Effects of drought-induced forest die-off

and species replacement on soil CO_2 effluxes

PhD Thesis

Josep Barba Ferrer

to be eligible for the doctor degree

Supervised by:

Dr. Francisco Lloret Maya

Dr. Jorge Curiel Yuste

Universitat Autònoma de Barcelona, March 2015

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4

" Recomano als biòlegs que, sense oblidar mai el nivell molecular, vagin de tant en tant a donar-se cops de cap al tronc d'un arbre i li demanin que els inspiri per tal de poder entendre el que passa al món. "

RAMON MARGALEF

(*i si poden fer 4 passes enrere i agafar perspectiva, millor que millor*)

Per la Irene

Abstract

The increase in frequency and intensity of drought events during the last decades has been considered responsible for the widespread forest mortality events observed abroad and particularly in the Mediterranean Basin. Predictions suggest that future climate conditions will be even warmer and drier for this region, but little is known about how this drought-induced forest die-off could affect carbon emissions and carbon sink capacity of terrestrial ecosystems. Soils, storing two thirds of C of terrestrial ecosystems, will play a crucial role in forest C balance. This thesis addresses soil CO_2 effluxes in a mixed Mediterranean forest, where several drought events since 1990's have resulted in Scots pine (*Pinus sylvestris* L.) defoliation and mortality, with a subsequent replacement by Holm oak (*Quercus ilex* L.). The study focuses on how this die-off and species replacement affects soil respiration (SR) and its heterotrophic and autotrophic components. It deals with SR dependency on abiotic (i.e. soil temperature, soil water content [SWC], stoniness, soil pH) and biotic (i.e. trees photosynthetic activity, forest structure, litter inputs on soil, fine roots biomass) controls at different temporal and spatial scales. Also the study determines rates of litter decomposition and nitrogen mineralization in different litter types (leaves and fine roots of *P. sylvestris* and *Q. ilex*) along the drought-induced die-off and replacement gradient. Mapping of SR spatial heterogeneity, partitioning of the sources of SR, litterbags decomposition experiments and field-experiments about the influence of plant photosynthetic activity on SR were done considering four habitats: non-defoliated pines [NDP], defoliated pines [DFP], dead pines [DP] and Holm oaks [HO]. Species identities had direct effect on litter decomposition by determining the specific chemistry of litter. Indirect effects were also found, likely due to soil decomposer communities associated at a given vegetation that modify rates of litter decomposition. Soil temperature and SWC strongly regulated temporal (from daily to seasonal) variability of SR (including both autotrophic and heterotrophic components). Forest structure and species identity better explained SR at tree-stand spatial scale. Photosynthetic activity exerted strong control over temporal variability of SR, with higher influence on living pines at daily time scales but it had stronger effect on SR under HO at seasonal scale. SR and its heterotrophic and autotrophic components remained apparently unaffected by drought-induced Scots pine die-off denoting a high functional resilience of the studied plant-and-soil system. Both spatial and temporal data indicated that this functional resilience of SR was the result of colonization by HO of the gaps created by the dead of pines. Despite this resilience, the replacement of Scots pine by Holm oak may result in a strong reduction in SR due to the observed low heterotrophic rates of SR under HO compared to NDP, at least at the considered successional scale. Since the studied phenomenon is expected to increase in the next decades, changes in soil respiration and in litter decomposition should have great impacts on the carbon balance of this type of forests.

Resum

L'increment en la freqüència i la intensitat de les sequeres en les últimes dècades s'ha considerat responsable de molts episodis de mortalitat forestal a nivell global, particularment a la regió mediterrània. Els models prediuen un futur encara més càlid i sec per aquesta regió, però es desconeix com aquest decaïment forestal per sequera pot afectar a les emissions de CO_2 i a la capacitat d'embornal de carboni (C) dels ecosistemes terrestres. Els sòls, que emmagatzemen dos terços del C dels ecosistemes terrestre, tindran un paper clau en el balanç de C. Aquesta tesi estudia els fluxos de CO_2 del sòl en un bosc mixt mediterrani que ha patit diversos episodis de sequera des dels anys 90, produint un decaïment i una mortalitat del pi roig (*Pinus sylvestris* L.) i conseqüentment una substitució per alzina (*Quercus ilex* L.). L'estudi vol respondre com aquest decaïment i substitució afecta la respiració del sòl (RS) i les seves fraccions autotròfiques i heterotròfiques. Estudia la dependència de la RS a variables abiòtiques (temperatura i humitat del sòl, pedregositat, pH del sòl) i biòtiques (fotosíntesi dels arbres, estructura forestal, entrades de matèria orgànica, biomassa d'arrels fines) a diferents escales temporals i espacials. L'estudi també vol determinar les taxes de descomposició i la mineralització del nitrogen en fulles i arrels fines de *P. sylvestris* i *Q. ilex* en un gradient del procés de decaïment forestal i substitució induït per sequera. Es va fer un mapeig de l'heterogeneïtat espacial de la RS, experiments de partició de fluxos de la RS, de descomposició de matèria orgànica i per mirar la influència de l'activitat fotosintètica en la RS, considerant quatre hàbitats: pins no defoliats (PND), pins defoliats (PDF), pins morts (PM) i alzines (A). La identitat de les espècies va influir directament les taxes de descomposició de la matèria orgànica, determinant-ne la química, però també de manera indirecta, determinant possiblement una comunitat descomponedora específica per cada espècie. La temperatura i la humitat del sòl regulaven la variabilitat temporal (diària i estacional) de la RS, incloent les components autotròfiques i heterotròfiques. L'estructura forestal i les espècies d'arbres explicaven millor la variabilitat espacial de la RS. L'activitat fotosintètica va exercir un gran control de la RS a escales temporals, amb una influència gran sobre els pins vius a escala diària i una influència gran sobre les alzines a escala estacional. El decaïment i mortalitat dels pins sembla que no va tenir efecte en la RS ni en les seves components, demostrant una gran resiliència funcional del sistema planta-sòl. Les dades espacials i temporals indicaven que la resiliència funcional era resultat d'una colonització per part de les alzines del sòl alliberat per la mort dels pins. Malgrat aquesta resiliència, la substitució de pins per alzines va comportar una reducció important de la RS produïda per una disminució de la component heterotròfica de pins a alzines. Tenint en compte que un augment de les sequeres podria incrementar el decaïment forestal en les properes dècades, canvis en la RS i en la descomposició de la matèria orgànica podrien tenir gran impacte en el balanç de C d'aquests ecosistemes.

10

Table of contents

Chapter 1. General introduction

1. SOIL RESPIRATION, COMPONENTS AND DRIVERS

1.1. Soil respiration: the black box output

Soils store two thirds of carbon (C) of forest ecosystems worldwide (Dixon et al., 1994) and the CO_2 emissions, mainly originated from soil biotic activity, are the second larger C flux of terrestrial ecosystems, only after gross primary production (GPP) (Schlesinger and Andrews, 2000). However, many uncertainties exist about how soil respiration (SR), which accounts for all the CO_2 efflux originated from soils, could respond under climate change scenarios, since small changes in drivers regulating SR could have enormous consequences in the total C balance of forests ecosystems (Valentini et al., 2000). Since there is still a limited knowledge of how abiotic and biotic drivers could regulate SR at different spatial and temporal scales, any improvement in determining the mechanisms and drivers responsible for SR variability could be useful to reduce the uncertainties estimated C emissions for terrestrial ecosystems (Meir et al., 2006).

Historically SR has been usually considered as a black box, viewed in terms of its inputs and outputs without considering the internal components and processes, and the mechanisms that regulate them. Far from that, SR is the sum of soil CO_2 effluxes produced by several biological processes, which Kuzyakov (2006) arbitrary summarized in six main sources (Fig. 1):

- *Root respiration*: respiration of the living root tissues, excluding symbionts such as mycorryzal hyphae.
- *Rhizomicrobial respiration*: respiration of rhizodeposits and plant assimilate supplies by microorganisms in the rhizosphere, not including mycorrhiza.
- *Mycorrhizal respiration*: respiration of mycorrhizal fungi, including carbohydrates derived from plant roots.
- *Microbial respiration of dead plant tissues*: respiration of microbes and saprophytic fungi of dead plant tissues, mainly leaves and fine roots.
- *Additional SOM-derived CO_2, priming effect*: stimulation of microbial decomposition of SOM (soil organic matter) affected by recent input rhizodeposits or/and fresh plant residues. The rhizodeposition of easily available C sources can greatly enhance the microbial activity in the rhizosphere. This increment in microbial activity is known as a 'rhizosphere priming effect' (Kuzyakov, 2002).

- *SOM-derived CO_2, basal respiration*: microbial decomposition of SOM in root free soil, frequently referred as 'basal soil respiration'.
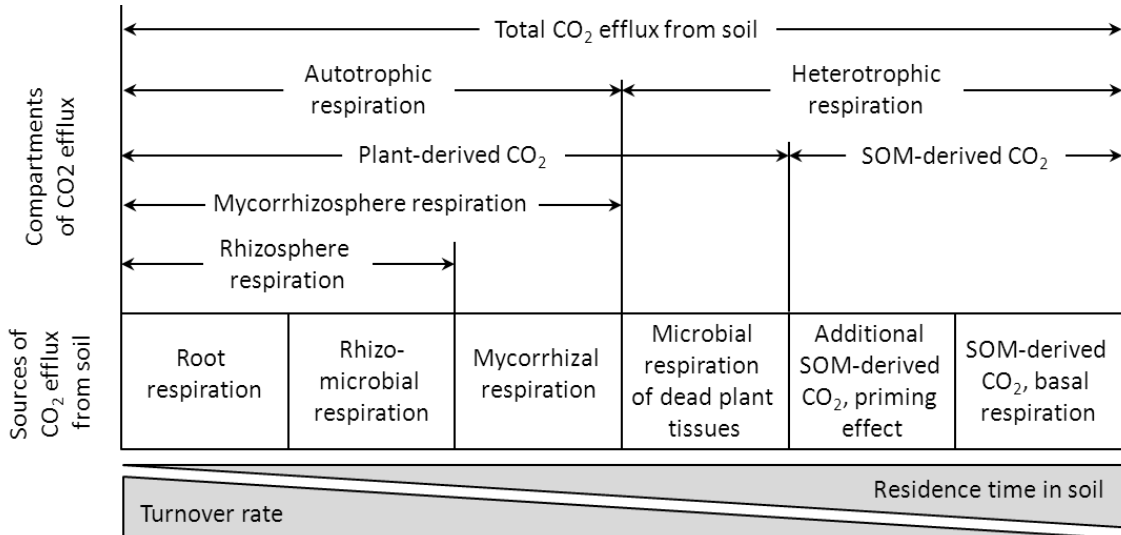

Figure 1. Six main sources of biogenic soil CO_2 efflux (modified after Kuzyakov, 2006). Sources were ordered according the turnover rates and residence times of C in soil.

Other terms combining the previous detailed SR sources have been commonly used due to the difficulty to measure each fraction separately. For instance, rhizosphere respiration has been defined as the sum of root and rhizomicrobial respiration or mycorrhizosphere respiration, which has been defined as rhizosphere (microorganisms directly dependent on root exudation and rhizodeposition) plus mycorrhizal respiration. Additionally, autotrophic and heterotrophic respiration are the most commonly used concepts, describing the root-derived and non-root-derived CO_2, respectively. However, these terms are sometimes used differently by different authors (Kuzyakov, 2006; Moyano et al., 2010).

In this thesis, we used the autotrophic respiration concept as the sum of root respiration (strictly root respiration plus rhizomicrobial respiration), priming effect and mycorrhizal respiration; and heterotrophic respiration concept as the sum of microbial respiration of dead plant tissues and basal respiration rates.

1.2. Soil respiration and its drivers: scale matters

Soil respiration in forests is mainly regulated by soil microclimatic conditions (temperature and soil moisture) (e.g. Jenkinson et al., 1992; Raich and Schlesinger, 1992; Raich et al., 2002) as well

as by the activity of the vegetation, responsible for the supply of carbohydrates to the different biological compartments of soil (Cook and Orchard, 2008; Högberg et al., 2001; Janssens et al., 2001). Microclimatic conditions have a strong effect on SR since the enzyme kinetics involved in carbohydrates oxidation by roots or in microbial decomposition are extremely sensitive to both, water and temperature (Davidson and Janssens, 2006). SR, in the absence of other limiting factors, increases with temperature. The temperature sensitivity of SR has been attributed to the temperature dependence of the biogeochemical processes, described in the 19th century (Arrhenius, 1889). However, the effect of temperature on SR strongly interacts with soil moisture, which may have a bi-modal effect (Davidson et al., 2006). Under very wet conditions, SR decreases due to limitations in O_2 soil diffusivity (Janssens et al., 2001b); but under drought conditions SR decreases due to moisture stress (Asensio et al., 2007b; Rey et al., 2002). The effect of desiccation in the soil metabolism has been mainly attributed to the limitation of diffusion of substrate in water films (Cook and Orchard, 2008; Davidson and Janssens, 2006; Skopp et al., 1990), the inability of most microorganisms, as aquatic organisms, to survive under water limiting conditions (Curiel Yuste et al., 2011) or the reduction of the substrate supply for mycorrhizosphere activity through stomatal closure (Aber et al., 1991). However, the capacity of the different SR components to adapt at local conditions and modulate their responses according to climatic conditions may also vary (Atkin et al., 2000; Curiel Yuste et al., 2014; Curiel Yuste et al., 2010).

Vegetation influences SR directly, via transported assimilated C (Ekblad and Högberg, 2001) or adding organic matter into the soil (Couteaux et al., 1995) and indirectly, by modifying environmental conditions such as light irradiance, soil temperature or soil water content (SWC) (Binkley and Giardina, 1998; Yuan et al., 2012) as well as by determining the composition and diversity of the soil decomposer community (Curiel Yuste et al., 2012; Grayston et al., 1998) which can alter rates of SOM decomposition (Strickland et al., 2009) and their sensitivity to climate (Curiel Yuste et al., 2011).

Importantly, the influence of each one of these biotic and abiotic variables on SR depends on the spatial and temporal scales at which the involved processes are considered (Levin, 1992). Regarding spatial variability, drivers of SR may strongly differ from micro (cm) to regional scales. SR at micro-scale can be explained by SWC and by vegetation-determined properties, such as litter quality and quantity or fine roots attributes (biomass and C:N ratio) (Fóti et al., 2009; Martin and Bolstad, 2009; Stoyan et al., 2000). At ecosystem scales SR variability could be more determined by the interaction between SWC and temperature and by the proximity to vegetation (Søe and Buchmann, 2005). At regional scales, climate (SWC and soil temperature) but specially gross primary production (GPP) are the main drivers of SR (Janssens et al., 2001;

Vargas et al., 2010a). In turn, drivers of temporal variability of SR also vary with the temporal scale at which process is considered. For instance diel variations of SR are mainly explained by soil temperature oscillations or recent photosynthetic activity (Kuzyakov and Gavrichkova, 2010; Ruehr et al., 2010; Tang et al., 2005)) whereas in seasonal or inter-annual variability of SR, SWC also plays a very important role together with soil temperature or plant productivity (Cook and Orchard, 2008; Irvine et al., 2008)). Even more, the different environmental drivers influence differently the different biotic soil CO_2 sources described in the previous section. In general, spatial and temporal variation in heterotrophic respiration is mainly dependant on micro-climatic variables (soil temperature and SWC) (Davidson et al., 1998), whereas autotrophic respiration, apart from being affected by climate, and specially by temperature (Pregitzer et al., 2000), is also usually strongly influenced by photosynthetic-related variables (Högberg et al., 2001).

Because the periods of optimal temperature and optimal moisture conditions hardly coincide, Mediterranean ecosystems add an extra degree of complexity to the temperature-moisture-vegetation regulation of SR (Curiel Yuste et al., 2007; Leon et al., 2014; Rey et al., 2005; Tedeschi et al., 2006). During the long summer droughts the lack of water drastically reduces soil enzymes activity (Sardans and Peñuelas, 2005) and nutrient diffusivity (Davidson and Janssens, 2006), limiting both soil autotrophic (Brunner et al., 2009; Nikolova et al., 2009) and heterotrophic respiration (Curiel Yuste et al., 2007; Reichstein et al., 2002), and thus, total SR (Asensio et al., 2007a; Cotrufo et al., 2011; De Dato et al., 2010). In the same line, controls of plant productivity over SR are particularly intricate, given that the seasonal change of vegetation photosynthetic activity in Mediterranean systems shows complex patterns, strongly subjected to synoptic variations in soil moisture and temperature (Tang and Baldocchi, 2005).

Thus, the mechanisms and controls of vegetation over SR are probably less well known than its climatic controls. The role of vegetation on SR is crucial from local (Tang et al., 2005) to regional scales (Janssens et al., 2001) but drivers regulating this SR-vegetation relationship have been reported to vary drastically over time (Moyano et al., 2008; Tang et al., 2005; Vargas et al., 2011). There is a great consensus about the existence of direct and indirect influences of vegetation on SR, via assimilated C transport to soil or by modifying environmental conditions (see above). But there is large uncertainty about how assimilated C is transported into the soil, which mechanisms are involved on, and how long the photosynthetic signal lasts to determine SR (Kuzyakov and Gavrichkova, 2010; Mencuccini and Holtta, 2010).

At seasonal and inter-annual timescales, vegetation influence over SR corresponds to the changes in belowground allocation (Curiel Yuste et al., 2004) and organic matter input into the

soil through litter deposition (leaves, fine roots, coarse debris, branches) (Bowden et al., 1993; Montero et al., 2005). In this regard, microbial-mediated litter decomposition is a major flux that is determined by climate (temperature and SWC) (Davidson and Janssens, 2006) and litter chemistry (i.e. high N or P content enhance decomposition rates, whereas lignin or holocellulose inhibits them) (Bonanomi et al., 2010; Gallardo and Merino, 1993). Microbial communities are generally able to adapt and optimize the decomposition rates of litter originated from standing vegetation, independently of the chemical characteristics of the litter, in which is known as "home field advantage" (HFA) (Austin et al., 2014; Ayres et al., 2009b). Accordingly, soil communities are generally able to decompose better litter from its own environment (8% on average (Ayres et al., 2009b)). However, this effect is still under debate since empirical evidences are highly variable and the reasons for the occurrence of HFA are little understood (Freschet et al., 2012; Keiser et al., 2014; Veen et al., 2015). Concurrent with the wide extended cases of episodes of climate-change induced forest die-off and subsequent species replacement (e.g. drought-induced secondary succession) several studies have speculated about the possible impact of climate-change-induced vegetation shifts on C dynamics (Ayres et al., 2009a; Ball et al., 2009; Cornwell et al., 2008; Freschet et al., 2013), but there is a lack of field studies testing how climate-induced vegetation shifts may affect the capacity of microbial communities to adapt to new conditions and new litter chemistry.

Beyond the clear influence that seasonal (e.g. Curiel Yuste et al. 2004) and between-year (e.g. Irvine et al., 2008) changes in plant/ecosystem resource availability have over SR, several authors have proposed two different mechanisms by which plant photosynthesis regulates SR (Kuzyakov and Gavrichkova, 2010; Mencuccini and Holtta, 2010). First, C assimilated in leaves may be transported by the phloem into roots to be respired in the rhizosphere. This is a relatively slow type of transport (0.5 to 1.0 m s^{-1}) that may take from several hours to days, depending on the size of the phloem pipeline (i.e. height of the vegetation). Second, fast propagation of waves of turgor and osmotic pressure of phloem due to the upload of the phloem with recently synthesised photosynthates could promote a fast response in SR (after few minutes to hours). This propagation would not necessarily depend on vegetation height. The two mechanisms operate independently and their relative effect over SR variability could differ among species and over seasons (Kuzyakov and Gavrichkova, 2010; Mencuccini and Holtta, 2010).

There exists a critical mass of studies evidencing the strong control that those two mechanisms exert over synoptic variations in biotic soil CO_2 emissions (Ekblad and Högberg, 2001; Högberg et al., 2001; Vargas et al., 2010b). Regarding time-series analyses of the effect of plant photosynthetic activity over SR, there exist several inherent difficulties and we can recognize at

least three limitations that hinder the properly interpretation of the magnitude of the control and the degree of causal-effect relations of those two mechanisms over SR. The first limitation is that solar radiation determines daily and seasonal cycles of different potential drivers of SR (i.e. soil temperature, photosynthesis or vapour pressure deficit) (Baldocchi et al., 2006), resulting in a high degree of autocorrelation between all these explicative and dependent variables (see an illustrative example in Fig. 2). If this autocorrelation is not properly assessed, we could overestimate the effect of photosynthesis or soil temperature on SR.

Figure 2. Example of daily cycles of soil respiration (SR), soil temperature (Temp), solar radiation (Rad), sap flow (SF) and relative air humidity (RH). Scales and units of different variables are not represented (J. Barba, unpublished data).
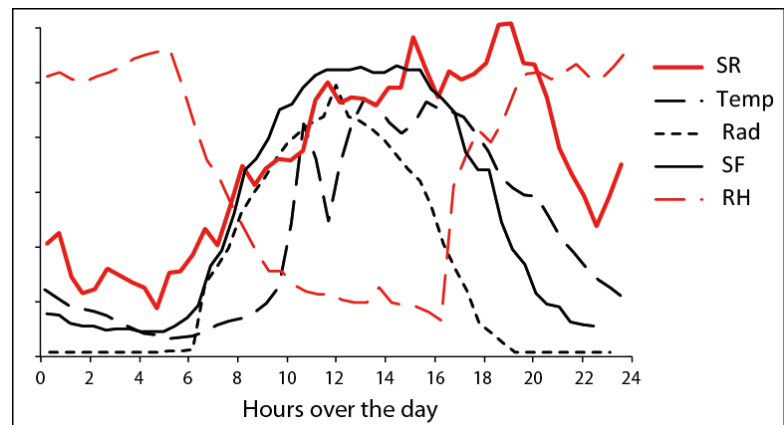
The second limitation is the difficulty to infer causality among variables when there exist a delayed correlation between them, i.e. usually the influence of temperature or photosynthesis on SR are not immediately reflected in SR changes. The time required for the transport of the assimilated C to the soil, the transport of the turgor signal through the phloem to fine roots, the time required to stimulate microbial growth and soil enzyme activity or the time-delay associated with variations in soil diffusivity could produce a time lag between changes in the predictor variables and the response (Baldocchi et al., 2006). Whereas most of the existing methods commonly used to analyse correlations among time series allow assessing the delay of two variables over time (Fourier transformations, cross-correlation analyses or hysteresis analyses (Kuzyakov and Gavrichkova, 2010)) they are not able to study changes over time in the delay and strength of correlations. To our knowledge, only Wavelet coherence analysis could assess not-constant delays between two variables over time, but its use in soil respiration studies has been very limited until now (Vargas et al., 2011, 2010b).

Finally, the third limitation in many studies is that photosynthetic activity is usually estimated at ecosystem level, whereas SR is always determined in a very discreet number of points that can hardly represent a marginal part of the ecosystem soil surface. Variables measured from eddy covariance and/or meteorological towers for a given area, such as Gross primary production

18

(GPP), vapour pressure deficit (VPD), photosynthetic active radiation (PAR), are generally used as surrogates of photosynthesis (Ekblad et al., 2005; Gaumont-Guay et al., 2008; Tang et al., 2005). The use of these variables is useful in biochemical-cycles studies at ecosystem level, but the assumption of constant photosynthesis rates and patterns in all trees growing in a given area introduces noise in the study of the mechanisms with regard to the controls of photosynthetic activity over SR. This error could be even greater in mixed forests or in forests with trees showing different degree of damage after a perturbation within the same footprint area. Thus we need further studies assessing photosynthesis (or its surrogates) and SR measured at tree level in order to improve the knowledge of the mechanisms linking photosynthesis and SR.

2. SOIL RESPIRATION UNDER GLOBAL CHANGE AND FOREST DIE-OFF

As briefly mentioned above, the increase of frequency and intensity of drought events and heat waves in the last decades (Stocker et al., 2013) has been considered responsible for drought-induced mortality events observed worldwide (Allen et al., 2010; Breshears et al., 2005; van Mantgem et al., 2009), being the South of Europe and Mediterranean region particularly affected (Bréda et al., 2006; Briffa et al., 2009; Carnicer et al., 2011; Della-Marta et al., 2007; Martínez-Vilalta and Piñol, 2002; Peñuelas et al., 2001). In the Mediterranean Basin, the intensification of droughts induced by climate change has affected specially to keystone species that meets their Southern limit of distribution in this part of Europe (Carnicer et al., 2011; Lenoir et al., 2010; Vayreda et al., 2013). A paradigmatic case is Scots pine (*Pinus sylvestris* L.), the conifer with broadest distribution in Europe (Critchfield and Little, 1966), that finds its southernmost distribution and driest limit of distribution in the Iberian Peninsula (Jalas and Suominen, 1976). Several drought events in the last decades have resulted in Scots pine die-off at different regions in Europe (Bigler et al., 2006; Galiano et al., 2010), including the Iberian Peninsula (Coll et al., 2013; Martínez-Vilalta et al., 2012; Vilà-Cabrera et al., 2011), where the die-off events are often accompanied by an expansion of *Quercus* species (Carnicer et al., 2014), specially Holm oak (*Quercus ilex* L.) (Galiano et al., 2013; Vilà-Cabrera et al., 2013), which is a typical Mediterranean species (Terradas, 1999) and has its optimal distribution in the west of the Mediterranean Basin (Barbero and Loisel, 1992).

Drought-induced mortality is characterized by a rapid canopy defoliation and by a progressive increase in mortality (Bréda et al., 2006). The phenomenon allows the coexistence of standing dead trees, partially-defoliated trees and apparently-unaffected, healthy trees. If the dominant species has been affected by die-off events, the trajectory of ecosystem C cycling is uncertain (Edburg et al., 2012; Reed et al., 2014), depending on the disturbance intensity, the degree of

overstorey canopy loss and its spatial pattern (clustered or diffuse), the compensatory responses of the surviving trees and the response of the understorey plants to competition release (Amiro et al., 2010; Brown et al., 2010; Gough et al., 2013). Tree mortality could further result on vegetation shift if it is not compensated by the recruitment of the dominant affected species (Lloret et al., 2012). In water-limited environment as the Mediterranean basin, the successful species will likely be those more resistant to drought conditions, such as *Quercus ilex*.

Predictions of Global Circulation Models further suggest that future trends of increasing temperature and decreasing precipitations, will increase drought-induced forest die-off in Mediterranean region (Giorgi and Lionello, 2008; Mariotti, 2010; Stocker et al., 2013). Additionally, land abandonment experienced during the last century in these areas may further accentuate climate change-induced mortality (Vayreda et al., 2011), because the increase in stems density (Vilà-Cabrera et al., 2012) may intensify competition for water. Widespread drought-induced die-off may have important effects on the whole C cycling (Reichstein et al., 2013) and several studies have reported great die-off impacts on soil C fluxes (Anderegg et al., 2013; Curiel Yuste et al., 2012; Moore et al., 2013; Xiong et al., 2011). However, other studies have described some soil compensatory mechanisms after forest perturbation that could results in some degree of functional soil resilience such as resources redistribution, increases of litter inputs, decoupling between C and N biochemical cycles or patterns of C allocation (Gough et al., 2013; Levy-Varon et al., 2014; Nave et al., 2011). Nevertheless, the mechanisms of resilience greatly depend on the species and on the degree of perturbation, which complicates the post-disturbance predictions of dynamics of SR and their components as well as their responses to different environmental drivers (van der Molen et al., 2011). It is expected that the functional resilience after drought events in these studied Mediterranean mixed forests (see below) could be high since firstly, it has been observed for this very same forest that species diversity reduced drought stress (Lebourgeois et al., 2013; Pretzsch et al., 2013) and secondly, because drought-prone environments tend to be more resilient after drought events than ecosystems not adapted to seasonal droughts(Grossiord et al., 2014).

3. SOIL RESPIRATION: PRADES MOUNTAINS AS THE STUDY CASE

The experiments detailed in the current thesis were performed in a mixed Mediterranean forest, at 1010 m a.s.l. on the North face of Titllar valley at Poblet Natural Reserve (Prades Mountains, NE Iberian Peninsula). The substrate consisted on fractured metamorphic schist that outcropped on the 44% of the study area. This great stoniness combined with high steepness (33° on average) resulted in frequent surface mass movements. For more information about the

study site, see Hereter and Sánchez (1999). The forest, unmanaged for the last 40 years (Hereş et al., 2012), and the overstorey was mainly dominated by Scots pine (*Pinus sylvestris* L.) with an important presence of Holm oak (*Quesrus ilex* L.) in the understorey and occasionally achieving the overstorey. Other coexisting species were *Quercus cerrioides* Willk. et Costa, *Taxus baccata* L., *Prunus mahaleb* L., *Ilex aquifolium* L., *Sorbus aria* L., *Sorbus aucuparia* L. and *Sorbus torminalis* L. In Prades mountains, Scots pine populations have suffered drought-induced die-off since the nineties resulting in a 20% of standing mortality (Hereş et al., 2012; Martínez-Vilalta and Piñol, 2002). The abundance of Holm oak in the understorey and the low recruitment of Scots pine in comparison to Holm oak, indicate that Scots pine is being replaced by Holm oaks (Vilà-Cabrera et al., 2013). Although several studies have addressed the causes of die-off and mortality, as well as the resulting stand dynamics, our knowledge is limited about the implications for biogeochemical cycles and the involved mechanisms (Galiano et al., 2011; Poyatos et al., 2013). Particularly, no studies has been performed to assess how Scots pine die-off and replacement by Holm oak may affect soil respiration and its different components. Scots pine and Holm oak are two major coexisting species in the Mediterranean-Eurosiberian ecotone in Europe and future models projections predict the Mediterranean Basin to be largely affected by climate change (Stocker et al., 2013). Therefore, the study of the effect on soil biogeochemical cycles of this species replacement will provide valuable information about how climate-driven shifts on vegetation impact on ecosystem functioning in a changing climate, particularly on biogeochemical cycles of forests.

4. THESIS OBJECTIVES

The general objective of this thesis is to study how vegetation transformations driven by climate change may induce changes in key functional process of the soil compartment, that in turn scale-up to the whole ecosystem. Concretely, we address the question of how drought-induced Scots pine die-off and replacement by Holm oak may affect soil CO_2 effluxes. The study was performed at stand scale in a mixed Mediterranean forest located in Prades mountains (NE Iberian Peninsula), and non-defoliated healthy Scots pine, defoliated Scots pine, dead Scots pine and Holm oak were selected as different stages of this die-off and replacement process. More specifically, the objectives of this thesis are:

Objective 1. Characterize the drivers (soil water content, soil temperature, fine roots biomass and C:N ratio or forest stand structure) of ecosystem-scale spatial variability of soil respiration under drought-induced forest die-off and species replacement (*Ch. 2*).

Objective 2. Determine how environmental drivers and photosynthetic activity regulate soil respiration at different temporal scales (*Ch. 3 and 4*). We are especially interested on determining how vegetation activity influence on soil respiration could shift along the different stages of the Scots pine die-off and replacement by Holm oak from daily to seasonal scales (*Ch. 4*).

Objective 3. Discriminate the contribution of the autotrophic and heterotrophic fractions to soil respiration and determine their seasonal dependence on environmental variables (*Ch. 3*).

Objective 4. Determine how drought-induced die-off and species replacement affect the rates of litter decomposition and nitrogen mineralization of different litter types along the successional gradient (leaves and fine roots of *P. sylvestris* and *Q. ilex*) (*Ch. 5*).

5. REFERENCES

Aber, J.D., Melillo, J.M., Nadelhoffer, K.J., Pastor, J., Boone, R.D., 1991. Factors controlling nitrogen cycling and nitrogen saturation in northern temperate forest ecosystems, Ecol. App. 1, 303-315.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N.G., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H.T., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manage. 259, 660–684.

Amiro, B.D., Barr, A.G., Barr, J.G., Black, T.A., Bracho, R., Brown, M., Chen, J., Clark, K.L., Davis, K.J., Desai, A.R., Dore, S., Engel, V., Fuentes, J.D., Goldstein, A.H., Goulden, M.L., Kolb, T.E., Lavigne, M.B., Law, B.E., Margolis, H.A., Martin, T., McCaughey, J.H., Misson, L., Montes-Helu, M., Noormets, A., Randerson, J.T., Starr, G., Xiao, J., 2010. Ecosystem carbon dioxide fluxes after disturbance in forests of North America. J. Geophys. Res. 115.

Anderegg, W.R.L., Kane, J.M., Anderegg, L.D.L., 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. Nat. Clim. Chang. 3, 30–36.

Arrhenius, S., 1889. ber die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Säuren. Zeitschrift für Phys. Chemie 4, 226.

Asensio, D., Peñuelas, J., Llusia, J., Ogaya, R., Filella, I., 2007a. Interannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought. Soil Biol. Biochem. 39, 2471–2484.

Asensio, D., Peñuelas, J., Ogaya, R., Llusia, J., 2007b. Seasonal soil and leaf CO_2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmos. Environ. 41, 2447–2455.

Atkin, O.K., Edwards, E.J., Loveys, B.R., 2000. Response of root respiration to changes in temperature and its relevance to global warming. New Phytol. 147, 141–154.

Austin, A.T., Vivanco, L., González-Arzac, A., Pérez, L.I., 2014. There's no place like home? An exploration of the mechanisms behind plant litter-decomposer affinity in terrestrial ecosystems. New Phytol. 204, 207–214.

Ayres, E., Steltzer, H., Berg, S., Wall, D.H., 2009a. Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. J. Ecol. 97, 901–912.

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009b. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biol. Biochem. 41, 606–610.

Baldocchi, D., Tang, J., Xu, L., 2006. How switches and lags in biophysical regulators affect spatial-temporal variation of soil respiration in an oak-grass savanna. J. Geophys. Res. 111, 1–13.

Ball, B.A., Bradford, M.A., Coleman, D.C., Hunter, M.D., 2009. Linkages between below and aboveground communities: Decomposer responses to simulated tree species loss are largely additive. Soil Biol. Biochem. 41, 1155–1163.

Barbero, M., Loisel, R., 1992. Biogeography, ecology and history of Mediterranean Quercus ilex ecosystems. Vegetatio 99-100, 19–34.

Bigler, C., Bräker, O.U., Bugmann, H., Dobbertin, M., Rigling, A., 2006. Drought as an inciting mortality factor in scots pine stands of the Valais, Switzerland. Ecosystems 9, 330–343.

Binkley, D., Giardina, C., 1998. Why do tree species affect soils? The warp and woof of tree-soil interactions. Biogeochemistry 42, 89–106.

Bonanomi, G., Incerti, G., Antignani, V., Capodilupo, M., Mazzoleni, S., 2010. Decomposition and nutrient dynamics in mixed litter of Mediterranean species. Plant Soil 331, 481–496.

Bowden, R.D., Nadelhoffer, K.J., Boone, R.D., Melillo, J.M., Garrison, J.B., 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. Can. J. For. Res. 23, 1402-1407.

Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought : a review of ecophysiological responses , adaptation processes and long-term consequences. Rev. Lit. Arts Am. 63, 625–644.

Breshears, D.D., Cobb, N.S., Rich, P.M., Price, K.P., Allen, C.D., Balice, R.G., Romme, W.H., Kastens, J.H., Floyd, M.L., Belnap, J., Anderson, J.J., Myers, O.B., Meyer, C.W., 2005. Regional vegetation die-off in response to global-change-type drought. Proc. Natl. Acad. Sci. U. S. A. 102, 15144–15148.

Briffa, K.R., van der Schrier, G., Jones, P.D., 2009. Wet and dry summers in europe since 1750: Evidence of increasing drought. Int. J. Climatol. 29, 1894–1905.

Brown, M., Black, T.A., Nesic, Z., Foord, V.N., Spittlehouse, D.L., Fredeen, A.L., Grant, N.J., Burton, P.J., Trofymow, J.A., 2010. Impact of mountain pine beetle on the net ecosystem production of lodgepole pine stands in British Columbia. Agric. For. Meteorol. 150, 254–264.

Brunner, I., Pannatier, E.G., Frey, B., Rigling, A., Landolt, W., Zimmermann, S., Dobbertin, M., 2009. Morphological and physiological responses of Scots pine fine roots to water supply in a dry climatic region in Switzerland. Tree Physiol. 29, 541–550.

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., Peñuelas, J., 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proc. Natl. Acad. Sci. U. S. A. 108, 1474–1478.

Carnicer, J., Coll, M., Pons, X., Ninyerola, M., Vayreda, J., Peñuelas, J., 2014. Large-scale recruitment limitation in Mediterranean pines : the role of Quercus ilex and forest successional advance as key regional drivers. Glob. Ecol. Biogeogr. 23, 371–384.

Coll, M., Peñuelas, J., Ninyerola, M., Pons, X., Carnicer, J., 2013. Multivariate effect gradients driving forest demographic responses in the Iberian Peninsula. For. Ecol. Manage. 303, 195–209.

Cook, F.J., Orchard, V.A., 2008. Relationships between soil respiration and soil moisture. Soil Biol. Biochem. 40, 1013–1018.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T. V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V., Westoby, M., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett. 11, 1065–1071.

Cotrufo, M.F., Alberti, G., Inglima, I., Marjanović, H., LeCain, D., Zaldei, A., Peressotti, A., Miglietta, F., 2011. Decreased summer drought affects plant productivity and soil carbon dynamics in a Mediterranean woodland. Biogeosciences 8, 2729–2739.

Couteaux, M.M., Bottner, P., Berg, B., 1995. Litter decomposition climate and litter quality. Trends Ecol. Evol. 10, 63–66.

Critchfield, W.B., Little, E.L., 1966. Geographic distribution of the pines of the world. US Department of Agriculture, Washington.

Curiel Yuste, J., Baldocchi, D.D., Gershenson, A., Goldstein, A., Misson, L., Wong, S., 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. Glob. Chang. Biol. 13, 2018–2035.

Curiel Yuste, J., Barba, J., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Mattana, S., Martínez-Vilalta, J., Nolis, P., Lloret, F., 2012. Changes in soil bacterial community triggered by drought-induced gap succession preceded changes in soil C stocks and quality. Ecol. Evol. 2, 3016–3031.

Curiel Yuste, J., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Ogaya, R., Penuelas, J., Sardans, J., Lloret, F., 2014. Strong functional stability of soil microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline precipitation. Soil Biol. Biochem. 69, 223–233.

Curiel Yuste, J., Janssens, I.A., Carrara, A., Ceulemans, R., 2004. Annual Q10 of soil respiration reflects plant phenological patterns as well as temperature sensitivity. Glob. Chang. Biol. 10, 161–169.

Curiel Yuste, J., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M., Sardans, J., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob. Chang. Biol. 17, 1475–1486.

Curiel Yuste, J., Ma, S., Baldocchi, D.D., 2010. Plant-soil interactions and acclimation to temperature of microbial-mediated soil respiration may affect predictions of soil CO2 efflux. Biogeochemistry 98, 127–138.

Davidson, E.A., Belk, E., Boone, R.D., 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Glob. Chang. Biol. 4, 217–227.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

Davidson, E.A., Janssens, I.A., Luo, Y., 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q10. Glob. Chang. Biol. 12, 154–164.

De Dato, G.D., De Angelis, P., Sirca, C., Beier, C., 2010. Impact of drought and increasing temperatures on soil CO2 emissions in a Mediterranean shrubland (gariga). Plant Soil 327, 153–166.

Della-Marta, P.M., Haylock, M.R., Luterbacher, J., Wanner, H., 2007. Doubled length of western European summer heat waves since 1880. J. Geophys. Res. D Atmos. 112.

Dixon, R.K., Solomon, A.M., Brown, S.E., Houghton, R.A., Trexier, M.C., Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. Science (80-.). 263, 185–90.

Edburg, S.L., Hicke, J.A., Brooks, P.D., Pendall, E.G., Ewers, B.E., Norton, U., Gochis, D., Gutmann, E.D., Meddens, A.J.H., 2012. Cascading impacts of bark beetle-caused tree mortality on coupled biogeophysical and biogeochemical processes. Front. Ecol. Environ. 10, 416–424.

Ekblad, A., Boström, B., Holm, A., Comstedt, D., 2005. Forest soil respiration rate and delta13C is regulated by recent above ground weather conditions. Oecologia 143, 136–142.

Ekblad, A., Högberg, P., 2001. Natural abundance of 13 C in CO 2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia 127, 305–308.

Fóti, S., Nagy, Z., Balogh, J., Bartha, S., Acosta, M., Czóbel, S., Péli, E., Marek, M., Tuba, Z., 2009. Small scale spatial variability and pattern of soil respiration and water content in wet and a dry temperate grasslands and bare soil. Ekologia 28, 389–398.

Freschet, G.T., Aerts, R., Cornelissen, J.H.C., 2012. Multiple mechanisms for trait effects on litter decomposition: Moving beyond home-field advantage with a new hypothesis. J. Ecol. 100, 619–630.

Freschet, G.T., Cornwell, W.K., Wardle, D.A., Elumeeva, T.G., Liu, W., Jackson, B.G., Onipchenko, V.G., Soudzilovskaia, N.A., Tao, J., Cornelissen, J.H.C., 2013. Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. J. Ecol. 101, 943–952.

Galiano, L., Martínez-Vilalta, J., Eugenio, M., Granzow-de La Cerda, Í., Lloret, F., 2013. Seedling emergence and growth of Quercus spp. following severe drought effects on a Pinus sylvestris canopy. J. Veg. Sci. 24, 580–588.

Galiano, L., Martínez-Vilalta, J., Lloret, F., 2010. Drought-Induced Multifactor Decline of Scots Pine in the Pyrenees and Potential Vegetation Change by the Expansion of Co-occurring Oak Species. Ecosystems 13, 978–991.

Galiano, L., Martínez-Vilalta, J., Lloret, F., 2011. Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. New Phytol. 190, 750–759.

Gallardo, A., Merino, J., 1993. Leaf Decomposition in Two Mediterranean Ecosystems of Southwest Spain: Influence of Substrate Quality. Ecology 74, 152–161.

Gaumont-Guay, D., Black, T.A., Barr, A.G., Jassal, R.S., Nesic, Z., 2008. Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand. Tree Physiol. 28, 161–171.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Glob. Planet. Change 63, 90–104.

Gough, C.M., Hardiman, B.S., Nave, L.E., Bohrer, G., Maurer, K.D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., 2013. Sustained carbon uptake and storage following moderate disturbance in a Great Lakes forest. Ecol. Appl. 23, 1202–1215.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem. 30, 369–378.

Grossiord, C., Granier, A., Ratcliffe, S., Bouriaud, O., Bruelheide, H., Chećko, E., Forrester, D.I., Dawud, S.M., Finér, L., Pollastrini, M., Scherer-Lorenzen, M., Valladares, F., Bonal, D., Gessler, A., 2014. Tree diversity does not always improve resistance of forest ecosystems to drought. Proc. Natl. Acad. Sci. U. S. A. 111, 14812–14815.

Hereş, A.M., Martínez-Vilalta, J., Claramunt López, B., 2012. Growth patterns in relation to drought-induced mortality at two Scots pine (Pinus sylvestris L.) sites in NE Iberian Peninsula. Trees 26, 621–630.

Hereter, A., Sánchez, J.R., 1999. Experimental areas of Prades and Montseny, in: Rodà, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.), Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin, pp. 15–27.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–92.

Irvine, J., Law, B.E., Martin, J.G., Vickers, D., 2008. Interannual variation in soil CO_2 efflux and the response of root respiration to climate and canopy gas exchange in mature ponderosa pine. Glob. Chang. Biol. 14, 2848–2859.

Jalas, J., Suominen, J., 1976. Atlas Florae Europaeae, vol 3. Societas Biologica Fennica Vanamo, Helsinki.

Janssens, I.A., Dore, S., Epron, D., Lankreijer, H., Buchmann, N., Longdoz, B., Brossaud, J., Montagnani, L., 2001. Climatic influences on seasonal and spatial differences in soil CO_2 efflux, in: Valentini, R. (Ed.), Canopy Fluxes of Energy, Water and Carbon Dioxide of European Forests. Springer-Verlag, Berlin, Germany, pp. 233–253.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grunwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, U., Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Glob. Chang. Biol. 7, 269–278.

Jenkinson, D.S., Harkness, D.D., Vance, E.D., Adams, D.E., Harrison, A.F., 1992. Calculating net primary production and annual input of organic matter to soil from the amount and radiocarbon content of soil organic matter. Soil Biol. Biochem.

Keiser, A.D., Keiser, D.A., Strickland, M.S., Bradford, M.A., 2014. Disentangling the mechanisms underlying functional differences among decomposer communities. J. Ecol. 102, 603–609.

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects, in: Journal of Plant Nutrition and Soil Science. pp. 382–396.

Kuzyakov, Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biol. Biochem. 38, 425–448.

Kuzyakov, Y., Gavrichkova, O., 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: A review of mechanisms and controls. Glob. Chang. Biol. 16, 3386–3406.

Lebourgeois, F., Gomez, N., Pinto, P., Mérian, P., 2013. Mixed stands reduce Abies alba tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. For. Ecol. Manage. 303, 61–71.

Lenoir, J., Gégout, J.C., Dupouey, J.L., Bert, D., Svenning, J.C., 2010. Forest plant community changes during 1989-2007 in response to climate warming in the Jura Mountains (France and Switzerland). J. Veg. Sci. 21, 949–964.

Leon, E., Vargas, R., Bullock, S., Lopez, E., Panosso, A.R., La Scala, N., 2014. Hot spots, hot moments, and spatio-temporal controls on soil CO_2 efflux in a water-limited ecosystem. Soil Biol. Biochem. 77, 12–21.

Levy-Varon, J.H., Schuster, W.S.F., Griffin, K.L., 2014. Rapid rebound of soil respiration following partial stand disturbance by tree girdling in a temperate deciduous forest. Oecologia 174, 1415–1424.

Lloret, F., Escudero, A., Iriondo, J.M., Martínez-Vilalta, J., Valladares, F., 2012. Extreme climatic events and vegetation: the role of stabilizing processes. Glob. Chang. Biol. 18, 797–805.

Mariotti, A., 2010. Recent Changes in the Mediterranean Water Cycle: A Pathway toward Long-Term Regional Hydroclimatic Change? J. Clim. 23, 1513–1525.

Martin, J.G., Bolstad, P. V, 2009. Variation of soil respiration at three spatial scales: Components within measurements, intra-site variation and patterns on the landscape. Soil Biol. Biochem. 41, 530–543.

Martínez-Vilalta, J., Aguadé, D., Banqué, M., Barba, J., Curiel Yuste, J., Galiano, L., Garcia, N., Gómez, M., Hereş, A.M., López, B.C., Lloret, F., Poyatos, R., Retana, J., Sus, O., Vayreda, J., Vilà-Cabrera, A., 2012. Las poblaciones ibéricas de pino albar ante el cambio climático: con la muerte en los talones. Rev. Ecosistemas 21, 15–21.

Martínez-Vilalta, J., Piñol, J., 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage. 161, 247–256.

Meir, P., Cox, P.M., Grace, J., 2006. The influence of terrestrial ecosystems on climate. Trends Ecol. Evol. 21, 254–260.

Mencuccini, M., Holtta, T., 2010. The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytol. 185, 189–203.

Montero, G., Ruiz-Peinado, R., Muñoz, M., 2005. Producción de biomasa y fijación de CO2 por los bosques españoles. Monografías del INIA. Serie Forestal nº 13., Madrid.

Moore, D.J.P., Trahan, N.A., Wilkes, P., Quaife, T., Stephens, B.B., Elder, K., Desai, A.R., Negron, J., Monson, R.K., 2013. Persistent reduced ecosystem respiration after insect disturbance in high elevation forests. Ecol. Lett. 16, 731–737.

Moyano, F.E., Atkin, O.K., Bahn, M., Bruhn, D., Burton, A.J., Heinemeyer, A., Kutsch, W.L., Wieser, G., 2010. Respiration from roots to mycorrhizosphere, in: Kutsch, W.L., Bahn, M., Heinemeyer, A. (Eds.), Soil Carbon Dynamics. An Integrated Methodology. Cambridge University Press, Cambridge, pp. 127–156.

Moyano, F.E., Kutsch, W.L., Rebmann, C., 2008. Soil respiration fluxes in relation to photosynthetic activity in broad-leaf and needle-leaf forest stands. Agric. For. Meteorol. 148, 135–143.

Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. J. Geophys. Res. Biogeosciences 116.

Nikolova, P.S., Raspe, S., Andersen, C.P., Mainiero, R., Blaschke, H., Matyssek, R., Häberle, K.-H., 2009. Effects of the extreme drought in 2003 on soil respiration in a mixed forest. Eur. J. For. Res. 128, 87–98.

Peñuelas, J., Lloret, F., Montoya, R., 2001. Severe drought effects on Mediterranean woody flora in Spain. For. Sci. 47, 214–218.

Poyatos, R., Aguadé, D., Galiano, L., Mencuccini, M., Martínez-Vilalta, J., 2013. Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytol. 200, 388–401.

Pregitzer, K.S., King, J.S., Burton, A.J., Brown, S.E., 2000. Responses of tree fine roots to temperature. New Phytol. 147, 105–115.

Pretzsch, H., Schütze, G., Uhl, E., 2013. Resistance of European tree species to drought stress in mixed versus pure forests: Evidence of stress release by inter-specific facilitation. Plant Biol. 15, 483–495.

Raich, J.W., Potter, C.S., Bhagawati, D., 2002. Interannual variability in global soil respiration, 1980-94. Glob. Chang. Biol. 8, 800–812.

Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus Ser. B Chem. Phys. Meteorol. 44, 81-99.

Reed, D.E., Ewers, B.E., Pendall, E., 2014. Impact of mountain pine beetle induced mortality on forest carbon and water fluxes. Environ. Res. Lett. 9, 105004.

Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M.D., Seneviratne, S.I., Zscheischler, J., Beer, C., Buchmann, N., Frank, D.C., Papale, D., Rammig, A., Smith, P., Thonicke, K., van der Velde, M., Vicca, S., Walz, A., Wattenbach, M., 2013. Climate extremes and the carbon cycle. Nature 500, 287–95.

Reichstein, M., Tenhunen, J.D., Roupsard, O., Ourcival, J., Rambal, S., Miglietta, F., Peressotti, A., Pecchiari, M., Tirone, G., Valentini, R., 2002. Severe drought effects on ecosystem CO2 and H2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? Glob. Chang. Biol. 8, 999–1017.

Rey, A., Pegoraro, E., Tedeschi, V., De Parri, I., Jarvis, P.G., Valentini, R., 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Glob. Chang. Biol. 8, 851–866.

Rey, A., Petsikos, C., Jarvis, P.G., Grace, J., 2005. Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. Eur. J. Soil Sci. 56, 589–599.

Ruehr, N.K., Knohl, A., Buchmann, N., 2010. Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry 98, 153–170.

Sardans, J., Peñuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biol. Biochem. 37, 455–461.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20.

Skopp, J., Jawson, M.D., Doran, J.W., 1990. Steady-State Aerobic Microbial Activity as a Function of Soil Water Content. Soil Sci. Soc. Am. J.

Søe, A.R.B., Buchmann, N., 2005. Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. Tree Physiol. 25, 1427–1436.

Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Stoyan, H., De-polli, H., Böhm, S., Robertson, G.P., Paul, E.A., 2000. Spatial heterogeneity of soil respiration and related properties at the plant scale. Plant Soil 222, 203–214.

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441–451.

Tang, J., Baldocchi, D.D., 2005. Spatial–temporal variation in soil respiration in an oak–grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. Biogeochemistry 73, 183–207.

Tang, J., Baldocchi, D.D., Xu, L., 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Glob. Chang. Biol. 11, 1298–1304.

Tedeschi, V., Rey, A., Manca, G., Valentini, R., Jarvis, P.G., Borghetti, M., 2006. Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. Glob. Chang. Biol. 12, 110–121.

Terradas, J., 1999. Introduction, in: Roda, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.), Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin, pp. 3–14.

Valentini, R., Matteucci, G., Dolman, A.J., Schulze, E.D., Rebmann, C., Moors, E.J., Granier, A., Gross, P., Jensen, N.O., Pilegaard, K., Lindroth, A., Grelle, A., Bernhofer, C., Grünwald, T., Aubinet, M., Ceulemans, R., Kowalski, A.S., Vesala, T., Rannik, U., Berbigier, P., Loustau, D., Gudmundsson, J., Thorgeirsson, H., Ibrom, A., Morgenstern, K., Clement, R., 2000. Respiration as the main determinant of carbon balance in European forests. Nature 404, 861–865.

Van der Molen, M.K., Dolman, A.J., Ciais, P., Eglin, T., Gobron, N., Law, B.E., Meir, P., Peters, W., Phillips, O.L., Reichstein, M., Chen, T., Dekker, S.C., Doubková, M., Friedl, M.A., Jung, M., van den Hurk, B.J.J.M., de Jeu, R.A.M., Kruijt, B., Ohta, T., Rebel, K.T., Plummer, S., Seneviratne, S.I., Sitch, S., Teuling, A.J., van der Werf, G.R., Wang, G., 2011. Drought and ecosystem carbon cycling. Agric. For. Meteorol. 151, 765–773.

Van Mantgem, P.J., Stephenson, N.L., Byrne, J.C., Daniels, L.D., Franklin, J.F., Fulé, P.Z., Harmon, M.E., Larson, A.J., Smith, J.M., Taylor, A.H., Veblen, T.T., 2009. Widespread increase of tree mortality rates in the western United States. Science 323, 521–524.

Vargas, R., Baldocchi, D.D., Allen, M.F., Bahn, M., Black, T.A., Collins, S.L., Yuste, J.C., Hirano, T., Jassal, R.S., Pumpanen, J., Tang, J., 2010a. Looking deeper into the soil: Biophysical controls and seasonal lags of soil CO2 production and efflux. Ecol. Appl. 20, 1569–1582.

Vargas, R., Baldocchi, D.D., Bahn, M., Hanson, P.J., Hosman, K.P., Kulmala, L., Pumpanen, J., Yang, B., 2011. On the multi-temporal correlation between photosynthesis and soil CO(2) efflux: reconciling lags and observations. New Phytol. 191, 1006–1017.

Vargas, R., Detto, M., Baldocchi, D.D., Allen, M.F., 2010b. Multiscale analysis of temporal variability of soil CO 2 production as influenced by weather and vegetation. Glob. Chang. Biol. 16, 1589–1605.

Vayreda, J., Gracia, M., Martinez-Vilalta, J., Retana, J., 2013. Patterns and drivers of regeneration of tree species in forests of peninsular Spain. J. Biogeogr. 40, 1252–1265.

Vayreda, J., Martínez-Vilalta, J., Gracia, M., Retana, J., 2011. Recent climate changes interact with stand structure and management to determine changes in tree carbon stocks in Spanish forests. Glob. Chang. Biol. 18, 1028–1041.

Veen, C.G.F., Sundqvist, M.K., Wardle, D.A., 2015. Environmental factors and traits that drive plant litter decomposition do not determine home-field advantage effects. Funct. Ecol. n/a–n/a.

Vilà-Cabrera, A., Martínez-Vilalta, J., Galiano, L., Retana, J., 2013. Patterns of Forest Decline and Regeneration Across Scots Pine Populations. Ecosystems 16, 323–335.

Vilà-Cabrera, A., Martínez-Vilalta, J., Vayreda, J., Retana, J., 2011. Structural and climatic determinants of demographic rates of Scots pine forests across the Iberian Peninsula. Ecol. Appl. 21, 1162–1172.

Xiong, Y., D'Atri, J.J., Fu, S., Xia, H., Seastedt, T.R., 2011. Rapid soil organic matter loss from forest dieback in a subalpine coniferous ecosystem. Soil Biol. Biochem. 43, 2450–2456.

Yuan, Z., Gazol, A., Wang, X., Xing, D., Lin, F., Bai, X., Zhao, Y., Li, B., Hao, Z., 2012. What happens below the canopy? Direct and indirect influences of the dominant species on forest vertical layers. Oikos 121, 1145–1153.

Chapter 2

Drought-induced tree species replacement is reflected in the spatial
variability of soil respiration in a mixed Mediterranean forest

Josep Barba, Jorge Curiel Yuste, Jordi Martínez-Vilalta and Francisco Lloret

ABSTRACT

As episodes of drought-induced forest mortality are being increasingly reported worldwide and may become more frequent in the future as a result of climate change, it is essential to characterize their functional implications in terms of ecosystem carbon and water fluxes. We investigated the spatial variability of soil respiration in a mixed Mediterranean forest located on rugged terrain, where Scots pine (*Pinus sylvestris*) is affected by drought-induced dieback and appears to have been replaced by Holm oak (*Quercus ilex*) as the dominant tree species. Soil respiration was measured in spring 2010 on two plots (16.2 X 16.2 m) using a static closed chamber method (soda lime technique) and a systematic sampling (1.8-m grid) including 100 points per plot. Biotic and abiotic variables, such as soil moisture, soil temperature, soil organic matter content, stoniness, pH, fine root C:N ratio and biomass, tree basal area and tree species and health condition of nearest neighbouring tree were also recorded. Our results showed that the spatial variability of soil respiration under optimal environmental conditions (spring) was high and showed no spatial autocorrelation on the scale studied (1-18 m). A mixed-effects model applied to explain the spatial variability of soil respiration indicated that only the variables related to forest structure (i.e., health condition and basal area) explained any of the observed variability of soil respiration (R^2= 0.45). Our model revealed that soil respiration was highest in soils close to dead pines and under Holm oak trees, suggesting that tree mortality and species replacement of pine trees by Holm oak may lead to higher soil respiration fluxes. The direct effect of tree mortality on soil respiration may be a transitory response caused by fine root mortality. Furthermore, the fact that tree species replacement as a result of drought-induced die-off is accompanied by concomitant changes in soil respiration has important implications for soil and ecosystem carbon balance.

1. INTRODUCTION

Soil CO_2 fluxes, resulting from soil heterotrophic (microbes) and autotrophic (root and rhizosphere) respiration, are the second largest flux in the carbon balance of terrestrial ecosystems, after gross primary production (GPP) (Schlesinger and Andrews, 2000), and they play a crucial role in the global carbon cycle. However, given the large temporal and spatial variability of soil respiration, and our limited knowledge of the mechanisms underlying its variability (Curiel Yuste et al., 2007), it is still unclear how soil CO_2 efflux will respond to climate change. Understanding the factors controlling the large spatial variability in soil respiration will help improve future predictions of local-to-global C emissions.

One of the major questions that remain unanswered is the high spatial variability in soil respiration, from the scale of cm (Janssens and Ceulemans, 1998) to larger scales such as meso-scale/ecosystem (Søe and Buchmann, 2005; Rodeghiero and Cescatti, 2008) and macro-scales/regional (Janssens et al., 2001; Vargas et al., 2010). While climatic variables, e.g. soil temperature or soil moisture, determine most of the temporal variability in soil respiration at different scales (Pregitzer et al., 2000; Davidson and Janssens, 2006), the spatial variability of soil respiration is determined by other factors, such as plant community composition, stand structure and/or soil organic matter content (Stoyan et al., 2000; Søe and Buchmann, 2005; Davi et al., 2006). The role of these variables also depends on the spatial scale under study. For instance, at the scale of cm (microscale) most of the variability in soil respiration is usually explained by soil moisture, litter biomass, fine root biomass and C:N ratio (Stoyan et al., 2000; Fóti et al., 2009; Martin and Bolstad, 2009), while at the scale of meters (mesoscale) plant proximity seems to play a very important role (Søe and Buchmann, 2005; Tang and Baldocchi, 2005). At the regional scale, ecosystem productivity coupled with temperature-moisture interactions control soil respiration across ecosystems (Janssens et al., 2001; Reichstein et al., 2003).

Most studies on the spatial variability in soil respiration in ecosystems have been conducted at the meso-scale level on flat terrains with relatively homogeneous conditions (Buchmann, 2000; Søe and Buchmann, 2005; Rodeghiero and Cescatti, 2008; Pickles et al., 2010). Much fewer studies have been carried out on rugged/heterogeneous terrains (Casals et al., 2000; Stoyan et al., 2000) which, even though these on the other hand, account for a substantial portion of the forested areas in the northern hemisphere, particularly in Mediterranean areas such as the Iberian Peninsula (Vallejo, 1983).

In addition to the complexity associated with rugged terrains, summer drought in the Mediterranean climate adds yet another level of complexity to our understanding of variability

in soil respiration (Asensio, Penuelas, Ogaya, et al., 2007; De Dato et al., 2010; Cotrufo et al., 2011). Drought limits the physiological performance of both plants (Brunner et al., 2009; Nikolova et al., 2009) and microbes (Reichstein et al., 2002; Rey et al., 2002; Curiel Yuste et al., 2007), as well as the release of nutrients (Davidson and Janssens, 2006) and enzymes performance (e.g. Sardans and Peñuelas, 2005; Sardans et al., 2008) in the soil pore space. Moreover, the increased frequency and severity of drought events and heat waves (Carnicer et al., 2011) has been considered responsible for widespread events of drought-induced mortality (Allen et al., 2010), including several in the Mediterranean basin (Peñuelas et al., 2001; Martínez-Vilalta and Piñol, 2002; Bréda et al., 2006). This die-off is characterized by rapid defoliation and progressive increase in the mortality of over-storey trees (Bréda et al., 2006). Such widespread mortality may modify regional landscapes on a sub-decadal timescale, with significant implications for stand structure and dynamics and for ecosystem function (Royer et al., 2011). At the ecosystem scale, forest decline may trigger ecological succession and hence modify spatial patterns of plant distribution, which may also strongly affect soil respiration (Tedeschi et al., 2006; Wang and Epstein, 2012). An increase in the frequency, duration and severity of drought and heat stress associated with climate change could, therefore, influence spatial variation in soil respiration either directly (e.g. via limiting water for microbial/root respiration) or indirectly (e.g. via changing the composition, structure and distribution of forests) (Janssens et al., 2001).

We studied the spatial variability in soil respiration in a mixed forest of Scots pine (*Pinus sylvestris* L) and Holm oak (*Quercus ilex* L) in the Prades Mountains (NE Iberian Peninsula). Scots pine is one of the most widely distributed tree species on Earth (Critchfield and Little, 1966) and its southernmost and dry distribution limit lies on the Iberian Peninsula (Jalas and Suominen, 1976). In contrast, Holm oak, which is one of the most common Mediterranean tree species (Terradas, 1999), has its optimal distribution in the west of the Mediterranean Basin, including the Iberian Peninsula (Barbero and Loisel, 1992). The Scots pine population in Prades is affected by climatic drought-induced die-off (Martínez-Vilalta and Piñol, 2002; Hereş et al., 2011) and is seemingly being slowly replaced by the more-drought-adapted Holm oak (Vila-Cabrera et al., 2012).

In more detail, the specific objectives were:

(1) To characterize the spatial variability (i.e. autocorrelation pattern) in soil respiration in a highly rugged mixed forest affected by drought-induced dieback. (2) To determine the minimum number of measurements necessary to estimate average soil respiration with a specific

predefined precision. (3) To identify the role of biotic and abiotic factors determining spatial variability in soil respiration at the stand scale.

2. MATERIALS AND METHODS

2.1. Site description and experimental design

The study was carried out in a mixed forest in Titllar Valley, Prades Mountains (NE Iberian Peninsula; 41°13'N, 0°55'E), at an elevation of between 1,010 and 1,033 m a.s.l. The climate is typically Mediterranean with a mean temperature of 11.2°C and annual mean rainfall of 720 mm (Climatic Digital Atlas of Catalonia (CDAC); (Ninyerola et al., 2000). The substrate consists of fractured metamorphic schist that outcrops on to a large part of the study area. As a consequence of great stoniness combination with steepness (33° on average), the surface of these plots is very unstable, resulting in frequent movements surface mass. Additional information about the study area can be found in Hereter and Sánchez (1999).

The soils are xerochrepts with clay loam texture. However, they are only present in 56% of the study area, whereas the rest of the surface is directly covered by schist outcrops or stones that are just under the organic horizons. Organic horizons cover most of the soil surface with a variable thickness. The mixed forest is composed of Scots pine (*Pinus sylvestris* L) (54% of the total basal area [BA] and mean diameter at breast height [DBH] of 0.32 m) and the evergreen Holm oak (*Quercus ilex* L) (41% of the total BA and mean DBH of 0.15 m). The remaining woody vegetation includes *Quercus cerrioides* Willk. et Costa*, Taxus baccata* L*, Prunus mahaleb* L*, Ilex aquifolium* L*, Amelanchier ovalis* Medik.*, Sorbus aria* L*, Sorbus torminalis* L and *Cistus laurifolius* L*. The Scots pine population is affected by drought-induced die-off, with approximately 20% standing mortality and a varying degree of defoliation in surviving trees (Martínez-Vilalta and Piñol, 2002; Vilà-Cabrera et al., 2012).

We aimed to survey a large number of sampling points, but it was not possible to measure more than 100 points per week. Thus, we measured soil respiration on different weeks and our experimental layout consisted of two plots (called A and B) of about 260 m² (16.2 x 16.2 m) separated by 65 meters. Each plot had 100 measurement points on a regular grid (consecutive points separated by 1.8 m). Both plots were similar in slope, stoniness and stand structure (Table 2). When, due to the great stoniness, it was impossible to take the soil measurements at the exact coordinates on the grid, the measurements were taken within a radius of 20 cm around the grid point, in suitable places. Despite this procedure, it was still impossible to obtain data

from three points on plot A and two points on plot B. All the measurements were made in spring 2010 between April and May.

2.2. Soil respiration and soil parameters

Soil respiration was measured using the soda lime technique, which enabled us to take a large number of measurements simultaneously. Respiration was measured over two consecutive weeks in spring 2010, during the growing season, when temperature and moisture were not limiting factors. On each plot, soil respiration was measured twice at the sampling points in 24 hour-cycles over two consecutive days. The two daily measurements were then averaged for each individual sampling point. In between the sampling weeks of the two plots, a storm of 75 mm occurred (Meteorological Service of Catalonia, www.meteocat.cat); this changed the environmental conditions and air temperature dropped by 11°C.

To measure soil respiration we followed the protocol proposed by Keith and Wong (2006). We used soda lime in granules of 2-4 mm mesh size. Approximately 8 g of soda lime per dish (inert glass) from a total of 200 Petri dishes were oven-dried at 105°C for 14 hours. The dishes were then weighed in order to record the exact initial dry mass of soda lime. The soda lime was then remoisturized using a fine spray (moisture is required in CO_2 absorption) and the dishes were covered with the lids and sealed with electrical PVC insulation tape, placed in airtight plastic bags and transported to the field site. To measure soil respiration we enclosed each Petri dish without the lid in a chamber with a volume of 0.4 L and internal diameter of 10.3cm. Thus, the sample area on which soil respiration was measured was 83.3 cm^2 and soil respiration data had to be referenced to that area. The dimensions of this chamber were smaller than normally recommended in protocols but larger diameters could not be used due to the great stoniness on the site. To minimize chamber heating, the chambers were made of white plastic inert to CO_2. To avoid any interference between Petri dishes and fluxes coming out of the ground, a metal structure was designed to support the Petri dishes 1 cm above the ground within the chamber.

Chambers were inserted 1 cm into the ground to avoid contamination with atmospheric CO_2 and horizontal fluxes of gases within the soil matrix. Deeper insertions were not possible because of the stoniness. Before starting the measurements the mosses and aerial parts of live vegetation were removed to prevent CO_2 uptake. The opening and closing time of each chamber was recorded to exactly determine the absorption period (ca. 24 h). When the measurements were complete, the Petri dishes were sealed again, transported to the laboratory, oven-dried at 105°C for 14 hours and finally weighted to get the final dry mass. Blank measurements were taken into

account for CO_2 absorbed by soda lime not released by soil respiration during the experimental procedure: we intercalated one blank in every 10 measurements, for a total of 40 blanks for the whole survey.

Soda lime measurements were calibrated in the field with an open-path infra-red gas analyzer (IRGA) (EGM-4, pp-Systems, HITCHING, UK) coupled to a closed dynamic chamber. Accordingly, soil respiration was measured at 50 points on two consecutive days: 15 points were measured on the first day with the soda lime technique and on the second with the IRGA system, and another 15 points were measured in reverse order. Additionally, 10 points were measured on both days with the soda lime technique and 10 more were measured with the IRGA system to verify that there were no differences in soil respiration between these two consecutive days (t-test, $P=0.49$). For each point, six soil respiration measurements were made with the IRGA every four hours over a 24 hour period, and these were averaged to calculate the daily estimate and to compare it with the soda lime measurements. A polynomial regression was then applied to estimate the possible underestimation of fluxes by the soda lime technique at high respiration values (Janssens and Ceulemans, 1998).

$$SR = 0.059 \cdot SL^2 + 0.537 \cdot SL + 1.094 \qquad \text{Equation 1}$$

where SR is soil respiration rate ($\mu mol\ m^{-2}\ s^{-1}$) measured with the IRGA and SL is soil respiration rate ($\mu mol\ m^{-2}\ s^{-1}$) measured with the soda lime technique. The equation fit was 0.83 (R^2).

2.3. Ancillary data recorded

Apart from the soil respiration measurements, additional environmental variables were also monitored during the sampling periods. Soil temperature was recorded instantaneously at each point, at a depth of four cm (the most important soil activity is concentrated in the top of soil), five times per day, using a thermometer (OMEGA, HH806AU, Stamford, USA). The five measurements were averaged to get the daily soil temperature at each point of the grid. Stoniness was estimated by two independent observers as the percentage of soil surface occupied by stones in a square (60 X 60 cm with a regular grid of 7.5 X 7.5 cm) around each sampled point.

Soil beneath the chambers was collected the day after the second sampling at each point (1 core of 10.4 cm in diameter for organic horizons and 1-4 cores, depending on the total depth, 4.5 cm in diameter, 0-20 cm depth for mineral horizons). The organic and mineral horizons were separated and their thickness was recorded. Stones were removed manually in the laboratory and samples were sieved with a mesh of 2mm. Soil moisture was determined separately in the soil organic horizons and soil mineral horizons as the difference between fresh and dry mass related to the dry mass in the fine fraction (<2mm). The mass of sieved organic soil was considered a proxy for soil organic matter content. Fine roots were manually removed from all organic horizon samples in the laboratory, following the method proposed by Metcalfe et al. (2007). Fine root carbon and nitrogen concentrations were determined by CHNS organic elemental microanalysis, using combustion coupled with gas chromatography (EUROVECTOR, EA3011, Milano, Italy). pH from the organic horizons of all samples were also measured with an Ag/AgCl combined glass electrode (CRISON, GLP21, Alella, Barcelona, Spain). The samples were mixed with distilled water in a 1:5 proportion.

For each sampling point on the grid we also recorded tree canopy and health state of trees (defoliation) in the zenithal projection. Scots pine coverage was considered a categorical variable ("Cov_SP") covering three levels: healthy pines (defoliation < 30%), defoliated pines (defoliation > 30%) and absence of living pines. Two independent observers visually estimated the degree of pine defoliation as the percentage of green needles of Scots pine trees in relation to an equivalent but completely healthy canopy. Holm-oak coverage ("Cov_HO") was considered as another additional categorical variable, with two levels indicating absence or presence of this species. No signs of defoliation were observed in individuals from this species. Furthermore, different stand structural parameters were determined for each sampling point: cumulative basal area of Scots pine and Holm oak (BA_SP and BA_HO, respectively) located within 3-meters of each point, and the nearest-neighbour tree species type (NN) to each sampling point (healthy pine, defoliated pine, dead pine, healthy Holm-oak).

Tree coordinates and diameter at breast height (DBH) of both living and dead trees were recorded on the two plots. To avoid any edge effects, trees located within a three-meter buffer belt around the plots were also geo-referenced and included in the spatial analyses.

2.4. Spatial and statistical analyses

From our gridded measurements, semi-variograms were used to determine the presence of spatial autocorrelation of the different variables measured during the sampling period.

Table 1.
Summary of the minimum adequate model of soil respiration. NN is the categorical variable "Nearest neighbour type" in three meters belt which has four levels: HP (healthy pine), DFP (defoliated pine), DP (dead pine), and Holm oak (HO). BA_HO is the accumulated basal area of Holm oak in three meters belt. Dead pine is used as the reference category and is included in the intercept.

Variables	Coefficient	Std. error	t-value	p-value
Intercept (NN = DP)	3.021	0.415	7.283	0.000
BA_HO	468.302	174.286	2.687	0.008
NN				
HP	-0.095	0.395	-0.240	0.811
DFP	-0.152	0.370	-0.412	0.682
HO	-0.496	0.288	-1.720	0.090
BA_HO * NN				
BA_HO * HP	-573.213	302.300	-1.896	0.061
BA_HO * DFP	-561.478	230.363	-2.437	0.017
BA_HO * HO	-369.716	178.244	-2.074	0.041

Interpolation maps were created to visualize the distribution of soil respiration, soil temperature, soil moisture, stoniness, soil organic matter, pH and fine roots biomass and C:N ratio at the plot scale.

Mixed-effects models with the 200 measurement points were used to study the influence of abiotic and biotic factors on the spatial variability of soil respiration. Soil moisture (continuous variable [cv]), soil temperature (cv), soil organic matter (cv), stoniness (cv) and pH (cv) were the abiotic explanatory variables. Fine root biomass (cv), fine root C:N ratio (cv), basal area of Scots pine (cv), basal area of Holm oak (cv), Scots pine coverage (discrete variable [dv]), Holm oak coverage (dv) and the nearest neighbour type (dv) were the biotic variables (Table 1). All the models had a random factor that included plot effects and also covered the variation resulting from measuring soil respiration over two weeks under different environmental conditions. We also considered that different measurement points around each tree were not independent, so soil respiration was nested within trees in the models. All the variables and their second-order interactions were added to obtain the saturated model, and they were then removed one by one following a step-wise procedure until the minimum adequate model (MAM) was found. The model's fit was estimated with the AIC (Akaike information criterion) statistic.

All the predictor variables included in the saturated model had an overall correlation lower than 0.4 (see supplementary material). Soil organic horizon moisture was included in the models instead of mineral horizons moisture because both these moisture values were autocorrelated

($R^2=0.51$) and the organic horizons had more samples than the mineral horizons (186 and 123 respectively).

Table 2.
Summary of variables included in the model of soil respiration. BA_SP and BA_HO are cumulative basal area of Scots pine and Holm oak respectively within 3-meters of each sampling point. Cov_SP is the categorical variable "Scots pine coverage" which has three levels: Healthy pine coverage, Defoliated pine coverage and Absence of pine coverage. Cov_HO is the categorical variable "Holm oak coverage" which has two levels: Presence and Absence of Holm oak coverage. NN is the categorical variable "Nearest neighbour type" in three meters belt which has four levels according with the different replacement stages: HP (healthy pine), DFP (defoliated pine), DP (dead pine), and Holm oak (HO). Abbreviations: C.V. = the coefficient of variation; n = number of measurements. Asterisks indicate significant differences between plots (one-way ANOVA. ***: P<0.001; **: P<0.01; *: P<0.05)

Variables	Plot A			Plot B			
	Mean	C.V.	n	Mean	C.V.	n	
Respiration (µmol C m^{-2} s^{-1})	3.04	0.25	97	2.45	0.19	98	***
Soil moisture (%)	36.11	0.87	97	88.06	0.51	98	***
Soil temperature (°C)	13.9	0.05	100	6.5	0.08	100	***
Soil organic matter content (g cm^{-2})	2.29	0.33	97	2.36	0.95	98	
Stoniness (%)	25.82	0.65	100	16.11	1.00	100	*
pH	6.25	0.09	78	6.37	0.11	82	*
Fine roots (g cm^{-2})	15.51	0.34	80	20.88	0.27	90	
C:N roots	37.92	0.47	53	34.05	0.54	69	*
BA_SP (m^2 m^{-2})	0.0015	1.78	100	0.0018	1.57	100	
BA_HO (m^2 m^{-2})	0.0015	0.93	100	0.0014	1.03	100	
Coverage Scots Pine (Cov_SP)			100			100	
Healthy pine (HP)			12			32	
Defoliated pine (DFP)			37			36	
Absence of pine (A_P)			51			32	
Coverage Holm oak (Cov_HO)			100			100	
Presence of Holm oak (P_HO)			72			83	
Absence of Holm oak (A_HO)			28			17	
Nearest neighbour type (NN)			93			88	
Healthy pine (HP)			9			8	
Defoliated pine (DFP)			7			6	
Dead pine (DP)			7			5	
Holm oak (HO)			70			69	

To determine the minimum number of measurements required to estimate an average of soil respiration rate at the plot level with a specified confidence interval, we used the expression:

$$n = \frac{s^2 \cdot t_\alpha^2}{D^2} \qquad \text{Equation 2}$$

where n is the number of samples required; s^2 is the sample variance; t_α is the Student's t-statistic at the α probability level; and D is the specified error limit (Adachi et al., 2005; Rodeghiero and Cescatti, 2008). Furthermore, a resampling routine was implemented to calculate average soil respiration and variance on each plot using an increasing number of samples (n) from our own data set, from n=3 to n=90 (with reposition). For each n, 20 different replicates were obtained by randomly selecting n of our samples. The replicates were then averaged and their variance was calculated as an estimate of precision.

All the analyses were carried out using R 2.9.2 (R Foundation for Statistical Computing, Vienna, Austria). Semi-variograms and interpolation maps were created with the R package gstat (Pebesma, 2004). The mixed-effects model was performed using the R package nlme (Pinheiro et al., 2009).
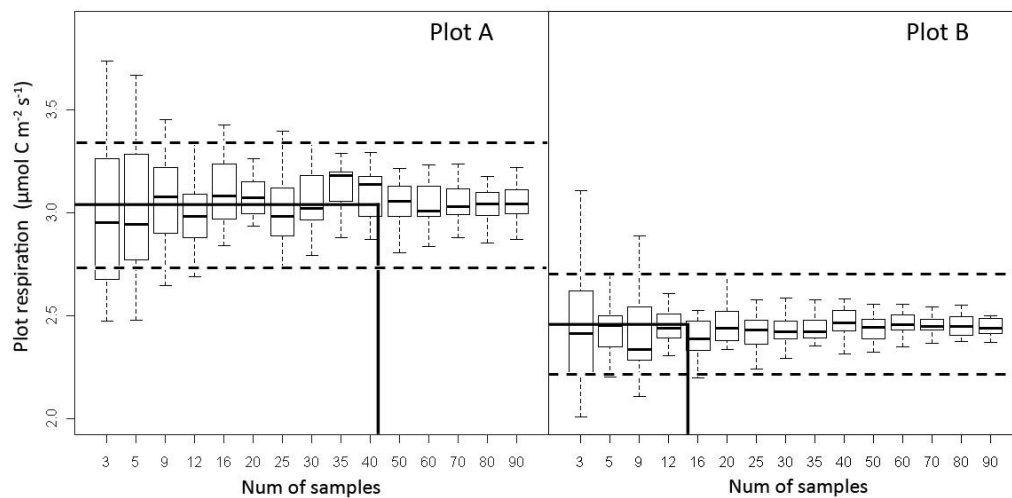
3. RESULTS

Figure 1. Median, quartiles and data range of soil respiration are represented in box-and-whisker plots as a function of sample size. The minimum number of random samples necessary to estimate the mean plot respiration within a specified confidence interval of 20% is indicated by solid lines. The confidence interval is indicated by dashed lines.

3.1. Spatial variability of soil respiration and environmental variables

The mean soil temperature and soil moisture during the sampling period were, respectively, 13.9°C and 23.4% on plot A and 6.5°C and 43.2% on plot B. The storm that occurred between the plot sampling modified the environmental conditions (air temperature decreased 11°C),
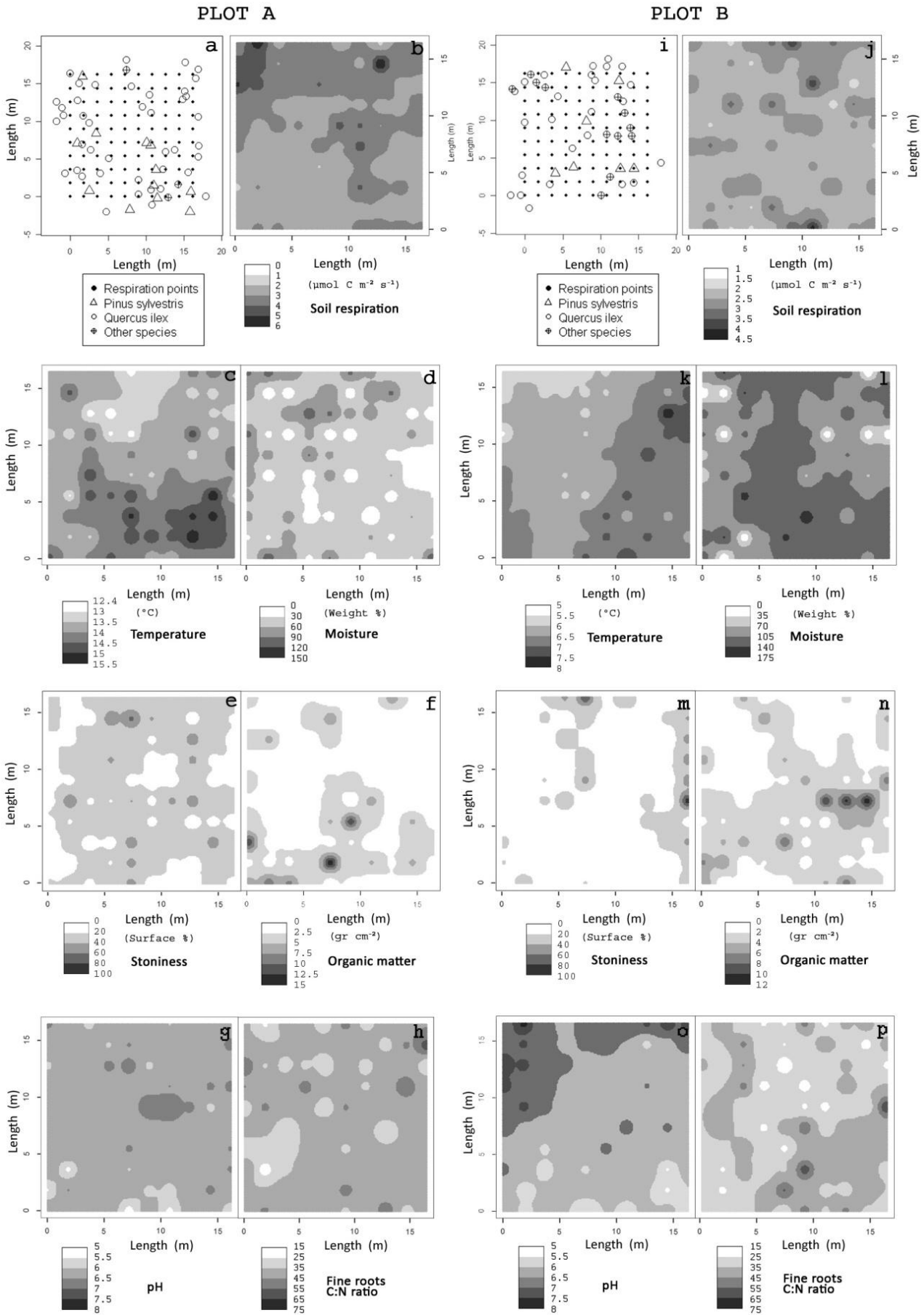
PLOT A

PLOT B

Figure 2. Spatial patterns of measured variables at the study plots. Locations of all measurement points and all trees within the 16.2 x 16.2 m (262 m^2) plots are shown in panels (a) and (i). Black dots

indicate locations where variables (soil respiration, soil temperature, soil moisture, soil organic matter, stoniness, pH, fine roots biomass and C:N ratio) were measured. Tree species are also represented in a two-meter buffer around plot limits. Soil respiration distribution is shown in panels (b) and (j), soil temperature is represented in panels (c) and (k), soil moisture is represented in panels (d) and (l), stoniness is represented in panels (e) and (m), the amount of soil organic matter is shown in panels (f) and (n), organic horizons pH is represented in panels (g) and (o) and fine roots C:N ratio is represented in panels (h) and (p). Variables were measured at 100 locations in a regular grid with a mesh size of 1.8 m. Please note that the data ranges for each variable are not always the same between plots.

producing these large differences between plots, which probably also affected soil respiration, which was 24% higher on plot A than on plot B. The other variables considered in the study were not statistically different between plots (36 stems, 44.5 m^2 ha^{-1} of basal area, 33° of slope and 26% ± 16 of stoniness for plot A; 32 stems, 41.3 m^2 ha^{-1} of basal area, 32° of slope and 16% ± 16 of stoniness for plot B; Table 2). The spatial distribution of environmental variables was highly heterogeneous within plots (Figure 2a and 2i), as supported by the absence of spatial autocorrelation for soil moisture (Figure 2d and 2l), stoniness (Figure 2e and 2m), soil organic matter content (Figure 2f and 2n), fine root biomass, fine roots C:N ratio (Figure 2h and 2p) and pH (Figure 2g and 2o) (see supplementary material).

Soil respiration rates were 3.04 and 2.45 μmol C m^{-2} s^{-1} in plot A and B, respectively, during the measurement campaign (Table 2). The minimum number of samples required to estimate soil respiration as calculated by equation 2 (confidence interval of 20%) was 42 and 15 in plots A and B, respectively (Figure 1). The number of sampling points increased considerably as the CI threshold diminished (in plot A more than 150 sampling points would have been needed to reach a CI of 10%).

Within plots, soil respiration did not exhibit any evident spatial pattern (Figure 2b and 2j), as shown by the absence of spatial autocorrelation: semi-variance remained constant regardless of the distance between measurement points, so two neighbouring measurements were as different as two distant measurements (Figure 3).

3.2. Factors controlling spatial variability of soil respiration

The absence of autocorrelation observed in soil respiration (Figure 3) allowed us to build statistical models while considering our individual measurements as independent (but assuming some degree of correlation between sampling points with the same nearest neighbour tree).

When a mixed-effects model was applied to soil respiration data, the only significant variables ($P<0.05$) in the minimum adequate model (MAM) were the continuous BA_HO (accumulated basal area of Holm oak in the 3 meters belt) and the categorical NN (nearest neighbour type in 3 meters belt), and the interaction between the two (Table 1). BA_HO was positively correlated with soil respiration. The interaction results indicate that, for the same BA_HO, soil respiration was significantly higher for those points located under dead pines than for those located under defoliated pines or Holm oaks, and that the effect of Holm-oak basal area increased more under dead pines than under the other vegetation categories ($P<0.05$) and was marginally higher than for those located under healthy pines ($P<0.07$; Table 1). For a given BA_HO, no differences were found in soil respiration between healthy pines, defoliated pines and Holm oaks. Variables contained in MAM were able to explain almost half of the variance in soil respiration, with a coefficient of determination of 0.45 (R^2).

4. DISCUSSION

4.1. Spatial variability in soil respiration

Soil respiration values measured at plot level were similar to those reported for springtime in other Mediterranean forests (Rey et al., 2002; Joffre et al., 2003; Tang and Baldocchi, 2005) and were particularly close to those measured in nearby valleys in the same natural reserve by Asensio et al. (2007) (from 1.35 to 3.7 μmol C m^{-2} s^{-1}), but higher than those reported by Piñol et al. (1995) (0.63 μmol C m^{-2} s^{-1}). The great difference between plots in the minimum number of samples required to estimate the average soil respiration is most probably the result of the storm that occurred between the plot measurements. A decrease of 7.5°C in soil temperature between plots could produce not only a significant decrease in soil respiration but also a reduction in soil respiration variability (CV decreased from 0.25 to 0.19).

Other studies on plots with similar or larger dimensions required lower minimum sampling size to reach similar C soil respiration estimates (Yim et al., 2003) or similar sampling size (Adachi et al., 2005; Rodeghiero and Cescatti, 2008). Although the minimum sampling size might not be a perfect proxy for heterogeneity because of the lack of a standardized methodology to measure the spatial variability of soil respiration (i.e. small chambers do not integrate the variability at small scale in the way that large chambers may do (Keith and Wong, 2006; Rodeghiero and Cescatti, 2008)), our finding shows that

although the ecosystem under study is very complex and highly heterogeneous, the variation in soil respiration is not greater than previous studies on more homogeneous terrains (Buchmann, 2000; Rodeghiero and Cescatti, 2008; Merbold et al., 2011).

Spatial distribution maps (Figure 2), high coefficients of variation (Table 2) and the absence of autocorrelation for soil respiration and most of the predictor variables (Figure 3 and supplementary material (A.1)) all support the idea that at the plot scale (1 – 16 m) the studied soils presented a high spatial heterogeneity. Studies in spatial variability of soil respiration indicate, however, that spatial patterns of soil respiration are strongly dependant on site and conditions. While several studies from tropical rainforests to Mediterranean mountains have described spatial autocorrelation of soil respiration at the scale of meters (from 2 to 10m) (Kosugi et al., 2007; Herbst et al., 2009; Chatterjee and Jenerette, 2011), which are scales similar to those applied in our study, others have found autocorrelation to occur only at smaller scales (from cm to 1m) (in grassland or plantation) (Stoyan et al., 2000; Fóti et al., 2009). Similar lack of autocorrelation to our study has been observed in a Mediterranean forest with a canopy at different development stages (Tedeschi et al., 2006). In our case, the high surface and sub-surface stoniness and the high ground instability as a result of the steep slope may partially explain the lack of spatial structure. Our results do not, however, allow us to rule out the existence of spatial structure of soil respiration at smaller (micro-local, cm) or even larger scales (landscape, from hectares to km^2); these concepts merit further exploration.
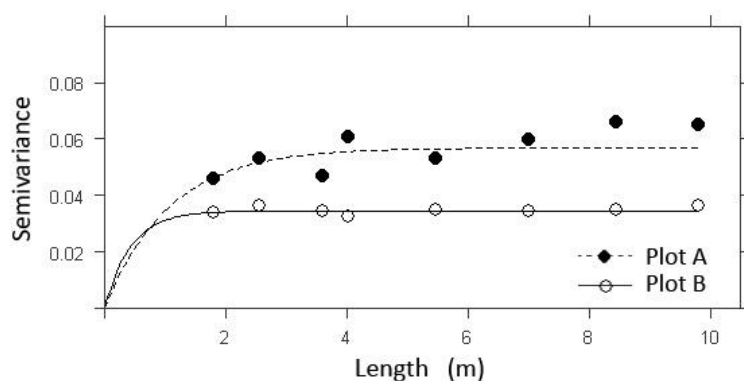

Figure 3. Semi-variograms of the transformed soil respiration data at different spatial scales within the plots.

4.2. Factors controlling spatial variability in soil respiration

Soil temperature has been reported as the main factor controlling soil respiration at a regional scale (Janssens et al., 2001; Reichstein et al., 2003; Davidson and Janssens, 2006; Wei et al., 2010), but at smaller spatial scales, the low variability in soil temperature might

be responsible for soil temperature's of the lack of effect on soil respiration variability. Soil moisture, which is usually the most limiting factor in Mediterranean ecosystems (Orchard and Cook, 1983; Chaves et al., 2002), did not demonstrate any effect on soil respiration, despite its high variability (Table 2). Water availability, despite showing a certain correlation with soil respiration (see supplementary material, (B)), may not have been limiting soil respiration in spring at the scale under study. Similar lack of abiotic control over the spatial variability of soil respiration has previously been observed in a Mediterranean oak forest in central Italy (Tedeschi et al., 2006) and a temperate beech forest in Germany (Buchmann, 2000). In our case, the great differences in soil respiration rates observed between plots can probably be explained by the differences in soil moisture and soil temperature between the sampling dates. However, the explanatory power of climate was negligible at the spatial scale. Although plot A was not as wet as plot B, soil respiration was higher on plot A, probably because plot A had enough soil moisture and much higher soil temperature than plot B.

Our best model (MAM) revealed the strong link between soils and canopy. Soil respiration was best explained by variables associated with vegetation structure: soil respiration was positively correlated with Holm-oak basal area (BA_HO) and its interaction with the nearest neighbour type (NN) (particularly with dead Scots pines (Table 1)). Our results highlight the strong cause-effect relationship between these two different ecological compartments, soil and aboveground canopy, which explains soil respiration variability at the ecosystem scale. In our study, the strong climatic constraints that resulted in canopy die-off were therefore reflected in soil C dynamics, at least in spring during the growing season. These results agree with previous studies showing that, at the ecosystem level, variability of soil respiration is strongly influenced by vegetation distribution and activity (Buchmann, 2000; Reichstein et al., 2003; Søe and Buchmann, 2005; Vargas et al., 2011). Vegetation affects soil respiration indirectly, e.g. by modifying soil microclimate (soil moisture and soil temperature) due to sun-radiation interception and soil water evapo-transpiration (Binkley and Giardina, 1998), or directly, e.g. by continuously providing photosynthates to the radical organs (e.g. Höberg et al. 2001) and to soil microbial communities, in the form of dead organic matter (litter-fall and roots) (Vogt and Persson, 1991; Schmidt et al., 2011) or labile C by rhizodeposition (e.g. exudation) (Grayston et al., 1997; Kuzyakov and Domanski, 2000), which feeds both symbiotic-forming fungi (e.g. mycorrhiza) (Phillips and Fahey, 2005) and free living microbes (Kuzyakov, 2002). In particular it seems that the autotrophic component of soil respiration (roots and mycorrhizes respiration), which generally accounts for 30 to 65% of total soil respiration, is strongly dependent on the supply of new photosynthates (Craine et al., 1999; Ekblad

and Högberg, 2001; Högberg et al., 2001, 2008; Heinemeyer et al., 2007; Subke et al., 2011; Vargas et al., 2011). Thus, in our case it is difficult to understand the lack of explicatory power of C:N of fine roots in the upper soil, which is a variable that is typically well correlated with radical metabolic activity (Pregitzer et al., 1998; Burton et al., 2002). It may be because we underestimated the amount of fine root biomass. We only sampled roots from the soil organic horizons (it was not possible to take samples from the soil mineral horizons at most points due to the great stoniness). However, fine roots on Mediterranean soils are not necessarily concentrated in the upper cm of the soil profile due to high exposure to strong fluctuations in water availability (Lopez et al., 2001).

The significant positive effect of HO basal area on soil respiration indicates that soil metabolic activity was clearly stimulated by the presence of this particular tree species. Holm oak is a species well adapted to seasonally dry Mediterranean conditions (Terradas, 1999), and it has not been as affected by increasing summer droughts as the Scots pine (Aguadé, 2011). It has also demonstrated a much better regeneration rate than Scots pine in the study area (Martínez-Vilalta and Piñol, 2002; Vilà-Cabrera et al., 2012) and it is therefore expected that the declining Scots pine populations will be progressively replaced by this more drought-resistant species. We propose that the observed positive effect of Holm oak basal area on soil respiration under dead pines responds to the positive effect that forest species replacement may exert on soil metabolic activity in gaps left by pines. Once a pine tree has died, the gap created could become susceptible to colonization by rhizospheres from other tree species better adapted to drought conditions and their associated microbial communities. Both roots and microbes could compete for the space and pool of nutrients and organic matter made relatively accessible for exploitation once the pine rizhosphere is no longer (Curiel Yuste et al., 2012). Indeed, the values of fine root (<2 mm) biomass and C:N ratio, the latter an indicator of fine root activity (Pregitzer et al., 1998; Burton et al., 2002), were not significantly different beneath dead pines as compared to living trees (P= 0.9 for fine root biomass; P= 0.5 for C:N ratio), suggesting that gap colonization by living vegetation was already taking place below ground on the sampling date. The convergence of key abundant bacterial taxa between soils beneath dead pines and Holm-oaks, which has been observed in a parallel study (Curiel Yuste et al., 2012), further supports this idea.

5. CONCLUSIONS

Our study highlights the complexity of the ecosystem-scale spatial patterns of soil respiration in this mountainous Mediterranean mixed forest subject to drought-induced species replacement. The lack of autocorrelation between the soil respiration measurements could be attributed to high spatial heterogeneity at the sampling scale under study (around 2-16 meters). Despite this large heterogeneity and the poor explicatory power of most environmental and abiotic factors, it was found that variables associated with forest structure, particularly the interaction between the basal area of HO and proximity to dead pines, were able to explain almost half of the observed variability in soil respiration. At ecosystem level, our results indicate that the spatial variability of soil respiration might not be regulated solely by variables typically involved in regulating soil metabolic activity (e.g. climate and plant productivity) but also by other ecologically driven processes involved in drought-induced secondary succession, e.g. below-ground gap colonization and resource exploitation. Although it is difficult to make generalizations about the overall ecosystem response to climate change from measurements made in a brief period, our results suggested that these drought-induced changes could imply substantial changes in soil respiration fluxes, not only because of the different species composition but also as a result of the intermediate phases of the species replacement process. Since the number of drought-induced forest die-backs is likely to increase (Allen et al., 2010), the impact of drought-induced forest die-off and subsequent ecological succession on soil C cycle merits further exploration at a local scale.

7. REFERENCES

Adachi, M., Bekku, Y., Konuma, A., Kadir, W., Okuda, T., Koizumi, H., 2005. Required sample size for estimating soil respiration rates in large areas of two tropical forests and of two types of plantation in Malaysia. Forest Ecology and Management 210, 455–459.

Aguadé, D., 2011. Water relations of Scots pine at its dry limit: effects of crown condition and competition by co-occurring Holm oak. Master project. Autonomous University of Barcelona.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N.G., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H.T., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259, 660–684.

Asensio, D., Penuelas, J., Llusia, J., Ogaya, R., Filella, I., 2007. Interannual and interseasonal soil CO_2 efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought. Soil Biology Biochemistry 39, 2471–2484.

Asensio, D., Penuelas, J., Ogaya, R., Llusia, J., 2007. Seasonal soil and leaf CO_2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmospheric Environment 41, 2447–2455.

Barbero, M., Loisel, R., 1992. Biogeography, ecology and history of Mediterranean Quercus ilex ecosystems. Vegetatio 99-100, 19–34.

Binkley, D., Giardina, C., 1998. Why do tree species affect soils? The warp and woof of tree-soil interactions. Biogeochemistry 42, 89–106.

Brunner, I., Pannatier, E.G., Frey, B., Rigling, A., Landolt, W., Zimmermann, S., Dobbertin, M., 2009. Morphological and physiological responses of Scots pine fine roots to water supply in a dry climatic region in Switzerland. Tree Physiology 29, 541–550.

Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Review Literature And Arts Of The Americas 63, 625–644.

Buchmann, N., 2000. Biotic and abiotic factors controlling soil respiration rates in Picea abies stands. Soil Biology and Biochemistry 32, 1625–1635.

Burton, A.J., Pregitzer, K.S., Ruess, R., Hendrick, R., Allen, M.F., 2002. Root respiration in North American forests: effects of nitrogen concentration and temperature across biomes. Oecologia 131, 559–568.

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., Peñuelas, J., 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences of the United States of America 108, 1474–1478.

Casals, P., Romanya, J., Cortina, J., Bottner, P., Couteaux, M.M., Vallejo, V.R., 2000. CO 2 efflux from a Mediterranean semi-arid forest soil. I. Seasonality and effects of stoniness. Biogeochemistry 48, 261–281.

Chatterjee, A., Jenerette, G.D., 2011. Spatial variability of soil metabolic rate along a dryland elevation gradient. Landscape Ecology 26, 1111–1123.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osória, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How Plants Cope with Water Stress in the Field? Photosynthesis and Growth. Annals of Botany 89, 907–916.

Cotrufo, M.F., Alberti, G., Inglima, I., Marjanović, H., LeCain, D., Zaldei, A., Peressotti, A., Miglietta, F., 2011. Decreased summer drought affects plant productivity and soil carbon dynamics in a Mediterranean woodland. Biogeosciences 8, 2729–2739.

Craine, J.M., Wedin, D.A., Chapin, F.S., 1999. Predominance of ecophysiological controls on soil CO2 flux in a Minnesota grassland. Plant and Soil 207, 77–86.

Critchfield, W.B., Little, E.L., 1966. Geographic distribution of the pines of the world. US Department of Agriculture, Washington.

Curiel Yuste, J., Baldocchi, D.D., Gershenson, A., Goldstein, A., Misson, L., Wong, S., 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. Global Change Biology 13, 2018–2035.

Curiel Yuste, J., Barba, J., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Mattana, S., Martínez-Vilalta, J., Nolis, P., Lloret, F., 2012. Changes in soil bacterial community triggered by drought-induced gap succession preceded changes in soil C stocks and quality. Ecology and Evolution 2, 3016–3031.

De Dato, G.D., De Angelis, P., Sirca, C., Beier, C., 2010. Impact of drought and increasing temperatures on soil CO2 emissions in a Mediterranean shrubland (gariga). Plant and Soil 327, 153–166.

Davi, H., Dufrene, E., Francois, C., Lemaire, G., Loustau, D., Bosc, A., Rambal, S., Granier, A., Moors, E.J., 2006. Sensitivity of water and carbon fluxes to climate changes from 1960 to 2100 in European forest ecosystems. Agricultural and Forest Meteorology 141, 35–56.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

Ekblad, A., Högberg, P., 2001. Natural abundance of 13 C in CO 2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia 127, 305–308.

Fóti, S., Nagy, Z., Balogh, J., Bartha, S., Acosta, M., Czóbel, S., Péli, E., Marek, M., Tuba, Z., 2009. Small scale spatial variability and pattern of soil respiration and water content in wet and a dry temperate grasslands and bare soil. Ekologia 28, 389–398.

Grayston, S.J., Vaughan, D., Jones, C.D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5, 29–56.

Heinemeyer, A., Hartley, I.P., Evans, S.P., De La Fuente, J.A.C., Ineson, P., 2007. Forest soil CO2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology 13, 1786–1797.

Herbst, M., Prolingheuer, N., Graf, A., Huisman, J.A., Weihermüller, L., Van derborghtJ, 2009. Characterization and Understanding of Bare Soil Respiration Spatial Variability at Plot Scale. Vadose Zone Journal 8, 762–771.

Hereter, A., Sánchez, J.R., 1999. Experimental areas of Prades and Montseny, in: Rodà, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.), Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin, pp. 15–27.

Hereş, A.-M., Martínez-Vilalta, J., Claramunt López, B., 2011. Growth patterns in relation to drought-induced mortality at two Scots pine (Pinus sylvestris L.) sites in NE Iberian Peninsula. Trees 26, 621–630.

Högberg, P., Högberg, M.N., Göttlicher, S.G., Betson, N.R., Keel, S.G., Metcalfe, D.B., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S., Näsholm, T., 2008. High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. New Phytologist 177, 220–228.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–92.

Jalas, J., Suominen, J., 1976. Atlas Florae Europaeae, vol 3. Societas Biologica Fennica Vanamo, Helsinki.

Janssens, I.A., Ceulemans, R., 1998. Spatial variability in forest soil CO_2 efflux assessed with a calibrated soda lime technique. Ecology Letters 1, 95–98.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grunwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, U., Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biology 7, 269–278.

Joffre, R., Ourcival, J.M., Rambal, S., Rocheteau, A., 2003. The key-role of topsoil moisture on CO_2 efflux from a Mediterranean Quercus ilex forest. Annals of Forest Science 60, 519–526.

Keith, H., Wong, S., 2006. Measurement of soil CO_2 efflux using soda lime absorption: both quantitative and reliable. Soil Biology and Biochemistry 38, 1121–1131.

Kosugi, Y., Mitani, T., Itoh, M., Noguchi, S., Tani, M., Matsuo, N., Takanashi, S., Ohkubo, S., Rahim Nik, A., 2007. Spatial and temporal variation in soil respiration in a Southeast Asian tropical rainforest. Agricultural and Forest Meteorology 147, 35–47.

Kuzyakov, Y., 2002. Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. Soil Biology and Biochemistry 34, 1621–1631.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. Journal Of Plant Nutrition And Soil Science 163, 421–431.

Lopez, B.C., Sabaté, S., Gracia, C.A., 2001. Vertical distribution of fine root density, length density, area index and mean diameter in a Quercus ilex forest. Tree Physiology 21, 555–560.

Martin, J.G., Bolstad, P.V., 2009. Variation of soil respiration at three spatial scales: Components within measurements, intra-site variation and patterns on the landscape. Soil Biology and Biochemistry 41, 530–543.

Martínez-Vilalta, J., Piñol, J., 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. Forest Ecology and Management 161, 247–256.

Merbold, L., Ziegler, W., Mukelabai, M.M., Kutsch, W., 2011. Spatial and temporal variation of CO_2 efflux along a disturbance gradient in a miombo woodland in Western Zambia. Biogeosciences 8, 147–164.

Metcalfe, D.B., Williams, M., Aragão, L.E.O.C., Da Costa, A.C.L., De Almeida, S.S., Braga, A.P., Gonçalves, P.H.L., De Athaydes, J., Junior, S., Malhi, Y., Meir, P., 2007. A method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy. New Phytologist 174, 697–703.

Nikolova, P.S., Raspe, S., Andersen, C.P., Mainiero, R., Blaschke, H., Matyssek, R., Häberle, K.-H., 2009. Effects of the extreme drought in 2003 on soil respiration in a mixed forest. European Journal of Forest Research 128, 87–98.

Ninyerola, M., Pons, X., Roure, J.M., 2000. A methodological approach of climatological modelling of air temperature and precipitation through GIS techniques. International Journal of Climatology 20, 1823–1841.

Orchard, V.A., Cook, F.J., 1983. Relationship between soil respiration and soil moisture. Soil Biology and Biochemistry 15, 447–453.

Pebesma, E., 2004. Multivariable geostatistics in S: the gstat package*1. Computers & Geosciences 30, 683–691.

Peñuelas, J., Lloret, F., Montoya, R., 2001. Severe drought effects on Mediterranean woody flora in Spain. Forest Science 47, 214–218.

Phillips, R.P., Fahey, T.J., 2005. Patterns of rhizosphere carbon flux in sugar maple (Acer saccharum) and yellow birch (Betula allegheniensis) saplings. Global Change Biology 11, 983–995.

Pickles, B.J., Genney, D.R., Potts, J.M., Lennon, J.J., Anderson, I.C., Alexander, I.J., 2010. Spatial and temporal ecology of Scots pine ectomycorrhizas. New Phytologist 186, 755–768.

Pinheiro, J., Bates, D., DepRoy, S., 2009. Linear and Nonlinear Mixed Effects Models. R package version 3.1-96.

Piñol, J., Alcaniz, J.M., Roda, F., 1995. Carbon dioxide efflux and pCO_2 in soils of three Quercus ilex montane forests. Biogeochemistry 30, 191–215.

Pregitzer, K.S., King, J.S., Burton, A.J., Brown, S.E., 2000. Responses of tree fine roots to temperature. New Phytologist 147, 105–115.

Pregitzer, K.S., Laskowski, M.J., Burton, A.J., Lessard, V.C., Zak, D.R., 1998. Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiology 18, 665–670.

Reichstein, M., Rey, A., Freibauer, A., Tenhunen, J., Valentini, R., Banza, J., Casals, P., Cheng, Y.F., Grunzweig, J.M., Irvine, J., Joffre, R., Law, B.E., Loustau, D., Miglietta, F., Oechel, W., Ourcival, J.M., Pereira, J.S., Peressotti, A., Ponti, F., Qi, Y., Rambal, S., Rayment, M., Romanya, J., Rossi, F., Tedeschi, V., 2003. Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices - art. no. 1104. Global Biogeochemical Cycles 17, 1104–1115.

Reichstein, M., Tenhunen, J.D., Roupsard, O., Ourcival, J., Rambal, S., Miglietta, F., Peressotti, A., Pecchiari, M., Tirone, G., Valentini, R., 2002. Severe drought effects on ecosystem CO_2 and H_2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? Global Change Biology 8, 999–1017.

Rey, A., Pegoraro, E., Tedeschi, V., De Parri, I., Jarvis, P.G., Valentini, R., 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biology 8, 851–866.

Rodeghiero, M., Cescatti, A., 2008. Spatial variability and optimal sampling strategy of soil respiration. Forest Ecology and Management 255, 106–112.

Royer, P.D., Cobb, N.S., Clifford, M.J., Huang, C.-Y., Breshears, D.D., Adams, H.D., Villegas, J.C., 2011. Extreme climatic event-triggered overstorey vegetation loss increases understorey solar input regionally: primary and secondary ecological implications. Journal of Ecology 99, 714–723.

Sardans, J., Penuelas, J., Ogaya, R., 2008. Experimental drought reduced acid and alkaline phosphatase activity and increased organic extractable P in soil in a Quercus ilex Mediterranean forest. European Journal of Soil Biology 44, 509–520.

Sardans, J., Peñuelas, J., 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biology and Biochemistry 37, 455–461.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.

Stoyan, H., De-polli, H., Böhm, S., Robertson, G.P., Paul, E.A., 2000. Spatial heterogeneity of soil respiration and related properties at the plant scale. Plant and Soil 222, 203–214.

Subke, J.-A., Voke, N.R., Leronni, V., Garnett, M.H., Ineson, P., 2011. Dynamics and pathways of autotrophic and heterotrophic soil CO2 efflux revealed by forest girdling. Journal of Ecology 99, no–no.

Søe, A.R.B., Buchmann, N., 2005. Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. Tree Physiology 25, 1427–1436.

Tang, J., Baldocchi, D.D., 2005. Spatial–temporal variation in soil respiration in an oak–grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. Biogeochemistry 73, 183–207.

Tedeschi, V., Rey, A., Manca, G., Valentini, R., Jarvis, P.G., Borghetti, M., 2006. Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. Global Change Biology 12, 110–121.

Terradas, J., 1999. Introduction, in: Roda, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.), Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin, pp. 3–14.

Vallejo, V.R., 1983. Estudio de los suelos forestales de la Depresión Central Catalana. PhD Thesis. Universitat de Barcelona.

Vargas, R., Baldocchi, D.D., Bahn, M., Hanson, P.J., Hosman, K.P., Kulmala, L., Pumpanen, J., Yang, B., 2011. On the multi-temporal correlation between photosynthesis and soil CO(2) efflux: reconciling lags and observations. New Phytologist 191, 1006–1017.

Vargas, R., Detto, M., Baldocchi, D.D., Allen, M.F., 2010. Multiscale analysis of temporal variability of soil CO 2 production as influenced by weather and vegetation. Global Change Biology 16, 1589–1605.

Vilà-Cabrera, A., Martínez-Vilalta, J., Retana, J., 2013. (in press) Patterns of forest decline and regeneration across Scots pine populations. Ecosystems 16, 323-335.

Vogt, K.A., Persson, H., 1991. Measuring growth and development of roots, in: Lassoie, J.P., Hinckley, T.M. (Eds.), Techniques and Approaches in Forest Tree Ecophysiology. CRC Press, pp. 477–501.

Wang, J., Epstein, H.E., 2013. Estimating carbon source-sink transition during secondary succession in a Virginia valley. Plant and Soil 362, 135-147.

Wei, W., Weile, C., Shaopeng, W., 2010. Forest soil respiration and its heterotrophic and autotrophic components: Global patterns and responses to temperature and precipitation. Soil Biology and Biochemistry 42, 1236–1244.

Yim, M.H., Joo, S.J., Shutou, K., Nakane, K., 2003. Spatial variability of soil respiration in a larch plantation: estimation of the number of sampling points required. Forest Ecology and Management 175, 585–588.

8. APPENDICES
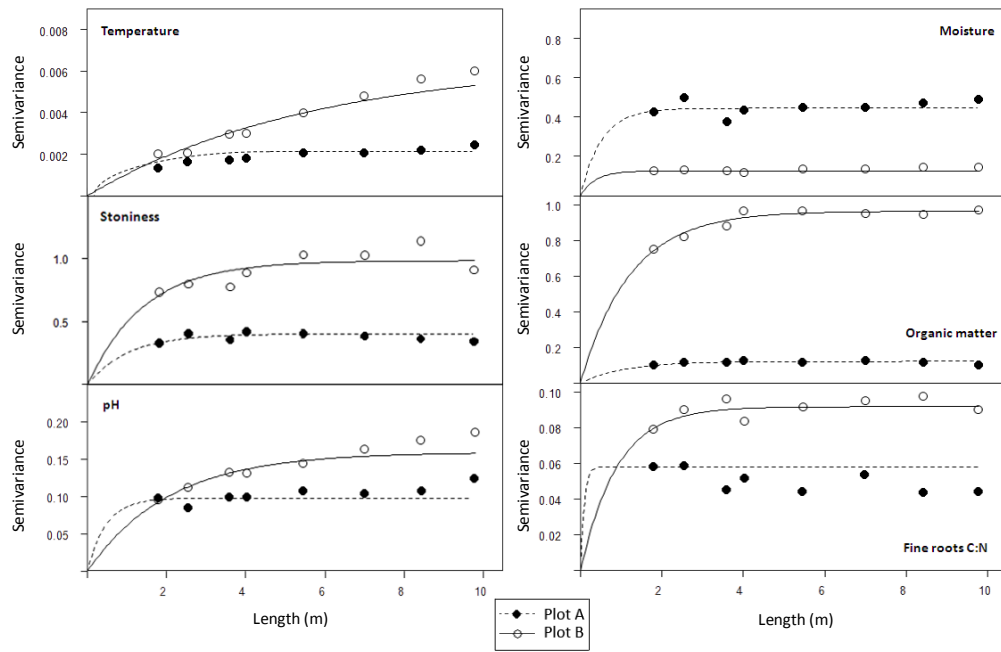
A. Figure 1. Semivariograms of the standardized variables data at different spatial scales within the plots.

B. Table 1. Correlation matrix from all continuous variables included in the analyses. BA_SP and BA_HO are cumulative basal area of Scots pine and Holm oak respectively in 3 meters belt around each measurement point. Respiration, moisture and temperature data are standardized by their plot means. Sample size of each variable is indicated in Table 1.

	Respiration (μmol C m² s⁻¹)	Moisture (%)	Temperature (°C)	Organic matter	Stoniness (%)	pH	Fine roots (g cm⁻¹)	C:N roots	BA_SP	BA_HO
Respiration (μmol C m² s⁻¹)	1									
Moisture (%)	0.262	1								
Temperature (°C)	-0.059	-0.139	1							
Organic matter	0.010	0.119	0.092	1						
Stoniness (%)	-0.007	0.019	0.003	-0.012	1					
pH	0.016	-0.145	-0.267	-0.088	-0.072	1				
Fine roots (g cm⁻¹)	0.138	0.354	-0.170	-0.008	-0.126	0.260	1			
C:N roots	0.023	0.017	0.071	0.036	-0.087	0.013	0.228	1		
BA_SP	-0.031	0.165	0.165	0.054	-0.152	-0.392	-0.051	-0.068	1	
BA_HO	0.116	0.010	-0.368	0.019	0.077	0.159	0.053	-0.055	-0.225	1

Chapter 3

Strong resilience of soil respiration components to drought-induced die-off resulting in forest secondary succession

Josep Barba, Jorge Curiel Yuste, Rafael Poyatos, Ivan Janssens and Francisco Lloret

ABSTRACT

How forests cope with drought-induced perturbations and how the dependence of soil respiration on environmental and biological drivers is affected in a warming and drying context are becoming key questions. We studied total soil respiration (R_S) and its heterotrophic (R_H) and autotrophic (R_A) (further split in fine root [R_R] and mycorrhizal respiration [R_M]) components in a mixed Mediterranean forest where Scots pine (*Pinus sylvestris* L.) is undergoing a drought-induced die-off and is being replaced by Holm oak (*Quercus ilex* L.). Soil respiration and its components were measured every two weeks during one year at four stages of the die-off and succession process (non-defoliated pines, defoliated pines, dead pines and Holm oak), using the mesh exclusion method. The aims were to determine whether die-off and forest succession were reflected in soil respiration and its components and to determine the influence of environmental and biotic variables on the soil respiration components.

Drought-induced pines die-off was not reflected in R_S nor in its components, which denotes a high functional resilience to pines die-off of the studied plant-and-soil system. Despite this resilience to die-off, the succession from Scots pines to holm oaks resulted in a reduction of R_H and thus in important decrease of total respiration (R_S was 36% lower in Holm oaks than in non-defoliated pines). R_S and all its components were strongly regulated by SWC-and-temperature interaction, which could have strong implications for the C fluxes under the drier and warmer conditions expected for the climate change scenarios. Since Scots pine die-off and *Quercus* species colonization seems to be widely occurring on the driest limit of the Scots pine distribution, the functional resilience of the soil system over die-off and the decrease of R_S from Scots pine to holm oak could have direct consequences on the carbon balance of these ecosystems.

KEYWORDS

Soil respiration, heterotrophic respiration, autotrophic respiration, partitioning fluxes, resilience, Mediterranean forest, *Pinus sylvestris*, *Quercus ilex*.

1. INTRODUCTION

Drought-induced forest die-off episodes are being increasingly reported globally (Allen et al., 2010), raising concerns on their possible association with recent global warming, at least in some parts of the globe (van Mantgem et al., 2009). Increased tree defoliation and mortality rates have been related to chronic and episodic drought in the Mediterranean region (Carnicer et al., 2013; Peñuelas et al., 2001), where the projected increase in the frequency and intensity of droughts (IPCC, 2013) may enhance the risk of extensive forest die-off. Widespread drought-induced tree mortality has dramatic effects on forest carbon cycling (Reichstein et al., 2013) which may differ from those reported for other disturbances such as fire or pest outbreaks (Anderegg et al., 2013). Soils store over two-thirds of the carbon in forest ecosystems (Dixon et al. 1994), but the impact of drought-induced tree mortality on soil carbon fluxes and their post-disturbance dynamics remains poorly understood (van der Molen et al., 2011).

The trajectory of ecosystem C cycling in stands where the dominant overstorey species has been severely affected by a die-off event is complex in space and time (Reed et al., 2014; Edburg et al., 2012). At subdecadal timescales, the trend in forest C uptake largely depends on disturbance intensity and the degree of overstorey canopy loss, the spatial pattern of tree dieback (i.e. clustered, diffuse), the compensatory responses by surviving dominant trees, and the response of understorey vegetation to competition release (Amiro et al., 2010; Brown et al., 2010; Gough et al., 2013). With regard to forest soil C losses, die-off episodes immediately curtail root and mycorrhizal respiration and reduce exudate supply from roots to soil, but they also stimulate decomposition of litter, roots and dead wood (Nave et al., 2011). Moreover, die-off episodes may increase soil moisture (Redding et al., 2008), which could enhance soil organic matter (SOM) decomposition rates in water limited ecosystems. Nevertheless, our knowledge on post-dieback ecosystem (and soil) C fluxes is still incomplete, because (1) it largely arises from studies where pests or wildfires, not drought, were the main disturbance drivers and (2) it lacks a detailed understanding of the post-dieback trajectories of soil respiration components (heterotrophic respiration, R_H; autotrophic respiration R_A; fine roots respiration, R_R; mycorrhizal respiration, R_M) and their responses to environmental drivers.

Drought-induced tree mortality episodes often result in a complex spatial pattern of standing dead trees and partially defoliated individuals, together with apparently unaffected trees. These episodes may cause vegetation shifts within years or decades if adult mortality and recruitment of the dominant and most affected species are not balanced through time (Lloret et al., 2012). Given that the substituting species will likely be more drought-resistant, such species replacement could have persistent effects on both stand C uptake and release processes. The

spatial distribution of soil CO_2 effluxes under non-limiting soil moisture conditions is sensitive to local changes in vegetation composition following tree mortality (Barba et al., 2013), but we do not know whether the response of soil CO_2 effluxes to the main abiotic drivers (i.e. soil temperature and moisture) differs along the stages of an ongoing vegetation shift. In Mediterranean forests, the co-limitation of R_S by soil moisture and temperature overlaps with a highly variable influence of plant productivity on R_S (Tedeschi et al., 2006) because of the inherent seasonal variability of plant photosynthetic activity and belowground C allocation (Keenan et al., 2009; Reichstein et al., 2002).

The variability of soil CO_2 efflux associated to the environmental drivers may also be mediated by the differential metabolic response of microbes, roots and rhizosphere to moisture and temperature changes (Kuzyakov, 2006; Moyano et al., 2010; Uren, 2000). Changes in microbial community composition have been observed following drought-induced forest die-off and succession (Curiel Yuste et al., 2012), but how these shifts in microbial community composition modify the contribution of autotrophic and heterotrophic respiration to total soil respiration remains poorly known.

Drought-induced dieback episodes, characterised by increased crown defoliation and mortality rates, have been reported in several Scots pine (*Pinus sylvestris* L.) populations in the northeast of the Iberian Peninsula (Galiano et al., 2010; Hereş et al., 2012; Martínez-Vilalta and Piñol, 2002). In particular, extreme drought events together with a lack of forest management have caused several Scots pine die-off episodes at the Prades Mountains since the 1990s (Hereş et al., 2012; Martínez-Vilalta and Piñol, 2002). In this study, we aim to quantify the effects of this Scots pine drought-related mortality process and the associated holm oak (*Quercus ilex* L.) colonisation (Vilà-Cabrera et al., 2013) on soil respiration and its components, at seasonal and annual timescales. During one year, we measured the seasonal variation of soil respiration and its heterotrophic and autotrophic (roots and mycorrhiza) components, associated with the different stages of this drought-induced die-off and species-replacement process: non-defoliated pines [NDP], partially defoliated pines [DFP], dead pines [DP] and holm oaks [HO].

We hypothesized that: 1a) Heterotrophic respiration would show a gradual increase from NPD to DFP and DP across this die-off gradient. In parallel, (1b) we would also expect autotrophic respiration to decrease, because of reduced above-and-belowground plant activity in DFP and DP. Therefore (1c) the relative contribution of heterotrophic respiration would increase from NDP to DFP and DP. Given that holm oak is a more drought-tolerant species compared to Scots pine, we expected (2a) generally higher soil respiration in holm oak in this drought-exposed site. We also hypothesized that (2b) soil respiration under Scots pine, especially the autotrophic

component, would be more sensitive to decreasing soil moisture during summer drought. Finally, we hypothesized that (3a) the spatial and temporal variation in heterotrophic soil respiration would be mainly dependent on environmental variables (soil temperature and moisture), whereas these variables would explain little variability for autotrophic respiration, usually more influenced by photosynthetic-related variables. Since vegetation has the capacity to modify soil environmental conditions, we also hypothesized that (3b) the vegetation die-off and the ongoing species succession would largely influence soil CO_2 effluxes via modifications in environmental conditions such as SWC.

2. MATERIALS AND METHODS

2.1. Study site

The study was conducted in a mixed forest in the Titllar Valley (Prades Mountains, NE Iberian Peninsula). The climate is Mediterranean, with a mean annual temperature of 11.2°C and precipitation of 664 mm (Ninyerola et al., 2007a, 2007b). The experimental area was located on the northwest face of the valley at an elevation between 1,010 and 1,030 m a.s.l. with a steep slope (33°). Soils are xerochrepts with clay loam texture and high gravel content (46 vol%). The substrate consists of fractured metamorphic schist that outcrops on a large part of the study area. For more information related to the study area, see Hereter and Sánchez, 1999; Barba et al., 2013.

This mixed forest is dominated by Scots pine (*Pinus sylvestris* L.) in the overstorey and holm oak (*Quercus ilex* L.) in the understorey. Severe drought events since the 1990s have affected specially the Scots pine populations (Martínez-Vilalta and Piñol, 2002), inducing a mean crown defoliation of 52% and standing mortality of 12% (Vilà-Cabrera et al., 2013). This situation, coupled to the low regeneration of pines (Vilà-Cabrera et al., 2013) is currently driving the replacement of pines by oaks as the dominant overstorey species.

2.2. Experimental design

2.2.1 Experiment scheme

Soil respiration fluxes were measured under 12 trees belonging to the different stages along the vegetation shift following the Scots pine die-off process (from now, type of tree): three non-defoliated Scots pines [NDP], three living defoliated Scots pines [DFP], three dead Scots pines

[DP] and three Holm oaks [HO]. The maximum distance between measuring points was *ca*. 200 m. Dead Scots pines were devoid of needles and small branchlets, and only the main bole and primary branches were still standing. Therefore we estimate that these trees have been dead for 8-10 years. The degree of pine defoliation was visually estimated as the percentage of green needles relative to a non-defoliated canopy of a similar sized tree from the same population (Galiano et al., 2010). Defoliated pines had less than 50% of green leaves. DBH in each type of tree were 37±7 cm in NDP, 59±9 cm in DFP, 58±7 cm in DP and 14±8 cm in HO (mean ± sd).
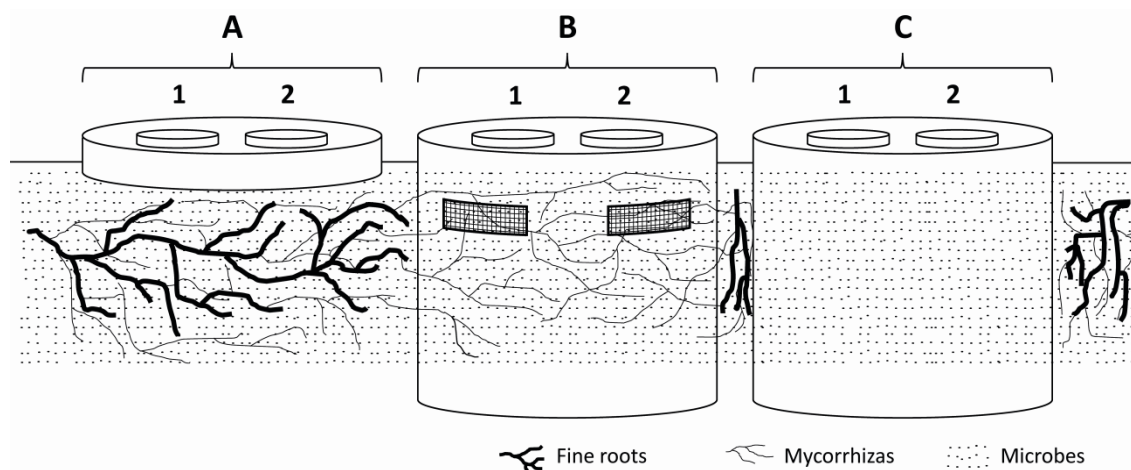

Figure 1. Root-exclusion experimental design.

The root excision method (Subke et al., 2006) was used for studying total, autotrophic and heterotrophic soil respiration, using the protocol proposed by Heinemeyer et al., (2007). Three different PVC collars (treatment collars) of 63 cm in diameter were installed within 3 m of each tree (Figure 1). The first type of collar (A) was 5 cm in height and was inserted only 2 cm into the ground and fixed with three metal sticks. Thus, these A-type collars did not interfere with fine roots, mycorrhizal or soil microbial dynamics and growth. The second and the third types of collar (B and C respectively) were 50 cm in height and were inserted up to a depth of 45 cm into the soil. Collar B had four rectangular windows (17 x 5 cm) at 10 cm from the top, covering 33% of the total collar perimeter. Windows were covered with nylon fabric of 41 μm mesh size, allowing ingrowth of fungal hyphae but not of roots (Ek, 1997). The deeper B-type collars prevented the ingrowth of fine roots (which are concentrated mainly in the upper centimetres of the soil (Jackson et al., 1997). C-type collars were the same size and were installed at the same depth as B-type collars, but they did not have windows, so it prevented the ingrowth of both, fine

roots and mycorrhizal hyphae. Hence, we assumed that in C-type collars, only the non-rhizospheric microbial community remained active.

Due to the great stoniness of the soil, two modifications were made to the Heinemeyer et al., (2007) experimental design. First, treatment collars were bigger (20 cm in diameter in the original protocol), and second, we dug a hole in the soil previous to the installation of the deeper collars. After placing the collar inside the hole, we filled the space inside the collar with the previously removed soil material, preserving the original soil-horizon order. Two smaller PVC collars (sampling collars; 10 cm in diameter and 4 cm of height) were installed as replicate collars within each treatment collar. Sampling collars were inserted 1 cm into the ground and fixed with polyurethane foam. These collars delimited the reference surface of our soil respiration measurements. Collar installation was made 14 months before the start of measurements in order to minimize the effects of the soil disturbance on soil respiration measures.

2.2.2 Measurements

Soil respiration rates were measured with a close-path infra-red gas analyser (IRGA) coupled to a closed dynamic chamber (EGM-4 and SRC-1, PP-Systems, HITCHING, UK) in the sampling collars (the soil chamber fitted well with the small collars because it had the same diameter). Soil respiration was measured every two weeks during one year, from June 2012 to June 2013 (25 campaigns). For each campaign, five rounds of measurements were made at all 72 small collars during 24 hours, in order to capture the soil respiration variability associated to daily cycles. One round was started two hours before sunrise, another was performed after sunset and the others were equally distributed during the daytime.

Soil temperature at 10 cm was measured once per big collar and per round of measurements using a thermometer (OMEGA, HH806AU, Stamford, USA). Soil water content (SWC) was measured each campaign at each tree by time domain reflectometry (TDR) (Tektronix 1502C, Beaverton, Oregon, USA). One 15 cm long TDR probe was permanently installed in the upper soil close to each tree throughout the experiment. In order to correct the SWC measurements for the stoniness, gravimetrical SWC measured in soil samples close to the TDR probes were regressed against TDR measurements (for more information, see Poyatos et al., 2013).

One litterfall trap (555 x 355 mm) was installed within 2 m of each tree and litterfall was collected during every campaign, dried during 24 h at 70°C and weighed. Soil depth beneath

each small collar was estimated as the average of five measurements with a fine metal stick at the end of the experiment.

Air temperature and relative humidity were continuously measured in a meteorological tower installed less than 100 m from the farthest tree. Continuous SWC was also recorded in the upper 30 cm of soil using six frequency domain reflectometers (CS616, Campbell Scientific INC) randomly distributed among the trees. A data acquisition system (CR1000 datalogger and AM16/32 multiplexers, Campbell Scientific Inc., Logan, UT, USA) was used to store 15-min means of soil moisture and meteorological variables sampled every 30 s.

2.3. Data analysis

2.3.1. Soil respiration calculation

The five soil respiration measurements recorded for each small collar were time-averaged in order to obtain mean daily soil respiration. Then, both replicates were averaged at the big collar level (A, B and C; Figure 1).

Total soil respiration (R_S), heterotrophic soil respiration (R_H), autotrophic soil respiration (R_A), fine roots respiration (R_R) and micorrhyzal respiration (R_M) were calculated following the protocol proposed by Heinemeyer et al., (2007). R_S and R_H were estimated directly as the soil respiration rates measured in collars A and C, respectively. R_A was calculated by subtracting C from A; R_R by subtracting B from A and R_M by subtracting C from B.

2.3.2. Soil respiration drivers.

Mixed-effects models were used to analyse the relationships between soil respiration and type of tree, soil temperature, SWC, litterfall, soil depth and season with campaign data. The limits of seasons were adjusted from environmental variables (i.e. Summer ended with the first rainfall events in Fall, which changed drastically SWC and temperature). Linear and exponential relationships between soil respiration and temperature were tested in the models, as well as linear and quadratic relationships between soil respiration and SWC. In all cases, the linear relationships performed better than the nonlinear transformations according to the AICc (corrected Akaike information criterion) statistic (data not shown).

Different models were fitted for each soil respiration component (R_S, R_H, R_A, R_M and R_R). As all variables were measured near the same trees throughout the campaigns, tree identity was

included as a random factor in all models. A step-wise procedure was used to select the best model starting from the saturated one, which contained all variables and their second-order interactions. Each time, the least significant variable was removed from the model until the AICc did not decrease further. Models within 2 AICc units of the best model were considered equivalent and we selected the simplest one. To determine the variability explained by each mixed model, we calculated the coefficient of determination using the log likelihood of both, the studied model and the null model (which did not include any predictor variables). In the root exclusion method, the PVC collars could interfere with soil temperature and moisture (Kuzyakov, 2006). Therefore, SWC was gravimetrically measured at 72 samples from the different kind of collars at the end of the experiment (July 2013) and one-way ANOVA was performed to test for differences in SWC between the different collars.

2.3.3. Annual SR

We used the models fitted in 2.3.2 to estimate daily values of R_S, R_H, R_A, R_M and R_R for a whole year (from mid-June 2012 to mid-June 2013). Apart from fixed predictors (soil depth, season, type of tree), daily-aggregated values of soil temperature, SWC and litterfall were needed as inputs for the model. For each tree and collar type, linear regressions were fitted between daily-averaged soil temperature measured during the campaigns and simultaneous air temperature measured at the meteorological tower ($R^2=0.93\pm0.02$, across-trees mean±sd). Likewise, daily SWC for each tree was estimated from linear regressions against mean SWC measured with the four frequency domain reflectometers near the meteorological tower ($R^2=0.66\pm0.27$, across-trees mean±sd). Daily litterfall across-trees was linearly interpolated from biweekly-measured litterfall. Modelled values of daily R_S, R_H, R_A, R_M and R_R were then aggregated to obtain annual values.

Mixed-effects models, including tree identity as a random factor, were then used to analyse the differences in annual soil respiration and its components between types of tree. Given the high spatial variability of soil respiration, especially in this ecologically complex site (Barba et al., 2013), and the limited number of replicates (3 trees per type), we considered marginally significant differences among means when $0.05 < p < 0.1$. Additionally, the non-parametric Friedman test and its post hoc analysis was applied to the daily-averaged data from the 25 campaigns, to test for possible differences in soil respiration and its components between types of tree.

Table 1.
Environmental variables during the study period summarized by type of tree (mean and standard deviation). Different lowercase letters indicate significant differences between type of trees ($p<0.05$, mixed effects model with tree as random factor). Units: soil temperature in °C; SWC in cm^3cm^{-3}; litterfall in $g\ m^{-2}\ d^{-1}$; soil depth in cm.

	NDP		DFP		DP		HO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Temperature	11.68	0.29	11.70	0.68	12.00	0.40	11.21	0.14
SWC	20.20	0.04	20.21	0.06	20.21	0.02	20.20	0.05
Litterfall	1.84	0.67	1.19	0.11	1.92	0.89	1.96	0.19
Soil depth	4.25	0.43 [ab]	5.27	0.94 [a]	3.64	0.72 [b]	3.44	0.32 [b]

To test whether the relative contribution of R_H to R_S increases along the die-off process, mixed-effects models with tree type (for testing annual differences) or with the interaction between tree type and season (for testing differences over seasons) were used, including tree identity as a random factor. Heterotrophic relative contribution data was log transformed to achieve normality.

All the analyses were carried out using R 3.0.3. (R Foundation for Statistical Computing, Vienna, Austria). The mixed-effects models were performed using the R package nlme (Pinheiro et al., 2009) and the step-wise model selection was performed with MuMIn package (Bartón, 2014).

3. RESULTS

3.1. Seasonal course of environmental variables and soil respiration components.

Over the study period, climate was typical of a low elevation Mediterranean mountain, with mean air temperature of 11.2°C, annual precipitation of 703 mm and relatively dry summer (93 mm from June to September and mean SWC below 10% from mid-July to the end of September) (Figure 2b and 2c). The seasonal pattern of litterfall was not as clear as those of temperature and SWC, but it seemed to peak at the end of fall (Figure 2d). No significant differences were found for soil temperature, SWC and litterfall among types of trees, although deeper soils were found under DFP than under DP or HO (less than 2 cm on average) (Table 1). While type of collar did not influence soil temperature during the experiment (p=0.87), SWC measured gravimetrically at the end of experiment (July 2013) was higher for deeper collars (B and C, see Figure 1) than for surface ones (A) (p=0.03, one-way ANOVA), 3.4% on average.
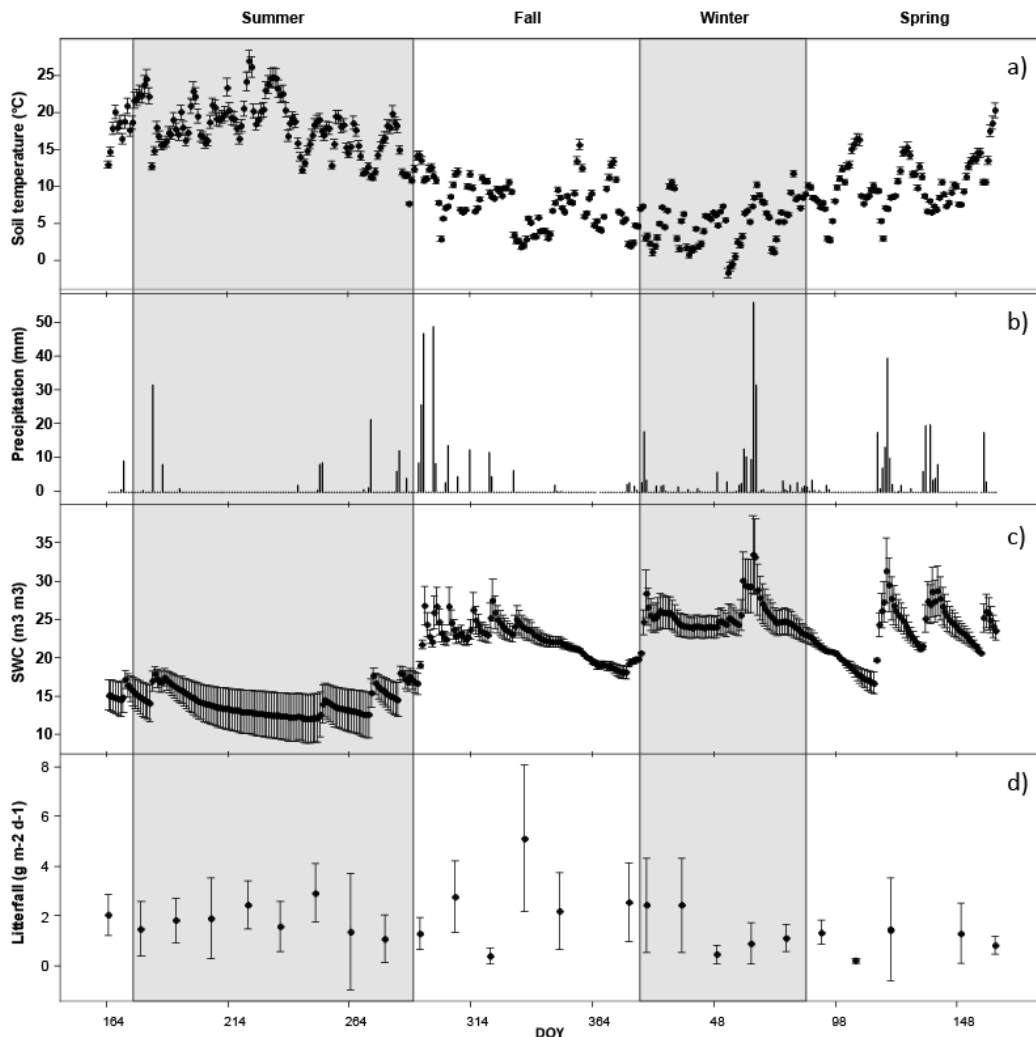
Figure 2. Seasonal course of environmental variables over the study period (June 2012 – June 2013). a) daily soil temperature (mean of all trees and treatments ± sd); b) daily precipitation; c) daily SWC (mean of all trees ± sd); d) daily litterfall (mean of all trees ± sd). Daily soil temperature for each tree and treatment was modelled with soil temperature campaign data and daily air temperature. Similarly, daily SWC for each tree was modelled with SWC campaign data and daily SWC measured continuously at 6 points randomly distributed in the study site (see section 2.2.2). Litterfall data showed at panel d) are the campaigns data.

Total soil respiration (R_S) flux peaked during late spring and early summer (up to 5 µmol m^{-2} s^{-1}; Fig 3, top panel) and then decreased over the course of the summer to 50 % of peak values. During fall and winter R_S was quite low (between 0 to 2 µmol C m^{-2} s^{-1}). R_H showed a similar annual pattern as R_S with values ranging between 1 and 4 µmol C m^{-2} s^{-1} (Figure 3, R_H panel). No seasonal pattern was observed neither for R_A, nor for its components (R_M and R_R) (Figure 3, bottom panels). Soil CO_2 efflux measured in HO was the lowest in 88% of the campaigns for R_S, 76% for R_H, 68% for R_A, 56% for R_R, but only in 24% of the campaigns for R_M (Figure 3). Indeed, the non-parametric Friedman test applied to the campaign data and its post hoc analysis

indicated that respiration rates under HO were lower than under the three types of pines for R_S, R_H, R_A and R_R (p<0.001 for R_S and R_H, p=0.001 for R_A and p=0.002 for R_R), but not for R_M.

The relative contribution of R_H to R_S did not show differences among tree types, neither at annual (p=0.968) nor at seasonal scales (p=0.325) (GLM models with tree identity as a random factor). Nonetheless, the relative contribution of the different soil respiration
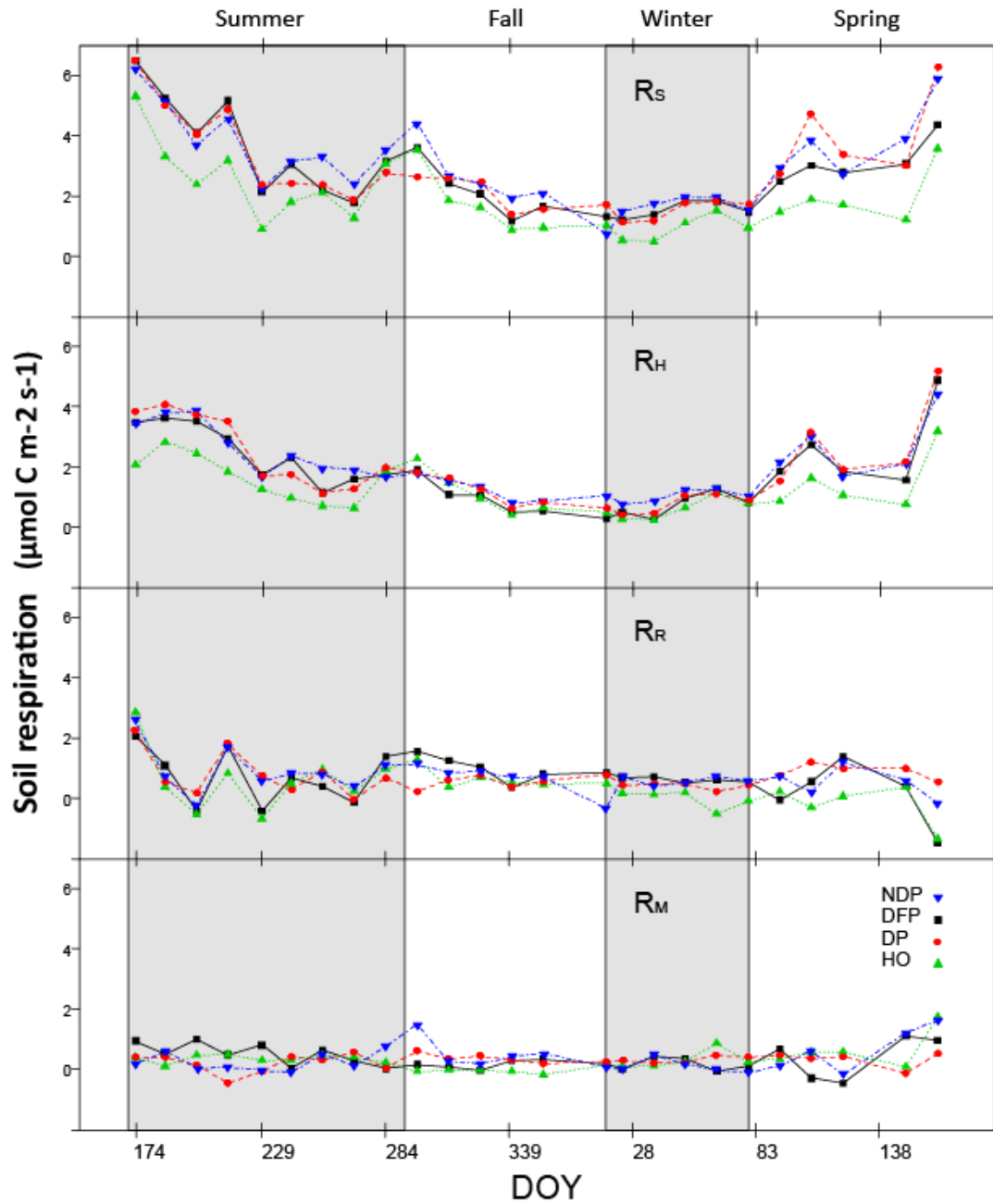

Figure 3. Annual soil respiration evolution and its components. Each dot represents the average of the 5 measurements within a 24-h cycle and the 3 trees of each type. Campaign-specific error bars were not drawn for better clarity.

components considering all tree types together varied during the year. The contribution of R_H to R_S was highest during mid-winter, spring and summer, and decreased during fall and mid-winter. In contrast, the contribution of R_R to R_S increased during fall and early winter. The contribution of R_M to R_S did not show any seasonal pattern, remaining low throughout the year (Figure 4).
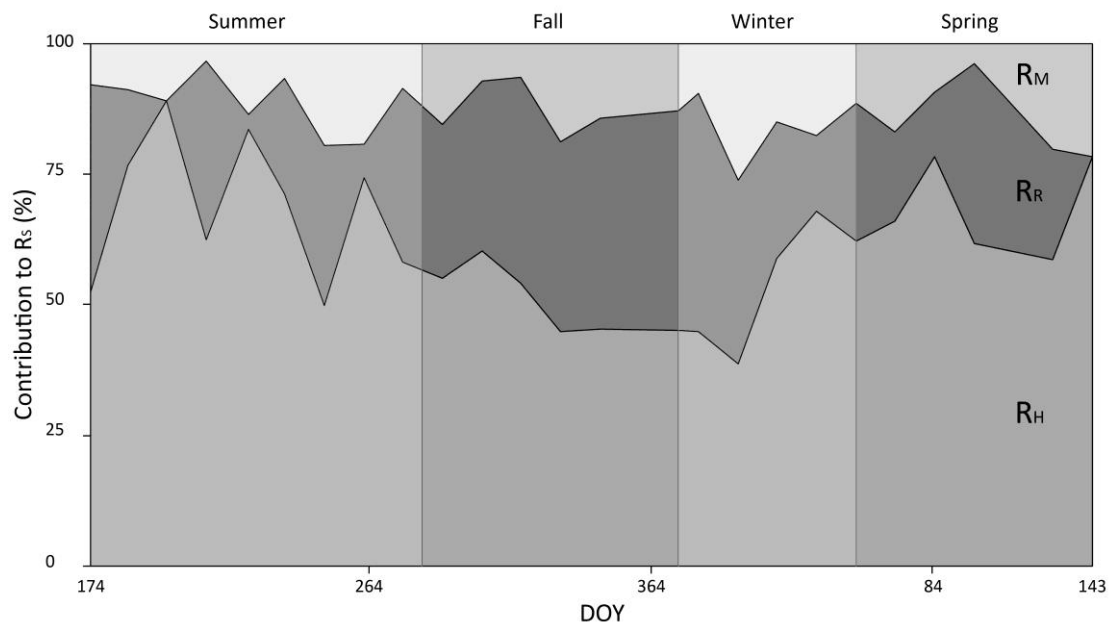

Figure 4. R_H, R_M and R_R contribution (in %) to R_S during campaigns (from June 2012 to June 2013) considering all tree types together.

3.2. Soil respiration drivers.

A mixed-effects model including soil depth (positive effect) and the interactions between SWC-temperature and season-temperature as predictor variables (Table 2, R_S) explained 54% of the seasonal variability in R_S. The higher the temperature, the larger the positive effect of SWC was on R_S. Temperature had a positive effect on R_S in all seasons, but had a higher effect during summer and spring than in fall and winter. However, at SWC values below 10%, the temperature effect on R_S was negligible or even negative in winter, spring and fall (Figure 5; a, b and d, respectively). No differences were detected in R_S seasonal variability or its environmental controls among types of tree. In contrast, tree type interacted with SWC to determine R_H, while the rest of the R_H predictors were the same (with the exception of soil depth) as those in the R_S model (Table 2, R_H and Figure 6) and an almost identical degree of explained variability was observed (56%). The interaction between SWC and temperature had the same positive effect than in the R_S model (higher effect of temperature at high SWC values). Temperature had the

lowest effect on R_H during fall and winter, a higher effect during summer and the highest effect during spring (Table 2, R_H).

Models of the autotrophic components of R_S explained much less variability than the ones fitted for R_S and R_H. The selected R_A model was able to explain only 16% of the autotrophic respiration variability, and included the positive effects of soil depth and litterfall and the positive interaction between SWC and temperature (Table 2, R_A). Likewise, the selected R_R model was able to explain only 13% of the variability in fine root respiration and contained the interactions season-SWC and season-temperature (Table 2, R_R). Finally, the selected R_M model was able to explain 24% of the variability in mycorrhizal respiration and contained only the interaction between SWC-temperature (Table 2, R_M). Type of tree had no effect on R_A, nor on its fractions (R_R and R_M).
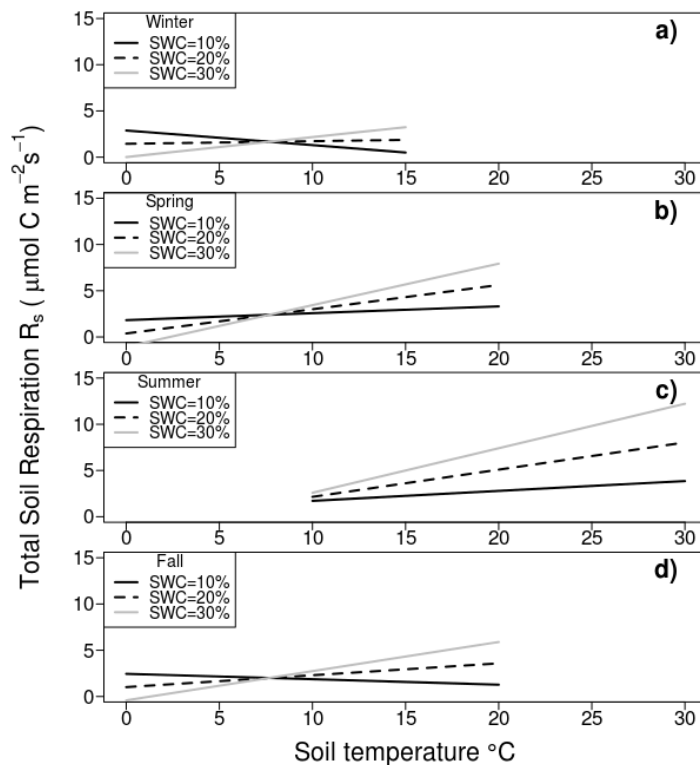

Figure 5. Modelled soil temperature responses of R_S at different levels of soil moisture, for each season, according to the R_S model in Table 2. Soil depth is also an explanatory variable in the model and its value has been assumed to be the average value across tree types. Data for all tree types have been pooled because no effect of tree type has been included in the model. Modelled responses have been drawn only for the observed soil temperature range for each season.

3.3. Annual soil respiration and its components.

For the overall set of sampled trees, modelled annual R_S (mean±SD) from July 2012 to July 2013 was 2.6±0.7 µmol m^{-2}s^{-1}, R_H was 1.7±0.4 µmol m^{-2}s^{-1}, representing 65% of R_S, while R_A was 1.0±0.5 µmol m^{-2}s^{-1} (36% of R_S). R_R and R_M, as components of R_A, were 0.6±0.5 µmol m^{-2}s^{-1} (23% of R_S) and 0.3±0.6 µmol m^{-2}s^{-1} (13% of R_S), respectively.

The mixed-effects models showed that annual R_S under holm oak trees (HO) was marginally significantly lower than under non-defoliated pines (NDP) (p-value: 0.074) (64% on average), whereas defoliated pines (DFP) and dead pines (DP) did not show differences with NDP or HO.
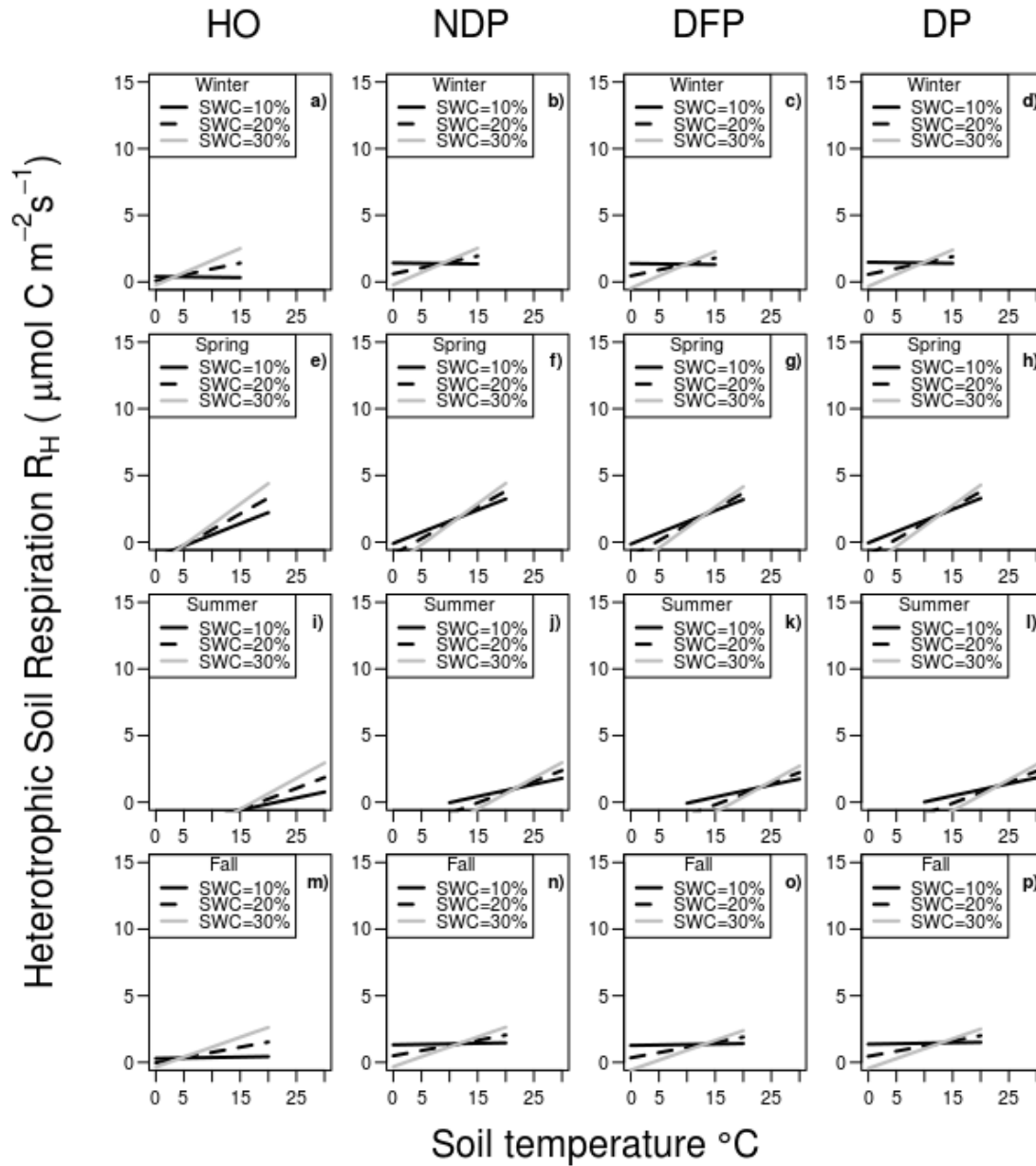

Figure 6. Modelled soil temperature responses of R_H at different levels of soil moisture, for each season and type of tree, according to the R_H model in Table 2. Modelled responses have been drawn only for the observed soil temperature range for each season.

Table 2.
Summary of the averaged model of R_S, R_H, R_A, R_R and R_M. Holm oak and Summer are used as the reference categories and are included in the intercept. Contribution of a variable is the proportion of set models that contain that variable. Different lowercase letters indicate significant differences between levels of predictor variables.

R_S

	Variables	Estimate	SE	t-value	p-value	
	Intercept	0.969	0.978	0.990	0.323	
	Depth	0.309	0.190	1,627	0.135	
	SWC	-0.144	0.037	-3,842	0.000	
	Temperature	-0.080	0.043	-1,875	0.062	
	SWC*Temp	0.019	0.003	6,318	0.000	
Season	Summer	-	-	-	-	a
	Fall	1,807	0.809	2,234	0.026	bc
	Winter	2,240	0.763	2,936	0.004	b
	Spring	1,169	0.775	1,508	0.133	ac
Season*Temp	Summer*Temp	-	-	-	-	a
	Fall*Temp	-0.166	0.067	-2,490	0.013	b
	Winter*Temp	-0.266	0.072	-3,686	0.000	b
	Spring*Temp	-0.032	0.053	-0.61	0.542	a

R_H

	Variables	Estimate	SE	t-value	p-value	
	Intercept	-0.734	0.58	-1,270	0.205	
	SWC	-0.032	0.03	-1,088	0.278	
	Temperature	0.027	0.03	0.826	0.410	
	SWC*Temp	0.009	0	1,146	0.000	
Season	Summer	-	-	-	-	a
	Fall	1,344	0.617	2,179	0.030	b
	Winter	1,455	0.57	2,536	0.012	b
	Spring	-0.079	0.586	-0.13	0.894	a
Type	HO	-	-	-	-	ab
	NDP	1,526	0.535	2,853	0.021	b
	DFP	1,586	0.511	3,102	0.015	b
	DP	1,667	0.479	3,480	0.008	b
Season*Temp	Summer*Temp	-	-	-	-	a
	Fall*Temp	-0.112	0.051	-2,185	0.030	b
	Winter*Temp	-0.127	0.054	-2,345	0.020	b
	Spring*Temp	0.101	0.040	2,535	0.012	c
SWC*Type	SWC*HO	-	-	-	-	a
	SWC*NDP	-0.050	0.022	-2,289	0.023	b
	SWC*DFP	-0.061	0.021	-2,960	0.003	b
	SWC*DP	-0.059	0.019	-3,191	0.002	b

R_A

Variables	Estimate	SE	t-value	p-value
Intercept	0.568	0.762	0.746	0.457
Depth	0.297	0.141	2,104	0.062
Litterfall	0.076	0.034	2,209	0.028
SWC	-0.063	0.026	-2,438	0.015
Temperature	-0.097	0.037	-2,640	0.009
SWC*Temp	0.008	0.002	3,922	0.000

R_R

	Variables	Estimate	SE	t-value	p-value	
	Intercept	-1,329	0.767	-1,731	0.085	
	SWC	0.093	0.028	3,331	0.001	
	Temperature	0.041	0.028	1,463	0.145	
Season	Summer	-	-	-	-	a
	Fall	2,213	1,236	1,790	0.075	ab
	Winter	1,868	1,109	1,684	0.093	ab
	Spring	3,978	1,216	3,271	0.001	b
Season*SWC	Summer*SWC	-	-	-	-	a
	Fall*SWC	-0.123	0.053	-2,329	0.021	b
	Winter*SWC	-0.093	0.045	-2,077	0.039	b
	Spring*SWC	-0.128	0.046	-2,789	0.006	b
Season*Temp	Summer*Temp	-	-	-	-	a
	Fall*Temp	0.043	0.060	0.709	0.479	ab
	Winter*Temp	-0.070	0.063	-1,097	0.273	ab
	Spring*Temp	-0.182	0.045	-4,036	0.000	b

R_M

Variables	Estimate	SE	t-value	p-value
Intercept	1,286	0.544	2,366	0.019
SWC	-0.054	0.023	-2,395	0.017
Temperature	-0.081	0.032	-2,511	0.013
SWC*Temp	0.005	0.002	3,076	0.002

Likewise, annual R_H under HO was significantly lower than under NDP (p-value: 0.030; 36% lower), and marginally significantly lower than under DFP (p-value: 0.089; 23% lower) and DP (p-value: 0.054; 33% lower). Mixed-effects models for annual R_A, R_R and R_M did not show differences among types of trees (Figure 7).

We used the model in section 3.2 to assess the effect of the higher soil moisture in deeper collars (B and C) due the collar effect (3.4% higher on average at July 2013), because this increased SWC could have stimulated R_H, overestimating the heterotrophic contribution to R_S and consequently, underestimating R_A. Since SWC was not measured at the different collar types during the campaigns, it was not possible to asses this effect over the year. However, if we assumed that the difference observed in July persisted throughout the year, annual R_H and its contribution on R_S would only have been 0.92% and 0.60% higher than the reported values, respectively (models estimations with an increment of 3.4% in SWC for all campaigns). Therefore, we considered the collar effect on the estimation of respiration fluxes to be negligible.
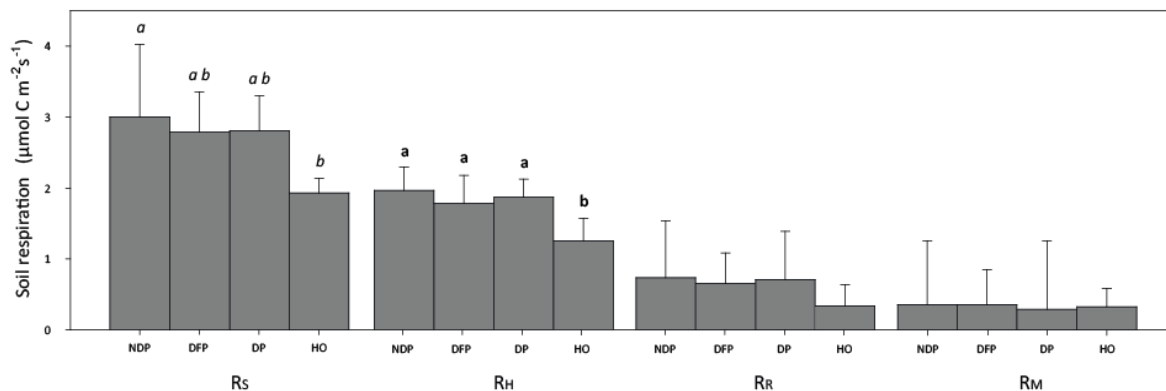

Figure 7. Annual R_S and its components (mean and SD) for each type of tree. Different normal font letters indicate significant differences between types of tree within each respiration component (p<0.05, mixed-effects model). Different letters in italics indicate marginally significant differences (0.05<P<0.1, mixed-effects model).

4. DISCUSSION

4.1. Annual soil respiration and its fractions

Mean annual R_S at the study site was 2.6 μmols m^{-2} s^{-1}, similar to values reported for a parallel valley in the same nature reserve (2.3 μmols m^{-2} s^{-1}, Asensio et al., 2007). R_H was the most important fraction of R_S, representing about 65%. This relative importance agrees well with estimates obtained in other forest ecosystems (Heinemeyer et al., 2007; Rey et al., 2002; Subke

et al., 2011; Tedeschi et al., 2006), confirming the important role of microbial soil respiration in R_S. The R_R and R_M contribution to R_S (23% and 13%, respectively) were also similar to values reported in other partitioning studies (Heinemeyer et al., 2007; Malhi et al., 1999; Rey et al., 2002; Ruehr and Buchmann, 2010; Subke et al., 2006).

4.2. Soil respiration and its components following Scots pine die-off

Scots pine die-off, from non-defoliated pines to defoliated pines and to dead pines, did not result in major changes in soil respiration (both heterotrophic and autotrophic activity, see Figure 7), contrary to what we had hypothesized (H1a, H1b). Therefore, also the relative contribution of heterotrophic respiration did not increase from NDP to DFP and DP. Defoliation did not affect R_S or its components with respect to non-defoliated pines (NDP). Moreover, 10 years after tree death, we observed that R_S (and all its components) associated with dead Scots pines has either completely recovered or remained unchanged (Figure 7). Even the autotrophic components (R_R and R_M) of R_S, directly dependent on the substrate inputs from plant photosynthetic activity (e.g. Högberg et al., 2001), were not affected along this die-off gradient. These effects are surprising, given that soil autotrophic respiration has been reported to be extremely sensitive to decreases in photosynthetic activity associated with defoliation and die-off (Binkley et al., 2006; Levy-Varon et al., 2014, 2012; Moore et al., 2013). Indeed, decreases in plant productivity have been associated with lower fine root biomass and hence lower root metabolic activity, lower belowground substrate allocation and lower root exudation, all contribution to lower R_A and its fractions (Högberg and Read, 2006).

We did not find higher heterotrophic respiration (R_H) under DP than under NDP, which is consistent with the fact that tree mortality did not apparently result in higher soil moisture, soil temperature or litterfall under dead pines, factors which are known to stimulate R_H. Although the relative contribution of R_H to R_S (and thus, also R_A contribution) showed a clear seasonal pattern, this pattern was preserved across the entire pine die-off gradient (NDP, DFP and DP).

The stability in R_S and in its fractions along the pine die-off gradient denotes a high degree of resilience of soil processes with respect to aboveground perturbations. While this has been partially shown in some studies (Binkley et al., 2006; Levy-Varon et al., 2014, 2012; Moore et al., 2013), here we present, to the best of our knowledge, the very first evidence that all the components of R_S remain apparently unaffected after drought-related mortality processes.

Our results might be partially explained by the disturbance recovery findings in Nave *et al.* (2011), which suggest that a short perturbation of forest C cycling due to partial canopy

disturbance could be rapid recovered (within a few years), thereby stabilizing the C cycle. They hypothesize that shortly after the perturbation, the expected decrease in ecosystem production and soil respiration (due to decrease fine root biomass and non-structural carbohydrate concentrations in roots) could be compensated by higher growth rates from the remaining healthy trees due to reduced competition for limiting resources. At the study site, after drought-induced pine mortality in the nineties higher growth rates of the remaining healthy trees were observed (Martínez-Vilalta and Piñol, 2002), consistent with this hypothesis. Soil water redistribution is likely to have been responsible for enhancing the system resilience, because pine die-off and mortality in this stand was induced by drought events (Martínez-Vilalta and Piñol, 2002; Poyatos et al., 2013).

The mechanism underlying the observed resilience of R_S following Scots pine die-off is likely more related to belowground colonization by HO than to the remaining, unaffected, pines. Results obtained from previous studies in the same site support this idea. Firstly, spatial variability of R_S close to dead pines has been mainly associated with the spatial distribution of HO basal area (Barba et al., 2013) suggesting a functional colonization by HO rhizosphere. Moreover, it has been observed that rates of R_R measured in living roots under DP were similar to those measured for HO (Pereira-Blanco, 2014), which suggests that fine roots colonization of the disturbed gap is taking place by HO. Finally, a convergence of the most abundant taxon in the soil bacterial community between HO and DP has also been reported in the study system (Curiel Yuste et al., 2012) indicating that HO colonization is also occurring at the microbial level.

4.3. Drought-induced substitution of Scots pine by holm oak causes a decrease in soil respiration

Changes in vegetation could produce changes in the whole plant-to-soil system, such as root biomass and distribution, nutrients and water balances, net primary production, carbohydrate allocation patterns, litter quantity and quality, decomposer community or microbial diversity (Binkley and Giardina, 1998; Curiel Yuste et al., 2012; Jackson et al., 1997; Palacio et al., 2007; Strickland et al., 2009), which in turn, could modify R_S and its heterotrophic and autotrophic fractions (Cornwell et al., 2008; Janssens et al., 2001; Kuzyakov, 2006; Uren, 2000; Vivanco and Austin, 2008). Despite the strong resilience of R_S and its components along the Scots pine die-off (NDP, DFP and DP) (see section 4.2), changes in R_S associated at the succession from Scots pine to Holm oak were observed. Annual R_S was 36% lower in HO compared to NDP, contrary to our hypothesis H2a. Although the analysis of annual values did not show differences in R_A, R_R or R_M between NDP and HO (Figure 7), seasonal data analysed with the non-parametric Friedman test suggested lower values for R_A, and R_R under HO. However, the magnitude of these differences

was small (Figure 3) and the differences in R_S between HO and NDP were apparently more related to differences in R_H (36% lower in HO than in NDP). But the vegetation effect on R_S was not indirect via modifications in the environmental conditions (as we expected in H3b), since no differences were found in SWC or soil temperature along the die-off stages or between species. These differences in R_S were probably driven by changes in in microbial community composition and functional diversity. Firstly, because specific microbial communities have been observed under each type of tree, but not differences in soil environmental conditions nor in soil C pools (both quality and quantity) at the different stages of tree succession were found at the study site (Curiel Yuste et al., 2012). Secondly, because HO decomposer community has shown lower functional diversity than NDP decomposer community in a litter decomposition experiment (Barba et al., 2015; under review).

This shift towards lower R_S under holm oak following Scots pine drought-induced mortality could have crucial implications for the carbon balance of this particular ecosystem and, by extension, for the carbon cycling in Mediterranean drought-exposed Scots pine forests where a gradual replacement by *Quercus* species is increasingly being reported (Carnicer et al., 2014; Galiano et al., 2013; Martínez-Vilalta et al., 2012; Vilà-Cabrera et al., 2013).

4.4. Environmental controls of autotrophic and heterotrophic soil respiration along a die-off gradient and species succession.

Soil depth and the interactions between SWC-temperature and season-temperature were able to explain 54% of the R_S variability. The positive interaction between SWC and temperature indicated that the higher the SWC, the higher the temperature effect, and had similar effects on R_H, R_R and R_M fluxes. However, only during part of the spring-time there were simultaneous high SWC and temperature values (see figure 1). We did not find support for our hypothesized higher sensitivity of R_S and R_A to SWC under Scots pine (H2b). On the contrary, we observe a higher sensitivity of R_H to SWC under HO (Table 2, R_H model; Figure 6), which could be due to higher adaptation to the strong seasonal changes in water availability of the microbial community associated with this typical Mediterranean species (Curiel Yuste et al., 2014) or to higher microbial biomass under HO which could respond higher to changes in SWC.

While environmental variables have been described as major drivers of R_A at global scale (Piao et al., 2010) vegetation activity has been usually described as the most determinant factor in R_S at ecosystem scale (Högberg et al., 2009; Janssens et al., 2001; Tang et al., 2005). Therefore, the limited explanatory power of environmental variables on autotrophic components obtained in

this study (and expected in H3a) could be explained by the lack of variables reflecting properly the photosynthetic activity of the site. Indeed a parallel study in the same area has shown that variations in R_R were well explained by plant photosynthetic activity (Pereira-Blanco, 2014). This highlights the need for further studies on the dependency of R_S and its components on aboveground plant productivity (and not only on environmental variables), both measured at stand level, to understand the ecosystem mechanisms to cope with climate-driven disturbances.

5. ACKNOWLEDGMENTS

6. REFERENCES

Amiro, B.D., Barr, A.G., Barr, J.G., Black, T.A., Bracho, R., Brown, M., Chen, J., Clark, K.L., Davis, K.J., Desai, A.R., Dore, S., Engel, V., Fuentes, J.D., Goldstein, A.H., Goulden, M.L., Kolb, T.E., Lavigne, M.B., Law, B.E., Margolis, H.A., Martin, T., McCaughey, J.H., Misson, L., Montes-Helu, M., Noormets, A., Randerson, J.T., Starr, G., Xiao, J., 2010. Ecosystem carbon dioxide fluxes after disturbance in forests of North America. J. Geophys. Res. 115.

Anderegg, W.R.L., Kane, J.M., Anderegg, L.D.L., 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. Nat. Clim. Chang. 3, 30–36.

Asensio, D., Peñuelas, J., Ogaya, R., Llusia, J., 2007. Seasonal soil and leaf CO_2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmos. Environ. 41, 2447–2455.

Barba, J., Curiel Yuste, J., Lloret, F., 2015. Changes in litter chemistry and functioning of decomposer communities associated with drought-induced species succession affect ecosystem carbon and nitrogen dynamics. Submitted at Plant and Soil.

Barba, J., Curiel Yuste, J., Martínez-Vilalta, J., Lloret, F., 2013. Drought-induced tree species replacement is reflected in the spatial variability of soil respiration in a mixed Mediterranean forest. For. Ecol. Manage. 306, 79–87.

Bartón, K., 2014. MuMIn: Multi-model inference. R package version 3.1-96.

Binkley, D., Giardina, C., 1998. Why do tree species affect soils? The warp and woof of tree-soil interactions. Biogeochemistry 42, 89–106.

Binkley, D., Stape, J.L., Takahashi, E.N., Ryan, M.G., 2006. Tree-girdling to separate root and heterotrophic respiration in two Eucalyptus stands in Brazil. Oecologia 148, 447–454.

Brown, M., Black, T.A., Nesic, Z., Foord, V.N., Spittlehouse, D.L., Fredeen, A.L., Grant, N.J., Burton, P.J., Trofymow, J.A., 2010. Impact of mountain pine beetle on the net ecosystem production of lodgepole pine stands in British Columbia. Agric. For. Meteorol. 150, 254-264.

Carnicer, J., Barbeta, A., Sperlich, D., Coll, M., Peñuelas, J., 2013. Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale. Front. Plant Sci. 4, 409.

Carnicer, J., Coll, M., Pons, X., Ninyerola, M., Vayreda, J., Peñuelas, J., 2014. Large-scale recruitment limitation in Mediterranean pines : the role of Quercus ilex and forest successional advance as key regional drivers. Glob. Ecol. Biogeogr. 23, 371–384.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T. V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V., Westoby, M., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett. 11, 1065–1071.

Curiel Yuste, J., Barba, J., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Mattana, S., Martínez-Vilalta, J., Nolis, P., Lloret, F., 2012. Changes in soil bacterial community triggered by drought-induced gap succession preceded changes in soil C stocks and quality. Ecol. Evol. 2, 3016–3031.

Curiel Yuste, J., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Ogaya, R., Penuelas, J., Sardans, J., Lloret, F., 2014. Strong functional stability of soil microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline precipitation. Soil Biol. Biochem. 69, 223–233.

Dixon, R.K., Solomon, A.M., Brown, S.E., Houghton, R.A., Trexier, M.C., Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. Science 263, 185–190.

Edburg, S.L., Hicke, J.A., Brooks, P.D., Pendall, E.G., Ewers, B.E., Norton, U., Gochis, D., Gutmann, E.D., Meddens, A.J.H., 2012. Cascading impacts of bark beetle-caused tree mortality on coupled biogeophysical and biogeochemical processes. Front. Ecol. Environ. 10, 416-424.

Ek, H., 1997. The influence of nitrogen fertilization on the carbon economy of Paxillus involutus in ectomycorrhizal association with Betula pendula. New Phytol. 135, 133–142.

Galiano, L., Martínez-Vilalta, J., Eugenio, M., Granzow-de La Cerda, Í., Lloret, F., 2013. Seedling emergence and growth of Quercus spp. following severe drought effects on a Pinus sylvestris canopy. J. Veg. Sci. 24, 580–588.

Galiano, L., Martínez-Vilalta, J., Lloret, F., 2010. Drought-Induced Multifactor Decline of Scots Pine in the Pyrenees and Potential Vegetation Change by the Expansion of Co-occurring Oak Species. Ecosystems 13, 978–991.

Gough, C.M., Hardiman, B.S., Nave, L.E., Bohrer, G., Maurer, K.D., Vogel, C.S., Nadelhoffer, K.J., Curtis, P.S., 2013. Sustained carbon uptake and storage following moderate disturbance in a Great Lakes forest. Ecol. Appl. 23, 1202–1215.

Heinemeyer, A., Hartley, I.P., Evans, S.P., De La Fuente, J.A.C., Ineson, P., 2007. Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Glob. Chang. Biol. 13, 1786–1797.

Hereș, A.M., Martínez-Vilalta, J., Claramunt López, B., 2012. Growth patterns in relation to drought-induced mortality at two Scots pine (Pinus sylvestris L.) sites in NE Iberian Peninsula. Trees 26, 621–630.

Hereter, A., Sánchez, J.R., 1999. Experimental areas of Prades and Montseny, in: Rodà, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.), Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin, pp. 15–27.

Högberg, P., Löfvenius, M.O., Nordgren, A., 2009. Partitioning of soil respiration into its autotrophic and heterotrophic components by means of tree-girdling in old boreal spruce forest. For. Ecol. Manage. 257, 1764–1767.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–92.

Högberg, P., Read, D.J., 2006. Towards a more plant physiological perspective on soil ecology. Trends Ecol. Evol. 21, 548–554.

Jackson, R.B., Mooney, H.A., Schulze, E.D., 1997. A global budget for fine root biomass, surface area, and nutrient contents. Proc. Natl. Acad. Sci. U. S. A. 94, 7362–7366.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grunwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, U., Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Glob. Chang. Biol. 7, 269–278.

Keenan, T., García, R., Friend, A.D., Zaehle, S., Gracia, C., Sabate, S., 2009. Improved understanding of drought controls on seasonal variation in Mediterranean forest canopy CO_2 and water fluxes through combined in situ measurements and ecosystem modelling. Biogeosciences 6, 1423–1444.

Kuzyakov, Y., 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biol. Biochem. 38, 425–448.

Levy-Varon, J.H., Schuster, W.S.F., Griffin, K.L., 2012. The autotrophic contribution to soil respiration in a northern temperate deciduous forest and its response to stand disturbance. Oecologia 169, 211–220.

Levy-Varon, J.H., Schuster, W.S.F., Griffin, K.L., 2014. Rapid rebound of soil respiration following partial stand disturbance by tree girdling in a temperate deciduous forest. Oecologia 174, 1415–1424.

Lloret, F., Escudero, A., Iriondo, J.M., Martínez-Vilalta, J., Valladares, F., 2012. Extreme climatic events and vegetation: the role of stabilizing processes. Glob. Chang. Biol. 18, 797–805.

Malhi, Y., Baldocchi, D.D., Jarvis, P.G., 1999. The carbon balance of tropical, temperate and boreal forests. Plant Cell Environ. 22, 715–740.

Martínez-Vilalta, J., Aguadé, D., Banqué, M., Barba, J., Curiel Yuste, J., Galiano, L., Garcia, N., Gómez, M., Hereş, A.M., López, B.C., Lloret, F., Poyatos, R., Retana, J., Sus, O., Vayreda, J., Vilà-Cabrera, A., 2012. Las poblaciones ibéricas de pino albar ante el cambio climático: con la muerte en los talones. Rev. Ecosistemas 21, 15-21.

Martínez-Vilalta, J., Piñol, J., 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage. 161, 247–256.

Moore, D.J.P., Trahan, N.A., Wilkes, P., Quaife, T., Stephens, B.B., Elder, K., Desai, A.R., Negron, J., Monson, R.K., 2013. Persistent reduced ecosystem respiration after insect disturbance in high elevation forests. Ecol. Lett. 16, 731–737.

Moyano, F.E., Atkin, O.K., Bahn, M., Bruhn, D., Burton, A.J., Heinemeyer, A., Kutsch, W.L., Wieser, G., 2010. Respiration from roots to mycorrhizosphere, in: Kutsch, W.L., Bahn, M., Heinemeyer, A. (Eds.), Soil Carbon Dynamics. An Integrated Methodology. Cambridge University Press, Cambridge, pp. 127–156.

Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. J. Geophys. Res. Biogeosciences 116.

Ninyerola, M., Pons, X., Roure, J.M., 2007a. Objective air temperature mapping for the Iberian Peninsula using spatial interpolation and GIS. Int. J. Climatol. 27, 1231–1242.

Ninyerola, M., Pons, X., Roure, J.M., 2007b. Monthly precipitation mapping of the Iberian Peninsula using spatial interpolation tools implemented in a Geographic Information System. Theor. Appl. Climatol. 89, 195–209.

Palacio, S., Maestro, M., Montserrat-Martí, G., 2007. Seasonal dynamics of non-structural carbohydrates in two species of mediterranean sub-shrubs with different leaf phenology. Environ. Exp. Bot. 59, 34–42.

Peñuelas, J., Lloret, F., Montoya, R., 2001. Severe drought effects on Mediterranean woody flora in Spain. For. Sci. 47, 214–218.

Pereira-Blanco, E., 2014. Response of fine root respiration to variations in biotic and abiotic factors in a mixed Mediterranean forest affected by drought induced secondary succession. Universitat Autònoma de Barcelona, Bellaterra.

Piao, S., Luyssaert, S., Ciais, P., Janssens, I.A., Chen, A., Cao, C., Fang, J., Friedlingstein, P., Luo, Y., Wang, S., 2010. Forest annual carbon cost: a global-scale analysis of autotrophic respiration 91, 652–661.

Pinheiro, J., Bates, D., DepRoy, S., 2009. Linear and Nonlinear Mixed Effects Models. R package version 3.1-96.

Poyatos, R., Aguadé, D., Galiano, L., Mencuccini, M., Martínez-Vilalta, J., 2013. Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytol. 200, 388–401.

Redding, T., Winkler, R., Teti, P., Spittlehouse, D., Boon, S., Rex, J., Dube, S., Moore, R.D., Wei, A., Carver, M., Schnorbus, M., Reese-Hansen, L., Chatwin, S., 2008. Mountain pine beetle and watershed hydrology. BC J. Ecosyst. Manag. 9, 33–50.

Reed, D.E., Ewers, B.E., Pendall, E., 2014. Impact of mountain pine beetle induced mortality on forest carbon and water fluxes. Environ. Res. Lett. 9, 105004.

Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M.D., Seneviratne, S.I., Zscheischler, J., Beer, C., Buchmann, N., Frank, D.C., Papale, D., Rammig, A., Smith, P., Thonicke, K., van der Velde, M., Vicca, S., Walz, A., Wattenbach, M., 2013. Climate extremes and the carbon cycle. Nature 500, 287–95.

Reichstein, M., Tenhunen, J.D., Roupsard, O., Ourcival, J., Rambal, S., Miglietta, F., Peressotti, A., Pecchiari, M., Tirone, G., Valentini, R., 2002. Severe drought effects on ecosystem CO2 and H2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? Glob. Chang. Biol. 8, 999–1017.

Rey, A., Pegoraro, E., Tedeschi, V., De Parri, I., Jarvis, P.G., Valentini, R., 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Glob. Chang. Biol. 8, 851–866.

Ruehr, N.K., Buchmann, N., 2010. Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration. Tree Physiol. 30, 165–176.

Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441–451.

Subke, J.-A., Inglima, I., Francesca Cotrufo, M., 2006. Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review. Glob. Chang. Biol. 12, 921–943.

Subke, J.-A., Voke, N.R., Leronni, V., Garnett, M.H., Ineson, P., 2011. Dynamics and pathways of autotrophic and heterotrophic soil CO2 efflux revealed by forest girdling. J. Ecol. 99, 186–193.

Tang, J., Baldocchi, D.D., Xu, L., 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Glob. Chang. Biol. 11, 1298–1304.

Tedeschi, V., Rey, A., Manca, G., Valentini, R., Jarvis, P.G., Borghetti, M., 2006. Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. Glob. Chang. Biol. 12, 110–121.

Uren, N.C., 2000. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants, in: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 19–40.

Van der Molen, M.K., Dolman, A.J., Ciais, P., Eglin, T., Gobron, N., Law, B.E., Meir, P., Peters, W., Phillips, O.L., Reichstein, M., Chen, T., Dekker, S.C., Doubková, M., Friedl, M.A., Jung, M., van den Hurk, B.J.J.M., de Jeu, R.A.M., Kruijt, B., Ohta, T., Rebel, K.T., Plummer, S., Seneviratne, S.I., Sitch, S., Teuling, A.J., van der Werf, G.R., Wang, G., 2011. Drought and ecosystem carbon cycling. Agric. For. Meteorol. 151, 765-773.

Van Mantgem, P.J., Stephenson, N.L., Byrne, J.C., Daniels, L.D., Franklin, J.F., Fulé, P.Z., Harmon, M.E., Larson, A.J., Smith, J.M., Taylor, A.H., Veblen, T.T., 2009. Widespread increase of tree mortality rates in the western United States. Science 323, 521–524.

Vilà-Cabrera, A., Martínez-Vilalta, J., Galiano, L., Retana, J., 2013. Patterns of Forest Decline and Regeneration Across Scots Pine Populations. Ecosystems 16, 323–335.

Vivanco, L., Austin, A.T., 2008. Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. J. Ecol. 96, 727–736.

Chapter 4

Multi-temporal influence of vegetation on forest soil respiration under drought-induced tree replacement of Scots pine by Holm oak

Josep Barba, Jorge Curiel Yuste, Rafael Poyatos, Ivan Janssens and Francisco Lloret

ABSTRACT

Plant photosynthetic activity influences soil respiration (SR) because, among other things, it supplies fine roots and rhizosphere with the photosynthates needed for their activity. However, how drought-induced die-off and tree species replacement could modify this plant photosynthetic influence over SR remains poorly understood. To determine the photosynthesis-SR relationship at different timescales, we measured every 30 min SR using solid-state CO_2 sensors and sap flow (SF) as a proxy of photosynthesis under (i) non-defoliated pine [NDP], (ii) defoliated pine [DFP], (iii) dead pine [DP] and (iv) Holm oak [HO] in a mixed Mediterranean forest where Scots pines (*Pinus sylvestris* L.) are undergoing a drought-induced die-off and are being replaced by Holm oaks (*Quercus ilex* L.). Direct plant photosynthetic effect on SR was not constant over the die-off and replacement process and varied across timescales. At the diel time-scale, changes in SF resulted in immediate changes in SR. This diel SF influence on SR was stronger for living pines [NDP and DFP] than for HO. At seasonal scale, however, HO was more determined by SF than in living pines, suggesting that the mechanisms of plant photosynthetic control over SR differed markedly among those two phylogenetically distant tree species. Both diel and seasonal analyses of the relation between photosynthesis of living pines and oaks with the SR under dead pines showed evidences of colonization by HO and not by the remaining living pines of gaps created after the dead of pines. This colonization would result in increased functional soil resilience.

1. INTRODUCTION

It is well known that variability of soil respiration (SR) from local to regional scales are tightly correlated with vegetation activity (Janssens et al., 2001; Tang et al., 2005). This effect of plant photosynthetic activity over SR could be direct, via the strong dependence of the autotrophic component of SR (roots and mycorrhiza) on the continuous supply of photosynthates produced by plants (Högberg et al., 2009, 2001) but also by stimulation of heterotrophic respiration throughout organic matter (litter) and exudates continuously added into the soil. Vegetation can also indirectly control soil respiration, since it has the capacity to modify soil abiotic conditions (Binkley and Giardina, 1998; Yuan et al., 2012). Despite being a large consensus on the crucial role that plant photosynthetic activity plays on SR, there are several issues that are still poorly understood, and hence hindering the properly interpretation of the mechanisms driving this multi-scalar above-belowground functional relation. Firstly, the influence of plant photosynthetic activity on SR and its different components could vary depending on the temporal scale at which they are considered (Moyano et al., 2008; Tang and Baldocchi, 2005; Vargas et al., 2011). For instance, it has been shown that seasonal changes in plant leaf area are strongly coupled to seasonal changes in SR (Curiel Yuste et al., 2004) because at this time scale soil metabolic activity is strongly dependent on the capacity of plants to supply carbohydrates to belowground compartments. However, the possible controls of plant photosynthetic activity over day-to-day variations in SR seems to be very site-dependent and subject to phloem transport lags (Kuzyakov and Gavrichkova, 2010; Mencuccini and Holtta, 2010). Nevertheless, it is important to highlight the difficulty, when working with temporal series, to infer causality between variables that show time lags between their signals. Several studies have reported that the relationship between plant photosynthetic activity and SR varies from few hours to several days, but there is not a consensus about how those time lags are related to uncertainties associated with the different techniques employed to determine mechanisms of carbohydrates-or-signal transport from leaves to fine roots (Kuzyakov and Gavrichkova, 2010; Mencuccini and Holtta, 2010). Additionally, methods commonly used to analyse high frequency time series data (Fourier transformation or cross-correlation analysis) does not allow to discern this relation at multi-temporal scales. Secondly, solar radiation determines, directly or indirectly, the daily cycle of many variables such as soil respiration, photosynthetic activity or soil temperature (Baldocchi et al., 2006); thus autocorrelation between these variables can hinder the interpretation of the relationship between SR and its drivers (Janssens et al., 2001). Particularly, the distinction of the temperature effect is essential in order to determine direct influence of photosynthesis on SR. Finally, and due to methodological and technical constraints, it is difficult to obtain simultaneous, high-resolution data of SR and photosynthesis at tree level, which hinders the possibility to study in detail the possible causal-effect relations between both set of processes. Time series

analysis (TSA), one of the most used approaches for studying this above-belowground relation at high-resolution frequency measurements (Kuzyakov and Gavrichkova, 2010), has been usually performed to analyse variations in SR in relation to ecosystem-scale proxies for photosynthesis (gross primary production [GPP] obtained from eddy covariance measurements) or to ecosystem-scale variations in physical parameters directly related to photosynthesis (photosynthetically active radiation [PAR], vapor pressure deficit [VPD]) (Ekblad et al., 2005; Gaumont-Guay et al., 2008; Tang et al., 2005). In brief, the absence of photosynthetic time series at tree level hinders the mechanistic understanding of this above-belowground relation. This problem is especially relevant in ecosystems with different tree species or in forests where tree diseases, pests or climate-induced die-off produces differential photosynthesis activity.

In Mediterranean basin, the increment of frequency and intensity of drought events during the last decades (Stocker et al., 2013) are currently producing an increment of drought-induced forest die-off (Carnicer et al., 2011). This increment of drought conditions concurs with the progressive abandonment of forest management (Vayreda et al., 2011), resulting in an increase of stems density, thus reducing water availability (Vilà-Cabrera et al., 2013). Therefore, drought-induced mortality could lead to a vegetation shift if the recruitment of dominant species and their adult mortality were not balanced through time (Lloret et al., 2012). These species replacement could have important consequences on photosynthesis activity and SR, that could in turn modify the forest capability to act as net carbon sink or source (Janisch and Harmon, 2002).

We studied soil respiration in a mixed forest dominated by Scots pine (*Pinus sylvestris* L.) in the overstorey, and Holm oak (*Quercus ilex* L.) in the understorey in the Prades Mountains (NE Iberian Peninsula). Forest structural data suggests that Holm oak is replacing Scots pine as the overstorey dominant species (Vilà-Cabrera et al., 2013) as consequence of two processes. Firstly, Scots pine population is suffering the extreme drought events during the last decades more than Holm oak trees (Martínez-Vilalta and Piñol, 2002). Secondly, the abandonment of forest management over 30 years ago (Hereş et al., 2012) has produced an increment of stems density which have increased competition for water (Vilà-Cabrera et al., 2013) and have created more shadowed conditions, benefiting Holm oak recruitment more than Scots pine seedling emergence (Galiano et al., 2013; Vilà-Cabrera et al., 2013). Previous studies in the area suggested that there is a functional resilience in SR following Scots pine die-off (along the sequence from non-defoliated pines to defoliated pines and dead pines). However important changes appeared when considering species replacement from Scots pine to Holm oak (Barba et al., 2015, n.d.). Here we study how changes in vegetation composition influence the relationship between plant photosynthesis and SR at high-resolution temporal scale. For this purpose, we measured SR and

sap flow at four stages of the drought-induced Scots pine die-off and replacement by Holm oak: non-defoliated pine [NDP], defoliated pine [DFP], dead pine [DP] and Holm oak [HO]). Our specific aims were:

1) To determine the direct influence of sap flow (SF, as a proxy of photosynthesis) on SR regardless of temperature variations from diel to seasonal scales.

2) To determine the time lags between soil respirations and sap flow.

3) To study the changes in the controls of SF over SR along the Scots pine die-off and Holm oak replacement gradient, paying particular attention to the influence that sap flow from healthy trees (NDP and HO) may have on SR beneath dead pine canopy.

2. MATERIALS AND METHODS

2.1. Study site

The study was conducted on a mixed Mediterranean forest in the northwest face of the Titllar Valley (Prades Mountains, NE Iberian Peninsula). The climate is Mediterranean, with a mean annual temperature of 11.2°C and precipitation of 664 mm (Ninyerola et al., 2007a, 2007b). The experimental area was located at an elevation of between 1,010 and 1,030 m a.s.l. with a great steepness (33°). Soils are xerochrespts with clay loam texture and a high gravel content of 46%. Fractured metamorphic schist outcrops on to a large part of the study area. For more information related to the study area, see (Barba et al., 2013; Hereter and Sánchez, 1999).

The mixed forest, which has not been managed for the last 30 yr (Hereş et al., 2012), is dominated by Scots pine (*Pinus sylvestris* L) in the overstorey and Holm oak (*Quercus ilex* L) in the understorey. Severe drought events since 1990s have produced Scots pine population die-off, with a mean crown defoliation of 52% and standing mortality of 12% (Vilà-Cabrera et al., 2013). This situation coupled to the low regeneration of pines (Vilà-Cabrera et al., 2013) and the much lower impact of drought on Holm oak is enhancing a replacement of pines by oaks as the overstorey dominant specie.

In order to study at high temporal resolution SR along the sequence of Scots pine die-off and replacement by Holm oak, four trees were selected (one non-defoliated pine [NDP], one defoliated pine [DFP], one dead pine [DP] and one Holm oak [HO]) within a maximum distance between them of 25 m. Defoliated pine had less than 50% of green leaves, visually estimated as

the percentage of green needles relative to a healthy canopy of a similar sized tree from the same population (Galiano et al., 2010).

2.2. Soil environmental and meteorological measurements

A data acquisition system (CR1000datalogger and AM16/32 multiplexers, Campbell Scientific Inc., Logan, UT, USA) was used to store 30 min means of soil temperature, volumetric soil water content (θ), sap flow (SF) and meteorological variables sampled every 30 s. Means of CO_2 concentrations measured twice per hour during 5 min were stored with the same acquisition system. A 16-m tower was installed less than 100 m from the farthest tree with sensors for measuring air temperature and air relative humidity (CS215, Campbell Scientific Inc.), precipitation (52203, R.M. Young Company, Traverse City, MI, USA), total solar radiation (SP1110, Skye Instruments Ltd, Llandrindod Wells, Powys, UK) and wind speed (05103-5, R.M. Young Company). Soil temperature at 5 cm depth was measured within 2-meters of each tree with thermocouple sensors. Average volumetric soil water content (θ) in the upper 30 cm of soil was monitored using a frequency domain reflectometer (CS616, Campbell Scientific INC.) installed among the studied trees. In order to correct θ, automatic measurements likely affected by the great stoniness, volumetric soil water content measured in soil samples were regressed against monitored θ (see Poyatos et al. 2013 (Poyatos et al., 2013) for more details).

2.3. Soil CO_2 efflux

Soil CO_2 concentrations were measured within 2-meters of each tree with Vaisala CARBOCAP CO_2 sensors (models GMT220; Vaisala, Helsinki, Finland) from April 2010 to December 2010. At each tree, two CO_2 sensors were installed at different depths (1 and 5 cm). The use of these sensors hinders measuring a large number of trees, but allows us to obtain CO_2 data at high temporal resolution and describe SR variability from daily to seasonal scales. The CO_2 concentrations reading from the Vaisala proves were continuously corrected for variations in soil temperature and air pressure (Tang et al., 2003). Soil CO_2 efflux was estimated for each tree from soil CO_2 concentrations measured at 1 and 5 cm depths using a flux gradient method based on Flick's law of diffusion:

$$F = -D_s \frac{\partial C}{\partial z}, \qquad\qquad\qquad\qquad Eqn1$$

where F is the CO_2 efflux (μmol m^{-2} s^{-1}), D_s is the gaseous diffusion coefficient of CO_2 in the soil and $\partial C/\partial z$ the vertical soil CO_2 gradient. D_s can be estimated as

$$D_s = \xi\, D_a ,$$ <div align="right">Eqn 2</div>

where ξ is the gas tortuosity factor and D_a is the CO_2 molecular diffusivity of CO_2 in the free air.

The effect of temperature and pressure on D_a is given by

$$D_a = D_{a0} \left(\frac{T}{T_0}\right)^{1.75} \frac{P_0}{P} ,$$ <div align="right">Eqn 3</div>

where D_{a0} is the reference value of D_a at T_0 (293.15 K) and at P_0 (1.013 X 10^5 Pa), and is given as 1.47 X 10^{-5} m²s⁻¹ (Jones, 1992).

To calculate the tortuosity factor, the most commonly used Millington-Quirk model (Millington and Quirk, 1961)was applied:

$$\xi = \frac{\alpha^{10/3}}{\phi^2},$$ <div align="right">Eqn 4</div>

where α is the volumetric air content (air-filled porosity), φ the porosity (sum of α and the volumetric water content (θ)).

$$\phi = \alpha + \theta = 1 - \frac{\rho_b}{\rho_m},$$ <div align="right">Eqn 5</div>

where ρ_b is the bulk density and ρ_m the particle density for the mineral soil calculated for soil samples close to each pair of CO_2 sensors.

Absolute values of SR, that could be used to calibrate the estimated fluxes, could not be obtained since no direct chamber SR measures were done close to CO_2 sensors. However, these kinds of calibrations only affect the absolute values but not the temporal patterns of SR, because the SR data is usually corrected with the intercept and the slope of linear regressions generated from the correlation between SR data inferred from diffusivity models and chamber-based measures. Since we aimed to focus on the temporal patterns, CO_2 fluxes directly estimated from CO_2 concentrations were analysed.

2.4. Sap flow measurements

Additionally, sap flow per unit leaf area (SF) was recorded as a proxy of photosynthetic activity in the same living trees (one non-defoliated pine, one defoliated pine and one Holm oak) for the same period than SR measurements. SF was measured with constant heat dissipation sensors (Granier, 1985) manufactured in our laboratory. Prove pairs were installed in April 2010, inserted into xylem (depth of 2 cm in Scots pines and 1.5 cm in Holm oaks) at breast height with

a vertical separation of 12 cm and covered with reflective bubble wrap to avoid the natural temperature gradients. See (Poyatos et al., 2013) for additional information about the applied corrections (e.g. natural temperature gradients in stem, sap flow density in the outer xylem, radial corrections to obtain whole tree sap flow on a sapwood area).

Despite SF has been reported as a good proxy of photosynthesis rates (Irvine et al., 2005), we assume that this relation is not complete under drought conditions due changes in the water use efficiency during carbon assimilation (Reichstein et al., 2002).

2.5. Data analysis

The direct inference of causality from SF to SR could overestimate the contribution of photosynthetic activity on SR because diurnal solar radiation cycle drives changes in temperature, which in turn, influences many variables including SR, SF or air diffusivity, producing auto-correlation effect (Vargas et al., 2011). Thus, in our analysis of the relationship between SF and SR we removed the effect of changes in temperature both on SR and SF. The direct effect of temperature on SR was removed for each tree using the exponential fit equation:

$$SR_c = SR - B_1 exp^{(B_2 * T_s)} \qquad \qquad \text{Eqn 6}$$

where SR_c is soil respiration corrected by temperature, SR is soil respiration estimated with Flick's law of diffusion from CO_2 measurements (Eqn 1) and B_1 and B_2 are parameters evaluated for each single day based on half hour T_s measurements. Additionally, temperature also influences SR indirectly, modifying the gaseous diffusion coefficient (D_s) of CO_2 in the soil. So we removed for each tree the effect of changes in D_s on SRr,

$$SR_r = SR_c - (B_3 + B_4 * D_s) \qquad \qquad \text{Eqn 7}$$

where SR_r is soil respiration corrected by T_s and CO_2 diffusivity, SR_c is soil respiration corrected by T_s (residuals from Eqn 6) and B_3 and B_4 are parameters calculated for each day based on half hour D_s estimations obtained with the Eqn 2.

SF was also temperature-corrected by fitting simple linear regression for each tree:

$$SF_r = SF - (B_5 + B_6 T_s) \qquad \qquad \text{Eqn 8}$$

where SF_r is the corrected sap flow, SF is the measured sap flow and B_5 and B_6 are parameters evaluated for each single day based on half hour soil temperature (T_s) measurements.

Figure 1. Seasonal course of environmental variables over the study period (April 29th 2010 - December 10th 2010). a) daily precipitation; b) soil water content (SWC) measured every 30 min; c) soil temperature measured every 30 min. Note that the labels in the *x* axis represent day of the year (DOY).
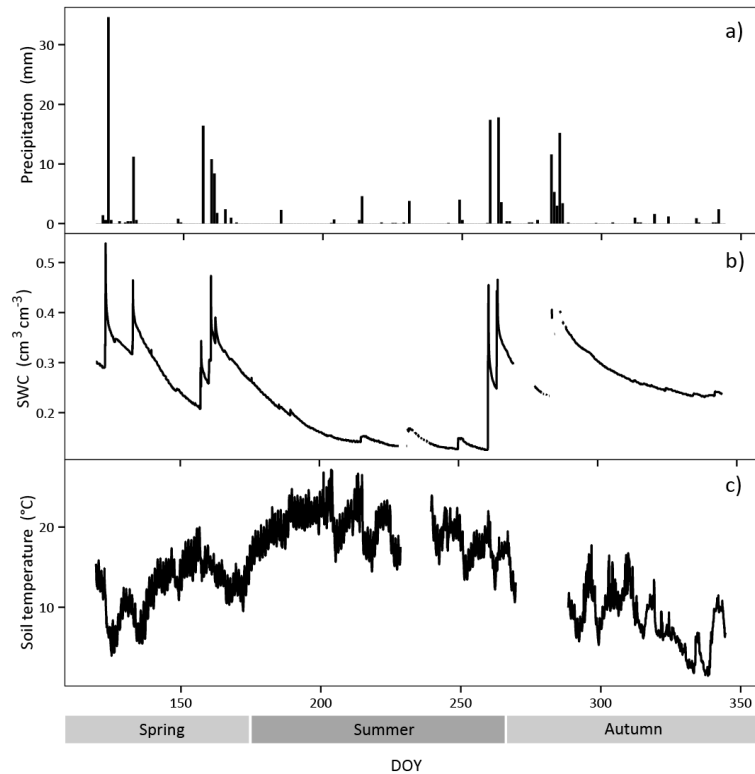
Wavelet coherence analyses were applied to analyse the relationship between SRr and SFr for each tree at multi-temporal scales. Wavelet coherence analysis is best suited for studying transient signals (oscillatory or non-oscillatory), or signals whose amplitude varies in time. This method is widely used in the field of general signal processing, in cases where the signal is localized both in time and frequency (Torrence and Compo, 1998). Since we are interested in the relationship between transient signals that may be present in two different time series, we have calculated the cross-wavelet transform and wavelet coherence pairwise to the dataset (for an introduction to this topic, see (Grinsted et al., 2004)). The wavelet coherence can be thought as a "localized correlation coefficient in time frequency space" (Grinsted et al., 2004). The calculations have been performed in MATLAB R2009b (MathWorks, Natick, MA, USA).

The wavelet analysis was calculated for each living tree (NDP, DFP and HO) between its temperature detrended time series soil respiration (SRr) and its temperature detrended time series sap flow (SFr). Additionally, wavelet analysis was calculated between DP SRr and NDP SFr, and DP SRr and HO SFr to test separately the influence of non-defoliated pine or Holm oak activity on SRr beneath dead pines. For wavelet analysis between SR-Temp, SR-SF, SF-Temp and SR-SWC, see Supplementary Material.

To assess the dependency of SR to SF at seasonal scale, linear models for each tree with daily averaged data were performed between SR, SF, soil temperature and SWC. Daily SR and SF were

not temperature detrended at these seasonal analyses, so soil temperature and SWC were included in models as predictor variables in order to not overestimate the SF contribution on SR. SR, soil temperature and SWC daily data were directly calculated averaging the 30-min frequency data. SF used in the models were the midday average (from 11 to 13h), since this variable is more dependent on environmental conditions than whole day average. For each tree, all models combining SF, soil temperature, SWC and their first-order interactions were evaluated to achieve the minimum adequate model according to the corrected Akaike information criterion (AICc), using the MuMIn R package (Bartón, 2014). SF from both NDP and HO were included in the DP saturated model to test for the non-defoliated pine or Holm oak influence on seasonal variability of DP. SR, SF and SWC were log-transformed to introduce a SR-exponential response to soil temperature in daily-averaged data analysis. Analyses of linear models at daily level were carried out using R 3.0.3. (R Foundation for Statistical Computing, Vienna, Austria).
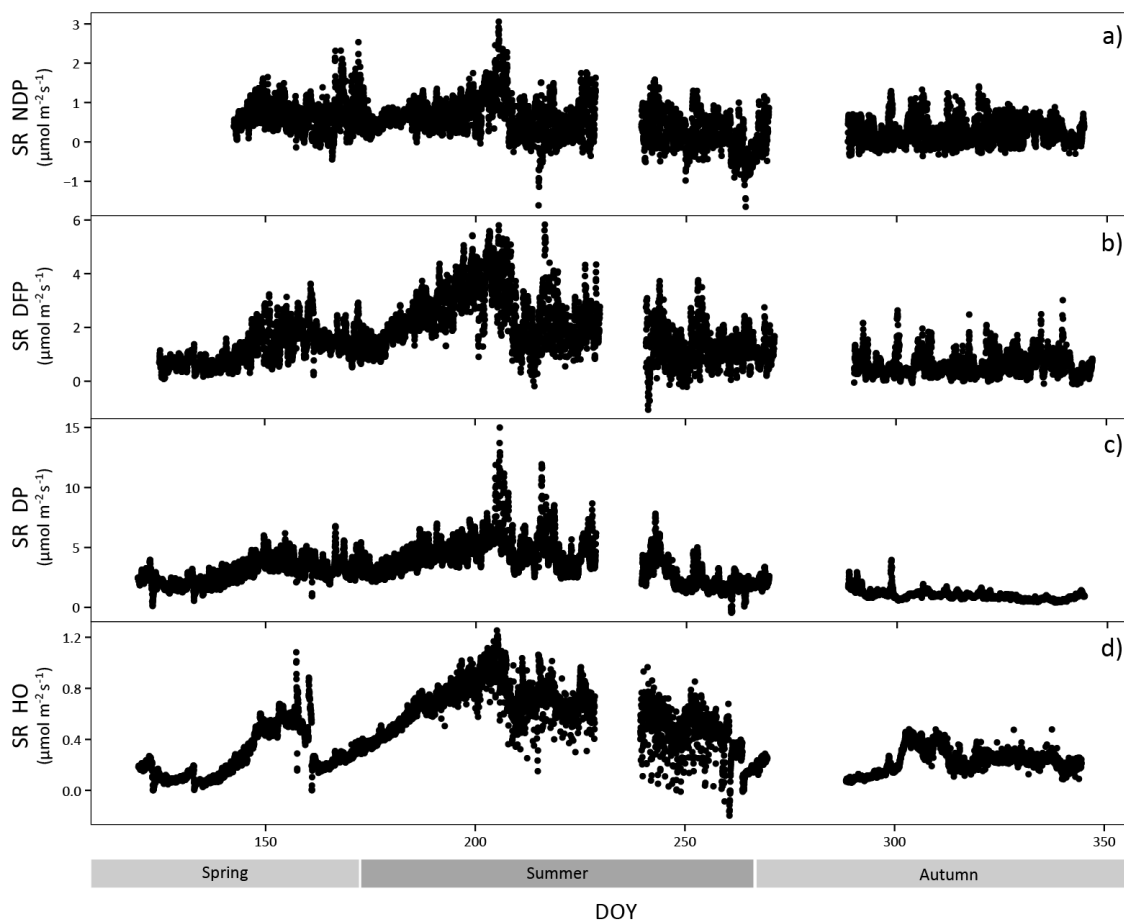

Figure 2. Seasonal course of soil respiration of non-defoliated pine (SR NDP) (a), defoliated pine (SR DFP) (b), dead pine (SR DP) (c) and Holm oak (SR HO) (d) measured every 30 min from DOY 142 to 228 (April 29th 2010 - December 10th 2010).
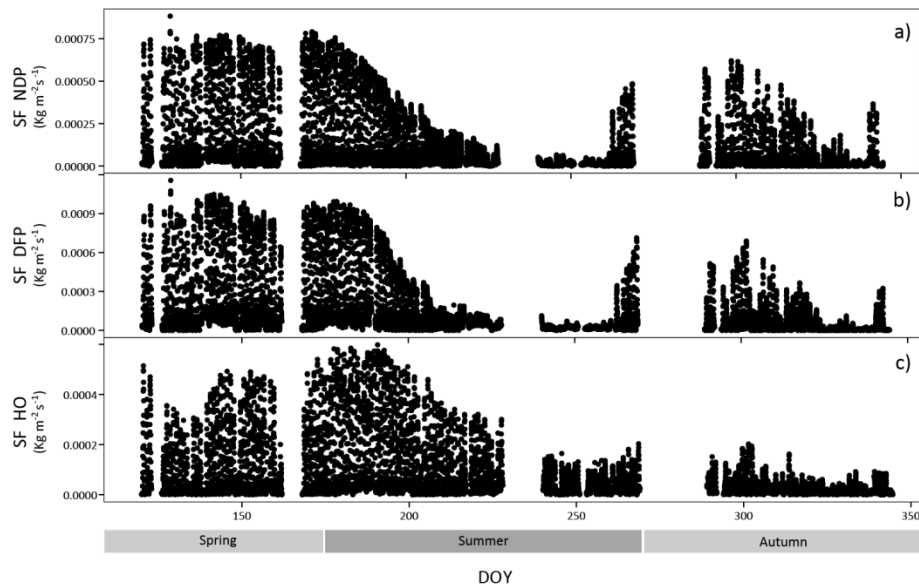
Figure 3. Seasonal course of sap flow of non-defoliated pine (SF NDP) (a), defoliated pine (SF DFP) (b) and Holm oak (SF HO) (c) measured every 30 min from DOY 142 to 228 (April 29th 2010 - December 10th 2010). The high dispersion in daily data is due the daily course of SF, corresponding the lowest values at night and the highest at midday SF. Midday values indicate seasonal pattern of SF.

3. RESULTS

3.1. Seasonal course of environmental variables, soil respiration and sap flow

Over the study period, accumulated precipitation was 197.5 mm, peaking in spring and autumn (Fig. 1a), which determined the observed SWC seasonal pattern (mean SWC was 23.8%) (Fig. 1b). Soil temperature ranged from 0 to 29.5°C with a mean of 14.3°C (Fig. 1c). SR increased along the spring, peaking in mid-July, when soil temperature achieved maximum values (more than 20°C) and SWC was near its minimum (Fig. 2). From mid-summer to the beginning of autumn rains, variations in SR were strongly coupled with fluctuations in SWC. The low temperature from the end of September concurred with low SR rates until the end of the experiment (December 2010). All trees showed similar seasonal pattern, although it was clearer in DFP, DP and HO than in NDP, where seasonal variability of SR was not so accentuated (Fig. 2). NDP and DFP showed similar temporal patterns of SF, maintaining high values during spring and decreasing drastically along the summer, following the reduction in SWC (Fig. 3). Autumn rains stimulated pines SF, which decreased again at the end of autumn. In HO the decrease of SF during summer was not as steep as in pines, and there was a very small response of SF to autumn rains. These seasonal variations in SF patterns were thoroughly discussed in (Poyatos et al., 2013) for the same study site.
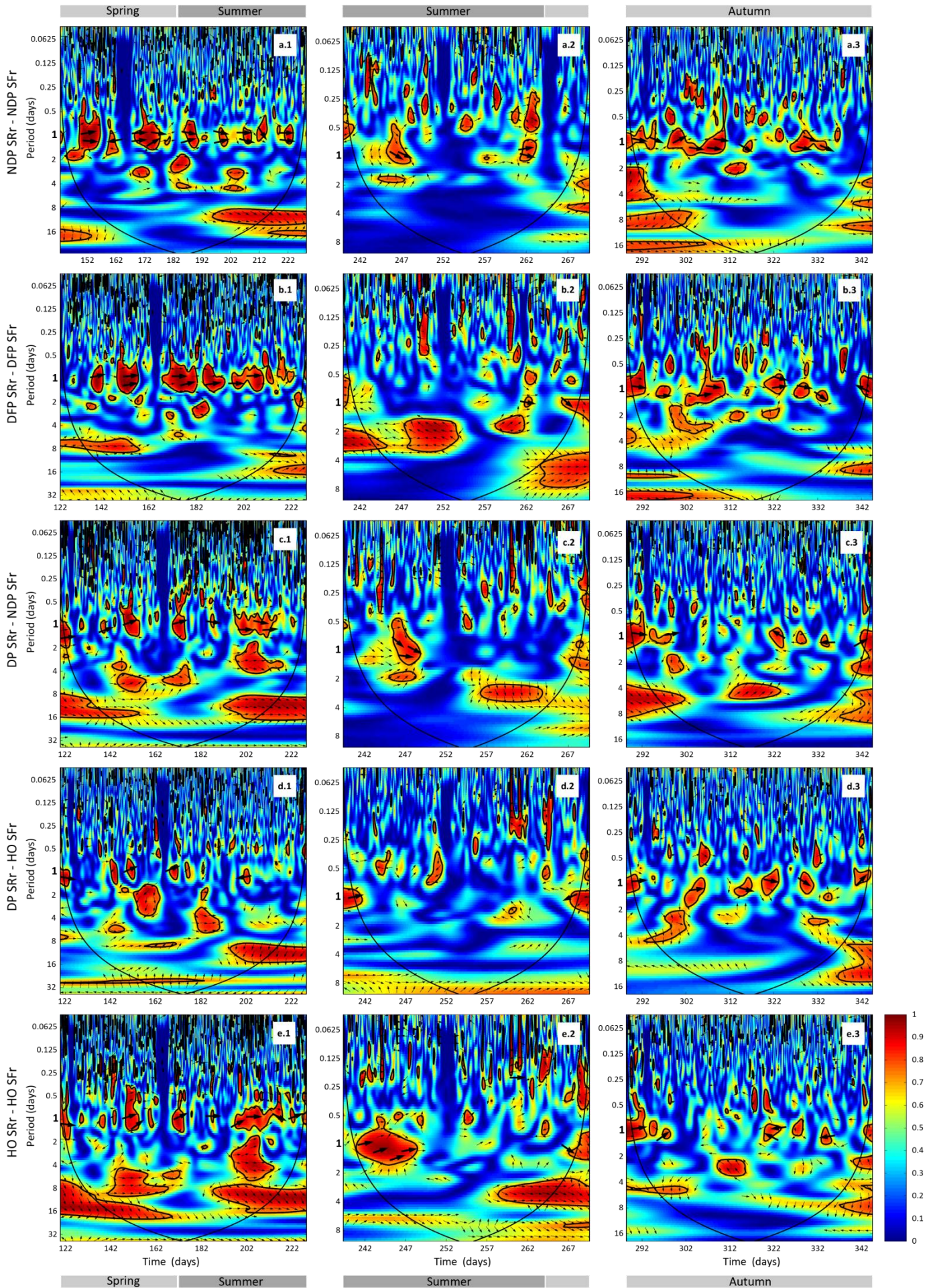
Figure 4. Cross wavelet analyses output and phase differences between corrected soil respiration (SRr) and corrected sap flow (SFr) from DOY 142 to 228 (April 29th 2010 - December 10th 2010). Two gaps in the time series (from DOY 228 to 239 and 269 to 288), resulting from power failures, required splitting each wavelet coherence analysis in three time series (1, 2 and 3). SRr and SFr from non-defoliated pines analysis are represented in panels a.1, a.2 and a.3. SRr and SFr from defoliated pines analysis are represented in panels b.1, b.2 and b.3. SRr from dead pines and SFr from non-defoliated pines analysis are represented in panels c.1, c.2 and c.3. SRr from dead pines and SFr from Holm oak analysis are represented in panels d.1, d.2 and d.3. SRr and SFr from Holm oak were represented in panels e.1, e.2 and e.3. The phase difference is shown by arrows. An arrow pointing to right corresponds to two signals which are in phase. If the arrows points downward, SRr delays the other variable by 90°, whereas if it points upward, SRr leads the other variable by 90°. The colour codes for power values are from dark blue (low values) to dark red (high values). Black lines contouring red areas represent the 5% significance level as calculated by 1000 Monte Carlo simulations (Grinsted et al., 2004). The other black lines indicate the cone influence that delimits the region not influenced by edge effects.

3.2. Daily patterns of SFr and SRr relationship with high-resolution data

The wavelet analysis of SRr and SFr (temperature-detrended soil respiration and sap flow time series) during the study period showed the strongest synchrony between the two signals with a period of one day (Figure 4). For these variables, NDP and DFP showed similar wavelet patterns over the study period at daily scale. These trees showed high daily correlation during spring and part of the summer (Fig. 4, panels a.1 and b.1) when SF fluxes were high, but there were a lack of correlation at the end of summer (Fig. 4, panels a.2 and b.2), when SFr fluxes drastically decreased. In living pines, autumn rains stimulated SFr, and thus, the correlation between SRr and SFr increased again (Fig. 4 panels a.3 and b.3), but it was not as high as in spring. The SRr and SFr signals for NDP and DFP were in phase over the whole study period (arrows pointing to right in Fig. 4), which means that these variables varied simultaneously. The wavelet analysis between SRr and SFr for HO showed almost no correlation over the study period at daily scale (Fig. 4 panels e), except for some short periods during spring and mid-summer. At these few periods, the two signals were in phase. Similar results were found when wavelet analyses were performed between HO SRr and SFr (both SFr from NDP or HO) (Fig. 4, panels c and d, respectively).

3.3. Sap flow seasonal influence on soil respiration with daily data

In NDP, the SR linear model including the interactions between soil temperature and SF and between SWC and SF (Table 1, NDP) explained 23% of the seasonal variability considering daily data. In this tree type, the effect of SF on SR was higher at both high soil temperature and SWC. The best model for DFP SR included SWC and the interaction between soil temperature and DFP

NDP

Variables	Estimate	SE	t-value	p-value
Intercept	1.624	2.467	0.658	0.512
Temp	0.410	0.095	4.328	< 0.001
SWC	4.291	2.130	2.014	0.046
SF_NDP	0.471	0.281	1.676	0.096
Temp * SF_NDP	0.044	0.010	4.223	< 0.001
SWC * SF_NDP	0.558	0.235	2.373	0.019

DFP

Variables	Estimate	SE	t-value	p-value
Intercept	-3.361	0.432	-7.777	< 0.001
SWC	-0.810	0.176	-4.611	< 0.001
Temp	0.241	0.030	7.932	< 0.001
SF_DFP	-0.153	0.047	-3.265	0.001
Temp * SF_DFP	0.021	0.003	6.684	< 0.001

DP

Variables	Estimate	SE	t-value	p-value
Intercept	1.289	0.284	4.540	< 0.001
Temp	0.072	0.005	14.606	< 0.001
SF_HO	0.171	0.026	6.617	< 0.001

HO

Variables	Estimate	SE	t-value	p-value
Intercept	-7.765	1.404	-5.532	< 0.001
Temp	0.231	0.050	4.650	< 0.001
SWC	-2.742	1.204	-2.278	0.024
SF_HO	-0.477	0.154	-3.104	< 0.001
Temp * SF_HO	0.021	0.005	4.090	< 0.001
SWC * SF_HO	-0.181	0.128	-1.415	< 0.001

Table 1. Summary of the minimum adequate model of non-defoliated pine (NDP), defoliated pine (DFP), dead pine (DP) and Holm oak (HO) daily soil respiration. Soil water content (SWC), sap flow of non-defoliated pine (SF_NDP), defoliated pine (SF_DFP) and Holm oak (SF_HO), as well as daily soil respiration were log transformed (see Materials and Methods for more details).

SF as predictor variables and was able to explain 64% of the seasonal variability. This interaction reflected that the higher temperature, the larger positive effect of SF on SR.

For DP, the best linear model for SR included the positive effect of soil temperature and HO SF (and not NDP SF) as predictor variables and was able to explain 76% of the seasonal variability.

For HO the best linear model for SR included the interactions between soil temperature and HO SF (positive effect) and between SWC and HO SF (negative effect) and explained 76% of the seasonal variability.

4. DISCUSSION

4.1. Sap flow influence on soil respiration

Sap flow, a reliable estimator of photosynthesis (Irvine et al., 2005), determined SR at diel timescale, supporting previous observations obtained from the application of similar methodologies (Vargas et al., 2011) or from other observations based on experimental field (destructive and stable isotope labelling) methods (Högberg et al., 2008; Subke et al., 2009). This direct and immediate effect was observed despite the fact that the photosynthesis-SR relationship was partially masked by other biotic sources of CO_2 independent or at least partially independent from photosynthesis, such as microbial decomposition of SOM and dead tissues (Kuzyakov, 2006). It is important to emphasize that the observed photosynthetic influence on SR occurred regardless of changes in soil temperature and gas diffusivity, which may act as confounding factors, obscuring the photosynthetic influence on SR (Curiel Yuste et al., 2004; Janssens et al., 2001; Vargas et al., 2011). In fact, the temperature corrections applied to SR and SF were so restrictive (all the potential effects of temperature were removed) that we are probably underestimating the real contribution of photosynthesis on SR.

The strong correlation of SF and SR at the diel time-scale, specially observed under living pines (NDP and DFP), was, however, not constant throughout time. Vargas et al. 2010 (Vargas et al., 2010) also found in a similar study system (mixed Mediterranean forest with *Quercus* and coniferous species) that the photosynthetic diel effect on SR was not constant over time, being stronger when both SR and SF showed higher rates during Spring and after the first rains in fall, as observed in this study. However, the periods of the strongest diel correlation between plant photosynthetic and SR did not necessarily coincided with periods of high autotrophic contribution to total SR. Indeed, the autotrophic SR contribution to total SR is generally higher during mid-summer and autumn, as observed in a parallel work carried out in the same study system (Barba et al., n.d.), which coincides with the period of lowest SF-SR correlations found in this study. Our results, therefore, suggests that during unfavourable periods (less C fixation and higher water stress), when SR reaches its minimum values and the autotrophic contribution to total SR peaks, this autotrophic contribution seems predominantly decoupled from concurrent photosynthesis, hence, it probably depended more on the mobilization of existing C reserves.

Other studies have described stronger positive relationship between soil autotrophic respiration and the total SR at high rates of SR (Kuzyakov and Gavrichkova, 2010; Subke et al., 2006) at diel scales which could partially agree with our results. From a mechanistic point of view, the seasonal and synoptic variations in the strength of the relationship between plant photosynthetic activity and SR could be explained by variations in processes controlling this

relation such as stomatal conductance (and thus in drought stress (Ruehr et al., 2009)), variations in carbon storage patterns or in photosynthetic rates (Hartley et al., 2006).

But photosynthetic activity not only determined SR at diel- but also at seasonal-time scale. Using daily averaged data we observed that SF strongly influenced SR at all tree types. This SF influence was affecting SR directly or in interaction with soil temperature or SF, which agrees with many studies that have shown the strong impact of the interactive effect of photosynthesis with other environmental over SR (Hartley et al., 2006; Janssens et al., 2001; Reichstein et al., 2003). Additionally, SF was also strongly regulated by temperature and SWC (Poyatos et al., 2013).

4.2. Lag between soil respiration and sap flow at diel scale

Wavelet coherence analysis showed no lag between SR and SF at diel time scale, which means that changes in SF resulted in immediate changes in SR. Since the phloem transport rate usually ranges between 0.5 to 1.0 m h^{-1} (Kuzyakov and Gavrichkova, 2010), the observed simultaneous photosynthetic influence on SR was more likely related to the fast propagation of waves of turgor and osmotic pressure in phloem produced by the addition of recently assimilated carbohydrates into the phloem (as suggested in (Mencuccini and Holtta, 2010)) than to the direct transport of assimilated carbon. Other studies of time series analysis using vapour pressure deficit (VPD), photosynthetically active radiation (PAR) or gross primary production (GPP) as proxies of photosynthesis have, however, reported lags of a few hours in the relationship between photosynthesis and SR at the diel time-scale (Misson et al., 2010; Tang et al., 2005; Vargas et al., 2011). The absence of SR-SF lag in our study, in contrast with the delay observed when using ecosystem-scale proxies for plant photosythentic activity, could be partially explained by an existing delay between these proxies and tree-based SF at diel scale. This delay would correspond to lags between evaporative demand and evapotranspiration above the canopy and SF measured at breast height at trunk (Ford et al., 2004). At the study site, lags up to 45 min were found between solar radiation and SF at breast height at trunk (Rafael Poyatos, unpublished data). In this regard, the use of SF provides an enormous advantage comparing with VPD, PAR, GPP, other meteorological, eddy-covariance or satellite derived variables, because SF is a variable tightly correlated with the capacity to obtain photosynthesis at the tree level. Having data of SR and photosynthesis at tree level is, therefore, essential in order to study the mechanism involved in this relation, especially in mixed forests or in systems with trees at different healthy states.

4.3. Soil respiration and sap flow relationship over the die-off and species replacement process

The photosynthesis influence on SR at diel and seasonal scales differed along the drought-induced die-off and species-replacement gradient. Interestingly, the diel influence of SF over SR differed among species, being higher for SR beneath living Scots pines (both NDP and DFP) than beneath Holm oaks. Pressure concentration waves (the fast signal transmission mechanism) depends on the osmotic pressure relative to turgor differences in the phloem, which has been reported to be highly variable among species, depending on the phloem structure and the connectivity between xylem and phloem (Thompson and Holbrook, 2003). Fast signal transmission mechanism seemed, therefore, to be more determining on regulating the photosynthesis-SR relationship for living Scots pines than for Holm oak at daily scale. This difference between species indicates that mechanisms and controls of autotrophic metabolic activity strongly differs between those two phylogenetically distant species, partially agreeing with differences in phloem conductivity between gymnosperms and angiosperms found in (Jensen et al., 2012). In this regard, our results suggest that the pine autotrophic compartment would be, apparently, more dependable upon recent carbon assimilation than oaks autotrophic compartment. Diel SR in HO should be more controlled by the mobilization of stored carbon than by the fast signal transmission mechanism (propagation of waves of turgor and osmotic pressure). Mencuccini et al. (2010) suggested that when the stored carbon is mobilized, the osmotic potential decreases and thus, the signal transmission decreases diminishes too (Mencuccini and Holtta, 2010).

The degree of leaf loss associated with drought did not affect the daily influence of SF on SR, since NDP and DFP showed very similar patterns (Figure 4), with high correlation from spring to mid-summer and during the second half of the autumn, when SF showed high daily rates.

At seasonal scale, photosynthesis daily-averaged data influenced SR at all ecotypes in the die-off/replacement gradient. Despite their similar diel behaviour, environmental drivers and SF could explain much more seasonal variability of SR under DFP than under NDP (64 and 23%, respectively). Although explanations behind these observed differences in the predictability of seasonal SR under NDP and DFP are not clear, differences between NDP and DFP in SF sensitivity to drought, in vulnerability to roots embolism or in the stored non-structural carbohydrates patterns across the tree organs found in the same study system (Aguadé et al., 2015; Poyatos et al., 2013) could be related to these differences in SR sensitivity to SF. In contrast with the results obtained at the diel time scale, SF exerted a stronger effect over seasonal variability of SR beneath HO than beneath living Scots pines. This could imply that the direct carbon assimilated transport (slow transport mechanism) played higher preponderance

control on SR beneath HO than beneath living pines, which was consistent with the daily scale findings.

A major finding of daily and seasonal analyses was that SR beneath DP was strongly determined by SF from Holm oaks, hence showing that, as speculated before (Curiel Yuste et al., 2012), the colonization of the gaps left by dead pines by Holm-oaks starts belowground. Wavelet analysis showed similar daily patterns in the SR-SF relationship between DP and HO, when SR of DP was regressed with SF of HO. At seasonal scale, SF of HO (and not SF of living pines) explained SR in this DP habitat. Indirect evidences of the HO colonization of gaps created by pines dead have also been reported for this study site, including convergence in soil bacterial diversity and community structure between DP and HO (Curiel Yuste et al., 2012), changes in organic matter decomposition rates over the die-off and replacement process (Barba et al., 2015) and in SR fluxes (Barba et al., 2013, n.d.). However, our results clearly showed that in the studied mixed forest of Prades mountains, HO is responsible of the colonization of gaps created by Scots pines dead after drought-induced die-off, promoting soil functional resilience.

5. ACKNOWLEDGMENTS

6. REFERENCES

Aguadé, D., Poyatos, R., Gómez, M., Oliva, J., Martínez-Vilalta, J., 2015. The role of defoliation and root rot pathogen infection in driving the mode of drought-related physiological decline in Scots pine (Pinus sylvestris L.). Tree Physiol. tpv005–. doi:10.1093/treephys/tpv005

Baldocchi, D., Tang, J., Xu, L., 2006. How switches and lags in biophysical regulators affect spatial-temporal variation of soil respiration in an oak-grass savanna. J. Geophys. Res. 111, 1–13. doi:10.1029/2005JG000063

Barba, J., Curiel Yuste, J., Lloret, F., 2015. Changes in litter chemistry and functioning of decomposer communities associated with drought-induced species succession affect ecosystem carbon and nitrogen dynamics. Soil Biol. Biochem.

Barba, J., Curiel Yuste, J., Martínez-Vilalta, J., Lloret, F., 2013. Drought-induced tree species replacement is reflected in the spatial variability of soil respiration in a mixed Mediterranean forest. For. Ecol. Manage. 306, 79–87. doi:http://dx.doi.org/10.1016/j.foreco.2013.06.025

Barba, J., Curiel Yuste, J., Poyatos, R., Janssens, I.A., Lloret, F., n.d. Strong resilience of soil respiration components to drought-induced die-off resulting in forest secondary succession. Submitt. Glob. Chang. Biol.

Bartón, K., 2014. MuMIn: Multi-model inference. R package version 3.1-96.

Binkley, D., Giardina, C., 1998. Why do tree species affect soils? The warp and woof of tree-soil interactions. Biogeochemistry 42, 89–106. doi:10.1023/A:1005948126251

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., Peñuelas, J., 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proc. Natl. Acad. Sci. U. S. A. 108, 1474–1478.

Curiel Yuste, J., Barba, J., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Mattana, S., Martínez-Vilalta, J., Nolis, P., Lloret, F., 2012. Changes in soil bacterial community triggered by drought-induced gap succession preceded changes in soil C stocks and quality. Ecol. Evol. 2, 3016–3031. doi:10.1002/ece3.409

Curiel Yuste, J., Janssens, I.A., Carrara, A., Ceulemans, R., 2004. Annual Q(10) of soil respiration reflects plant phenological patterns as well as temperature sensitivity. Glob. Chang. Biol. 10, 161–169. doi:10.1111/j.1529-8817.2003.00727.x

Ekblad, A., Boström, B., Holm, A., Comstedt, D., 2005. Forest soil respiration rate and delta13C is regulated by recent above ground weather conditions. Oecologia 143, 136–142.

Ford, C.R., Goranson, C.E., Mitchell, R.J., Will, R.E., Teskey, R.O., 2004. Diurnal and seasonal variability in the radial distribution of sap flow: predicting total stem flow in Pinus taeda trees. Tree Physiol. 24, 941–950.

Galiano, L., Martínez-Vilalta, J., Eugenio, M., Granzow-de La Cerda, Í., Lloret, F., 2013. Seedling emergence and growth of Quercus spp. following severe drought effects on a Pinus sylvestris canopy. J. Veg. Sci. 24, 580–588. doi:10.1111/j.1654-1103.2012.01485.x

Galiano, L., Martínez-Vilalta, J., Lloret, F., 2010. Drought-Induced Multifactor Decline of Scots Pine in the Pyrenees and Potential Vegetation Change by the Expansion of Co-occurring Oak Species. Ecosystems 13, 978–991. doi:10.1007/s10021-010-9368-8

Gaumont-Guay, D., Black, T.A., Barr, A.G., Jassal, R.S., Nesic, Z., 2008. Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand. Tree Physiol. 28, 161–171.

Granier, A., 1985. Une nouvelle methode pour la measure du flux de seve brute dans le tronc des arbres. Ann. des Sci. For. 42(2), 193–200.

Grinsted, A., Moore, J.C., Jevrejeva, S., 2004. Application of the cross wavelet transform and wavelet coherence to geophysical time series. Nonlinear Process. Geophys.

Hartley, I.P., Armstrong, A.F., Murthy, R., Barron-Gafford, G., Ineson, P., Atkin, O.K., 2006. The dependence of respiration on photosynthetic substrate supply and temperature: Integrating leaf, soil and ecosystem measurements. Glob. Chang. Biol. 12, 1954–1968.

Hereş, A.M., Martínez-Vilalta, J., Claramunt López, B., 2012. Growth patterns in relation to drought-induced mortality at two Scots pine (Pinus sylvestris L.) sites in NE Iberian Peninsula. Trees 26, 621–630. doi:10.1007/s00468-011-0628-9

Hereter, A., Sánchez, J.R., 1999. Experimental areas of Prades and Montseny, in: Rodà, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.), Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin, pp. 15–27.

Högberg, P., Högberg, M.N., Göttlicher, S.G., Betson, N.R., Keel, S.G., Metcalfe, D.B., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S., Näsholm, T., 2008. High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. New Phytol. 177, 220–228.

Högberg, P., Löfvenius, M.O., Nordgren, A., 2009. Partitioning of soil respiration into its autotrophic and heterotrophic components by means of tree-girdling in old boreal spruce forest. For. Ecol. Manage. 257, 1764–1767. doi:10.1016/j.foreco.2009.01.036

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–92. doi:10.1038/35081058

Irvine, J., Law, B.E., Kurpius, M.R., 2005. Coupling of canopy gas exchange with root and rhizosphere respiration in a semi-arid forest. Biogeochemistry 73, 271–282. doi:10.1007/s10533-004-2564-x

Janisch, J.E., Harmon, M.E., 2002. Successional changes in live and dead wood carbon stores: implications for net ecosystem productivity. Tree Physiol. 22, 77–89.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grunwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, U., Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Glob. Chang. Biol. 7, 269–278. doi:10.1046/j.1365-2486.2001.00412.x

Jensen, K.H., Liesche, J., Bohr, T., Schulz, A., 2012. Universality of phloem transport in seed plants. Plant, Cell Environ. 35, 1065–1076.

Jones, H., 1992. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology. Cambridge University Press, New York.

Kuzyakov, Y., 2006. Sources of CO2 efflux from soil and review of partitioning methods. Soil Biol. Biochem. 38, 425–448.

Kuzyakov, Y., Gavrichkova, O., 2010. Time lag between photosynthesis and carbon dioxide efflux from soil: A review of mechanisms and controls. Glob. Chang. Biol. 16, 3386–3406.

Lloret, F., Escudero, A., Iriondo, J.M., Martínez-Vilalta, J., Valladares, F., 2012. Extreme climatic events and vegetation: the role of stabilizing processes. Glob. Chang. Biol. 18, 797–805. doi:10.1111/j.1365-2486.2011.02624.x

Martínez-Vilalta, J., Piñol, J., 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage. 161, 247–256. doi:10.1016/S0378-1127(01)00495-9

Mencuccini, M., Holtta, T., 2010. The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. New Phytol. 185, 189–203. doi:10.1111/j.1469-8137.2009.03050.x

Millington, R., Quirk, J., 1961. Permeability of porous solids. Trans. Faraday Soc. 54, 1200–1207.

Misson, L., Rocheteau, A., Rambal, S., Ourcival, J.-M., Limousin, J.-M., Rodriguez, R., 2010. Functional changes in the control of carbon fluxes after 3 years of increased drought in a Mediterranean evergreen forest? Glob. Chang. Biol. 16, 2461–2475. doi:10.1111/j.1365-2486.2009.02121.x

Moyano, F.E., Kutsch, W.L., Rebmann, C., 2008. Soil respiration fluxes in relation to photosynthetic activity in broad-leaf and needle-leaf forest stands. Agric. For. Meteorol. 148, 135–143.

Ninyerola, M., Pons, X., Roure, J.M., 2007a. Objective air temperature mapping for the Iberian Peninsula using spatial interpolation and GIS. Int. J. Climatol. 27, 1231–1242. doi:10.1002/joc.1462

Ninyerola, M., Pons, X., Roure, J.M., 2007b. Monthly precipitation mapping of the Iberian Peninsula using spatial interpolation tools implemented in a Geographic Information System. Theor. Appl. Climatol. 89, 195–209. doi:10.1007/s00704-006-0264-2

Poyatos, R., Aguadé, D., Galiano, L., Mencuccini, M., Martínez-Vilalta, J., 2013. Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytol. 200, 388–401. doi:10.1111/nph.12278

Reichstein, M., Rey, A., Freibauer, A., Tenhunen, J., Valentini, R., Banza, J., Casals, P., Cheng, Y.F., Grunzweig, J.M., Irvine, J., Joffre, R., Law, B.E., Loustau, D., Miglietta, F., Oechel, W., Ourcival, J.M., Pereira, J.S., Peressotti, A., Ponti, F., Qi, Y., Rambal, S., Rayment, M., Romanya, J., Rossi, F., Tedeschi, V., 2003. Modeling temporal and large-scale spatial variability of soil respiration from soil water availability, temperature and vegetation productivity indices - art. no. 1104. Global Biogeochem. Cycles 17, 1104–1115.

Reichstein, M., Tenhunen, J.D., Roupsard, O., Ourcival, J., Rambal, S., Miglietta, F., Peressotti, A., Pecchiari, M., Tirone, G., Valentini, R., 2002. Severe drought effects on ecosystem CO_2 and H_2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? Glob. Chang. Biol. 8, 999–1017. doi:10.1046/j.1365-2486.2002.00530.x

Ruehr, N.K., Offermann, C.A., Gessler, A., Winkler, J.B., Ferrio, J.P., Buchmann, N., Barnard, R.L., 2009. Drought effects on allocation of recent carbon: From beech leaves to soil CO_2 efflux. New Phytol. 184, 950–961.

Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. doi:10.1017/CBO9781107415324

Subke, J.-A., Inglima, I., Francesca Cotrufo, M., 2006. Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review. Glob. Chang. Biol. 12, 921–943. doi:10.1111/j.1365-2486.2006.01117.x

Subke, J.A., Vallack, H.W., Magnusson, T., Keel, S.G., Metcalfe, D.B., Högberg, P., Ineson, P., 2009. Short-term dynamics of abiotic and biotic soil 13CO2 effluxes after in situ 13CO2 pulse labelling of a boreal pine forest. New Phytol. 183, 349–357.

Tang, J., Baldocchi, D.D., 2005. Spatial–temporal variation in soil respiration in an oak–grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. Biogeochemistry 73, 183–207. doi:10.1007/s10533-004-5889-6

Tang, J., Baldocchi, D.D., Qi, Y., Xu, L., 2003. Assessing soil CO2 efflux using continuous measurements of CO2 profiles in soils with small solid-state sensors. Agric. For. Meteorol. 118, 207–220.

Tang, J., Baldocchi, D.D., Xu, L., 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Glob. Chang. Biol. 11, 1298–1304. doi:10.1111/j.1365-2486.2005.00978.x

Thompson, M. V., Holbrook, N.M., 2003. Scaling phloem transport: Water potential equilibrium and osmoregulatory flow. Plant, Cell Environ. 26, 1561–1577.

Torrence, C., Compo, G.P., 1998. A Practical Guide to Wavelet Analysis. Bull. Am. Meteorol. Soc. 79, 61–78.

Vargas, R., Baldocchi, D.D., Bahn, M., Hanson, P.J., Hosman, K.P., Kulmala, L., Pumpanen, J., Yang, B., 2011. On the multi-temporal correlation between photosynthesis and soil CO(2) efflux: reconciling lags and observations. New Phytol. 191, 1006–1017. doi:10.1111/j.1469-8137.2011.03771.x

Vargas, R., Detto, M., Baldocchi, D.D., Allen, M.F., 2010. Multiscale analysis of temporal variability of soil CO 2 production as influenced by weather and vegetation. Glob. Chang. Biol. 16, 1589–1605. doi:10.1111/j.1365-2486.2009.02111.x

Vayreda, J., Martínez-Vilalta, J., Gracia, M., Retana, J., 2011. Recent climate changes interact with stand structure and management to determine changes in tree carbon stocks in Spanish forests. Glob. Chang. Biol. 18, 1028–1041. doi:10.1111/j.1365-2486.2011.02606.x

Vilà-Cabrera, A., Martínez-Vilalta, J., Galiano, L., Retana, J., 2013. Patterns of Forest Decline and Regeneration Across Scots Pine Populations. Ecosystems 16, 323–335. doi:10.1007/s10021-012-9615-2

Yuan, Z., Gazol, A., Wang, X., Xing, D., Lin, F., Bai, X., Zhao, Y., Li, B., Hao, Z., 2012. What happens below the canopy? Direct and indirect influences of the dominant species on forest vertical layers. Oikos 121, 1145–1153.
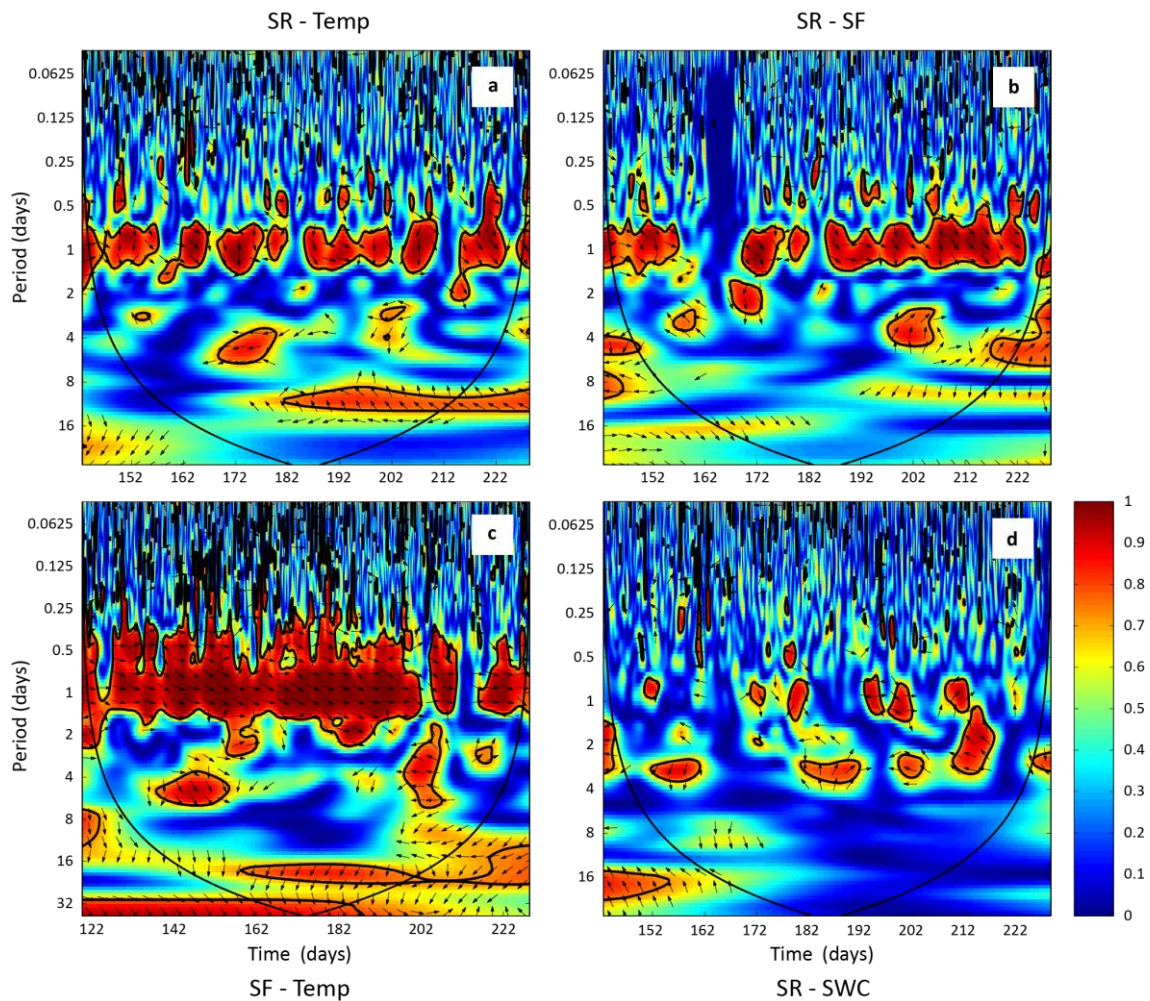
7. APPENDICES

Figure S1. Cross wavelet analyses output between soil respiration (SR) and soil temperature (Temp) (a), soil respiration and sap flow (SF) (b), sap flow and soil temperature (c) and soil respiration and soil water content (SWC) (d) at non-defoliated pine from DOY 142 to 228 (May 22nd 2010 to August 16th 2010). The colour codes for power values are from dark blue (low values) to dark red (high values). Black lines contouring red areas represent the 5% significance level, as calculated via 1000 Monte Carlo simulations (Grinsted et al., 2004). The other black lines indicate the cone influence that delimits the region not influenced by edge effects. Since solar radiation drives soil respiration, sap flow and soil temperature, these variables present clear daily patterns and the wavelet analyses show a strong correlation between them (a, b and c). Therefore, no direct causality inferences inference could be done between this pairs of variables without removing the autocorrelated signal. On the other hand, when the analysed temporal series do not have transient signals or signals whose amplitude do not vary harmonically in time (such as daily SWC variations), power values show low values (d).

Chapter 5

Changes in litter chemistry and functioning of decomposer communities associated with drought-induced species succession affect ecosystem carbon and nitrogen dynamics

Josep Barba, Francisco Lloret and Jorge Curiel Yuste

ABSTRACT

One major challenge in the prediction of ecological functioning in forest ecosystems is understanding the links between above- and below-ground processes. Litter decomposition, which plays a key role in these processes, could be altered by to changes in environmental or species composition. We studied litter decomposition and N dynamics in a forest experiencing drought-induced die-off and subsequent succession from *Pinus sylvestris* to *Quercus ilex* dominance to disentangle the effects of tree species and the tree-specific soil community on litter decomposition. Litter bags with leaves and fine roots from *P. sylvestris* and *Q. ilex* were placed under canopies representing three habitats of the die-off and succession process (healthy and dead *P. sylvestris* and *Q. ilex*). Mass loss and N were assessed over three years. We found that the decomposition rate of the different litter varied between habitats, highlighting the species-specific correspondence between above- and below-ground communities and their effect on ecosystem functioning. Differences in nitrogen dynamics were found between litter types, but no habitat effects were observed. When litter decomposition was compared in the two forest successional extremes (healthy *P. sylvestris* and *Q. ilex*), a 44% increase in decomposition rate for leaves and a 46% decrease for fine roots were detected. Our results show that forest die-off and tree-species succession are reflected in litter decomposition and N dynamics, which could have great implications in these type of forests, since *P. sylvestris* die-off and *Q. ilex* replacement have been reported in recent years and are expected to worsen in the next decades.

KEYWORDS

Litter decomposition, forest die-off, home field advantage, carbon cycle, nitrogen cycle, Mediterranean forest.

1. INTRODUCTION

The aerobic decomposition of dead organic matter is one of the main sources of CO_2 emission from terrestrial ecosystems, consequently playing a critical role in their carbon (C) and nutrient balances at both local (Bonanomi et al., 2010; Santa Regina, 2001) and global scales (Prentice, Farquhar & Fasham 2001; Canadell *et al.* 2007; IPCC 2013). Therefore, in order to improve current predictions of ecosystem responses to climate change, it is crucial to understand the drivers controlling litter decomposition dynamics (Cao and Woodward, 1998). Since the first half of the twentieth century, temperature, moisture and vegetation have been described as the main drivers of litter decomposition (Waksman and Gerretsen, 1931). The key role of temperature and moisture is based on the well-known fact that the enzyme kinetics involved in microbial decomposition are very sensitive to both water and temperature (Davidson and Janssens, 2006).

Furthermore, vegetation controls organic carbon decomposition in different ways. Firstly, vegetation determines decomposition via species-specific litter quality, because the differences in chemical litter composition between plant species imply different litter degradability, and hence different rates of decomposition. This influence of litter quality on litter decomposition has been described from local (Saura-Mas et al., 2012; Wang et al., 2014) to regional scales (Cornwell et al., 2008; Melillo et al., 1982; Vivanco and Austin, 2006). Accordingly, different indicators of litter quality such as C:N ratio, nutrient (N and/or P) content, and the content of some structural molecules (e.g. lignin or holocellulose), have been correlated with litter decomposability (Bonanomi et al., 2010; Couteaux et al., 1995; Gallardo and Merino, 1993; Gholz et al., 2000; Vivanco and Austin, 2006)). More specifically, litter's initial C:N ratio has been identified as one of the best chemicals predictors of litter decomposition (Berg and McClaugherty, 2008; Bonanomi et al., 2010; Melillo et al., 1982; Parton et al., 2007). Moreover, for a given species, different organs (i.e. leaves, fine roots and twigs) present a different chemical composition and, therefore, different rates of decomposition (Freschet et al., 2013; Vivanco and Austin, 2006; Wang et al., 2014). Secondly, vegetation has the ability to modify environmental conditions, such as temperature and moisture (Binkley and Giardina, 1998; Yuan et al., 2012), thus indirectly determining decomposition by affecting enzyme kinetics (Cornwell et al., 2008; Freschet et al., 2012) or photodegradation rates associated with exposure to radiation (Austin and Vivanco, 2006). Thirdly, vegetation can influence litter decomposition via its co-evolution with the soil decomposer community (Ayres et al., 2009a; Vivanco and Austin, 2008), resulting in specific tree-species soil communities (Curiel Yuste et al., 2012; Grayston and Prescott, 2005; Waldrop and Firestone, 2006) with different functional diversity (Waldrop and Zak, 2004; Wallenstein et al., 2013). This co-evolution between tree species and their soil communities is

reflected by a given microbial community's common capacity to decompose more efficiently the litter of the plant species from which is derived (Austin et al., 2014). This effect, called home-field-advantage (HFA) (Austin et al., 2014; Ayres et al., 2009a, 2009b), is widespread in forest ecosystems, enhancing litter decomposition by 8% on average (Ayres et al., 2009b). However, the extent to which soil communities are able to efficiently decompose different substrates and to which differences in litter degradability could influence the correct interpretation of HFA is still under debate (Freschet et al., 2012; Keiser et al., 2014). This highlights the complexity of the controls of litter decomposition dynamics and the paucity of our knowledge of above-below-ground interactions. For instance, most research has focused on the decomposition patterns of above-ground litter (needles and/or leaves), whereas the decomposition of fine roots, which accounts for at least half of the litter produced by vegetation (Clemmensen et al., 2013; Montero et al., 2005), has been only marginally studied.

Moreover, it is important to understand how climate change-induced shifts in vegetation health (Lloret et al., 2012) may alter above-below-ground interactions and hence rates of organic matter decomposition and nutrient turnover. More specifically, studies of the effect of litter quality and soil community, and their interaction, on organic matter decomposition and nutrient turnover have speculated about their possible significant impact on C and nutrients dynamics in vegetation shifts induced by climate change (Ayres et al., 2009a, 2009b; Ball et al., 2009; Cornwell et al., 2008; Freschet et al., 2013; Mclaren and Turkington, 2010). No studies have been directly designed, however, to estimate how climate-change-induced secondary succession may affect forest C dynamics and the capacity of terrestrial ecosystems to sequester C.

Drought- and heat-induced tree die-off and mortality have been reported over the last few decades around the world (Allen *et al.* 2010), particularly in South Europe and the Mediterranean Basin (Briffa et al., 2009; Carnicer et al., 2011; Della-Marta et al., 2007; Lloret et al., 2004). In this region, various models have consistently predicted a high impact from drought in the following decades (Giorgi and Lionello, 2008; Mariotti, 2010). These changes towards ecosystems with a more limited supply of water have been associated with the decline of keystone species that have their southern limit of distribution in the Mediterranean Basin (Carnicer et al., 2014; Lenoir et al., 2010; Vayreda et al., 2013). This is the case with *Pinus sylvestris* L., which, in some areas of the Iberian peninsula, is being replaced by other species such as *Quercus ilex* L., which are better adapted to drought (Carnicer et al., 2014; Vilà-Cabrera et al., 2013).

In this study we aim to assess how changes in litter quality associated with these climate-change-induced vegetation shifts and consequent secondary succession affect litter

110

decomposition rates and N dynamics. We address the climate-driven forest succession from *P. sylvestris* to *Q. ilex* occurring in the Prades Mountains (NE Iberian Peninsula). This information on litter decomposition and N dynamics in *P. sylvestris* forests is particularly relevant for regional assessment of the C and nutrient balance because this widely distributed species is experiencing severe die-off episodes in different regions (Bigler et al., 2006; Martínez-Vilalta and Piñol, 2002). Leaf and fine-root litter bags from both species were placed beneath healthy *P. sylvestris*, dead *P. sylvestris* and *Q. ilex* canopies in a fully crossed factorial design, and we measured decomposition and C and N content over three years. Specifically, we hypothesized that drought-induced forest succession could modify litter decomposition and N dynamics through changes in litter quality (litter effects), changes in the soil environment (habitat effects) and their interaction.

2. MATERIALS AND METHODS

Our experiment was performed in a mixed forest on the northwest-facing hillside in Titllar Valley, Prades Mountains (NE Iberian Peninsula; 41°13'N, 0°55'E; 1015 m asl). The climate is Mediterranean, with a mean annual temperature of 11.3°C and mean annual precipitation of 664 mm (period 1951-2010) (Ninyerola et al., 2007a, 2007b). The experimental area was located on a 35° hill slope, on metamorphic schist substrate that outcrops onto a large part of the study site. Soils are xerochrepts with clay loam texture and high gravel content (46% volume). Organic horizons cover most of the soil and outcrops with variable thickness. For more information about the studied area, see Hereter & Sánchez (1999) and Barba *et al.* (2013). The mixed forest, which has not been managed for the last 30 years (Hereş et al., 2012), is mainly composed of *Pinus sylvestris* L. (Scots pine) (54% of the forest basal area and mean diameter at breast height [DBH] of 0.32 m) and *Quercus ilex* L. (Holm oak) (41% of the total BA and DBH of 0.15 m). The study area has been affected by several drought events since the 1990s, particularly the *P. sylvestris* population (Martínez-Vilalta and Piñol, 2002), producing an average mortality of 12% of standing trees and mean crown defoliation of 52% (Vilà-Cabrera et al., 2013). This situation, coupled with contrasted recruitment rates between both species (low rates in *P. sylvestris* and high rates in *Q. ilex*) (Vilà-Cabrera et al., 2013), will lead to a progressive replacement of *P. sylvestris* by *Q. ilex* as the dominant over-storey species.

The decomposition experiment considered three types of trees, representing different stages of the ongoing forest succession (Healty *P. sylvestris* [HPs], Dead *P. sylvestris* [DPs] and *Q. ilex* [Qi]). We established two meters around trees as the respective rhyzosphere-influence area on soil environment (hereafter, habitat). Five replicates (hereafter, microsites) of each of these three

habitat types were selected on a 1-ha study site. Microsites of the three habitat types were spatially randomized since die-off pattern was diffused. Selected dead pines had died in the nineties as consequence of serve drought events (Martínez-Vilalta and Piñol, 2002).

Freshly senescent leaves and living fine roots (diameter thinner than 2 mm) from *P. sylvestris* and *Q. ilex* were collected from the same study area and oven-dried at 60°C for 24h. Litter bags (0.5 mm nylon mesh and size 7.5 X 8.5 cm) were filled with a known dry-weight amount of litter (0.5-1 g) (Ps needles, Qi leaves, Ps roots and Qi roots). Mesh size was large enough to allow microbial and fungi activity as well as small access by arthropods, but small enough to avoid major losses of the smallest litter portions (Killham, 1994). Six litter bags containing each litter type were placed on each microsite, with a total of 360 litter bags (3 habitat types X 5 microsite replicates X 4 litter types X 6 litter-type replicates). A square metal fence (1 X 1 m) was installed on each microsite and litter bags were placed inside to avoid disturbances from wild boars during the experiment. Leaf litter bags were placed on the surface and fine-root litter bags were buried at a depth of 5-10 cm. We did not remove either the organic horizons underneath the litter bags at the beginning of the experiment or the litterfall during the experiment - as commonly done (i.e. Vivanco & Austin 2008) - since we wanted to approximate the decomposition process to natural conditions as much as possible. Litter bags were placed in the field in July 2011 and collected at 0.16, 0.5, 1, 1.5, 2 and 3 yrs and oven-dried at 60°C for 24h. The remaining litter was dry cleaned with a brush and weighed. Each individual sample was ground and analysed for total nitrogen [%N] and carbon [%C] content by CHNS organic microanalysis, using combustion coupled with gas chromatography (EUROVECTOR, EA3011, Milano, Italy). Similarly, initial litter quality (%N and %C) was assessed for three samples of each litter type.

To control the possible effects of the microsites' environmental differences on decomposition process, soil water content (SWC) and soil temperature were measured every two weeks from January 2012 to July 2013 at each microsite. SWC was measured by time domain reflectometry (TDR) (Tektronix 1502C, Beaverton, Oregon, USA). One TDR probe 15cm long was permanently installed in the upper soil on each microsite. In order to correct the SWC measurements for stoniness, gravimetrical SWC was regressed against TDR measurements (for more information, see Poyatos et al., 2013). Soil temperature was measured once a day every two weeks at 10 cm, using a thermometer (OMEGA, HH806AU, Stamford, USA). Additional information about soil properties such as pH, N availability, SOM content, decomposer community composition at the different habitats could be found in Curiel Yuste and others (2012).

To assess the mass loss for litter and habitat types, we used a general linear model (GLM) coupled with an exponential decay equation (expressed as ln (M_t / M_o), where M_t is the remaining dry mass of each sample on the sampled date and M_0 is the initial dry mass) for each litter type (fixed factor) and habitat type (fixed factor) (Saura-Mas et al., 2012), and we included time as an additional variable in the model. Since the ln (M_t / M_0) divided by time has been defined as the decomposition constant (k) (Olson, 1963), the modelled slope for each pair of combinations (4 litter types X 3 habitat types) represents the decomposition constant k of each combination. As all the litterbags were collected on the same microsites throughout the experiment, microsite was also included in the model as a random factor. The model also contained the interactions between litter type, habitat type and time. Since this interaction was significant, the effect of litter was analysed separately in an additional model that included microsite and habitat type as random factors and time. Similarly, a model for habitat type was built, including microsite and litter type as random factors and time.

Moreover, the remaining mass (%) and relative nitrogen content (%) were analysed by GLM models. The temporal pattern of remaining mass and N % in relation to remaining mass were analysed separately for litter and habitat types in different models, which also included microsite as a random factor and time. These models also tested differences between litter and habitat types, respectively, for a given time. Overall differences between litter or habitat types for the whole period of time were analysed with similar GLM models, but considering time as a random factor. A log-odd transformation was undertaken to achieve normality in the % of remaining mass (i.e. log[x/(1-x)]).

To test the possible effect of initial litter quality on the decomposition process, linear regression was fitted with the mean (± SE) of the k values obtained in each habitat type (n=3) and mean (± SE) C:N values obtained in five samples analysed at the start of the experiment.

All the analyses were carried out using R 3.0.3. (R Foundation for Statistical Computing, Vienna, Austria). The mixed-effects models were performed using the R packages nlme and lme4 (Bates et al., 2014; Pinheiro et al., 2009).

3. RESULTS

3.1. Litter decomposition rates

The decomposition rate of the different litter types varied between habitats, as supported by a significant interaction between litter and habitat effects (Table 1). All litter types were
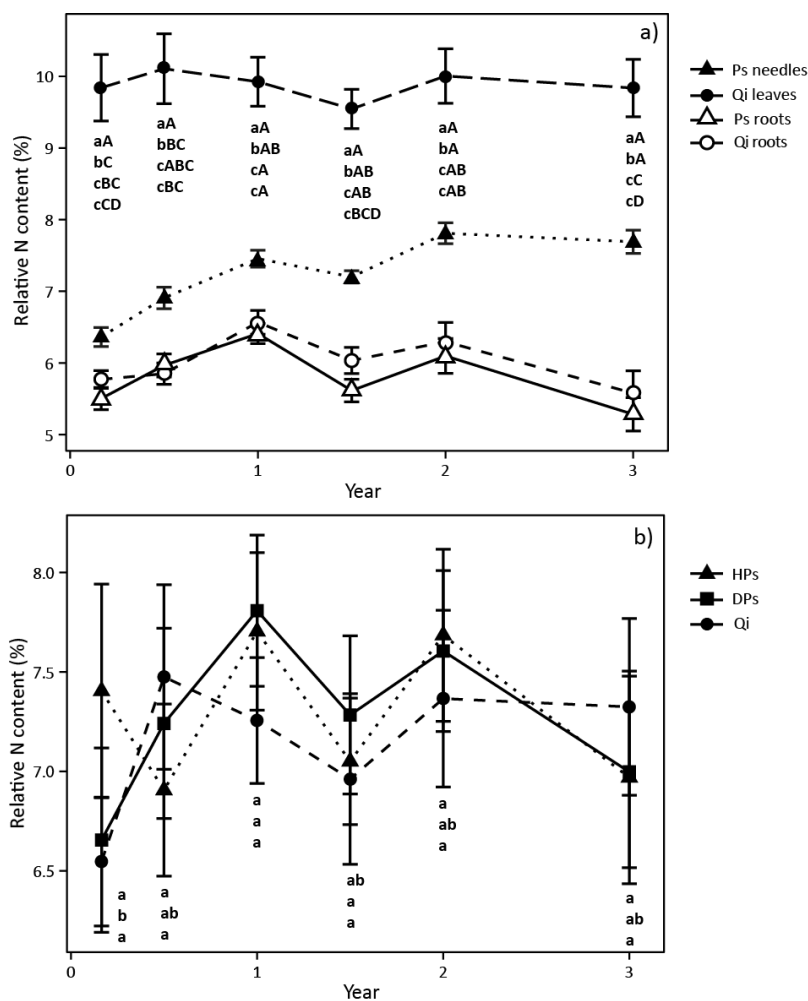
Figure 1. Decomposition constants (k) of *Pinus sylvestris* and *Quercus ilex* litter (leaves and fine roots) across three microsites obtained by the GLM. The lower case indicates significant differences in k between litter types within each microsite. The capital letters indicate significant differences in k between microsites within each litter type (p<0.05).

decomposed with a similar k in HPs habitat, but there were differences in the DPs or Qi habitats (Fig. 1). In both the DPs and Qi habitats, Qi leaves and Ps roots showed consistently higher decomposition rates than Ps needles and Qi roots. In the Qi habitat, Qi leaves showed the highest decomposition rate, followed by Ps roots, and Qi roots showed the lowest decomposition rates, whereas Ps needles showed intermediate rates between the Ps roots and Qi roots. The Ps needle k was higher in HPs than in DPs habitats and showed intermediate values in the Qi habitat. Similarly, Qi leaves were decomposed better in Qi habitats than in pine-determined habitats. The k of Ps roots did not show any significant differences in any of the habitats, and Qi roots showed higher k in both the pine-determined habitats (HPs and DPs) than in the Qi habitat. Overall, decomposition rates varied across the different litter origins: in pine litter, the decomposition rates were higher in needles (0.14 ± 0.01 yr^{-1}) than in roots (0.17 ± 0.01 yr^{-1}), while the opposite trend was observed for Holm-oak leaf (0.19 ± 0.02 yr^{-1}) and root (0.13 ± 0.01 yr^{-1}) litter (GLM with litter type as predictor and microsite and habitat type as random factors and time, p<0.05) (Fig. 2a). Furthermore, when considering all three habitats together, litter composition k almost significantly correlated (R^2=0.76; p=0.081) with the initial litter quality (C:N ratio) (Fig. 3).

When the four litter types were considered together, no differences appeared in decomposition rates between habitat types (Fig. 2b) (GLM with habitat type as predictor and microsite and litter type as random factors, p<0.05). No differences were found in the measured environmental conditions between habitat types (GLM with soil temperature or soil water content as independent variables, habitat type as predictor and microsite as random factor; p=0.94 for soil temperature and p=0.16 for soil water content).
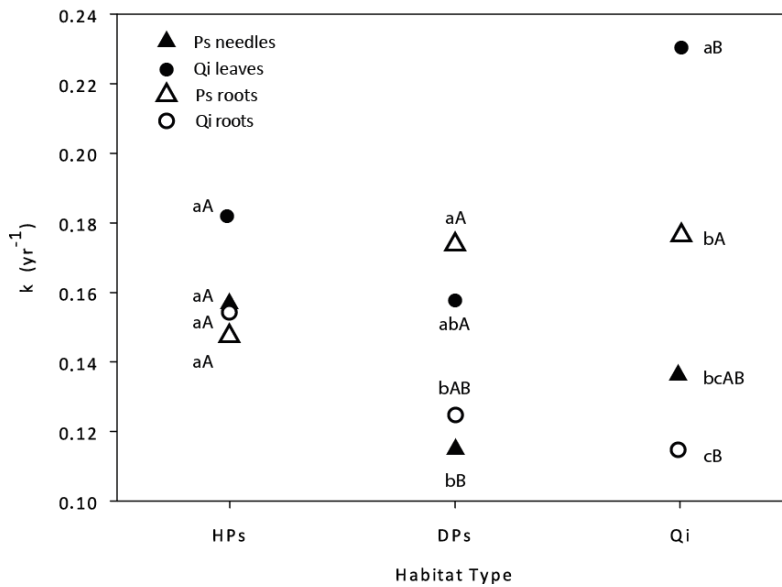
Figure 2. Effects of litter type (a) and microsite (b) on the decomposition constant (k) (mean ± SE) obtained by the GLM. The lower case indicates significant differences in k between litter types (a) and microsites (b) (p<0.05). Significant differences between litter types and habitat types were obtained from GLMs that considered only the respective variables as fixed factors (see text).
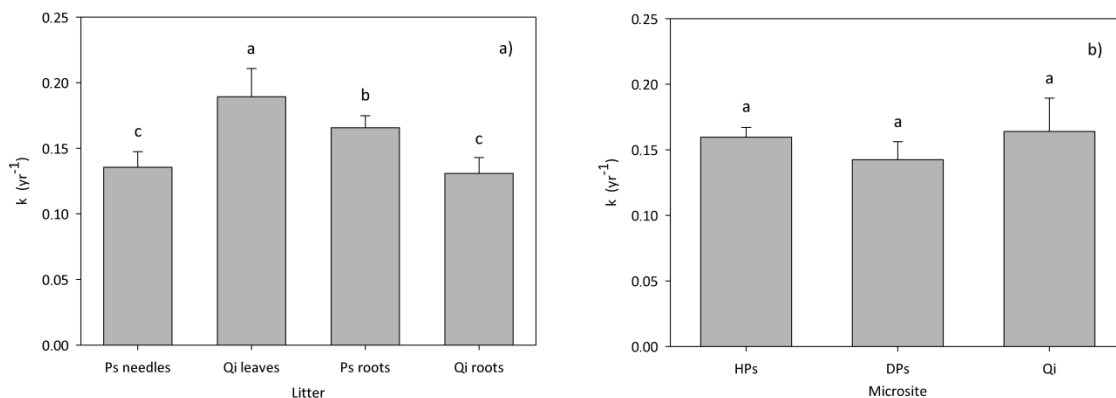

Figure 3. Relationship between the initial C:N ratio of litter (leaves and fine roots from *Pinus sylvestris* and *Quercus ilex*) and decomposition constant (k) (mean ± SE), calculated as the mean of k considering the three habitat types.

Since HPs and Qi represented the two forest successional extremes, the pine-needle and root decomposition rates beneath HPs were compared with the oak-leaf and root decomposition rates beneath Qi. Ps needles in the HPs habitat showed an average of 44% lower k than Qi leaves

in the Qi habitat (p=0.046). Ps roots in the HPs habitat showed an average of 46% higher k than Qi roots in the Qi habitat (p=0.046). All the differences in k between litter and habitat combinations were determined with the GLM, with the interaction between litter type and habitat type and with microsite as a random factor; p<0.05 (Fig. 1).

Figure 4. Mass remaining (mean ± SE) for each litter type (a) and habitat type (b) at 6 collection times (0.16, 0.5, 1, 1.5, 2 and 3 years, n=5). The lower case indicates significant differences in remaining mass between litter types (panel a) within collection times. The capital letters indicate significant differences in remaining mass significant over time within litter types (panel a) and within habitat types (panel b). No significant differences were found in remaining mass between microsites within collection times (b). Comparisons were made with GLMs, with microsite as a random factor (p<0.05). Statistical analyses were performed with remaing mass log-odd transformed to achieve normality.
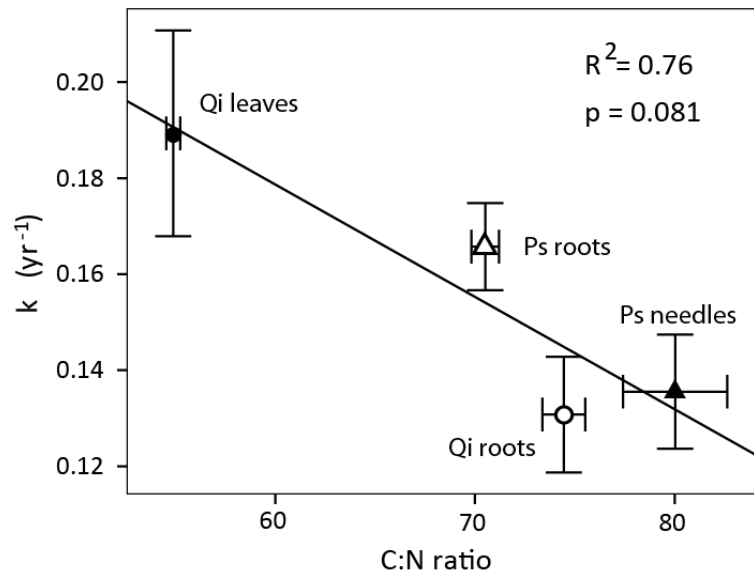
3.2. Mass remaining over time

The mass remaining over time decreased for all litter types (Fig. 4a) and habitats (Fig. 4b). Its temporal evolution varied between litter types (F=16.58, p<0.001) but no significant differences were found between habitat types for the whole time period (F=1.56, p=0.250, GLMs with litter types and habitat types as predictors and microsite and time as random factors). Qi roots maintained the highest remaining mass throughout the studied period, while Qi leaves presented an accelerated biomass loss in comparison to the other litter types around 1.5 years after starting. The biomass loss of the two types of pine litter remained quite similar over the three years, with a tendency towards an increase in roots after 1.5 years.

3.3. Litter N content over time

N content over time showed clear differences between litter types (Fig. 5a) (F=297.44, p<0.001; GLM with microsite and year as random factors). These differences were consistent with those initially found in the different litter types (Table 2). As expected, N content was always higher in

116

both types of leaves than in roots, and Qi leaves showed higher N content than Ps needles. These differences between species were absent in fine roots (Figure 5a). Qi leaves showed similar N content values over time while a slight increase in N content was observed in Ps needles. Roots from both species showed similar patterns over time, with similar N content values at the beginning and end of the experiment, and slightly higher values during the second year (Fig. 5a). No effects of habitat type effects on N content were detected over time (F=0.186, p=0.833).
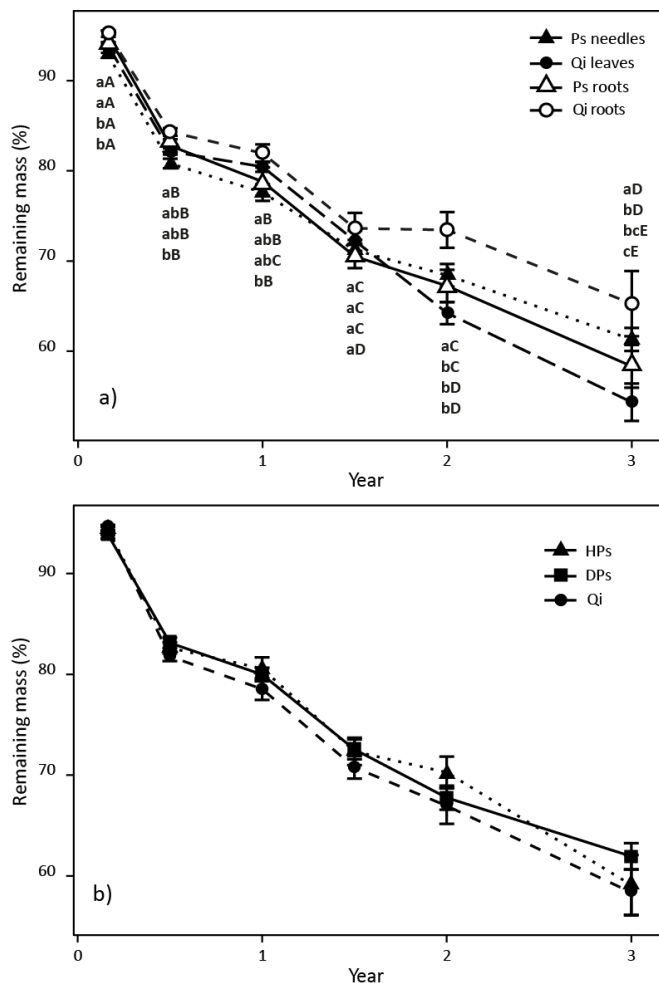

Figure 5. Relative N content (N % of sampled remaining biomass) (mean ± SE) for each litter type (a) and microsite (b) at 6 collection times (0.16, 0.5, 1, 1.5, 2 and 3 years, n=5). The lower case indicates significant differences in N content between litter types (panel a) and habitat type (panel b) within collection times. The capital letters indicate significant differences in N content over time within litter types (panel a). No significant differences were found in N content between habitat types within collection times (b). Comparisons were made with GLMs, with microsite as a random factor (p<0.05). Note that the slope around 0 indicates that C and N decay at a similar rate.

4. DISCUSSION

4.1. Forest succession and C dynamics

Here, we show that drought-induced secondary succession from *P. sylvestris* to *Q. ilex* may substantially alter patterns of litter decomposition and N dynamics in Mediterranean forests. This alteration is due to both differences in litter quality - and hence decomposition rates - between these two tree species and the differential capacities of the microbial communities

associated with the habitats - in turn modified by drought - to decompose the different litter types.

Litter quality exerted a major control over litter decomposition, which is something that has already been widely observed both at local (Aponte et al., 2012; Bonanomi et al., 2010; Gallardo and Merino, 1993) and regional scales (Cornwell et al., 2008; Couteaux et al., 1995; Vivanco and Austin, 2006). In our study, initial litter C:N ratio appeared as a good predictor of litter quality since it correlated quite well with the decomposition rate constant (k) of the litter types, independently of habitat. However, other chemical controls on litter decomposition may also be important - for example, initial lignin content, which is usually negatively correlated with decomposition constant (Cornwell et al., 2008). In fact, lignin content in Ps needles has been reported to be higher than in Qi leaves (Kattge et al., 2011; Mediavilla et al., 2011), in agreement with our observations of the lower decomposition rates of Ps needles. Other physical controls could also underlie the differences in litter decomposition observed (Cornwell et al., 2008). Qi leaves show higher area/volume ratio than Ps needles (Kattge et al., 2011), enhancing microbial accessibility and consequently decomposition rates. Leaf litter usually decomposes better than fine-root litter (Freschet et al., 2013; Gholz et al., 2000; Vivanco and Austin, 2006) due to its better quality (i.e. low C:N) (Bird and Torn, 2006; Wang et al., 2010), as we observed in *Q. ilex*, but not for *P. sylvestris*, although the latter's needles are sclerophyllous and showed lower C:N than fine roots (see also Wang *et al.* 2014).

The lack of any significant differences in the abiotic environmental variables between habitats points to the role of habitat specificities of soil decomposer communities as major controllers of the observed differences between habitats in decomposition rates (Curiel Yuste et al., 2012; Keiser et al., 2014). It has been hypothesized that soil decomposer communities associated with the distinct stages of forest succession exhibit a specific capacity to decompose litter of varying quality (Freschet et al., 2012; Keiser et al., 2014). In this particular case, we observed that soil communities under HPs habitats were able to decompose litter of differing quality to a similar degree, whereas soil microbial communities found under DPs and HO were more selective and significantly capable of decomposing better litter of higher quality. Thus, these functional differences along the drought-induced successional gradient endorse the existence of a concomitant microbial succession in such habitats, as reported in the studied forest (Curiel Yuste et al., 2012) and in other ecosystems (Keiser et al., 2014; Waldrop and Firestone, 2006; Wickings et al., 2012). Therefore, litter decomposition rates in forest subjected to drought-induced species replacement would be modified not only by alterations in litter quantity and quality, but also by changes in the decomposer community associated with tree replacement.

Our results only partially supported the home field advantage (HFA) hypothesis, which proposes higher litter mass loss under the species producing a given litter type (at *home*) than under other species (*away*). Only Qi leaves showed higher decomposition rates in Qi habitat than in HPs or DPs habitats (36% on average). This was probably because soil decomposer community under Qi was more specialized in the decomposition of higher quality organic matter (lower C:N ratio), such as Qi leaves. Ps needles also showed higher decomposition rates in the HPs habitat than in the Qi or DPs, but this result is not completely in accordance with HFA hypothesis since the decomposer community in the HPs habitat seemed to be generalist, and thus able to decompose litter of very different quality at similar rates. Consequently, the local adaptation of the decomposer communities, as suggested by HFA hypothesis, would not occur in the HPs habitat. In our case, Ps roots showed similar decomposition rates across habitats, and for Qi the root decomposition rates were actually higher in HPs than in Qi habitats. These results do not support the HFA hypothesis, whereby the HFA is more pronounced in more recalcitrant litter (Milcu and Manning, 2011; Strickland et al., 2013, 2009). Our results do concur, however, with 25% of the experiments that performed reciprocal litter transplants between tree species without observing any stimulation of decomposition at home (Ayres et al., 2009b).

The comparison of litter decomposition between the two forest succession extremes can help to predict future trends in the C dynamics and C accumulation of these forests. While holm-oak leaves decompose faster than pine needles beneath their respective species, for fine roots the opposite trend was observed: rates of fine-root decomposition in the HPs habitat were higher than the rates of Qi fine-root decomposition in the Qi habitat, suggesting that secondary succession may produce a substantial decoupling of above- and below-ground trends in organic matter decomposition. Thus, *P. sylvestris* replacement by *Q. ilex* would imply a faster decomposition of superficial leaf-derived soil layers but a slower decomposition of root-derived material, which is generally the major contributor to soil organic matter (Clemmensen et al., 2013). Nevertheless, the final C balance would ultimately depend on the contribution of the above- and below-ground biomass of the different species to the C pool (Bardgett et al., 2013; Berg, 2000). In fact, our observations correspond to decomposition rates and do not measure the amount of litter produced by the different species, which may be particularly high under defoliated trees.

4.2. Forest succession and N dynamics

We also observed strong effects of forest successional stages on N dynamics during litter decomposition. These effects can mainly be attributed to differences in litter chemistry and

palatability rather than differences in microbial communities associated with habitat shift. Differences in the initial relative N content between leaves types were maintained over time during the decomposition process. The relative lack of N mobilization in Qi roots and Ps needles and roots during the first year could be explained by the loss of C soluble compounds during the initial stages of the decomposition process (Berg, 2000). The net release of N observed in roots during the final year may reflect a much more intense mineralization of N-rich compounds once the labile C had been lost in the initial stages. The relative N content in decomposed litter remained quite stable over the three years, however, especially in Qi leaves, indicating that N was released from litter at rates similar to those of total C. The exception was found in Ps needles, which presented relative N immobilization over the three years. The greater lignin cover in Ps needles, which are low in N, and these needles' low area/volume rate may protect the internal N and avoid the rapid loss of C soluble compounds, enhancing a relative accumulation of N over time. It is therefore likely that the microbial communities might need more than three years to be able to access to the poor but well protected N pool of pine needles.

The lack of any clear pattern of relative N immobilization or release during the decomposition process or any effect of habitat over time could indicate that N turnover was not playing an active role in *P. sylvestris* die-off and, therefore, in *Q. ilex* colonization. If N had been a limiting factor for the forest tree growth on the study site, the soil communities associated with the tree rhyzosphere could have been selected to decompose N components more efficiently. The N soil content on the study site (Curiel Yuste et al., 2012) and the P soil content in the nearby valley of the Prades mountains with the same substrate composition (metamorphic substrate with neutral pH) also suggest that N and P do not limit forest growth.

5. CONCLUSIONS

In this study, we are the first, to our knowledge, to use a real-case scenario of the effects of die-off-driven forest succession on litter decomposition and N dynamics. Our study shows that drought-driven succession from *P. sylvestris* to *Q. ilex* is modifying leaf and fine-root decomposition through changes in the chemical nature of the litter (palatability) and the relationship between above-ground species and below-ground decomposers communities. This finding is relevant since these successional events involving two major coexisting species in the Mediterranean-Eurosiberian ecotone in Europe have recently been reported (Carnicer et al., 2014; Galiano et al., 2013; Vilà-Cabrera et al., 2013) and are expected to be more widespread in the coming decades (Carnicer et al., 2014; Vayreda et al., 2013). Global change could influence litter decomposition due to changes in soil temperature and moisture availability, but also

because of the existence of associated vegetation shifts. These vegetation changes could, in turn, influence litter decomposition by determining litter quality and the relationship between above-tree above-ground tree species and their below-ground soil decomposer community. In this particular study, drought-induced replacement of Scots pines by Holm oaks seems to provoke significant changes, firstly in the chemical composition of litter and secondly in the ability of different microbial communities to decompose organic matter. The result should be a net increase in the decomposition rates of the "above-ground" litter (moving from recalcitrant Ps needles to more palatable Qi leaves) but a net reduction in the rates of "below-ground" litter decay, largely due to the lower capacity of the microbial communities under colonizer Holm-oaks to decompose root material as compared with those under pines. Our results also suggest, therefore, that in order to correctly predict the effects of climate change effects on the capacity of terrestrial systems to sequester C, models should closely examine changes in both the chemical composition and functioning of the decomposer communities associated with drought-induced secondary successions.

6. CONCLUSIONS

7. REFERENCES

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N.G., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H.T., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manage. 259, 660–684.

Aponte, C., García, L. V., Marañón, T., 2012. Tree Species Effect on Litter Decomposition and Nutrient Release in Mediterranean Oak Forests Changes Over Time. Ecosystems 15, 1204–1218.

Austin, A.T., Vivanco, L., 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. Nature 442, 555–558.

Austin, A.T., Vivanco, L., González-Arzac, A., Pérez, L.I., 2014. There's no place like home? An exploration of the mechanisms behind plant litter-decomposer affinity in terrestrial ecosystems. New Phytol. 204, 207–214.

Ayres, E., Steltzer, H., Berg, S., Wall, D.H., 2009a. Soil biota accelerate decomposition in high-elevation forests by specializing in the breakdown of litter produced by the plant species above them. J. Ecol. 97, 901–912.

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009b. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biol. Biochem. 41, 606–610.

Ball, B.A., Bradford, M.A., Coleman, D.C., Hunter, M.D., 2009. Linkages between below and aboveground communities: Decomposer responses to simulated tree species loss are largely additive. Soil Biol. Biochem. 41, 1155–1163.

Barba, J., Curiel Yuste, J., Martínez-Vilalta, J., Lloret, F., 2013. Drought-induced tree species replacement is reflected in the spatial variability of soil respiration in a mixed Mediterranean forest. For. Ecol. Manage. 306, 79–87.

Bardgett, R.D., Manning, P., Morri??n, E., De Vries, F.T., 2013. Hierarchical responses of plant-soil interactions to climate change: Consequences for the global carbon cycle. J. Ecol. 101, 334–343.

Bates, D., Maechler, M., Bolker, D., Walker, S., 2014. lme4: Linear mixed-effects models using Eigen and S4.

Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. For. Ecol. Manage. 133, 13–22.

Berg, B., McClaugherty, C., 2008. Plant litter: decomposition, humus formation, carbon sequestration, Carbon. Springer-Verlag.

Bigler, C., Bräker, O.U., Bugmann, H., Dobbertin, M., Rigling, A., 2006. Drought as an inciting mortality factor in scots pine stands of the Valais, Switzerland. Ecosystems 9, 330–343.

Binkley, D., Giardina, C., 1998. Why do tree species affect soils? The warp and woof of tree-soil interactions. Biogeochemistry 42, 89–106.

Bird, J.A., Torn, M.S., 2006. Fine roots vs. needles: A comparison of 13C and 15N dynamics in a ponderosa pine forest soil. Biogeochemistry 79, 361–382.

Bonanomi, G., Incerti, G., Antignani, V., Capodilupo, M., Mazzoleni, S., 2010. Decomposition and nutrient dynamics in mixed litter of Mediterranean species. Plant Soil 331, 481–496.

Briffa, K.R., van der Schrier, G., Jones, P.D., 2009. Wet and dry summers in europe since 1750: Evidence of increasing drought. Int. J. Climatol. 29, 1894–1905.

Canadell, J.G., Le Quéré, C., Raupach, M.R., Field, C.B., Buitenhuis, E.T., Ciais, P., Conway, T.J., Gillett, N.P., Houghton, R.A., Marland, G., 2007. Contributions to accelerating atmospheric CO2 growth from

economic activity, carbon intensity, and efficiency of natural sinks. Proc. Natl. Acad. Sci. U. S. A. 104, 18866–18870.

Cao, M.K., Woodward, F.I., 1998. Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. Nature 393, 249–252.

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., Peñuelas, J., 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proc. Natl. Acad. Sci. U. S. A. 108, 1474–1478.

Carnicer, J., Coll, M., Pons, X., Ninyerola, M., Vayreda, J., Peñuelas, J., 2014. Large-scale recruitment limitation in Mediterranean pines : the role of Quercus ilex and forest successional advance as key regional drivers. Glob. Ecol. Biogeogr. 23, 371–384.

Clemmensen, K.E., Bahr, a, Ovaskainen, O., Dahlberg, A., Ekblad, a, Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D. a, Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–8.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T. V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V., Westoby, M., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett. 11, 1065–1071.

Couteaux, M.M., Bottner, P., Berg, B., 1995. Litter decomposition climate and litter quality. Trends Ecol. Evol. 10, 63–66.

Curiel Yuste, J., Barba, J., Fernandez-Gonzalez, A.J., Fernandez-Lopez, M., Mattana, S., Martínez-Vilalta, J., Nolis, P., Lloret, F., 2012. Changes in soil bacterial community triggered by drought-induced gap succession preceded changes in soil C stocks and quality. Ecol. Evol. 2, 3016–3031.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

Della-Marta, P.M., Haylock, M.R., Luterbacher, J., Wanner, H., 2007. Doubled length of western European summer heat waves since 1880. J. Geophys. Res. D Atmos. 112.

Freschet, G.T., Aerts, R., Cornelissen, J.H.C., 2012. Multiple mechanisms for trait effects on litter decomposition: Moving beyond home-field advantage with a new hypothesis. J. Ecol. 100, 619–630.

Freschet, G.T., Cornwell, W.K., Wardle, D.A., Elumeeva, T.G., Liu, W., Jackson, B.G., Onipchenko, V.G., Soudzilovskaia, N.A., Tao, J., Cornelissen, J.H.C., 2013. Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. J. Ecol. 101, 943–952.

Galiano, L., Martínez-Vilalta, J., Eugenio, M., Granzow-de La Cerda, Í., Lloret, F., 2013. Seedling emergence and growth of Quercus spp. following severe drought effects on a Pinus sylvestris canopy. J. Veg. Sci. 24, 580–588.

Gallardo, A., Merino, J., 1993. Leaf Decomposition in Two Mediterranean Ecosystems of Southwest Spain: Influence of Substrate Quality. Ecology 74, 152–161.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E., Parton, W.J., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: Toward a global model of decomposition. Glob. Chang. Biol. 6, 751–765.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. Glob. Planet. Change 63, 90–104.

Grayston, S.J., Prescott, C.E., 2005. Microbial communities in forest floors under four tree species in coastal British Columbia. Soil Biol. Biochem. 37, 1157–1167.

Hereş, A.M., Martínez-Vilalta, J., Claramunt López, B., 2012. Growth patterns in relation to drought-induced mortality at two Scots pine (Pinus sylvestris L.) sites in NE Iberian Peninsula. Trees 26, 621–630.

Hereter, A., Sánchez, J.R., 1999. Experimental areas of Prades and Montseny, in: Rodà, F., Retana, J., Gracia, C.A., Bellot, J. (Eds.), Ecology of Mediterranean Evergreen Oak Forests. Springer, Berlin, pp. 15–27.

Kattge, J., Díaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Bönisch, G., Garnier, E., Westoby, M., Reich, P.B., Wright, I.J., Cornelissen, J.H.C., Violle, C., Harrison, S.P., Van Bodegom, P.M., Reichstein, M., Enquist, B.J., Soudzilovskaia, N.A., Ackerly, D.D., Anand, M., Atkin, O., Bahn, M., Baker, T.R., Baldocchi, D., Bekker, R., Blanco, C.C., Blonder, B., Bond, W.J., Bradstock, R., Bunker, D.E., Casanoves, F., Cavender-Bares, J., Chambers, J.Q., Chapin, F.S., Chave, J., Coomes, D., Cornwell, W.K., Craine, J.M., Dobrin, B.H., Duarte, L., Durka, W., Elser, J., Esser, G., Estiarte, M., Fagan, W.F., Fang, J., Fernández-Méndez, F., Fidelis, A., Finegan, B., Flores, O., Ford, H., Frank, D., Freschet, G.T., Fyllas, N.M., Gallagher, R. V., Green, W.A., Gutierrez, A.G., Hickler, T., Higgins, S.I., Hodgson, J.G., Jalili, A., Jansen, S., Joly, C.A., Kerkhoff, A.J., Kirkup, D., Kitajima, K., Kleyer, M., Klotz, S., Knops, J.M.H., Kramer, K., Kühn, I., Kurokawa, H., Laughlin, D., Lee, T.D., Leishman, M., Lens, F., Lenz, T., Lewis, S.L., Lloyd, J., Llusià, J., Louault, F., Ma, S., Mahecha, M.D., Manning, P., Massad, T., Medlyn, B.E., Messier, J., Moles, A.T., Müller, S.C., Nadrowski, K., Naeem, S., Niinemets, Ü., Nöllert, S., Nüske, A., Ogaya, R., Oleksyn, J., Onipchenko, V.G., Onoda, Y., Ordoñez, J., Overbeck, G., Ozinga, W.A., Patiño, S., Paula, S., Pausas, J.G., Peñuelas, J., Phillips, O.L., Pillar, V., Poorter, H., Poorter, L., Poschlod, P., Prinzing, A., Proulx, R., Rammig, A., Reinsch, S., Reu, B., Sack, L., Salgado-Negret, B., Sardans, J., Shiodera, S., Shipley, B., Siefert, A., Sosinski, E., Soussana, J.F., Swaine, E., Swenson, N., Thompson, K., Thornton, P., Waldram, M., Weiher, E., White, M., White, S., Wright, S.J., Yguel, B., Zaehle, S., Zanne, A.E., Wirth, C., 2011. TRY - a global database of plant traits. Glob. Chang. Biol. 17, 2905–2935.

Keiser, A.D., Keiser, D.A., Strickland, M.S., Bradford, M.A., 2014. Disentangling the mechanisms underlying functional differences among decomposer communities. J. Ecol. 102, 603–609.

Killham, K., 1994. The soil biota, in: Killham, K. (Ed.), Soil Ecology. Cambridge University Press, p. 196.

Lenoir, J., Gégout, J.C., Dupouey, J.L., Bert, D., Svenning, J.C., 2010. Forest plant community changes during 1989-2007 in response to climate warming in the Jura Mountains (France and Switzerland). J. Veg. Sci. 21, 949–964.

Lloret, F., Escudero, A., Iriondo, J.M., Martínez-Vilalta, J., Valladares, F., 2012. Extreme climatic events and vegetation: the role of stabilizing processes. Glob. Chang. Biol. 18, 797–805.

Lloret, F., Siscart, D., Dalmases, C., 2004. Canopy recovery after drought dieback in holm-oak Mediterranean forests of Catalonia (NE Spain). Glob. Chang. Biol. 10, 2092–2099.

Mariotti, A., 2010. Recent Changes in the Mediterranean Water Cycle: A Pathway toward Long-Term Regional Hydroclimatic Change? J. Clim. 23, 1513–1525.

Martínez-Vilalta, J., Piñol, J., 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. For. Ecol. Manage. 161, 247–256.

Mclaren, J.R., Turkington, R., 2010. Plant functional group identity differentially affects leaf and root decomposition. Glob. Chang. Biol. 16, 3075–3084.

Mediavilla, S., González-Zurdo, P., García-Ciudad, A., Escudero, A., 2011. Morphological and chemical leaf composition of Mediterranean evergreen tree species according to leaf age. Trees - Struct. Funct. 25, 669–677.

Melillo, J.M., Aber, J., Muratore, J., 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 3, 621–626.

Milcu, A., Manning, P., 2011. All size classes of soil fauna and litter quality control the acceleration of litter decay in its home environment. Oikos 120, 1366–1370.

Montero, G., Ruiz-Peinado, R., Muñoz, M., 2005. Producción de biomasa y fijación de CO2 por los bosques españoles. Monografías del INIA. Serie Forestal nº 13., Madrid.

Ninyerola, M., Pons, X., Roure, J.M., 2007a. Objective air temperature mapping for the Iberian Peninsula using spatial interpolation and GIS. Int. J. Climatol. 27, 1231–1242.

Ninyerola, M., Pons, X., Roure, J.M., 2007b. Monthly precipitation mapping of the Iberian Peninsula using spatial interpolation tools implemented in a Geographic Information System. Theor. Appl. Climatol. 89, 195–209.

Olson, J.S., 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44, 322–331.

Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C., Fasth, B., 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315, 361–364.

Pinheiro, J., Bates, D., DepRoy, S., 2009. Linear and Nonlinear Mixed Effects Models. R package version 3.1-96.

Poyatos, R., Aguadé, D., Galiano, L., Mencuccini, M., Martínez-Vilalta, J., 2013. Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. New Phytol. 200, 388–401.

Prentice, I., Farquhar, G., Fasham, M., 2001. The carbon cycle and atmospheric carbon dioxide, in: Pitelka, L., Rojas, A.R. (Eds.), Climate Change 2001: The Scientific Basis. GRID-Arendal in 2003, pp. 183 – 237.

Santa Regina, I., 2001. Litter fall, decomposition and nutrient release in three semi-arid forests of the Duero basin, Spain. Forestry 74, 347–358.

Saura-Mas, S., Estiarte, M., Peñuelas, J., Lloret, F., 2012. Effects of climate change on leaf litter decomposition across post-fire plant regenerative groups. Environ. Exp. Bot. 77, 274–282.

Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441–451.

Strickland, M.S., McCulley, R.L., Bradford, M.A., 2013. The effect of a quorum-quenching enzyme on leaf litter decomposition. Soil Biol. Biochem. 64, 65–67.

Vayreda, J., Gracia, M., Martinez-Vilalta, J., Retana, J., 2013. Patterns and drivers of regeneration of tree species in forests of peninsular Spain. J. Biogeogr. 40, 1252–1265.

Vilà-Cabrera, A., Martínez-Vilalta, J., Galiano, L., Retana, J., 2013. Patterns of Forest Decline and Regeneration Across Scots Pine Populations. Ecosystems 16, 323–335.

Vivanco, L., Austin, A.T., 2006. Intrinsic effects of species on leaf litter and root decomposition: A comparison of temperate grasses from North and South America. Oecologia 150, 97–107.

Vivanco, L., Austin, A.T., 2008. Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. J. Ecol. 96, 727–736.

Waksman, S.A., Gerretsen, F.C., 1931. Influence of Temperature and Moisture Upon the Nature and Extent of Decomposition of Plant Residues by Microorganisms. Ecology 12, 33–60.

Waldrop, M.P., Firestone, M.K., 2006. Response of microbial community composition and function to soil climate change. Microb. Ecol. 52, 716–724.

Waldrop, M.P., Zak, D.R., 2004. Divergent responses of soil microbial activity and soil C storage to atmospheric N deposition suggests decomposer communities are not functionally redundant. Ecol. Soc. Am. Annu. Meet. Abstr. 89, 527.

Wallenstein, M.D., Haddix, M.L., Ayres, E., Steltzer, H., Magrini-Bair, K.A., Paul, E.A., 2013. Litter chemistry changes more rapidly when decomposed at home but converges during decomposition-transformation. Soil Biol. Biochem. 57, 311–319.

Wang, H., Liu, S., Mo, J., 2010. Correlation between leaf litter and fine root decomposition among subtropical tree species. Plant Soil 335, 289–298.

Wang, W., Zhang, X., Tao, N., Ao, D., Zeng, W., Qian, Y., Zeng, H., 2014. Effects of litter types, microsite and root diameters on litter decomposition in Pinus sylvestris plantations of northern China. Plant Soil 374, 677–688.

Wickings, K., Grandy, A.S., Reed, S.C., Cleveland, C.C., 2012. The origin of litter chemical complexity during decomposition. Ecol. Lett. 15, 1180–1188.

Yuan, Z., Gazol, A., Wang, X., Xing, D., Lin, F., Bai, X., Zhao, Y., Li, B., Hao, Z., 2012. What happens below the canopy? Direct and indirect influences of the dominant species on forest vertical layers. Oikos 121, 1145–1153.

Chapter 6. General conclusions

. Drought-induced forest Scots pine die-off and replacement by Holm oak was reflected in soil CO_2 effluxes at different spatial and temporal scales (*Ch. 2, 3, 4 and 5*).

. Abiotic variables such as soil temperature and soil water content strongly regulated temporal (from daily to seasonal) variability of soil respiration including both autotrophic and heterotrophic components (*Ch. 3 and 4*), whereas the spatial variability of soil respiration was better explained by forest structure and species identity than by abiotic factors (*Ch. 2*).

. Photosynthetic activity exerted a strong control over temporal variability of soil respiration. This influence was not constant across time scales or between tree species (*Ch. 4*). At daily time-scale, variations of soil respiration under Scots pines were more sensitive to photosynthetic activity than variations of soil respiration under Holm oaks. However, at seasonal scale plant photosynthetic activity exerted a stronger regulation over SR under Holm oaks than under Scots pines (*Ch. 4*). These findings denoted that the mechanisms of control of photosynthesis over soil respiration varied among those two phylogenetically distant tree species.

. Soil respiration and all its components (both autotrophic and heterotrophic) remained apparently unaffected by drought-induced Scots pine die-off and mortality (from non-defoliated to defoliated and dead pines) (*Ch. 3*), which denoted a high functional resilience to drought disturbances of the studied plant-soil system. However, the spatial and temporal fluctuations in SR indicated a soil Holm oak colonization of the gaps created by the dead of pines, compensating the expected changes in autotrophic and heterotrophic respiration due to die-off (*Ch. 2, 3 and* 4), and promoting functional resilience of the plant - soil system.

. Despite the soil functional resilience following Scots pine die-off, the replacement of Scots pine by Holm oak in the long term was associated with a strong reduction of the heterotrophic component of soil respiration, producing an important drop in total soil respiration (more than a third) (*Ch. 3*). Therefore, species replacement of Scots pine by Holm oak, may have more consequences, in terms of ecosystem functioning and CO_2 emissions of this drought-limited forest, than the Scots pine die-off and mortality by itself.

. Drought-induced Scots pine die-off and replacement by Holm oak may result on important changes in the rates of litter (both leaves and fine roots) decomposition and nitrogen (N) mineralization (*Ch. 5*). We found that species-specific chemistry of litter produced by Scots pines

and Holm oaks had a direct effect over decomposition rates and N dynamics of litter. But also indirect effects were found, since the decomposer communities associated with a given dominant tree are able to modify litter decomposition rates (*Ch. 5*). Therefore, the replacement of tree species associated with drought-induced species replacement may exert a profound impact over the functioning and capacity of microbial communities to decompose SOM.

. Since Scots pine replacement by *Quercus* species (specially by Holm oak) has been widely reported in the Mediterranean region (the southern-most limit of distribution of Scots pine forests), and given that climatic conditions for Scots pines are expected to worsen in the next decades, changes in soil respiration and in litter decomposition associated with Scots pine die-off and the subsequent successional replacement by Holm oak are expected to have great impacts on the carbon balance of this type of forests.

Agraïments