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Across-scale patterning of plant-soil-pathogen interactions in *Quercus* *suber* decline

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Abstract

Forests worldwide have been recently affected by severe decline and mortality, while our understanding about forest decline across spatial scale is still limited. In this work we study how *Quercus suber* trees adjust their physiology, in terms of water use efficiency and secondary growth, to pathogen-induced oak decline at the whole-tree, local and landscape scales. This work was carried out in Mediterranean mixed forests where their dominant key species *Q. suber* is affected by a severe decline and mortality induced by the exotic soil-borne pathogen *Phytophthora cinnamomi*. Significant differences were not observed between defoliated and healthy trees, either in terms of water use efficiency or growth at the whole-tree scale. We found that limiting conditions, such as low soil depth and high pathogen abundance, induced trees to higher water use efficiency at local and landscape scales. Overall our findings point out that *Q. suber* trees subjected to soil drought and root pathogens increase water use efficiency to some extent, while this response might not be enough for the trees to overcome the physiological stress associated to the pathogen-induced dieback. We discuss the complex way by which adult *Q. suber* trees physiologically respond to *P. cinnamomi*-induced mortality, improving our understanding of the likely consequences of chronic oak decline in the future.

Keywords

carbon isotope; cork oak; cross-scale relationship; defoliation; ecophysiology; exotic pathogen; forest decline; forest disease; root pathogen; secondary growth; soil heterogeneity; tree dieback

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Introduction

In the recent decades, forests worldwide have been affected by severe decline and mortality (e.g. Allen et al. 2015; Brouwers et al. 2013; van Mantgem et al. 2009). In these affected systems, environmental stress operating at individual scale (e.g. physiological tree stress) can directly cause tree mortality, but also can interact with other disturbance processes at local or intermediate scales (e.g. insect pests). The result of such interactions may trigger complex responses subjected to significant uncertainties (Chave 2013; Levin 1992) that can be key drivers of the ecosystem dynamics (Allen 2007; Peters et al. 2007). Nonetheless, our understanding of forest decline across spatial scales is constrained by limited knowledge of species-specific physiological thresholds, individual- and site-specific variation in these mortality thresholds and pathogen (or insect)-related feedbacks (Allen 2007).

In a context of worldwide tree mortality, oak forests have been severely impaired in a number of different systems in Europe and North America (e.g. Haavik et al. 2015; Thomas et al. 2002). Oak decline is considered of paramount importance in Mediterranean systems because this phenomenon involves evergreen oak species (e.g. *Quercus ilex*, *Quercus suber*, *Quercus agrifolia*) that dominate the typical agroforestry systems of the west Mediterranean Basin (i.e. *dehesas* and *montados*) and oak savannas of western North America. Evergreen oak trees are a major structural element in these forests and maintain ecosystem functions and services such as enhancing diversity, soil protection, provisioning of resources and cultural services (Marañón et al. 1999; Marañón et al. 2012; Olea and San Miguel-Ayán 2006). In the Mediterranean Basin the decline of these keystone species is frequently caused by soil-borne *Phytophthora* species (Brasier 1992; Jung et al. 2016; Pérez-Sierra et al. 2013). Although several species of *Phytophthora* have been isolated in soils from declining Mediterranean oak

forests, *Phytophthora cinnamomi* is by far the most aggressive one and has been consistently recognized as a main driver of oak mortality in central and southern Spain, Portugal and southern regions of France and Italy (Brasier 1992; Corcobado et al. 2014; Sánchez et al. 2002). This soil-borne pathogen that attacks tree root systems is considered one of the 100 most dangerous invasive species on earth (Lowe et al. 2000; Weste and Marks 1987). Oak decline involves the interaction of *P. cinnamomi* with abiotic drivers that can modulate the effects of the pathogen on trees at different spatial scales (Balci et al. 2010; Brasier 1996; Camilo-Alves et al. 2013). For example, climatic factors such as drought and heat waves have been identified as important drivers of oak decline that can accelerate the oak mortality process at regional scale (e.g. Bendixsen et al. 2015; Camilo-Alves et al. 2013; Corcobado et al. 2014). Moreover, other abiotic factors such as soil characteristics can influence trees at local scale, weakening and predisposing trees to defoliation and death (Camilo-Alves et al. 2013; Corcobado et al. 2013b; Costa et al. 2010). The high socio-economic and environmental value of evergreen oaks in Mediterranean systems highlights the need to understand the drivers of oak decline at different scales.

Tree mortality can be lower than expected when individuals have the capability to attenuate the detrimental effects of stress (Lloret et al. 2012). It has been shown that seedlings and saplings of evergreen *Quercus* species may respond physiologically to *P. cinnamomi* infection in ways similar to drought stress, at least in terms of water relationships. For instance, previous studies have found that infected *Q. suber* and *Q. ilex* seedlings showed lower stomatal conductance and, in turn, higher water use efficiency than non-infected ones (Luque et al. 1999; Maurel et al. 2001; Robin et al. 2001; Sghaier-Hammami et al. 2013). However, these studies were performed under controlled watering regimes in greenhouse experiments. To our knowledge, there is a

lack of information about physiological responses to *P. cinnamomi* infection in adult *Q. suber* trees under field conditions. If adult trees cannot show any responsive mechanisms or the effective physiological response is exceeded, a high mortality rate might be expected, which in turn could trigger cascading effects at ecosystem level (Anderegg et al. 2013; Edburg et al. 2012; Ellison et al. 2005). For example, a decrease in *Q. suber* recruitment has been detected in Mediterranean forests affected by *P. cinnamomi*, that might alter the successional trajectories of Mediterranean oak forests (Ibáñez et al. 2015b). *Q. suber* decline is also affecting ecosystem functioning, reducing soil respiration and decoupling limiting nutrients (Ávila et al. 2016), which might limit even further demographic and ecosystem processes in Mediterranean forests. Moreover, how trees respond physiologically to pathogen attack can trigger regional-scale massive decline (Allen 2007). These complex relationships require the study of ecological processes at different spatial scales. In this context, characterizing the response of key species to current pathogen-driven tree mortality processes may provide valuable information for predicting future community-level responses in a context of global change, as well as to improve conservation and management guidelines.

Here we aim to analyze how individuals of the dominant evergreen oak species *Quercus suber* adjust their physiology, in terms of water use efficiency and secondary growth, at different scales in Mediterranean oak forests affected by pathogen-induced oak decline. First, we explored whether defoliated trees have different water use efficiency and secondary growth than healthy trees at the whole tree-scale (i.e. differences attributable to each individual tree). Second, we assessed which abiotic (i.e. soil depth, organic matter, water content and texture) and biotic factors (i.e. pathogen abundance) explain tree water use efficiency and secondary growth for healthy and defoliated *Q. suber* trees at the local scale (i.e. comparing trees with different microsite

conditions). Finally, we analyzed differences in water use efficiency and secondary growth at landscape scale (i.e. comparing trees in different forest types). We hypothesized that (1) defoliated trees would show higher water use efficiency and lower secondary growth than healthy trees; (2) higher water use efficiency and lower secondary growth would be related to limiting soil abiotic conditions and pathogen abundance and (3) at landscape scale, water use efficiency and tree growth would vary among forest types differing in edaphoclimatic characteristics.

Methods

Study area and tree species

This study was carried out in mixed oak forests in Los Alcornocales Natural Park (Southern Spain), one of the largest *Q. suber* forests within the Mediterranean Basin (Urbieto et al. 2008). The climate is sub-humid Mediterranean with warm, dry summers and mild, humid winters. Annual mean temperature ranges from 15.4 to 17.3 °C and annual mean rainfall varies from 720 to 1100 mm (period 1951-1999, Ninyerola et al. 2005). These forests grow on acidic, nutrient-poor, sandy soils derived from Oligo-Miocene sandstone and sometimes they are interspersed with clayish soils derived from layers of marl sediments. Vegetation in the overstory is dominated by the evergreen *Quercus suber* L. In drier lowlands with clayish soils, *Q. suber* trees form open woodlands with the evergreen and shade-intolerant *Olea europaea* var. *sylvestris* Brot. In moister habitats, *Q. suber* coexists with the deciduous shade-tolerant *Quercus canariensis* Willd. forming closed forests (Ojeda et al. 2000). In these forests, *Q. suber* is extensively managed for cork production. Moreover, the adult trees of the species show important problems of defoliation and mortality. Extremely high abundances of *Phytophthora cinnamomi* have been found in soils of symptomatic *Q. suber* trees, and

therefore this pathogen has been suggested as a main driver of the species decline in the area (Gómez-Aparicio et al. 2012).

Study sites, stand structure and tree mortality data

We selected six 1-ha sites within the Natural Park, covering a gradient of climate and soil properties (Table 1). Three of the sites were situated in open woodlands of *Q. suber* and *O. europaea* and the other three in closed forests dominated by *Q. suber* and *Q. canariensis* (see Appendix S1). All selected sites are affected by *Q. suber* decline, with trees showing different levels of defoliation (Gómez-Aparicio et al. 2012). Topography was kept constant in order to avoid confounding effects of the studied variables. Annual precipitation for each plot was obtained from Ninyerola et al. (2005).

In each site we mapped and identified all live and dead trees higher than 1.5 m and with a diameter at breast height (dbh) > 2 cm using a total station Leica TC 407. We measured the dbh of each of the trees mapped (n = 1341 trees). In addition, we evaluated the health status of *Q. suber* trees by a visual estimation of crown defoliation on a standardized semi-quantitative scale widely used in the region to monitor oak decline (García et al. 2011; Gómez-Aparicio et al. 2012; Ibáñez et al. 2015b): healthy reference trees, slightly defoliated trees (<50% crown defoliation); highly defoliated trees (>50% crown defoliation) and dead trees. Tree conditions were evaluated twice (early spring 2010 and 2012) to ensure healthy status of sampled trees. We found no symptoms of defoliation or mortality in individuals of the coexistent tree species *Q. canariensis* or *O. europaea* in our studied plots.

Wood samples, secondary growth and intrinsic water-use efficiency

187 Sampling was conducted in autumn 2013. At each of the six study sites we randomly
188 selected five healthy *Q. suber* trees, five defoliated *Q. suber* trees (i.e. > 50% of crown
189 defoliation) and five trees of the coexistent species (*O. europaea* in woodlands or *Q.*
190 *canariensis* in closed forests) with average size in terms of dbh, height and canopy
191 diameter. For each tree we sampled three branches with similar age, based on the branch
192 diameter (aprox. 5 cm, 14 years), south-exposed and located 4-6 m from the ground (n =
193 270 sampled branches).

194 All sampled branches were analyzed for estimating secondary growth and
195 intrinsic water-use efficiency (iWUE). We cut transversely three 1-mm thick sections of
196 each branch and the samples were air-dried. One section per branch was polished with a
197 series of successively finer sand-paper grits until rings were clearly visible. We scanned
198 those sections at high resolution (600 dpi) and used imageJ v1.49 (Schneider et al.
199 2012) to measure the total number of annual growth rings and the width of each ring
200 (see Appendix S2). We analyzed secondary growth of branches to avoid the bias from
201 the tree inner rings when decline might have not started yet. We used the other two
202 sections of each branch to analyze intrinsic water-use efficiency (iWUE) from $^{13}\text{C}/^{12}\text{C}$
203 isotope ratios in the branch wood. Wood samples were carefully homogenized and
204 milled using a ball-mill (MM301, Retsch. Germany). Aliquots of 0.5-0.7 mg from each
205 wood section were weighed on a balance (AD6 Autobalance Controller, Perkin Elmer.,
206 USA) and placed into a tin capsule for isotopic analyses. Cellulose was not extracted,
207 since both whole wood and cellulose isotope show similar trends related to atmospheric
208 CO_2 concentration and climate (Saurer et al. 2004).

209 The isotope ratio $^{13}\text{C}/^{12}\text{C}$ was determined on a stable isotope mass spectrometer
210 (ThermoFinnigan MAT 251, CA) at the Stable Isotope Facility at the University of
211 California, Davis. Isotopic values were expressed relative to the international Vienna

Pee-Dee Belemnite (V-PDB) standard as $\delta^{13}\text{C}$. Two analytical standards were included for analysis after every 10 wood samples: cellulose ($\delta^{13}\text{C} = -24.72\text{‰}$) and phthalic acid ($\delta^{13}\text{C} = -30.63\text{‰}$). The repeated analysis of these two internal standards yielded a standard deviation $<0.1\text{‰}$ and the accuracy of analyses was 0.07‰ . The estimated precision of the measurements was $\pm 0.1\text{‰}$.

Isotopic discrimination between the C of atmospheric CO_2 and wood carbon (Δ) in plants is a consequence of the preferential fixation of the ^{12}C compared with ^{13}C by plants C_3 during photosynthesis and it was defined by Farquhar and Richards (1984) as:

$$\Delta = \frac{(\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_{\text{plant}})}{(1 + \delta^{13}\text{C}_{\text{plant}}/1000)} \quad (1)$$

where $\delta^{13}\text{C}_{\text{atm}}$ is the isotope ratio of C ($^{13}\text{C}/^{12}\text{C}$) in atmospheric CO_2 and $\delta^{13}\text{C}_{\text{plant}}$ is the isotope ratios of C in tree-ring wood, in both cases relative to the standard V-PDB and expressed in parts per thousand (‰). In turn, Δ is linearly related to the ratio of intercellular to atmospheric (C_i/C_a) CO_2 mole fractions (Farquhar et al. 1982):

$$\Delta = a + (b - a)C_i/C_a \quad (2)$$

where a is the fractionation caused by CO_2 diffusion through the stomata (4.4‰), and b is the fractionation caused by Rubisco and PEP carboxylase (27‰ ; Farquhar and Richards 1984). The values for C_a and $\delta^{13}\text{C}$ were obtained from McCarroll and Loader (2004). The linear relationship between (C_i/C_a) and Δ can be used to calculate the intrinsic water-use efficiency (iWUE), defined as the ratio of net assimilation to stomatal conductance to water vapor, which can be calculated as:

$$\text{iWUE} = \frac{C_a(b - \Delta)}{1.6(b - a)} \quad (3)$$

The iWUE, expressed in $\mu\text{mol mol}^{-1}$, has been widely related to trends in the internal regulation of carbon uptake and water loss in plants and is considered the main indirect

parameter to integrate over time the instantaneous water use efficiency (Farquhar and Richards 1984). More details on the calculation of iWUE from $\delta^{13}\text{C}$ can be found in McCarroll and Loader (2004).

Soil variables and pathogen analysis

We analyzed abiotic and biotic soil variables under trees in order to relate them to tree water use efficiency and secondary growth. We selected abiotic soil variables related to soil water balance and soil productivity such as soil water potential, texture, depth and organic matter. We analyzed *P. cinnamomi* abundance as a biotic factor because this pathogen is the main driver of the oak decline in these forests (Gómez-Aparicio et al. 2012). We took three soil samples (0-20 cm) under the canopy of each studied tree with a cylindrical auger and mixed to produce one composite soil sample per tree. Soil samples were transported in polyethylene bags in coolers to the laboratory. Total soil depth was recorded under each tree with a metal stick from the surface to the beginning of the bedrock and was calculated as the mean of three measurements under each individual.

Soil samples were air dried at room temperature and sieved at 2 mm to remove root material and stones. A complete particle size analysis was carried out using the Bouyoucos hydrometer method (Gee and Bauder 1986). From this method we selected sand ($0.0625\text{mm} < \text{sand} < 2\text{mm}$) as a representative measurement of soil texture. Previously, a subsample of 20 g was used to determine the water content gravimetrically by weighing the fresh and dried soil (105°C). A subsample of 2 g was then incinerated for 4 h at 550°C to determine the soil organic matter content by calcinations method (Sparks et al. 1996). Soil water potential was determined by

empirical relationships among soil water content and soil texture and organic matter (Rawls et al. 2003; Saxton et al. 1986).

We determined the abundance of *P. cinnamomi* following the method developed by Romero et al. (2007). Soil suspensions were prepared by adding 10 g of dry sieved soil in 100 ml of sterilized Water-Agar (0.2%), and aliquots of 1 ml were plated on Petri dishes, each containing 20 ml of the selective medium NARPH (Hüberli et al. 2000). For each soil sample, a total of 20 Petri dishes were prepared. Dishes were incubated at 24°C in the dark for 24h, then the agar surface of each plate was washed with sterile water and dishes were re-incubated at 24°C in the dark for 48h. Colonies obtained were identified (using an inverted microscope) by the clustered hyphal swellings typical of *P. cinnamomi* (Erwin and Ribeiro 1996; Sánchez et al. 2002) and counted. As soil samples were previously dried, it was assumed that each colony obtained resulted from the germination of, at least, one resistant spore (oospore or chlamydospore). Results were expressed as colony forming units per gram of dry soil (CFU g⁻¹).

Data analysis

To test our first hypothesis, we fitted Linear Mixed Models that compare water use efficiency and secondary branch growth of healthy vs. defoliated *Q. suber* trees. We included branch diameter as a covariate in the model for secondary branch growth. To answer our second hypothesis at local scale, we fitted Linear Mixed Models that explain water use efficiency and secondary growth of *Q. suber* trees as a function of abiotic and biotic microsite conditions (i.e. soil depth, soil water potential, sand content, soil organic matter content and *P. cinnamomi* abundance). We fitted separate models for healthy and defoliated trees. The saturated model was successively simplified until the minimal adequate model using Akaike Information Criterion corrected for small

samples (AICc, Burnham and Anderson, 2002). Models with a difference in $AIC_c < 2$ units are considered to have equivalent empirical support. We introduced plot as a random factor in all Linear Mixed Models.

To test our third hypothesis, we carried out Linear Mixed Models that compare physiological variables of trees and edaphoclimatic variables between forest types. In order to compare the physiological performance of trees of different species, we fitted Linear Mixed Models including species as a fixed factor (healthy *Q. suber* trees and coexistent *O. europaea* or *Q. canariensis*). As coexistent species differed between forest types, we run the models for all trees but separating *Q. suber* trees in two categories (*Q. suber* in woodlands or *Q. suber* in closed forests). We included branch diameter as a covariate in the model for secondary branch growth. Plot effect was used as a random factor except for annual precipitation. For this variable we carried out a General Linear Model due to the lack of variability within plots.

All models were fitted using the *nlme* library of the R statistical software v. 3.1.1 (R Core Team 2014). Parameters of the best model were estimated with restricted maximum likelihood (REML, Zuur et al. 2009) and model selection was carried out using MuMIn package (Bartoń 2016). Tuckey's tests were applied for multiple comparisons between all pairs of means when significant variables were observed in the LMM. Graphics were performed using R statistical software and Sigmaplot v. 12 (Systat Software Inc.).

Results

Our six study sites were severely affected by decline. The proportion of defoliated *Q. suber* trees varied from 14% to 64%, while the proportion of dead *Q. suber* trees varied from 5% to 38% (Table 1). In all studied forests, we found the presence of

Phytophthora cinnamomi resting spores in soil samples. The average abundance of this pathogen per plot ranged from $4.63 \pm 1.77 \text{ CFU} \cdot \text{g}^{-1}$ to $440.53 \pm 60.65 \text{ CFU} \cdot \text{g}^{-1}$ (Table 1), reaching densities as high as $1476 \text{ CFU} \cdot \text{g}^{-1}$ under some defoliated trees. It was found higher pathogen abundance under defoliated than under healthy *Q. suber* trees (LMM $F = 4.65$, $P = 0.036$).

The linear mixed models did not show any difference between defoliated and healthy *Q. suber* trees either in terms of water use efficiency (LMM $F = 0.086$, $P = 0.771$, Fig. 1a) or growth (LMM $F = 0.187$, $P = 0.667$, Fig. 1b). At the local scale, we found that variables explaining variance of water use efficiency and secondary growth differed between healthy and defoliated *Q. suber* trees. The best model of water use efficiency for healthy trees included a negative effect of soil depth, whereas for defoliated trees it contained a negative effect of soil depth and a positive effect of soil pathogen abundance (Table 2, Fig. 2). Regarding secondary branch growth, the null model was the best fit for healthy trees indicating that there was no detectable effect of the studied soil properties on this variable (Appendix S3). For defoliated trees, the best model of secondary growth included a positive effect of soil pathogen abundance (Table 2). This relationship did not have a strong empirical support though, since the difference in AIC with the null model was never larger than two units (see Appendix S3).

At the landscape scale, water use efficiency of *Q. suber* trees differed between forest types, with higher values in woodlands than in closed forests. *Q. suber* trees in woodlands had a 20% higher water use efficiency than *Q. suber* in closed forests (Table 3, Fig. 3a). However secondary branch growth of *Q. suber* trees was similar between forest types (Table 3, Fig. 3b). We also found marginally significant differences between forest types in terms of organic matter, texture and soil pathogen abundance ($P < 0.10$, Table 3, Fig. 3c,d,e), Woodlands had higher soil organic matter but lower sand

content than closed forests (Fig. 3c,d). The abundance of *P. cinnamomi* was higher in woodlands than in closed forests (Fig. 3e). Moreover, there was difference in terms of rainfall between forest types, with lower precipitation in woodlands than in closed forests (LM $F_{1,10} = 12.049$, $P = 0.006$, Fig. 3f). We found differences among *Q. suber* and coexistent non-affected species in terms of water use efficiency (LMM $F = 27.030$, $P < 0.0001$, Fig. 4a). We found that *Q. suber* had similar water use efficiency than *O. europaea* in woodlands ($P = 0.944$), and similar values than *Q. canariensis* in closed forests ($P = 0.199$). *Q. suber* in woodlands had a 20% higher water use efficiency than what they had in closed forests ($P < 0.001$, Fig. 4a). We did not find differences in the secondary branch growth among species in both forest types (LMM $F = 1.884$, $P = 0.144$, Fig. 4b).

Discussion

Our results illustrate complex relationships between spatial patterning and coupled ecological processes that help us to understand the phenomenon of *Q. suber* decline. At the whole-tree scale, we did not find that the process of pathogen-induced oak decline led to a straightforward response in water use efficiency and secondary branch growth, contradicting our first hypothesis (Fig. 1). Therefore, our results suggest a decoupling between whole-tree scale defoliation symptoms and the physiological response of trees.

We expected that defoliated trees under stress would show a physiological response to oak decline coupled with defoliation symptoms since trees in water-limited ecosystems have many adaptive mechanisms to cope with drought and biotic disturbances (Peñuelas et al. 2000). Trees usually respond to drought-induced water stress by closing their stomata to limit water loss and increasing water use efficiency (Serrano and Peñuelas 2005; Tenhunen et al. 1990). This is a mechanism also expected

in trees affected by root pathogens (Camilo-Alves et al. 2013; Fleischmann et al. 2002). However, the reduction in stomatal conductance also increases the risk of carbon starvation (Martínez-Vilalta et al. 2002) together with the risk of hydraulic failure enhanced by a reduced water transport capacity (McDowell et al. 2008; McDowell 2011). Both mechanisms may induce tree defoliation and mortality (Allen et al. 2015; McDowell 2011). Although trees native to water-limited ecosystems are able to respond to drought, their physiological phenotypic plasticity and adaptive capacity may be exceeded under intense climate stress in combination with biotic disturbances (Galiano et al. 2012; Sangüesa-Barreda et al. 2013). We also expected lower secondary branch growth in defoliated trees than in healthy trees, as a reduction in tree growth has been previously reported for other evergreen Mediterranean oaks affected by decline (Solla et al. 2009). However, we found that branches of healthy and defoliated trees can growth at similar rate, disregarding the general health status of the tree. It is possible that other variables related with vegetative and reproductive growth, such as trunk growth or seed production, would have better reflected the differences between healthy and defoliated trees. Our results showed that *Q. suber* trees might be unable to mitigate the negative effects of a decrease in water availability related to *P. cinnamomi*-induced decline through a reduction in stomatal conductance, resulting in defoliation and tree death.

At local scale, low soil depth and high pathogen abundance induced trees to stressful situations that led to higher water use efficiency (Fig. 2), which confirms our second hypothesis. Shallow soils can inhibit root growth into deep layers (Fisher and Binkley 2000), which in turn can limit the access to belowground water resources, especially during summer drought (Costa et al. 2008). Therefore, it should be expected that water use efficiency increases as soil depth decreases, as a consequence of a more efficient stomatal control of water loss by transpiration in shallow soils experimenting

water shortage (Maseyk et al. 2011; Ogaya and Penuelas 2003; Tenhunen et al. 1990). We also detected increasing water use efficiency in defoliated trees as soil pathogen abundance increased. It has been shown that *P. cinnamomi* causes a reduction in fine root abundance in defoliated oaks (Corcobado et al. 2013a), which might decrease their water absorption capacity affecting stomatal conductance and increasing water use efficiency (Osswald et al. 2014). For example, previous studies have found physiological responses of *Quercus* seedlings infected by *P. cinnamomi*, such as stomatal closure, and a more efficient water use and photochemical performance (Luque et al. 1999; Maurel et al. 2001; Robin et al. 2001; Sghaier-Hammami et al. 2013). This response may enable trees to tolerate water stress. However, although we detected an ecophysiological response of defoliated *Q. suber* trees to high pathogen loads, we did not find that those trees had on average higher water use efficiency than healthy *Q. suber* trees. Our results suggest that *Q. suber* trees subjected to soil drought and root pathogens increase water use efficiency to some extent, while this response might not be enough to overcome the physiological stress eventually leading to defoliation and death.

Results obtained at local scale seem to agree with patterns obtained at a landscape scale. Water use efficiency differed significantly between open woodlands and closed forests, as we hypothesized (Fig. 3). Open woodlands were characterized by lower precipitation but also their soils had lower sand and higher organic matter contents than closed forests. These soil differences are usually related to milder effects of water shortage on plants (Galiano et al. 2010; Sperry and Hacke 2002). However, closed forests were characterized by higher precipitation and frequent fogs, even during the summer dry season, which can reduce significantly the water stress of trees (Ojeda et al. 2000; Urbietta et al. 2008) and explain the lower water use efficiency of trees in

409 this forest type (see Appendix S1). It has been shown that changes in external drivers or
410 disturbances can alter pattern-process relationships (Allen 2007; Peters et al. 2007). For
411 example, we could expect that the links between water stress and water use efficiency
412 would be altered as a consequence of the introduction of *P. cinnamomi*. Despite new
413 processes and feedbacks might be observed as a result of the oak decline, our results did
414 not suggest any change in the relationship between water stress and the water use
415 efficiency at local and landscape scales.

416 Our findings showed at landscape scale a high plasticity of *Q. suber* to modify
417 its water use efficiency depending on the forest type where trees grow (Fig. 4). The
418 large range of values observed in carbon isotope discrimination and the differences in
419 this variable between forest types seems to suggest that *Q. suber* trees can adjust
420 efficiently the stomatal conductance in relation to local water availability (Gouveia and
421 Freitas 2009; Otieno et al. 2007). The water use efficiency variance obtained here is
422 within the range of those reported for other tree species with high plasticity in response
423 to water availability (Ferrio et al. 2003; Shestakova et al. 2014). In our study we
424 obtained that *Q. suber* modified their water use efficiency up to similar values of the
425 semi-deciduous *Q. canariensis* growing in closed forests. This convergent response can
426 be explained as evergreen and deciduous *Quercus* species may show similar responses
427 to water constraint in terms of stomatal conductance and net CO₂ assimilation under
428 mesic conditions (Damesin et al. 1998). However, *Q. suber* was able to increase even
429 more its water use efficiency, reaching in open woodlands similar values of than *O.*
430 *europaea*, a species with anatomical and hydraulic traits that enable high drought stress
431 tolerance (Rossi et al. 2013). Our results support that *Q. suber* trees are able to increase
432 water use efficiency in drier sites while they keep growth rates similar to those found in
433 wetter sites, at least in terms of branch secondary growth.

Conclusions

Oak decline from Mediterranean forest ecosystems is considered an important concern for managers, landowners and researchers because in the last decades a decrease in vigor and an increase in mortality of evergreen oak trees threaten these forests, and consequently, the goods and services these systems provide. Our results suggest that, although *Q. suber* is able to respond ecophysiologically to water stress at local and landscape scale, the overall response of defoliated trees is not enough to overcome the stress induced by *P. cinnamomi*. This fact might have been responsible of high mortality rates when *Q. suber* forests are affected by global change drivers such as drought and invasive pathogens. Particularly in our study area, more than the half of the basal area of *Q. suber* was affected by defoliation and high tree mortality rates. A lack of coevolutionary history between *Q. suber* and *P. cinnamomi* is probably causing the high vulnerability of this tree species to the pathogen. Overall, our results illustrate the complex way by which adult *Q. suber* trees physiologically respond to *P. cinnamomi*-induced mortality and may help to anticipate the subsequent consequences if the oak decline becomes widespread in the future.

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Table 1. Structural and edaphoclimatic attributes of the studied sites. Coexistent species are *O. europaea* in woodlands or *Q. canariensis* in closed forests. Defoliated and dead *Q. suber* is expressed as percentage of total *Q. suber* trees. Relative *Q. suber* basal area is expressed as percentage of total basal area of *Q. suber* trees. Data of soil properties and *P. cinnamomi* abundance are mean and standard error ($N = 30$). Ca, K and N analyses and data from Ibáñez et al. (2015a).

Variable	WOODLANDS			CLOSED FORESTS		
	S1	S2	S3	S1	S2	S3
Latitude (N)	36° 04' 38"	36° 18' 37"	36° 31' 69"	36° 06' 09"	36° 23' 10"	36° 28' 13"
Longitude (W)	05° 33' 05"	05° 41' 14"	05° 38' 08"	05° 30' 53"	05° 31' 52"	05° 35' 31"
Forest structure						
Coexistent species stand density (ind·ha ⁻¹)	48	49	53	277	36	99
<i>Q. suber</i> stand density (ind·ha ⁻¹)	69	200	265	331	93	78
Defoliated <i>Q. suber</i> (%)	36.23	14.00	63.77	26.59	39.78	26.92
Dead <i>Q. suber</i> (%)	37.68	7.00	5.28	33.23	15.05	14.10
Relative <i>Q. suber</i> basal area (%)						
Healthy	29.51	74.37	26.23	61.05	33.97	39.30
Defoliated	65.84	19.64	37.56	36.44	41.38	32.32
Dead	4.65	5.99	36.21	2.51	24.66	28.39
Site characteristics						
Precipitation (mm)	948.9	726.4	973.1	1067.1	1022.6	1097.0
Soil depth (cm)	35.82 ± 3.03	41.53 ± 3.50	45.02 ± 2.52	49.08 ± 2.27	57.24 ± 2.45	55.82 ± 2.35
Sand (%)	47.06 ± 1.75	68.60 ± 0.99	50.20 ± 1.66	72.90 ± 0.99	68.52 ± 1.04	75.26 ± 0.95
Silt (%)	22.01 ± 0.70	16.29 ± 0.49	17.92 ± 0.40	13.29 ± 0.44	17.78 ± 0.55	14.97 ± 0.53
Clay (%)	30.93 ± 1.30	15.12 ± 0.65	31.88 ± 1.51	13.81 ± 0.69	13.70 ± 0.68	9.77 ± 0.55
Ca (mg kg ⁻¹)	3397 ± 151	1455 ± 119	5181 ± 348	703 ± 39	961 ± 67	896 ± 57
K (mg kg ⁻¹)	261.6 ± 14.6	233.4 ± 10.4	299.5 ± 15.6	116.1 ± 6.3	154.5 ± 8.0	183.7 ± 11.4
N (mg g ⁻¹)	4.84 ± 0.30	3.53 ± 0.19	4.76 ± 0.27	2.49 ± 0.12	2.25 ± 0.14	2.27 ± 0.15
pH	5.94 ± 0.04	5.13 ± 0.03	5.92 ± 0.02	4.89 ± 0.05	5.04 ± 0.04	5.09 ± 0.04
<i>P. cinnamomi</i> abundance (CFU g ⁻¹)	440.5 ± 60.65	93.83 ± 33.36	355.87 ± 47.36	4.83 ± 1.50	80.17 ± 31.32	4.63 ± 1.77

Table 2. Linear mixed models for analysis of the water use efficiency (WUE) and secondary branch growth of healthy and defoliated *Q. suber*. Degrees of freedom (d.f.), F-ratios and P values of the best models are shown. For growth of healthy *Q. suber*, the best model was the null model (Appendix S3).

Variable	Tree category	Fixed factors	d.f.	F	P
WUE	Healthy	Depth	1	5.250	0.031
	Defoliated	<i>P. cinnamomi</i> abundance	1	13.411	0.001
		Depth	1	4.087	0.055
Growth	Healthy	NULL			
	Defoliated	<i>P. cinnamomi</i> abundance	1	11.733	0.002

Table 3. Linear mixed models for analysis of the differences between forest types (woodland and closed forest) in water use efficiency and secondary branch growth of *Q. suber* trees, soil organic matter, sand content and *P. cinnamomi* abundance. Degrees of freedom (d.f.), F-ratios and P values of the best models are shown.

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Variable	d.f.	<i>F</i>	<i>P</i>
Water use efficiency	1	52.035	0.002
Secondary branch growth	1	0.003	0.960
Soil organic matter	1	3.969	0.081
Sand content	1	6.489	0.064
<i>P. cinnamomi</i> abundance	1	5.310	0.083

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Figure captions

Fig. 1 Water use efficiency (a) and secondary branch growth (b) of healthy (HEA) and defoliated *Q. suber* trees (DEF). $N = 30$. n.s.= Non-significant ($P > 0.05$). Each boxplot shows the median, inter-quartiles and whiskers (i.e. 1.5 times inter-quartile rank). Dots outside the whiskers are outliers.

Fig. 2 Relationships between water use efficiency of healthy (HEA) and defoliated (DEF) *Q. suber* trees and soil depth (a); and soil pathogen abundance (b). Grey dots and lines are for defoliated *Q. suber* trees and black dots and lines are for healthy *Q. suber* trees. $N = 15$. Estimate (β), standard error (S.E.) and P value of the slopes are shown. n.s.= Non-significant ($P > 0.05$)

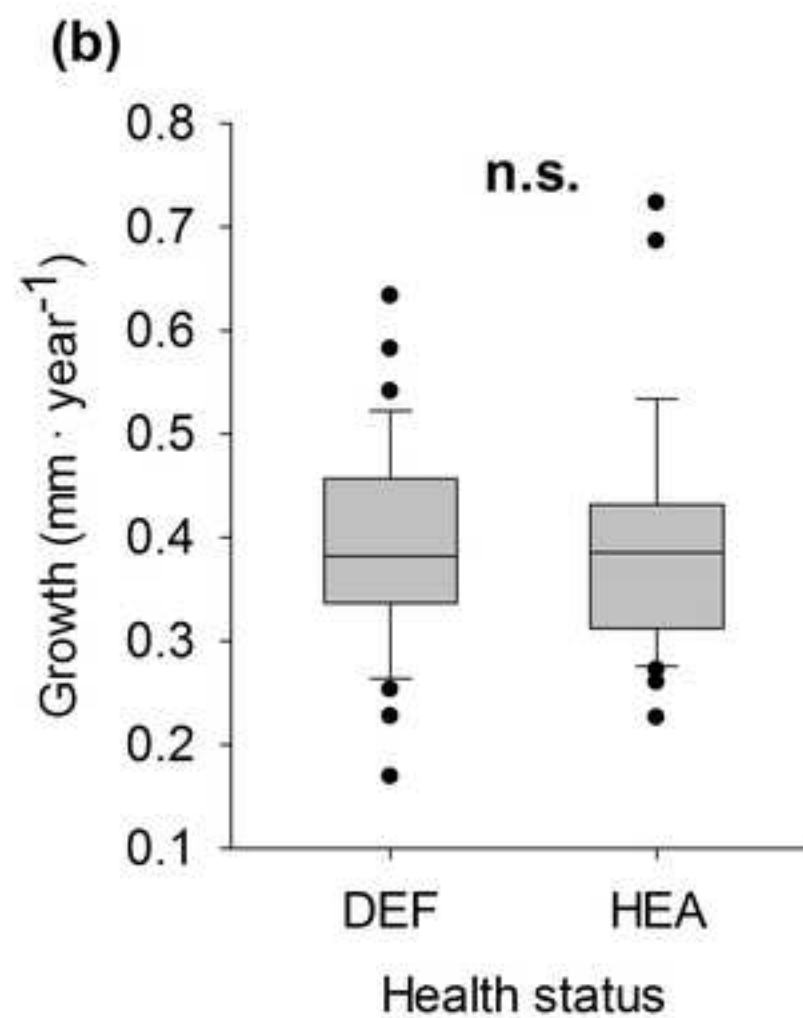
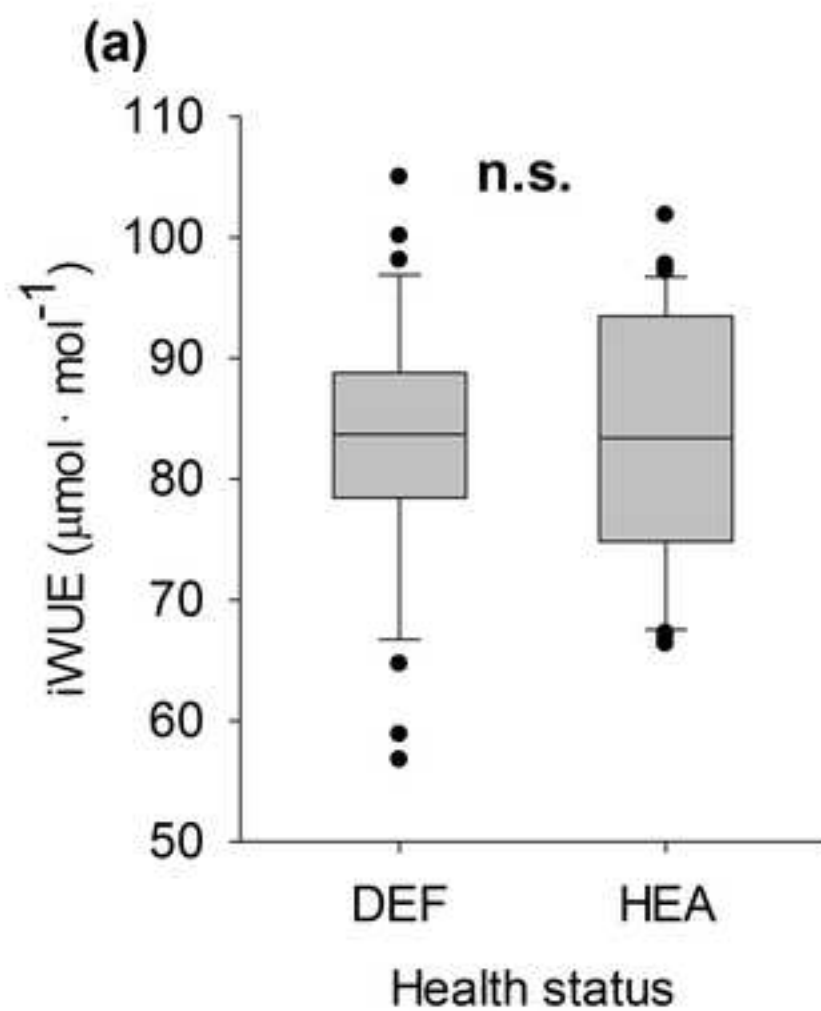
Fig. 3 Mean values (\pm standard error) of water use efficiency (a), secondary branch growth (b), organic matter (c), sand content (d), soil pathogen abundance (e) and precipitation (f) in the three sites (S1, S2, S3) within the two forest types (WL = Woodlands; CF = Closed forests). $N = 10$ for all figures except for (f) where $N = 3$. Significant differences between forest types were shown: point (\bullet) indicates $P < 0.10$, single asterisk (*) indicates $P < 0.05$; double asterisks (**) indicates $P < 0.01$; n.s. indicates $P > 0.10$

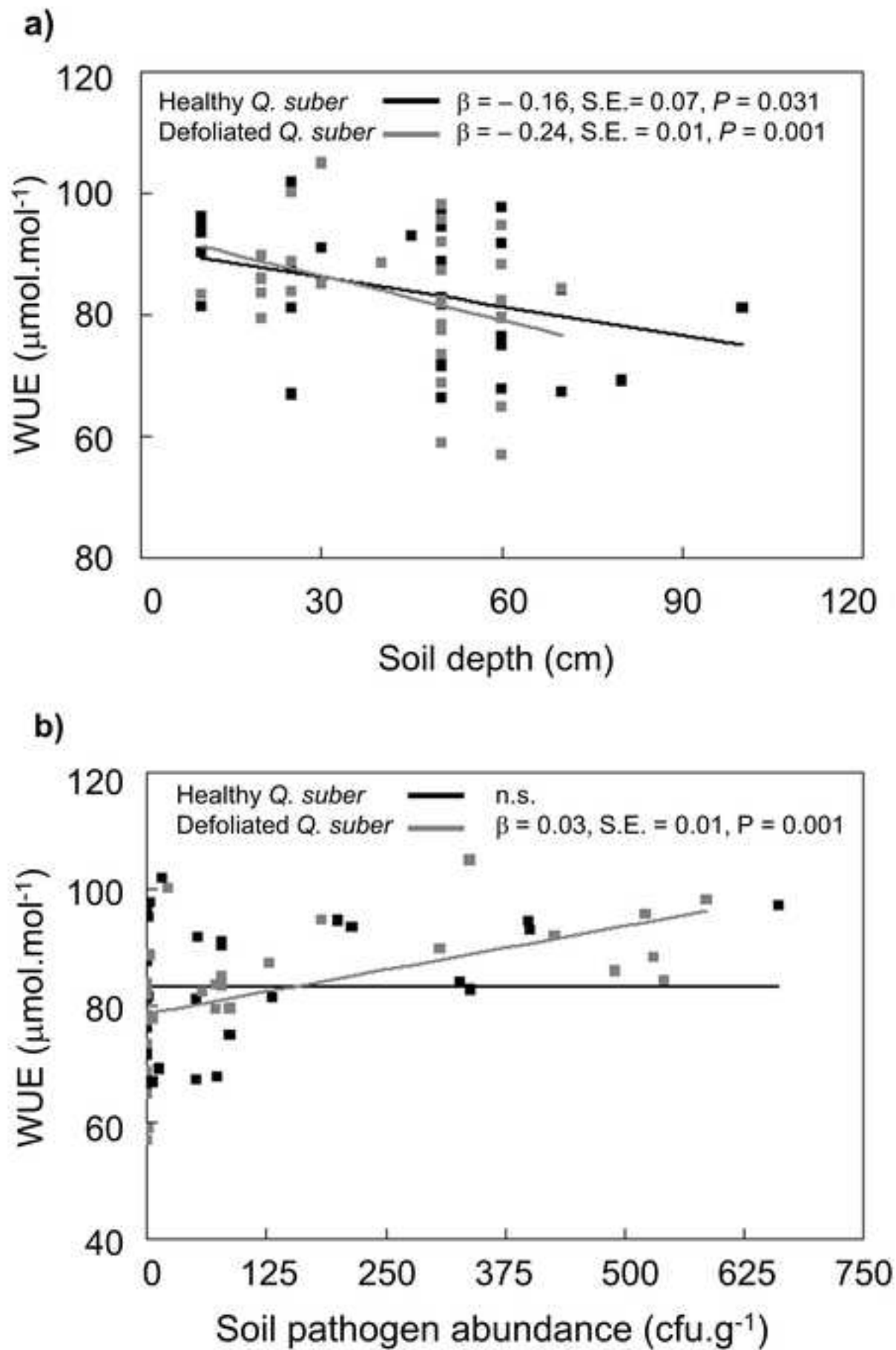
Fig. 4 Water use efficiency (iWUE) (a) and secondary branch growth (b) of healthy *Q. suber* trees in each forest type (woodland QS-WL, closed forest QS-CF) and the coexistent species *O. europaea* (OE-WL) and *Q. canariensis* (QC-CF). $N = 15$. The lower case indicates significant differences in water use efficiency among the tree categories ($P < 0.05$). n.s.= Non-significant ($P > 0.05$)

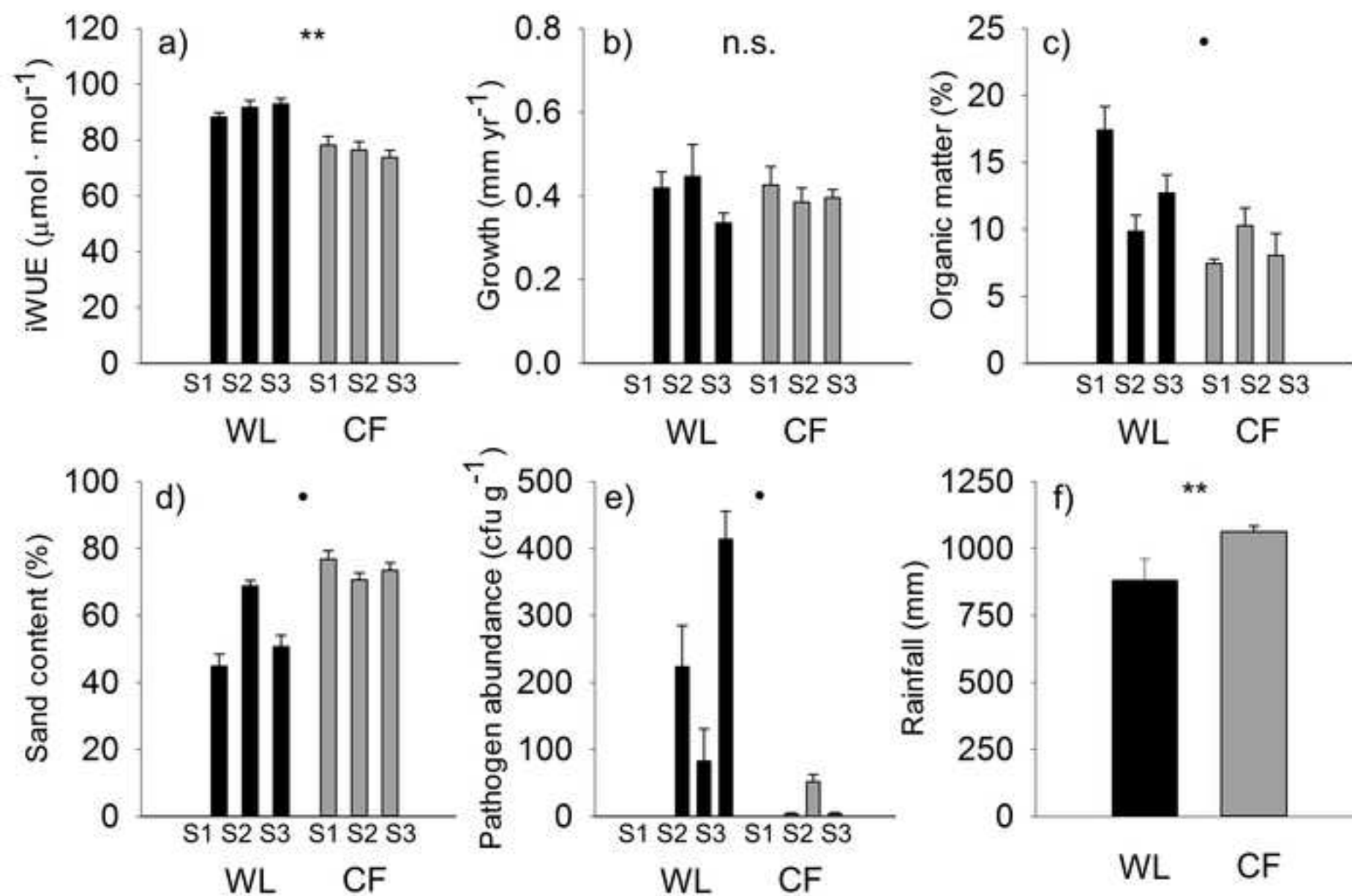
Online Resources

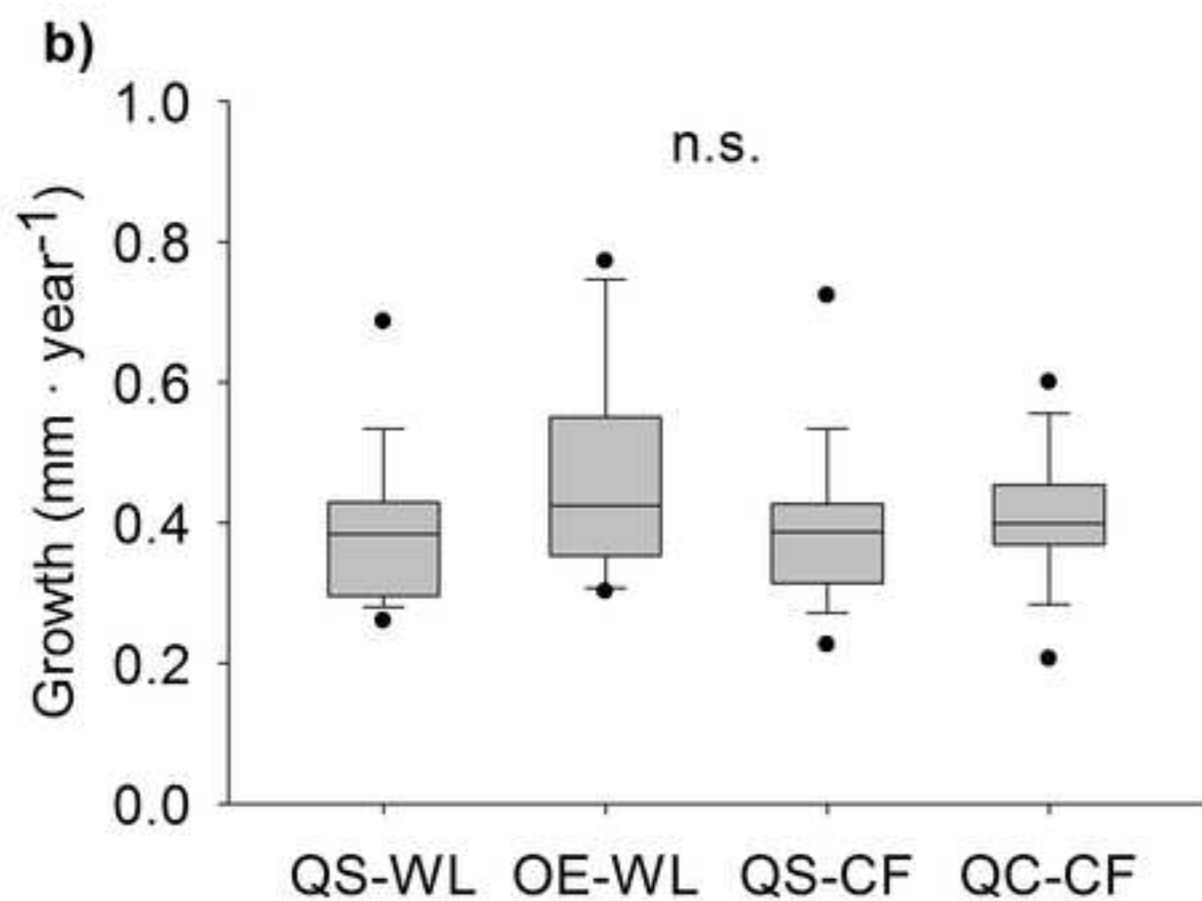
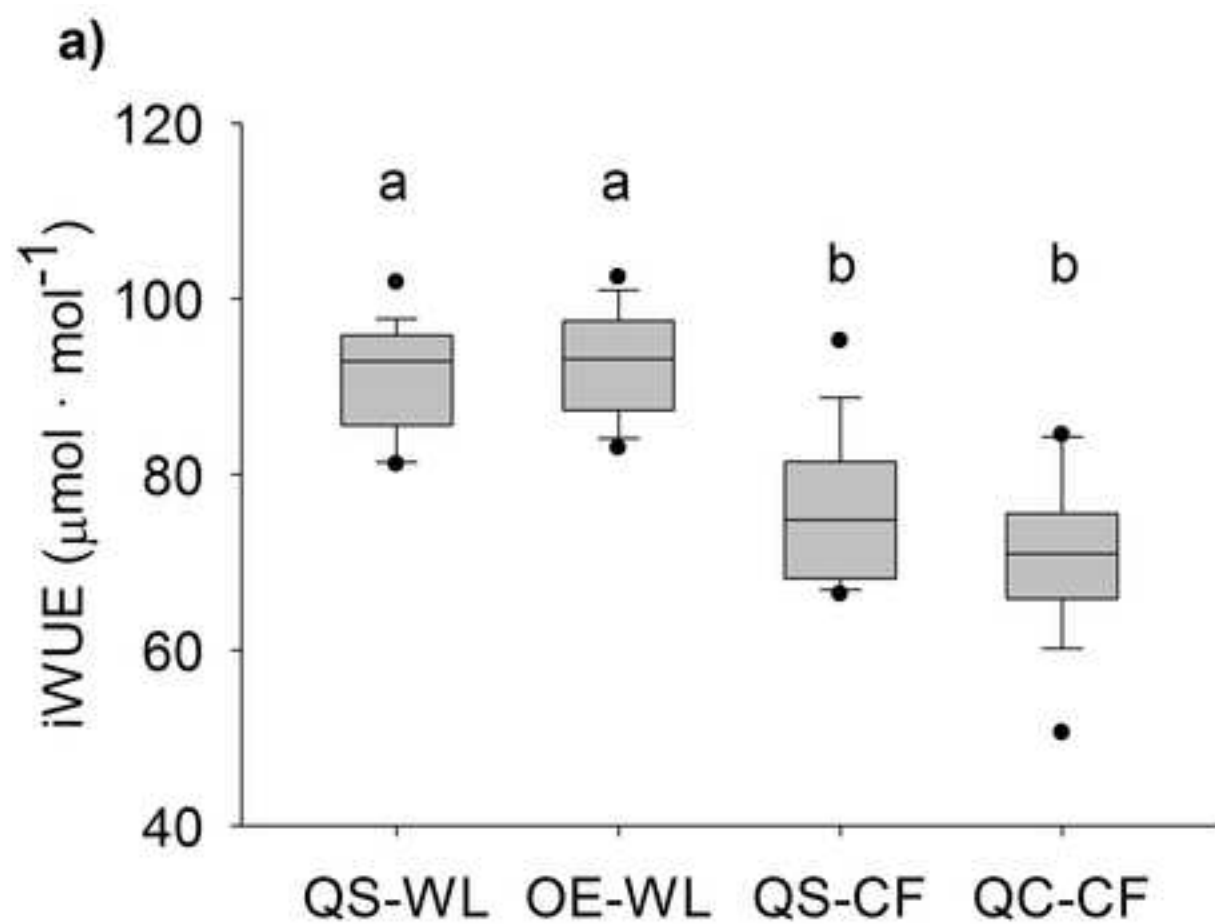
Appendix S1. Location map of the study sites and pictures of the forest types.

722 Appendix S2. Tree ring of trees and ean seasonal temperature and total seasonal
723 precipitation for the period 1999-2013.
724 Appendix S3. Best-fitting regression models of water use efficiency and secondary
725 branch growth for healthy and defoliated trees.









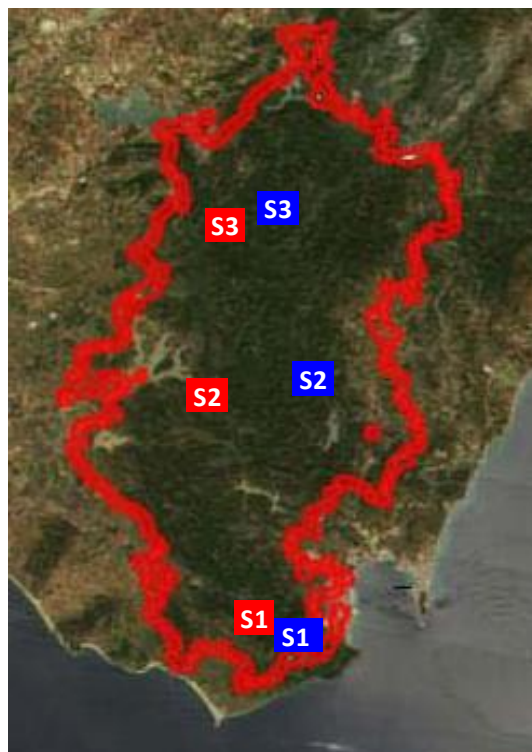
Supporting information to the paper:

Ávila, J.M. et al. Across-scale patterning of plant-soil-pathogen interactions in *Quercus suber* decline. European Journal of Forest Research. Supplementary Information.

Appendix S1 Location map of Los Alcornocales Natural Park and study sites. Pictures of both forest types (Woodlands and Closed Forests).



Los Alcornocales Natural Park



Appendix S2 Tree ring with (a) obtained in *Olea europaea*, Oe; healthy *Quercus suber*, Qs (h); defoliated *Quercus suber*, Qs (d); and *Quercus canariensis*, Qc, trees sampled within open woodland (OW) and closed forests (CF), respectively. Seasonal mean temperature (b) and seasonal total precipitation (c) obtained in the Meteorological Station from Jimena de la Frontera (Location 36° 24' 49" N, 05° 23' 01" W; 53 m above sea level and located in the center of the Natural Park) for the period 1999-2013. Seasonal temperature (T) and precipitation (P) were computed for winter (wi, december of the previous year, January and February), spring (sp, March, April, May), summer (su, June, July, August), and autumn (au, September, October, November).

