category studied.*

	SPRING	EARLY FALL	HO	D	DP	NDP
Soil Temperature (˚C)						
Mean	**9.32±0.90 A*** **	**16.94±0.54 B*** **	12.93±1.18 a	12.69±1.12 a	12.63±1.18 a	13.10±1.04 a
SWC-Gravimetric (%)						
Mean	**2.42±1.12 A** **	**1.54±0.75 B** **	1.80±0.27 ab	**1.60±0.20 b·**	2.33±0.26 a	2.18±0.34 ab
Max	5.01	3.49				
Min	0.56	0.53				

476 Data are absolute maxim and minimal values and mean±sd of two days. Statistical differences (lme, ML test)
477 between levels of each factor are assessed by different letters (lower case, tree category factor; upper case, season
478 factor).SWC, Soil Water Content; HO, Holm-oak; D, dead pines; DP, defoliated pines; NDP, non-defoliated pines.
479 Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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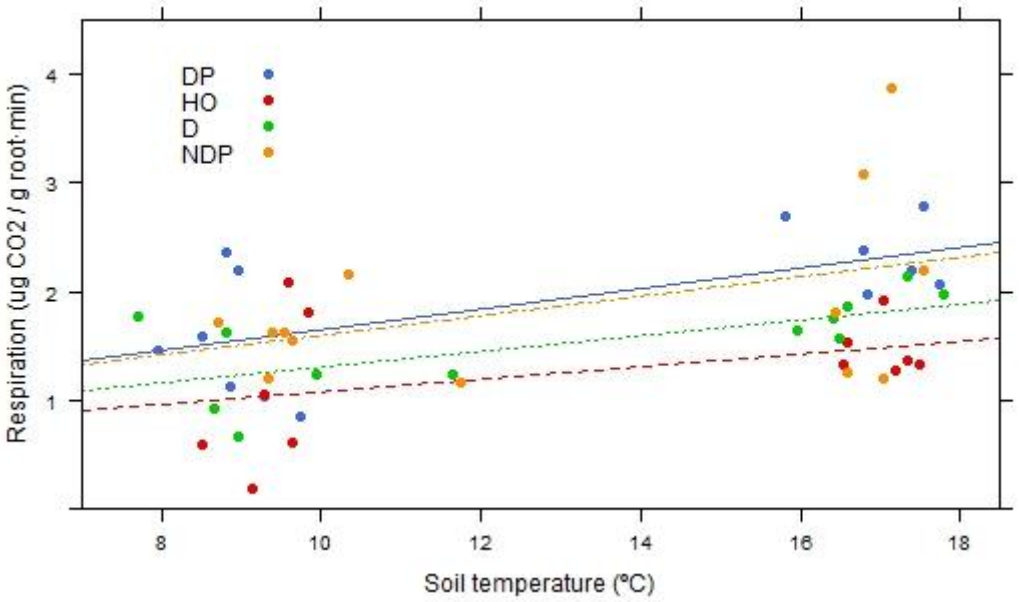

Figure 3. Response of fine root respiration (FRR) to soil temperature (ST) in the four studied tree categories: Holm-oaks (HO, dashed line); dead pines (D, dotted line); defoliated pines (DP, solid line) and non-defoliated pines (NDP, dot-dashed line). FRR under living pines (DP, NDP) were higher than under Holm-oak (lme, RML, $p<0.01$). Differences in FRR between dead and living pines were close to significance (lme, RML, $p<0.1$).

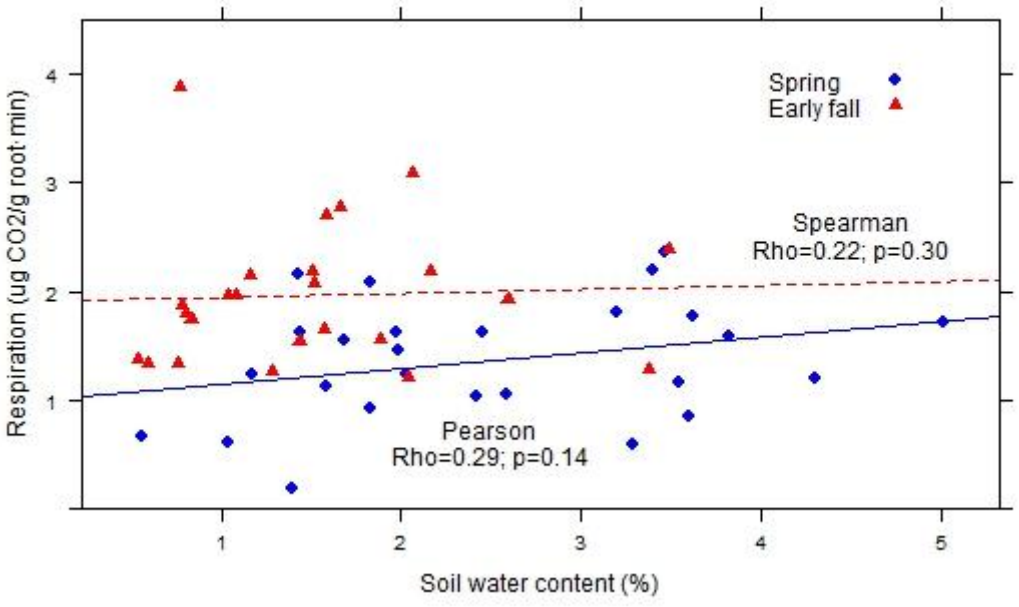

Figure 4. Response of fine root respiration (FRR) to soil water content (SWC) along both sampling seasons, spring (solid line) and early fall (dotted line).

Table 3. FRR values (µg CO_2/g_{root}·min) of Hol-oak (HO), dead pines (D), defoliated pines (DP) and non-defoliated pines (NDP) in the two seasons sampled, spring and early fall

Category / Season	HO	D	DP	NDP	TOTAL
Spring	1.06±0.30	1.24±0.17	1.52±0.22	1.58±0.13	**1.37±0.11 A****
Early fall	1.46±0.10	1.82±0.09	2.35±0.13	2.23±0.43	**1.97±0.13 B****
TOTAL	**1.26±0.16 a***	**1.53±0.12 a·**	1.90±0.17 b	1.88±0.22 b	

Data are means±sd of seven independent samples. Significant differences or marginally significant differences (LME, RML, p<0.1) between the levels of each factor are indicated by different letters (Tree category, lower case; Season, big case) and marked in black. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4. Results of most parsimonious lme models to test differences in FRR (µg CO_2/g_{root}·min) between tree categories: Holm-oak (HO), dead pines (D), defoliated pines (DP) and non-defoliated pines (NDP).

Model / Variables	M1: ST+Categories		M2: ST×Assim+Categories	
	Coefficent	t-value	Coefficent	t-value
Intercept (HO)	0.220 (0.304)	0.72	-0.453 (0.545)	-0.83
ST	**0.079*** (0.020)**	**4.00**	**0.145* (0.039)**	**3.69**
Categories				
DP	**0.686** (0.220)**	**3.11**	0.380 (0.259)	1.46
NDP	**0.626** (0.220)**	**2.84**	**0.486· (0.235)**	**2.06**
D	0.284 (0.224)	1.26		
Assim			**33.39** (13.48)**	**2.48**
ST × Assim			**-3.23** (1.25)**	**-2.59**

Coefficients are means ± 1se. ST, soil temperature; Assim, Assimilation. Interaction between factors is indicated by the symbol`×´. Significant or marginally significant results are marked in black.
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

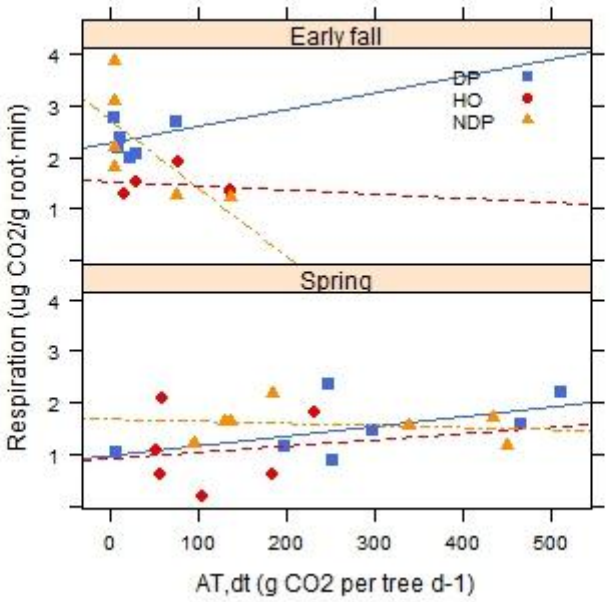

Figure 5. Relation between fine root respiration (FRR) and daily mean assimilation per tree ($A_{T,dm}$) in both sampling seasons for each tree category: Holm-oaks (HO, dashed line); defoliated pines (DP, solid line) and non-defoliated pines (NDP, dot-dashed line).

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Appendix

Appendix 1. Tables and graphics

Table S1. Stand characteristics of high-hill area of Titllar valley including the study area.

	Density (stems/Ha)	DBH (cm)	Basal area (m2/Ha)	Normal diameter (cm)	Ocupacion (% BA_{sp}/ BA_{total})
Pinus sylvestris	184±20	26.98±13.61	13.18±1.66	40.90±2.59	35.87
Quercus ilex	1636±441	11.16±4.51	18.63±3.93	48.44±5.17	50.70
Quercus sp.	165±194	15.20±5.42	3.37±3.83	17.20±11.58	9.18
Ilex aquifolium	189±24	7.87±3.03	1.04±0.26	11.43±1.44	2.84
Others	57±20	12.72±5.88	0.52±0.54	6.96±4.23	1.42

Variables were measured in three circular plots of 15 m radio in November 2012 (Paola Beltrán,Ferran Colomer&Eva Pereira). Data are mean±sd. DBH, diameter at breast height; BA, basal area.

Table S2. FRR correlations with different daily mean assimilation variables for the two sampled seasons (spring, early fall).

Season	Assim_m2	Assim_m3	Assim_m5	Assim_m6
Spring	**0.42·**	0.39	0.37	0.31
Early fall	-0.24	-0.41	**-0.47 ·**	**-0.51·**

Data are Rho values from the non parametric Spearman's rank correlation test. Assim_m2, mean assimilation of two FRR sampling days; Assim_m3, mean assimilation of two FRR sampling days and one day before; Assim_m5 and Assim_m6, include three and four days before respectively. Alternative hypothesis: true rho is not equal to 0. Significant results are in bold and marginal significance (0.05>p<0.1) is expressed by`·´

Table S3. Abbreviations and units list of measured variables and parameters managed in this study.

SIMBOL	**DESCRIPTION**	**UNITS**
R_r	Root respiration	µg CO_2 /g_{root}·min
Δ CO_2	CO_2 differential	µmol CO_2/mol
F	Air flow	µmol/s
A_L	Assimilation rate per unit leaf area	µmol/m^2·s
A_T	Whole tree assimilation rate	mmol CO_2/s
$A_{T,dm}$	Daily mean assimilation per tree	g CO_2/d
J_T	Instantaneous (15 min means) whole tree sap flow	dm^3 H_2O/s
$J_{T,dm}$	Day-time averaged whole tree sap flow (86400 s)	dm^3 H_2O/day
δ_c	Instantaneous (15 min means) sap flow per unit sapwood	dm^3 H_2O/m^2·s
WUE_L	Water use efficiency at leave level	mmol CO_2/mol H_2O
a_S	Tree basal area	cm^2
a_L	Tree leaf area	m^2
T_L	Leaf transpiration rate	mmol H_2O/m^2·s
m_r	Root mass	g
DBH	Tree diameter at breast height	cm
$D_{t,\,i}$	Distance between trees	m
ST	Soil temperature	˚C
SWC	Soil water content	% (cm^3 H_2O/cm^3 soil)

Table S4. Summary of results of different lme models applied to explain FRR.

Model	Variable	Df	AIC	BIC	logLik	Test	L.Ratio	P-value
1	Null	3	105.71	111.44	-49.85			
2	SWC	4	107.53	115.18	-49.76	1 vs 2	0.18	0.6732
3	**ST**	**5**	**95.42**	**104.98**	**-42.71**	**2 vs 3**	**14.11**	**<0.001*** **
4	**Category**	**8**	**91.43**	**106.72**	**-37.71**	**3 vs 4**	**9.99**	**0.0186***
5	Season	9	92.91	110.12	-37.45	4 vs 5	0.52	0.4717
6	Season x Category	12	97.68	120.63	-36.84	5 vs 6	1.23	0.7465
7	ST x Category	15	100.25	128.93	-35.12	6 vs 7	3.43	0.3294
8	SWC x Category	18	100.91	135.32	-32.45	7 vs 8	5.34	0.1486

Significant differences of factors were tested by ANOVA comparison of lme models with the method of Maximal Likelihood (ML). CL, class treatment; ST, soil temperature; SWC, soil water content. Interaction between factors is indicated by the symbol`x´. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table S5. Summary of results of different lme models applied to explain FRR including assimilation as explicative variable

Model	Variable	Df	AIC	BIC	logLik	Test	L.Ratio	P-value
1	Null	3	87.85	92.76	-40.92			
2	**Assimilation**	4	**86.68**	**93.23**	-39.34	1 vs 2	3.17	**0.0750·**
3	**ST**	5	**82.4**	**90.59**	-36.2	2 vs 3	6.28	**0.0122***
4	SWC	6	84.04	93.86	36.02	3 vs 4	0.36	0.5475
5	**Category**	8	**80.27**	**93.37**	-32.13	4 vs 5	7.77	**0.0206***
6	Season	9	82.18	96.92	32.09	5 vs 6	0.08	0.7708
7	**Assimilation x ST**	10	**76.52**	**92.9**	28.26	6 vs 7	7.66	**0.0056****
8	AssimxSTxCategory	16	83.46	109.66	-25.73	7 vs 8	5.06	0.5357

Significant differences of factors were tested by ANOVA comparison of lme models with the method of Maximal Likelihood (ML). CL, class treatment; ST, soil temperature; SWC, soil water content. Interaction between factors is indicated by the symbol`x´. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table S6. Daily mean assimilation per tree ($A_{T,dm}$; g CO2 /day) of Holm oak, dead pines (D), defoliated pines (DP) and non-defoliated pines (NDP) in the two seasons sampled.

Category / Season	HO	DP	NDP	TOTAL
Spring	106.56±25.29 A***a	269.72±60.89 D***b	241.15±54.75D ***b	205.81±31.42A***
Early fall	92.43±19.89 C***c	39.37±17.4 B***c	31.85±18.91 B***c	54.55±11.91 B***
TOTAL	99.50±15.58 a	154.55±44.11 b·	136.50±40.21 b*	

Data are means of seven independent samples. Significant differences or marginally significant differences (LME, RML, p<0.1) between the levels of each factor are indicated by different letters (Tree category, lower case; Season, big case). Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

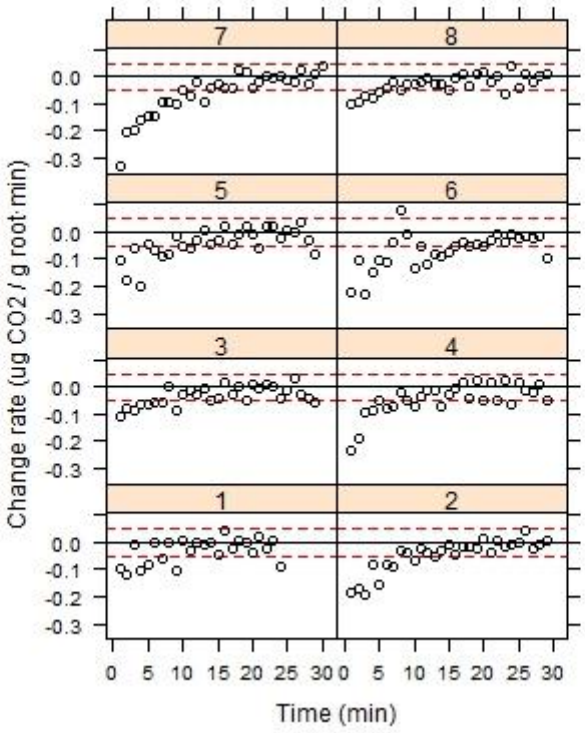

Figure S1. Fine root respiration change (µg CO2 /g root·min) rate of Ulmus sp. along 30 measuring minutes. Stable values are thus not significantly different from 0, between 0.05 and -0.05 (red dotted lines). Results from Anova test show stable values at minute 12 and from minute 16.

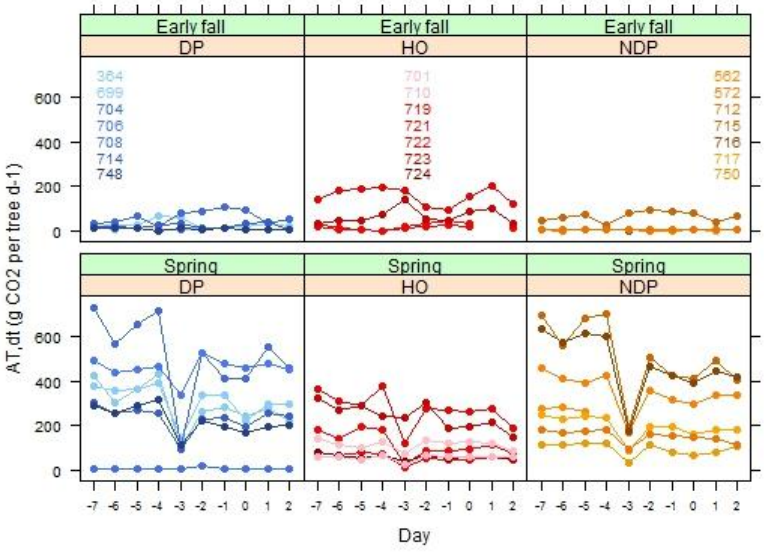

Figure S2. Daily mean assimilation per tree (numbers code) from seven days before sampling start for the both studied seasons. FRR were sampled at day 1 and 2. Photosynthesis data were recorded on day 0.

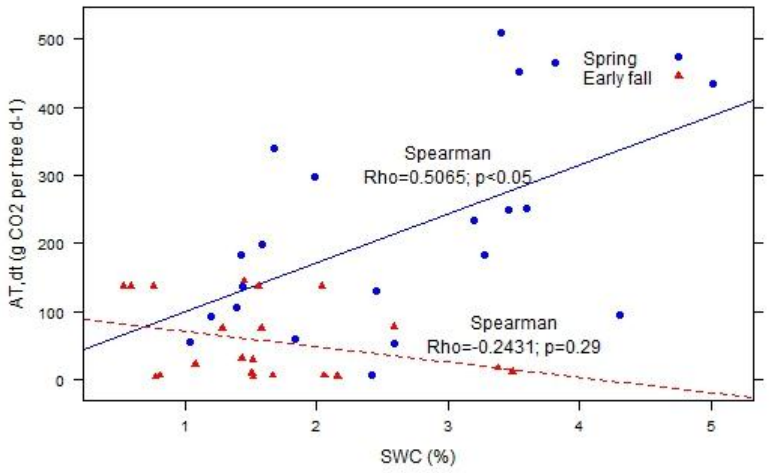

Figure S3. Relation between daily mean assimilation per tree (AT,dm; g CO2 /day) and soil water content (%) along both sampling seasons (spring, dashed line); (early fall, solid line). Assimilation positively related to SWC in spring (Spearman, rho=0.5064935, p<0.05) and not in early fall (Spearman, rho=-0.2431424, p=0.2882)

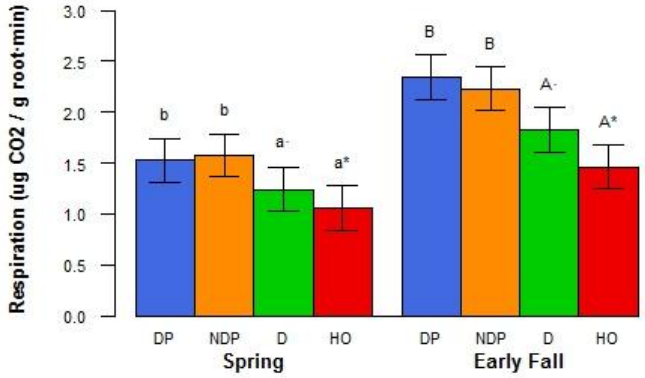

Figure S4. FRR values (µg CO_2 /g_{root}·min) of Holm oak (HO), Dead pines (D), Defoliated pines (DP) and Non-defoliated pines (NDP) in the two seasons sampled, spring and early fall. Data are means±se of seven independent samples. Significant differences or marginally significant differences (LME, RML, p<0.1) between the levels of each factor are indicated by different letters (Tree category, lower case; Season, big case). *Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Appendix 2. Measurement precision

The measurement precision depends on the CO_2 noise of the infrared gas analyzer (Irga), the flow rate and the CO_2 differential between both chambers. The later depends on the respiration rate of the roots and the amount of plant material enclosed in the chamber. Since the CO_2 noise is ca. ±0.1 ppm, for the lowest root respiration rates of 0.10 $\mu g/g \cdot min$ registered by Burton and Pregitzer (2003) we would reach a precision in CO_2 differential (ΔCO_2) measurement between 2-5% with the following parameters: a flow rate of 200 $\mu mol/s$ and a mean root weight between 1-2 g (Equation S1,S2; "Configuration topics," 2012). With such parameters we ensured a precision smaller than 5% even for very low respiration rates.

$$R(\mu g/g \cdot min) = \frac{44 \cdot \Delta CO^2(\mu mol/mol) \times 60 \cdot F(\mu mol/s)}{m_r(g) \cdot 10^6} \times \frac{1000 - [H_2O]_{reference}}{1000 - [H_2O]_{sample}}$$

Equation S1. Equation for the respiration rate (R). ΔCO_2 is the CO_2 differential between sample and reference chamber, F is the air flux and m_r is the root mass. The last term refers to the water concentration of the air in the reference and sample chambers and takes account for the dilution effect of the H_2O on the CO_2

$$Precision = \frac{CO_2\ noise}{\Delta CO_2}$$

Equation S2. Licor 6400 measure precision of the CO2 differential (ΔCO_2) created by the root sample enclosed in the chamber. The CO2 noise is ±1 ppm at 350 ppm with 4-second average signal.

Appendix 3. Equations

$$C_{t,i} = \sum_{t=1}^{n} \left(\frac{DBH_i}{DBH_t}\right) / D_{t,i}$$

Equation S3. Hegyi competence index ($C_{t,i}$) where DBH is the diameter at breast height in target tree (t) and competitors (i), n is the number of competitors and $D_{t,i}$ is the distance between target tree and competitor i.

$$SWC = \frac{W_{wet} - W_{dry}}{V_{total} - V_{stones}} \times 100$$

Equation S4. Direct measure of volumetric soil water content (SWC). W_{wet} and W_{dry} refers to dry and wet soil weight, V_t is the total volume of soil sample and V_{stones} is the volume of stones present in each sample.