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Exploring interactive effects of climate change and exotic pathogens on *Quercus suber* performance: Damage caused by *Phytophthora cinnamomi* varies across contrasting scenarios of soil moisture

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Abstract

Climate change and exotic pests and pathogens are causing alarming forest declines worldwide. However, we still lack a comprehensive understanding of how damage caused by exotic pests and pathogens might vary under the different scenarios of water availability imposed by a changing climate, particularly in water-limited forests as those that occupy Mediterranean areas. In this paper we aimed to experimentally analyse the interactive effects of the aggressive exotic pathogen *Phytophthora cinnamomi* and climate change-related reductions in soil moisture on seedling performance of the Mediterranean host *Quercus suber*. We conducted a full-factorial greenhouse experiment where the physiology and growth of *Q. suber* seedlings was measured in soils with different combinations of *P. cinnamomi* inoculum density (0, 30, 60 and 120 colony forming units per gram of dry soil) and soil moisture (15%, 40%, 50% and 100% soil water holding capacity) simulating different invasion and climate change scenarios. We found additive effects of *P. cinnamomi* and drought on *Q. suber* performance aboveground, although these effects were not always negative. In fact, seedlings showed a compensatory physiological response to *P. cinnamomi* infection by increasing their net photosynthetic rates. Our results also supported important interactive effects of pathogens and soil moisture on belowground performance. Thus, the inoculum density in the soil required to cause significant root damage in experimental seedlings decreased as soil moisture increased. From a climate change perspective, these results suggest that an average drier climate might imply sub-optimal conditions for *P. cinnamomi* infections allowing for a slower advance of the disease in invaded areas. However, this effect will be modulated by the also predicted more frequent extreme climatic events. A higher frequency of extreme rain events that saturate the soil might be particularly

beneficial for *P. cinnamomi*, boosting its soil density beyond any possible response capacity of susceptible hosts.

Keywords: climate change, drought, invasive pathogens, Mediterranean forests, oak decline, seedling performance.

Introduction

Global change drivers such as climate change and the invasion of exotic pests and pathogens are causing alarming forest declines worldwide (Allen et al. 2015, Anderegg et al. 2015). Several studies have related the increase in tree defoliation and mortality rates to extreme drought events and heat waves, as well as to the more gradual and continuous process of temperature increase and rainfall reduction experienced by large parts of the globe (van Mantgem et al. 2009, Carnicer et al. 2011, Sangüesa-Barreda et al. 2015). On the other hand, the number of exotic pests and pathogens and the magnitude of their damages to tree species seem to be increasing at unprecedented rate (Dukes et al. 2009, Santini et al. 2013, Hansen et al. 2015). Among the potential causes behind such increase, the expanding transport of goods and people together with indirect climate change alterations of host-pest and host-pathogen relationships have been proposed to play a fundamental role (Jactel et al. 2012, Trumbore et al. 2015). However, the experimental evidence showing interactive effects of climate change and exotic pests and pathogens on tree species is still very limited, particularly in water-limited forests as those that occupy semi-arid and Mediterranean areas (Pautasso et al. 2010, Sturrock et al. 2011).

The Mediterranean Basin is considered one of the most susceptible regions to global change around the world (Schröter et al. 2005, Doblas-Miranda et al. 2017). On

50 the one hand, climatic models predict for this region a 2-5ºC increase in mean annual
51 temperature by the end of the 21st century, as well as a 30% annual rainfall reduction
52 and a higher frequency of extreme climatic events such as droughts and floodings
53 (Giorgi and Lionello 2008, Lindner et al. 2010, IPCC 2013). On the other hand, exotic
54 pathogenic microbes are causing devastating epidemics throughout the Mediterranean
55 Basin, particularly those belonging to the genus *Phytophthora* (Hansen et al. 2015, Jung
56 et al. 2018). This oomycete genus includes some of the most aggressive pathogens of
57 woody species on earth, as *Phytophthora cinnamomi* Rands. This pathogen has a likely
58 origin in Papua New Guinea (Arentz and Simpson 1986), but it is already introduced in
59 forests worldwide (Sena et al. 2018). *Phytophthora cinnamomi* is a soil-borne pathogen
60 that destroys the fine roots of its hosts, impeding nutrient and water uptake, and leading
61 to defoliation, loss of vigour, and eventual death of infected trees. In the Mediterranean
62 Basin, *P. cinnamomi* is decimating natural populations of dominant evergreen oaks such
63 as *Quercus ilex* and *Quercus suber* (Brasier 1992, Sánchez et al. 2002, Camilo Alves et
64 al. 2013, Ávila et al. 2017). This high mortality rate represents a problem of paramount
65 ecological and social importance, since evergreen oaks are major structural elements in
66 Mediterranean forests and savannah-like ecosystems ("dehesas" and "montados") that
67 provide extremely valuable biodiversity, economic and cultural services (Marañón et al.
68 2012). Predicting the future viability of Mediterranean oak forests will undoubtedly
69 require a comprehensive understanding of how the severity of the disease caused by *P.*
70 *cinnamomi* might vary under the different scenarios of water availability (the main
71 limiting resource in these forests) imposed by a changing climate.
72 Understanding the interactive effects of exotic soil-borne pathogens and climate
73 change on tree health in water-limited forests is not an easy task, since it will depend on
74 complex responses of host resistance and pathogen growth to decreasing levels of soil

moisture. Low values of soil moisture associated with lower precipitation and extreme droughts cause a direct abiotic stress on tree species that strongly reduces their performance (Peñuelas et al. 2013, 2017) and that might make them more susceptible to pathogen attack (Marçais et al. 1993, Corcobado et al. 2014). However, most pathogenic soil-borne fungi and oomycetes require high soil moisture for the germination and dispersal of their spores (Lacey & Harper 1986, Desprez-Loustau et al. 2006). Moreover, low water potential of the host cortical tissues has been shown to have a negative direct effect on pathogen growth within the host (Tippett et al. 1987, Marçais et al. 1993). Therefore, it could happen that the severity of the damages caused by exotic soil-borne pathogens to susceptible hosts in a climate change scenario were lower than expected due to direct negative effects of lower soil moisture on pathogen population growth. Since the damage caused by soil-borne pathogens (and by *P. cinnamomi* in particular) on tree species is strongly dependent on soil inoculum density (Gómez-Aparicio et al. 2012, Serrano et al. 2015), its maintenance in the soil under the minimum threshold required for disease expression might indirectly favour performance of seedlings and adults of susceptible species.

In this paper we aimed to experimentally analyse the interactive effects of the exotic pathogen *P. cinnamomi* and climate change-related reductions in soil moisture on seedling performance of the highly susceptible host *Q. suber*. To achieve this aim, we conducted a full-factorial greenhouse experiment where the growth and physiology of *Q. suber* seedlings was measured in soils with different combinations of *P. cinnamomi* density and soil moisture. We simulated a range of increasing pathogen densities from zero inoculum to large inoculum densities (120 colony forming units per gram of dry soil, CFU/g) known to produce root symptoms in *Q. suber* seedlings under controlled conditions (Serrano et al. 2015). Such large densities, and even much larger, can be

found in declining *Q. suber* forests of the Mediterranean Basin (Gómez-Aparicio et al. 2012). We then reproduced different scenarios of soil moisture expected under contrasting scenarios of climate change, from very low levels (15% of soil water holding capacity) typical of springs in extremely dry years to saturated soils typical of wet years in Mediterranean forests. We tested three specific hypotheses: 1) pathogen infection and drought will act as multiple stressors of *Q. suber*, both having negative effects on seedling performance; 2) *Q. suber* seedlings will show physiological and morphological adaptive responses to the two stress factors, with the aim of increasing water use efficiency and compensating the root damages caused by *P. cinnamomi* (i.e. lower photosynthetic rate and stomata conductance, higher biomass allocation belowground); and 3) pathogens and drought will have interactive effects on performance of *Q. suber* seedlings, the severity of the damages caused by *P. cinnamomi* increasing with soil moisture. The results of this experiment will greatly contribute to achieving a better understanding of the future of Mediterranean oak forests under interactive effects of global change drivers of yet unclear consequences.

Material and Methods

Experimental design

The experiment was conducted from January to April 2016 in a greenhouse at the University of Córdoba (Córdoba, Spain, 37°51′N, 4°48′ W). Average air temperature and moisture during the experiment were 12.3 ± 3.1°C and 77 ± 13.2% (respectively), varying from 10.6± 2.9°C and 89.3±7.2% in January to 15.7±2.4ºC and 66.5±11.3% at the end of the experiment. On January 13th, one-year old seedlings of *Q. suber* provided by a local nursery (Viveros San Jerónimo, Sevilla, Junta de Andalucía) were individually planted in 3L pots filled with a mixture of sand, silt, clay, peat and soil

from a natural *Q. suber* forest free of *P. cinnamomi* (Marismillas de Doñana, Huelva, Spain) in proportions 55:20:10:10:5 v/v. With this substrate we aimed to reproduce the sandy texture and acidic pH typical of *Q. suber* forest soils (Gómez-Aparicio et al. 2012), as well as its native microbiota. The absence of *P. cinnamomi* in the soil was tested following Romero et al. (2007). Soil samples (10 g) from three different trees in the area were air dried, sieved and suspended in 100 ml 0.2% sterilized water-agar. Then, 1 ml aliquots were plated on Petri dishes containing NARPH selective medium and incubated. No *P. cinnamomi* colonies were detected under the inverted microscope.

Seedlings were assigned to different experimental groups following a full-factorial design with two factors: *P. cinnamomi* inoculum density and soil moisture. Each factor had four levels, resulting in 16 experimental treatments and 160 seedlings (4 inoculum densities × 4 moisture levels × 10 replicates). Inoculum consisted of *P. cinnamomi* chlamydospores in sterile water suspension (isolate PE90) prepared following Sánchez et al. (2002). The four levels of inoculum density were: 1) Non-inoculated seedlings, used as control treatment. These seedlings received 100 ml of inoculum free water; 2) Low inoculum density, corresponding to a soil inoculated with 100 ml of water suspension with 2.3×10^3 chlamydospores/ml equivalent to 30 colony forming units per gram of dry soil (CFU/g) (Serrano et al. 2015). This density represents half of the minimum experimental threshold identified in a previous study for root disease expression in *Q. suber* seedlings (Serrano et al. 2015); 3) Medium inoculum density, where each pot received 100 ml of inoculum with 4.6×10^3 chlamydospores/ml equivalent to 60 CFU/g, near the minimum inoculum for root disease expression in *Q. suber* seedlings (Serrano et al. 2015); and 4) High *P. cinnamomi* density, where each pot received 100 ml of inoculum with 10^4 chlamydospores/ml equivalent to 120 CFU/g, enough to cause severe root damage in *Q.*

150 *suber* seedlings (Serrano et al. 2015).

151 The four different soil moisture levels were chosen to simulate soil water
152 availability under contrasting climate change scenarios: 1) Saturated soil at 100% water
153 holding capacity (WHC), simulating a wet spring where soils remained saturated most
154 of the time; 2) 50% WHC, simulating the average soil moisture in spring in *Q. suber*
155 forests of southern Spain (Gómez-Aparicio et al., unpublished data); 3) 40% WHC,
156 simulating a 20% reduction over the previous treatment predicted for 2050 using an
157 ACGCM for the scenario SRES IS92a (Manabe et al. 2004). We used this scenario
158 because, as far as we know, it is the only one that has been used to make predictions in
159 soil moisture; and 4) 15% WHC, simulating an extremely dry spring in *Q. suber* forests
160 of southern Spain (Ávila et al. 2019). Soil moisture was controlled twice per week for
161 every pot, weighting them and watering those which needed it to maintain constant
162 moisture levels. Average volumetric soil water content along the study period was
163 22.8±0.4%, 11.3±0.1%, 9.1±0.1% and 3.4±0.1% for the 100% WHC, 50% WHC, 40%
164 WHC and 15% WHC treatments, respectively. Pots were randomly distributed within
165 the greenhouse and repositioned monthly to avoid the effect of possible small
166 differences in environmental conditions. The height of all seedlings was measured
167 before the application of the experimental treatments. Mean initial height of
168 experimental seedlings was 39.46±0.41 cm (n=160) and did not differ among levels of
169 pathogen density or soil moisture ($p>0.05$ in both cases).

170

171 *Physiological and morphological seedling measurements*

172 To evaluate the effect of the different treatments on *Q. suber* seedlings, physiological
173 traits were measured during the length of the experiment. Net photosynthetic rate (A_{max})
174 and stomatal conductance (g_s) were measured three times along the experiment (after 4,

9 and 14 weeks of the experimental treatments) using a portable infrared CO_2 gas analyzer (Li6400XT, Li-Cor, Inc., Lincoln, NE, USA) fitted with a 6-cm^2 cuvette. Environmental conditions were fixed during measurements at 400 ppm of ambient CO_2, air flow of 400 cm^3/min, saturating light conditions of 1000 μmol/m^2s, and leaf temperature of 20 ºC. All measurements were conducted between 11:00 am and 13:30 am (GMT). Physiological measurements were made in a subset of four seedlings per treatment (n = 4 seedlings × 16 treatments = 64 seedlings).

All seedlings were harvested on April 20th. No seedling died along the course of the experiment. Severity of leaf symptoms was assessed for each seedling using a 0-4 scale, according to the percentage of yellow or wilted foliage (0 = 0% necrotic tissue, 1 = 1–33%, 2 = 34–66%, 3 = > 67%, 4 = dead tissue) (Sánchez et al. 2002). Root damage was assessed by using the same scale referred to the percentage of necrotic roots (Sánchez et al. 2002). A subsample of necrotic fine roots of seedlings growing in the different treatments was plated on NARPH agar medium for re-isolation of the pathogen. Then each plant was divided into stems, leaves and roots for quantification of morphological traits. All plant material was dried at 70°C for a minimum of 48 h to estimate shoot biomass (sum of stem and leaf biomass), total root biomass, fine root biomass (roots < 0.2 mm of diameter), and root mass fraction (RMF, root dry mass per unit of total plant dry mass). Total biomass (i.e., shoot + root biomass) was strongly correlated ($r > 0.95$) with shoot biomass, and therefore it was not included in the statistical analyses.

Data analysis

Physiological traits (A_{max} and g_s) were analyzed using repeated measures ANOVA, including pathogen density, soil moisture and its interaction as between-subject factors.

Severity of damages and morphological traits were analyzed using Generalized Linear Models (GLMs), also including pathogen density, soil moisture and its interaction as fixed factors. The severity of root damage was not normally distributed, so it was modeled using a Gamma distribution with identity as the link function. The remaining physiological and morphological variables were modeled using a Gaussian distribution with identity as the link function. Initial seedling height was introduced as covariable in all the analyses. When a factor or interaction was significant, differences among levels were tested using post-hoc Tukey tests. All statistical analyses were performed using R version.3.3.2 software (R Core Team 2017), using the package "car" for the analyses (Fox and Weisberg 2011) and the package "ggplot2" for the graphs (Wickham 2009).

Results

Effects of pathogen density and soil moisture on physiological traits

We found a significant effect of pathogen density on net photosynthetic rates, as well as a marginal significant effect on stomatal conductance (Table 1). Seedlings inoculated with *P. cinnamomi* had in general higher net photosynthetic rates (A_{max}) and stomatal conductance (g_s) than non-inoculated seedlings (Figure 1a,b). The increase in the net photosynthetic rate was higher at intermediate inoculum level (60 CFU/g) than at the other two pathogen densities (30 and 120 CFU/g). We did not find any effect of soil moisture on the physiological traits of the experimental seedlings (Table 1).

Effects of pathogen density and soil moisture on aboveground morphological traits

Shoot biomass was not affected by either pathogen density or soil moisture (Table 2). However, the two experimental factors have additive effects on the severity of leaf damage (Table 2). Leaf damage increased with the density of *P. cinnamomi* in the soil,

being significantly higher at 60 CFU/g and 120 CFU/g than in control seedlings (Figure 2a). Leaf damage was higher at the two extreme soil moisture levels (15% and 100% WHC) than at the two intermediate levels (40% and 50%, Figure 2b).

Effects of pathogen density and soil moisture on belowground morphological traits

Soil pathogen density and soil moisture had significant interactive effects on the four belowground variables measured (total root biomass, fine root biomass, root mass fraction and root damage), although for fine root biomass the interaction was only marginally significant (Table 2). Total and fine root biomass were affected by soil pathogens only at the highest level of soil moisture, being lower at the highest pathogen density (120 CFU/g) than in control seedlings (Figure 3a,b). Root mass fraction (RMF) was affected by soil pathogens only at the two highest levels of soil moisture (50% and 100% WHC). It was highest in control seedlings than in those inoculated with low pathogen density (30 CFU/g), seedlings inoculated with medium (60 CFU/g) and high pathogen density (120 CFU/g) showing intermediate RMF values (Figure 3c). Root damage was influenced by pathogen density at the four soil moisture levels explored (Figure 3d). However, the pathogen density required to cause significant higher damage in experimental than control seedlings decreased as soil moisture increased. Thus, at the lowest soil moisture level (15% WHC), only seedlings inoculated with the highest *P. cinnamomi* density (120 CFU/g) showed significantly more root damage than control seedlings. At 40% WHC, significant root damage was detected for seedlings inoculated with 60 CFU/g and 120 CFU/g. Finally, at the highest soil moisture levels (50% and 100% WHC), all inoculated seedlings showed more damage than control seedlings independently of inoculum density. *P. cinnamomi* was re-isolated from the roots of seedlings growing in infested soil but never from control seedlings.

Discussion

Global change drivers are known to act simultaneously, although many of their combined effects on natural ecosystems still remain unknown (Didham et al. 2007, Tylianakis et al. 2008). The current increase of tree decline and mortality around the world and the increasing evidence of climate-change related drought and exotic pathogens as primary drivers of these processes makes it particularly important understanding the existence of independent vs. interactive effects of these drivers on tree health. In order to further this knowledge, in this study we used a complex experimental approach where physiological and morphological traits of *Q. suber* seedlings were measured under 16 different contrasted combinations of *P. cinnamomi* density and soil moisture simulating different invasion and climate change scenarios. Our results support the existence of additive negative effects of both exotic pathogens and drought on seedling performance aboveground, but also showed the existence of important interactive effects on belowground root performance that might strongly determine the rate of decline of *Q. suber* populations in Mediterranean forests and dehesas invaded by *P. cinnamomi*.

Additive negative effects of Phytophthora cinnamomi and soil moisture on physiological and aboveground morphological traits

We found additive effects of both *P. cinnamomi* density and soil moisture on ecophysiological and aboveground morphological traits of *Q. suber* seedlings. However, contrary to our first hypothesis, such effects were not always negative. Thus, seedlings infected by *P. cinnamomi* showed higher net photosynthetic rates and stomatal conductance than control seedlings. This result was quite unexpected, since the

few studies that have explored to date the physiological response of *Quercus* species to *P. cinnamomi* infection had generally found a reduction in stomatal conductance (Luque et al. 1999, Robin et al. 2001, Maurel et al. 2001) and net photosynthetic rate (Sghaier-Hammami et al. 2013), a response similar to that induced by drought and aimed to decrease water loss. Here, on the contrary, seedlings showed a compensatory physiological response to *P. cinnamomi* infection by increasing their net photosynthetic rates and to a lower extent their stomatal conductance, contrary to our second hypothesis. This physiological response could reflect an attempt of *Q. suber* seedlings to compensate the loss of root functionality by increasing carbon uptake in the presence of *P. cinnamomi*, as has been reported for other host-pathogen interactions (Walters 2015). However, the net photosynthetic rate of inoculated seedlings did not increase linearly with the density of *P. cinnamomi* soil inoculum. On the contrary, such compensatory response was maximum at intermediate pathogen density, seedlings inoculated with 60 CFU/g showing almost twice higher net photosynthetic rates than control seedlings, and decreased at higher pathogen densities (Figure 1a). Overall, these results suggest that susceptible hosts as *Q. suber* can have certain physiological plasticity to counteract the negative effects of an aggressive pathogen as *P. cinnamomi*, but that once overpassed certain thresholds of pathogen density the damage imposed to the root system might be too high for the seedlings to compensate it through adaptive physiological responses.

We did not find significant effects of soil moisture on the physiological performance of *Q. suber* seedlings, despite the fact that we reproduced four contrasted soil moisture scenarios ranging from a very low soil water content (15% WHC) to a saturated soil (100% WHC). This result likely reflects that *Q. suber* is a drought-tolerant Mediterranean species evolutionary adapted to cope with low levels of soil water

availability under long periods of time (Ramírez-Valiente et al. 2009, Gil-Pelegrín et al. 2017). Soil moisture did however affect the severity of leaf damage shown by the seedlings at the end of the experiment, although the severity of the damage did not show a linear response to the decrease in soil moisture. On the contrary, leaf damage was maximal at the two extremes of the soil moisture gradient, showing how the lack of water can be as detrimental as its excess (Gómez-Aparicio et al. 2008, Pérez-Ramos and Marañón 2009). Soil saturation can generate hypoxia conditions that translate into a poor root functioning and consequent wilting of some part of the foliage (Sairam et al. 2008). Overall, our results demonstrated that *P. cinnamomi* and soil moisture had additive and non-linear effects on aboveground seedling performance that varied in sign and magnitude depending on the trait considered. However, these effects were in general of much lower magnitude than those detected belowground, likely due to a delay between pathogen infection and aboveground symptoms (Robin et al. 1998, Moreira et al. 2000, Ruiz-Gómez et al. 2018), which suggests that understanding the interactive effects of global change drivers on plant performance requires a close look belowground.

Interactive effects of Phytophthora cinnamomi and soil moisture on belowground morphological traits

A main result of our study is that pathogen density and soil moisture had interactive effects on the belowground performance of *Q. suber* seedlings, in agreement with our third hypothesis. Thus, the severity of the root damage caused by the aggressive soil-borne pathogen *P. cinnamomi* to *Q. suber* seedlings differed substantially among the four soil moisture levels used as surrogates of different climate change scenarios. As predicted, the largest levels of root damage occurred in general at the highest levels of

soil moisture (50% and 100% WHC), translating into a somewhat lower RMF in infected seedlings compared to control seedlings. Pathogen effects on total and fine root biomass were detected only at the highest soil moisture level (100% WHC), where highly infected seedlings had on average 22% and 61% lower total and fine root biomass (respectively) than control seedlings (Figure 3a,b). Because of the poor ability of *P. cinnamomi* to degrade the lignocellulose complex of mature roots (Cahill and McComb 1992, Nicoski 1996), it is likely that our estimation of root biomass included damaged root tissue not yet totally decomposed, therefore representing a more conservative estimate of the belowground effects of *P. cinnamomi* than the direct visual estimation of necrotic root tissue.

The fact that the largest negative effects of *P. cinnamomi* on root biomass occurred under soil water saturation conditions is consistent with previous knowledge that shows high soil water levels to favor the infective inoculum build-up of *P. cinnamomi*. High soil moisture and free water favors sporangial production and zoospore release, and increases zoospore mobility through the soil to infect roots, allowing for rapid secondary cycles and multiple infections on the host roots (see Camilo-Alves et al. 2013 and references therein). However, our results also suggest that the pathogen capacity to cause root damage can be sensitive to moderate reductions in soil moisture, in agreement with previous studies that have shown water restriction to reduce root damage induced by *P. cinnamomi* (Tippett et al. 1987, 1989, Maurel et al. 2001). Thus, whereas in the 100% and 50% WHC scenarios a low density of *P. cinnamomi* (30 CFU/g) was enough to cause significant higher root damage in infected than control *Q. suber* seedlings, in the 40% WHC scenario only seedlings infected with moderate and high densities (>60 CFU/g) differed from control seedlings. This implies that even a slight decrease of soil moisture simulating a 30% reduction in annual

precipitation was enough to trigger differences in root damage caused by *P. cinnamomi*. Further decreasing the soil water content to 15% WHC increased even further (>120 CFU/g) the minimum threshold required to cause significant damage to the root system. This negative relationship between soil moisture and the inoculum threshold required to cause disease is consistent with the more acute decline of *Quercus* species observed in fine textured soils with high water retention compared to well-drained sandy soils (Gómez-Aparicio et al. 2012, Corcobado et al. 2013). From a global change perspective, these results imply that disease trajectories of forests already invaded by *P. cinnamomi* will strongly depend on rainfall regimes and associated variations in soil moisture that might exert a significant control on the growth rates of *P. cinnamomi* populations.

Conclusions

Understanding how climate change might affect plant disease epidemics is a current challenge, since the different components of climate change (i.e. changes in average temperature and precipitation, increase of extreme events) can have contrasting effects on the dynamics of pathogen populations. To date, the most frequently recognized consequence of climate change on plant diseases has been the positive effect of a warmer climate due to enhance reproductive capacity and survival of pathogenic species and their expansion towards cold areas (Trumbore et al. 2015, Burgess et al. 2017). Therefore, it is often assumed that plant disease epidemics might become more frequent as climate changes (Sturrock et al. 2011). However, in systems like southern European forests where the most severe abiotic stress is undoubtedly drought, changes in precipitation and associated levels of soil moisture might be the most relevant factors driving alterations in the rate and magnitude of tree diseases, with yet unforeseen consequences. To our knowledge, this study addressed for the first time the

consequences of different realistic scenarios of soil moisture on the damages caused by the aggressive exotic pathogen *P. cinnamomi* at different levels of soil inoculum that might represent different stages of invasion (from non-invaded soils to highly invaded areas). Although results involving experimental seedlings under controlled conditions need to be carefully extrapolated to adult trees under natural conditions, our results strongly suggest that the capacity of *P. cinnamomi* to cause disease is modulated by even small variations in soil moisture, and that a drier climate might imply sub-optimal conditions for root infections, lengthening the time required for disease expression in susceptible hosts. This finding, together with the physiological plasticity shown by *Q. suber* to counteract pathogen effects under moderate inoculum abundance, might allow for a slower advance of the root rot caused by *P. cinnamomi* in a drier future.

However, it is important to take into account that the consequences of an average drier climate on disease dynamics will be strongly modulated by the effects of the also predicted most frequent extreme climatic events (droughts and floodings). Together with previous studies (Robin et al. 2001, Corcobado et al. 2014), our results suggest that a higher frequency of extreme rain events that saturate the soil and cause temporal waterlogging will be particularly beneficial for *P. cinnamomi* infections, boosting its density beyond any possible response capacity of susceptible hosts. Because of this, there is an urgent need to adopt management strategies that contribute to maintain soil inoculum densities in already invaded forests as low as possible for the longest period of time. Once a high *P. cinnamomi* density is achieved in the soil, severe damage to susceptible hosts like the evergreen oaks that dominate Mediterranean forests and dehesas will be caused independently of the soil moisture level, with catastrophic consequences for the long term conservation of these valuable systems.

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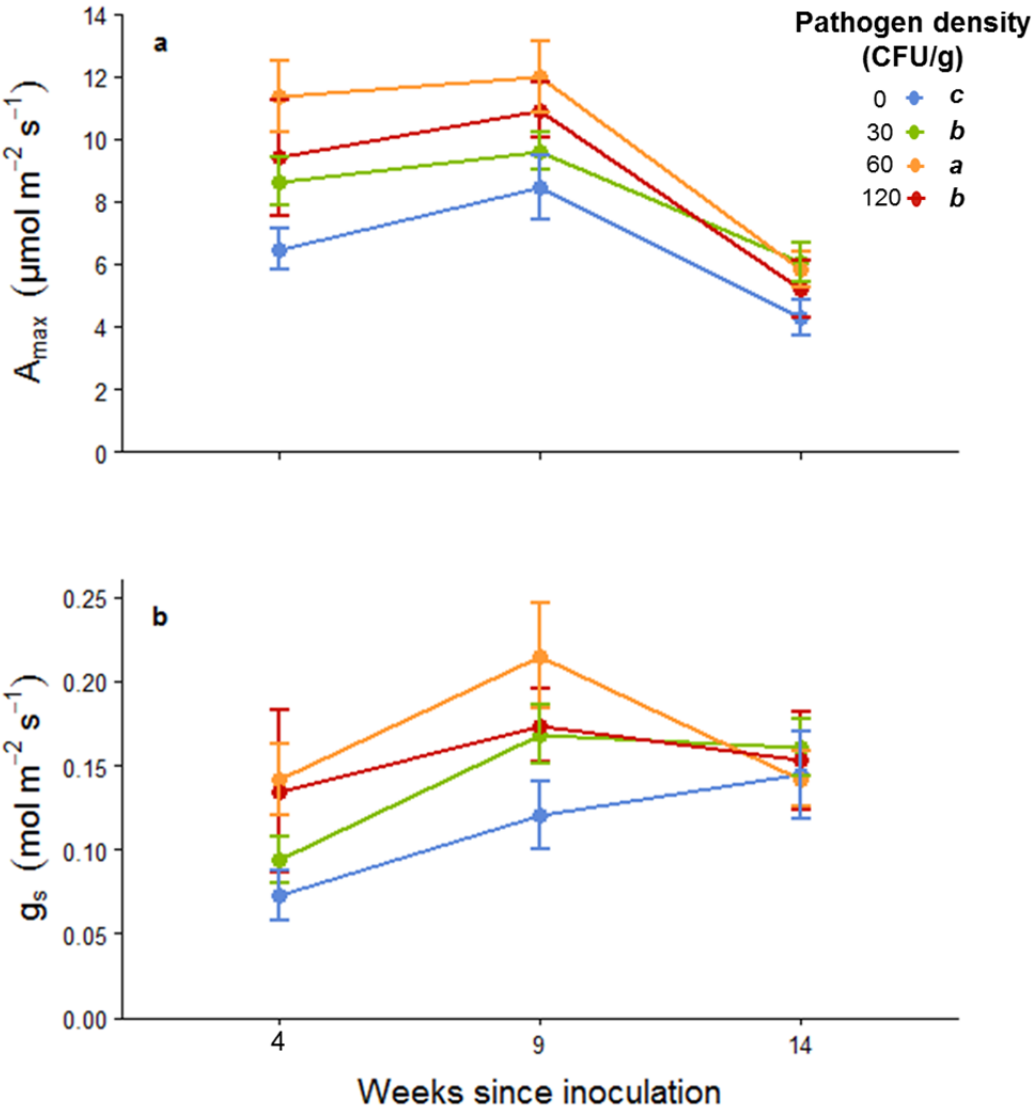

Figure 1. Net photosynthetic rate (A_{max}) and stomata conductance (g_s) of *Quercus suber* seedlings under the four different treatments of pathogen density (measured as number of colony forming units per gram of dry soil, CFU/g) along the course of the experiment. Different letters in the legend indicate significant differences among treatments after Tukey tests. The effect of pathogen density on stomata conductance was only marginally significant (Table 1). Bars represent means±SE (n=6).

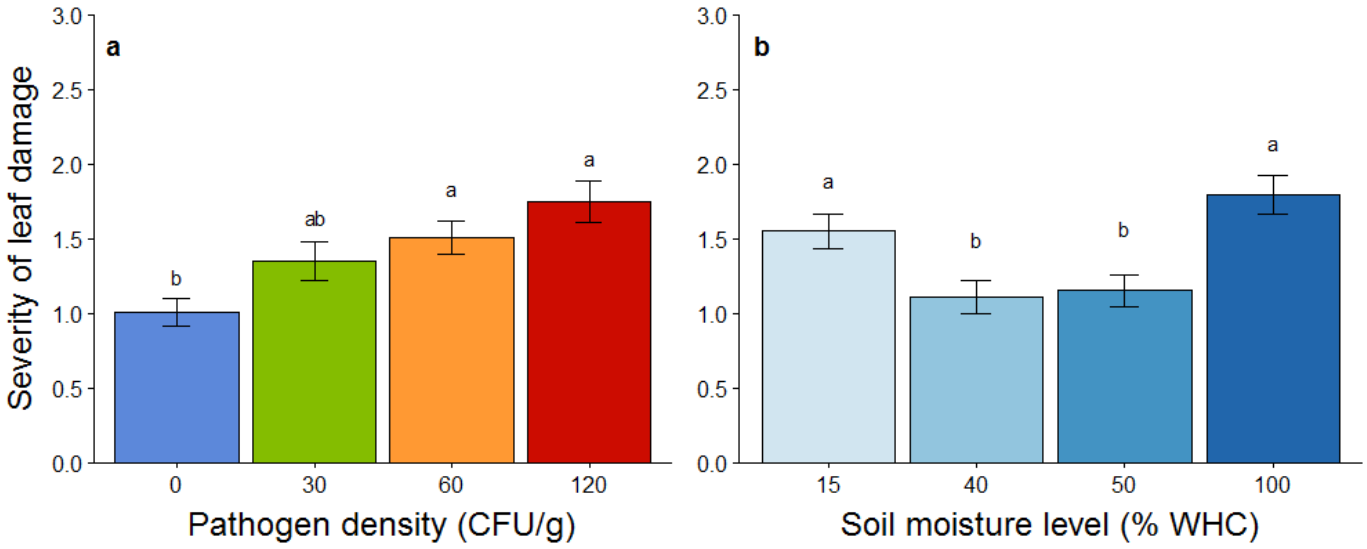

Figure 2. Effects of pathogen density (measured as number of colony forming units per gram of dry soil, CFU/g) and soil moisture (measured as % of water holding capacity, WHC) on the severity of leaf damage of *Quercus suber* seedlings. Different letters show significant differences after Tukey tests. Bars represent means±SE (n=10).

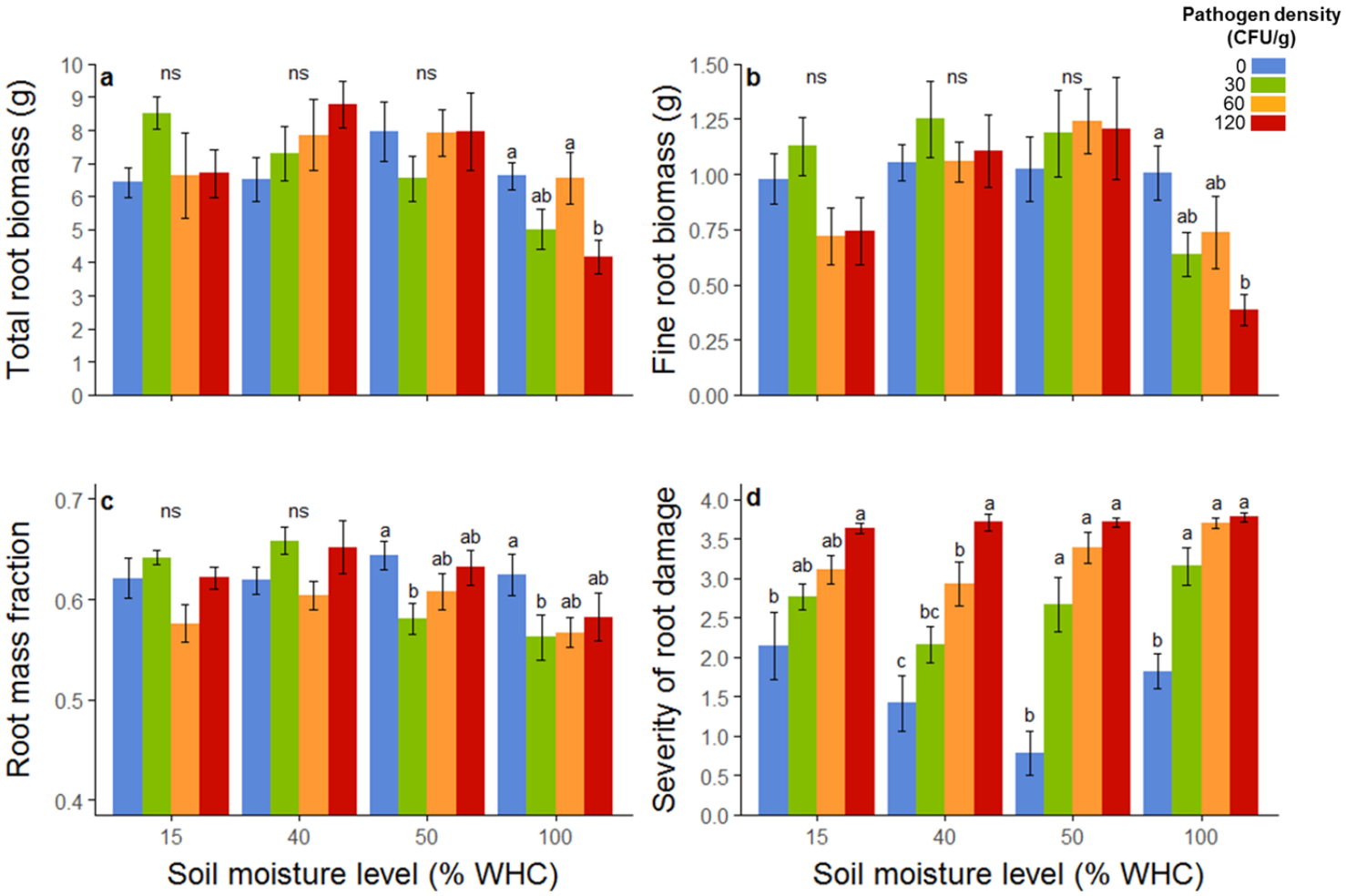

Figure 3. Interactive effects of pathogen density (measured as number of colony forming units per gram of dry soil, CFU/g) and soil moisture (measured as % of water holding capacity, WHC) on belowground morphological traits of *Quercus suber* seedlings. Different letters show significant differences after Tukey tests. Bars represent means±SE (n=10).

Table 1. Results of the repeated measures ANOVA (rmANOVA) analysis for the influence of pathogen density, soil moisture, and its interaction on physiological traits of *Q. suber* seedlings over time. Variables analyzed were net photosynthetic rate (A_{max}) and stomatal conductance (g_s).

Variable	Factors	df Num	df Den	F	P-value
A_{max}	*Between-subjects source*				
	Pathogen density (PD)	3	47	4.59	**0.007**
	Soil moisture (SM)	3	47	1.81	0.16
	PD x SM	9	47	0.67	0.73
	Error	15	47	1.65	0.10
	Within-subjects source				
	Time (T)	2	46	49.22	**<0.0001**
	T × PD	6	92	1.39	0.22
	T × SM	6	92	1.1	0.37
	T × PD × SM	6	92	0.6	0.89
	Error	30	92	0.87	0.65
g_s	*Between-subjects source*				
	PD	3	47	2.25	*0.09*
	SM	3	47	1.54	0.22
	PD × SM	9	47	0.6	0.79
	Error	15	47	1.10	0.38
	Within-subjects source				
	T	2	46	21.36	**<0.0001**
	T × PD	6	92	1.17	0.42
	T × SM	6	92	1.05	0.33
	T × PD × SM	6	92	0.64	0.85
	Error	30	92	0.86	0.68

Table 2. Results of the statistical analyses (GLMs) performed to test the effect of the experimental treatments and its interaction on the morphological traits of *Q. suber* seedlings. Significant differences among treatments ($p<0.05$) are highlighted in bold, and marginal differences ($p<0.10$) in italics.

Treatment		Morphological traits					
		Aboveground traits		Belowground traits			
		Shoot biomass	**Leaf damage**	**Total root biomass**	**Fine root biomass**	**Root mass fraction**	**Root damage**
Pathogen density (PD)	P-value	0.07	**<0.0001**	0.93	*0.08*	**0.008**	**<0.0001**
	df	3	3	3	3	3	3
	Deviance	15.34	1.99	0.09	0.39	0.03	16.36
Soil moisture (SM)	P-value	0.33	**<0.0001**	**0.0002**	**<0.0001**	**0.001**	0.16
	df	3	3	3	3	3	3
	Deviance	7.35	2.19	4.14	1.89	0.04	0.95
PD × SM	P-value	0.37	0.27	**0.012**	*0.07*	**0.03**	**0.0005**
	df	9	9	9	9	9	9
	Deviance	21.26	0.94	4.52	0.89	0.06	5.4
Initial Height	P-value	**0.01**	0.52	*0.07*	0.90	**0.03**	0.93
	df	1	1	1	1	1	1
	Deviance	12.46	0.03	0.71	0.17	0.01	0.01
	Explained deviance	0.15	0.30	0.24	0.29	0.27	0.23