Received Date: 19-Jan-2018

Accepted Date: 23-Oct-2018

Article type: Research paper

Editor: Melany Fisk

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. This article can be read <https://rdcu.be/baruH> and can be found in: <https://doi.org/10.1007/s10533-018-0521-3>. Please cite this article as: Avila, J.M., Gallardo, A. & Gómez-Aparicio, L. Biogeochemistry (2018). Article in press. doi: 10.1007/s10533-018-0521-3

Pathogen-induced tree mortality interacts with predicted climate change to alter soil respiration and nutrient availability in Mediterranean systems

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Abstract

Ecosystems worldwide must simultaneously cope with several global change drivers with potential strong effects on ecosystem functioning. These drivers might interact in unexpected ways, but our still limited understanding of these interactive effects precludes us from predicting the impact of global change on ecosystem functioning. In this study we assessed the direct effects of pathogen-induced tree mortality and predicted warming and drought on C, N and P in Mediterranean forest soils affected by the decline of their dominant tree species (i.e. *Quercus suber*) due to the invasive pathogen *Phytophthora cinnamomi*. We also explored the potential indirect effects due to species replacement after *Q. suber* mortality. To achieve our goal, we conducted a soil incubation experiment using soils collected under *Q. suber* trees with different health status (i.e. healthy, defoliated and dead trees) and from coexistent shrubs (i.e. pioneer and late successional shrubs). These soils were incubated under controlled temperatures and soil moistures, mimicking various climate change scenarios predicted for 2050 and 2100 in the Mediterranean Basin. Our results showed that *P. cinnamomi*-induced mortality and future warming and drought may interact to simultaneously alter biogeochemical cycles in *Q. suber* forest soils. Resistance of studied variables to changes in temperature and moisture tended to be lower for dead trees than for healthy and defoliated trees. Moreover, we found that soil respiration and nutrient availability might be affected indirectly by *P. cinnamomi*-induced mortality due to species replacement. Overall, our results support a high potential of invasive pathogen species for modifying the response of soil functioning to climatic stressors.

Keywords: Aridity; Climate Change; Drought; Forest disease; Global Change; Mediterranean shrubs; Non-additive effects; Oak decline; Pathogen; *Phytophthora cinnamomi*; *Quercus suber*; Soil nutrients; Soil respiration; Warming.

INTRODUCTION

Evidence of a strong effect of climate change on the structure and functioning of ecosystems have accumulated over the last decades (Anderegg et al. 2015; Cao & Woodward 1998; Grimm et al. 2013; Peñuelas et al. 2002; Petchey et al. 1999). Many regions of the world are experiencing a general trend towards warmer temperatures and drier conditions (Giorgi & Lionello 2008; IPCC 2014b). These increasingly arid conditions might trigger significant changes in soil functioning in the following decades (Emmett et al. 2004). For instance, it has been shown that predicted warming tends to increase carbon (C) and nutrient mineralization and nutrient availability as a consequence of higher microbial metabolism (Bai et al. 2013; Emmett et al. 2004), whereas drought usually reduces microbial activity and nutrient availability in soils (Emmett et al. 2004; Matías et al. 2012). However, other studies have found no effect of increased temperature (Auyeung et al. 2013; Dijkstra et al. 2012) or drier conditions (Auyeung et al. 2013; Curiel Yuste et al. 2014) on carbon mineralization and nutrient availability. These facts showed that the uncertainty associated with varying effects of warming and drought complicate forecasting predictions.

Besides climate change, ecosystems worldwide are faced with a variety of other global change-related drivers that affect ecosystem functioning such as invasive species, N deposition or changes in land-use (Trumbore et al. 2015; Vitousek 1994). More specifically, exotic pests and pathogens are considered a problem of paramount importance in forest systems, with profound direct and indirect impacts on plant communities and ecosystem functioning (e.g. Gandhi & Herms 2010; Loo 2009). Direct impacts, such as decrease in C stocks and soil respiration or increase in N availability, occur in the short-term (i.e. weeks to years) as a consequence of tree defoliation and mortality and the alteration of the abiotic and biotic environment under affected trees (Hicke et al. 2012; Lovett et al. 2006). Moreover, indirect impacts can occur in longer time scales (decades) due to species replacement as a consequences of differences among coexistent species in their vulnerability to mortality agents (Cobb et al. 2013; Lovett et al. 2010). The sign and magnitude of the indirect effects on biogeochemical

cycles will depend on the differences between replacement species in their footprint on the soil environment (Cobb et al. 2013; Lovett et al. 2010).

Soils in forests affected by exotic pests or pathogens might cope with climate change stressors in unexpected ways. Multiple stressors (e.g. pests/pathogens, warming and drought) may have simple additive effects on ecosystems. Alternatively, stressors might have interactive effects if one stressor increases or reduces the resistance of the ecosystem process to the other stressors (Paine et al. 1998). These interactive effects lead to cumulative effects greater (synergistic interactions) or lower (antagonistic interactions) than the additive sum of the effects predicted from single-factor experiments. The single-factor approach therefore risks over- or under-estimating the impact that multiple drivers of global change might have on ecosystem process (e.g. Luo et al. 2008; Morillas et al. 2015). In the last decades much effort has been directed to analyze the impact of predicted climate change on soil. However, to our knowledge no previous studies have attempted to experimentally assess the effect of climate change on soil functioning and nutrient availability in forests affected by pest- or pathogen-induced tree mortality.

Mediterranean ecosystems are among those more threatened by global change (Garbelotto & Pautasso 2012; Giorgi & Lionello 2008). The Mediterranean Basin has suffered a strong increase in temperature (+1.5°C) and drought severity (-15.6% precipitation; +7.4% evaporative demand) in the last five decades (Vicente-Serrano et al. 2014). Moreover, Mediterranean systems are facing the consequences of invasive pests and pathogens that are severely altering ecosystems (Brasier 1992; Garbelotto & Pautasso 2012). For example, the exotic soil-borne pathogen *Phytophthora cinnamomi* is responsible for the severe decline of evergreen *Quercus* species (e.g. *Quercus suber* and *Quercus ilex*) in southern Europe (Camilo-Alves et al. 2013; Sánchez et al. 2002). Furthermore, the impacts of this pathogen may expand to other regions in the southern and northern hemisphere as a consequence of its potential range expansion under a warmer climate (Burgess et al. 2017). Pathogen-driven tree mortality can have direct impacts on ecosystem functioning by reducing tree productivity, soil respiration rates and P availability, as well as by causing complex changes in the N cycle that are strongly

context-dependent (Ávila et al. 2016; Cherubini et al. 2002; Shearer et al. 2009). Moreover, gaps opened after tree death will be very likely occupied by coexistent drought-tolerant shrubs not affected by the pathogen (Ibáñez et al. 2017), which might lead the system to a condition of arrested succession for at least several decades (Acácio et al. 2007). The substitution of trees by shrubs might indirectly cause a substantial alteration of soil functioning as a consequence of the contrasting footprint of these two life forms on soils, with shrubs showing lower litter production and litter quality than trees (Rolo et al. 2012). Comparing the effects of replacement shrubs and *Q. suber* trees on soil variables might help us to understand the indirect effect of tree mortality. However, there is a lack of studies that have attempted to disentangle how Mediterranean forests disturbed by exotic pests or pathogens might direct or indirectly respond to increasing aridity, limiting our ability to predict the functioning of these systems under realistic global change scenarios.

In this study we aim to assess the effects of pathogen-driven tree mortality, warming and drought, and their interactions, on biogeochemical cycles in Mediterranean forests of southern Spain affected by the decline of their dominant species (i.e. *Q. suber*) due to the invasive pathogen *P. cinnamomi*. To achieve this goal, we conducted a soil incubation experiment using soils collected under *Q. suber* trees with different health status (i.e. healthy, defoliated and dead trees) and from coexistent shrubs (i.e. pioneer and late successional shrubs that occupy tree gaps after *Q. suber* death) that were incubated under controlled temperatures and soil moistures, mimicking various climate change scenarios predicted for the Mediterranean Basin. The specific objectives of this study were: 1) To analyze the direct effects of *P. cinnamomi*-driven tree mortality, predicted warming and drought and their interactions on soil respiration and nutrient availability (NH_4^+ , NO_3^- and PO_4^{3-}); 2) To quantify the resistance (i.e. the amount of change caused by a disturbance, *sensu* Orwin and Wardle, 2004) of soil respiration and nutrient availability to predicted changes in temperature and soil moisture under trees with different health status; and 3) To explore the indirect effects of *P. cinnamomi*-driven tree mortality for soils under predicted warming and drought scenarios, due to the substitution

of *Q. suber* trees by shrubs after tree death; we explored this by comparing the response to the climatic scenarios of soils under healthy *Q. suber* trees and successional shrubs.

MATERIALS AND METHODS

Study site

This study was conducted in mixed oak forests in Los Alcornocales Natural Park, southern Spain. The climate is sub-humid Mediterranean, characterized by warm and dry summers and cool, humid winters. Annual mean temperature ranges from 15.4 to 17.3 °C and annual mean rainfall ranges from 970 to 1100 mm (period 1951-1999, Ninyerola et al. 2005). Forests occur on acidic sandy soils interspersed with clay layers. The flora is dominated by mixed sclerophyll forests, with *Quercus suber* L. as the main species. In drier lowlands (hereafter open woodlands) *Q. suber* coexists with the evergreen and shade-intolerant *Olea europaea* var. *sylvestris* Brot. and a dense and diverse understory dominated by shrub species such as *Pistacia lentiscus* L. and *Teline linifolia* L. The moister habitats (hereafter closed forests), are formed by *Q. suber* intermingled with the winter-deciduous shade-tolerant *Quercus canariensis* Willd. These closed forests are characterized by a rich understory dominated by shrub species such as *Arbutus unedo* L., *Ulex borgiae* Rivas Mart., *Erica arborea* L. and *Erica scoparia* L. (Ojeda et al. 2000). In the study area, *Q. suber* adult trees show signs of defoliation and mortality. Extremely high abundances of *Phytophthora cinnamomi* Rands 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).

Sampling design

Soil sampling was carried out during spring of 2012, the most biologically active season in Mediterranean-type ecosystems (García et al. 2002). We selected two sites within the Natural Park, one in open woodlands (36° 4'N, 5°33'W) and another in closed forests (36° 23'N, 5°31'W). We included both forest types to increase the generality of our results and to explore

the inherent heterogeneity that exists in the studied Mediterranean forests affected by *P. cinnamomi*. These forests are affected by a severe oak decline induced by *P. cinnamomi*, where 55 to 74% of *Q. suber* trees were categorized as defoliated or dead (Ávila et al. 2017). In both forest sites, we selected soils from five different origins (hereafter soil origin) to analyze the direct and indirect effect of *P. cinnamomi*-induced tree mortality on soil variables: soils under *Q. suber* trees with different health status (i.e. healthy, defoliated and dead) and under pioneer and late-successional shrubs. At each site, we selected 5 individual trees of average size (i.e. 30-40 cm diameter at breast height) per health category. We also selected five individuals of each of the two most abundant shrub species at each forest type belonging to two different successional stages: *Teline linifolia* (pioneer) and *Pistacia lentiscus* (late-successional) in the open woodland, and *Ulex borgiae* (pioneer) and *Erica arborea* (late-successional) in the closed forest (See Figure S1 in Supplementary Material). We sampled soil under trees and shrubs avoiding overlapping crowns with individuals of different species or health status. Soil samples were collected within two meters from the bole of each individual tree and under the canopy of the shrubs. The samples were taken from the top 15 cm of the soil using a circular soil corer (5 cm diameter x 20 cm height). Each soil sample (2 sites x 5 soil origins x 5 individuals = 50 samples) was transported in polyethylene bags in coolers to the laboratory, and further maintained as independent samples (Reinhart & Rinella 2016). Soil was sieved at 2 mm and air-dried at room temperature until a constant weight was obtained (~ 4 weeks). Prior to soil incubations, we determined the water holding capacity (WHC) of each soil sample using a subsample of 50 g that was saturated by water, covered and allowed to drain for 48 h. The water content of the drained soil was considered 100% of the WHC (Agehara & Warncke 2005). Soil gravimetric water content was determined by oven drying samples at 105° C for 24 h.

Soil incubations: temperature and soil moisture scenarios

In order to simulate the effect of predicted warming and drought on soil variables, we selected different temperatures and soil moistures based on projections of climatic models. For temperature, we used three different scenarios. The first one (T = 16°C) is considered the mean

spring temperature in the study sites (Ninyerola et al., 2005). The other two scenarios (i.e. 19 and 22°C) were the projected spring mean temperatures for the study site for 2040-2070 and 2070-2100, respectively (Moreno & Obrador 2007). These projections were determined from the HadCM3 (an atmosphere-ocean coupled general circulation model, ACGCM) using the scenario SRES A2, the most common scenario from IPCC (Nakicenovic & Swart 2000). For soil moisture, we chose 4 different scenarios of soil water content (SWC) in terms of WHC: 80%, 40%, 32% and 15% of the WHC. The first scenario was 80% of the WHC simulating a wet spring, where soils remain saturated most of the time (as it was observed in 2010 at our study sites, J.M. Ávila, unpublished data). We did not use 100% of WHC to avoid problems with gas diffusion in soil respiration measurements. The second scenario was 40% of the WHC, which is the average soil moisture in spring for these sites (period 2010-2012; J.M. Ávila, unpublished data). The third scenario was a reduction of 20% in soil moisture predicted for 2050 (the same period than for the second scenario of temperature) using an ACGCM for the scenario SRES IS92a (Manabe et al. 2004; Wetherald & Manabe 2002). We used this scenario because it is comparable with the SRES A2 (Wang et al. 2004) and, as far as we know, it is the only one that has been used to make predictions in soil moisture. Soil moisture under this scenario was 32% of WHC. Finally, the last scenario was a simulation of an extremely dry spring (15% of WHC). We used soil moisture data from spring of 2012 as a reference because that year the precipitation was the lowest in the last 50 years (following data from the meteorological station Jerez de la Frontera Airport, 36° 45' 2" N and 6° 3' 20" W) and, specifically, the spring rainfall in the study area was extremely low (60% lower than in a normal year, 1951-1999, Ninyerola et al. 2005).

Air-dried soil samples (20 g) from the different five soil origins (i.e. soils underneath healthy, defoliated and dead *Q. suber*, as well as under pioneer and late successional shrubs) were introduced in polypropylene specimen cups and were incubated during 4 weeks using the different scenarios of temperature and soil moisture (TEM = 16, 19 and 22°C; SWC = 80, 40, 32, 15% of WHC) following a factorial design (2 forest sites, 5 levels of soil origin, 3 levels of TEM, 4 levels of SWC, 5 replicates per treatment combination = 600 incubations). This 4-week

incubation experiment represents a short-term assay that can estimate microbial activity and C, N and P fluxes rapidly and reliably (Franzluebbers 1999). Soil samples were incubated in growth chambers in dark conditions. The cups were closed with polyethylene film allowing gas exchange, but limiting water losses. Moisture was monitored weekly by weighing and it was corrected when reduced >5%.

Lab analyses

For each soil sample, we monitored C mineralization measuring CO₂ produced by soil microorganisms using a colorimetric method (García-Palacios et al. 2013). Once per week, one 4-well microstrip filled with cresol red agar (indicator) was attached to the cup side. Cresol red agar is a mixture of an indicator solution (18.75 ppm cresol red dye, 220 mM potassium chloride and 3.75 mM sodium bicarbonate) amended with melted 3% purified agar (2:1 indicator:agar). The jars were closed air tight for 6 hours and the absorbance of the microstrips was read at 595 nm before and after that period. The well absorbance after the 6-hour period was normalized, averaged in each jar and converted to CO₂ concentration using a calibration curve created for this method in the lab (Campbell et al., 2003). We calculated cumulative respiration over the entire length of the incubation (i.e. from the 4 weekly measures). To do this, we previously computed for each cup the average soil respiration rate per week (Milla et al. 2006).

For each soil sample we estimated N availability using ionic exchange membranes (Durán et al. 2013; Qian & Schoenau 2002; Subler et al. 1995). We chose this method because previous studies have demonstrated that it is an excellent approach for estimating soil nutrient availability, showing a strong correlation with plant nutrient uptake (Schoenau & Huang 1991; Ziadi et al. 1999). Anion and cation exchange membranes (types I-100 and I-200, Electropure Excellion, Laguna Hills, California) of 2.5 x 2.5 cm were incubated together with the soil samples during 4 weeks. After the incubation, the adsorbed nutrients in the membranes were extracted using 25 ml of 2 M KCl in 125 ml flasks by orbital spinning for 1h at 200 rpm.

Ammonium and nitrate concentrations were assessed with the indophenol-blue method (Sims et al. 1995).

P availability was determined as the labile inorganic fraction of P, and it was analyzed after soil incubation using ionic membranes following the method described by Schoenau and Huang (1991) and Kouno et al. (1995). A moist soil sample of 2 g was placed in a 50 ml centrifugation flask with an anionic exchange membrane (2.5 x 2.5 cm squares). Then 20 ml of distilled water was added, and the flasks were shaken on an orbital shaker for 16 hours. After extraction, the adsorbed phosphorus in the membranes was extracted using 15 ml of 0.5M HCl for 1 h added in 125 ml flasks by orbital spinning for 1 h at 200 rpm. Inorganic P in the extraction eluents was analyzed by the molybdenum-blue method (Allen et al. 1986).

Data analyses

Generalized Linear Models (GLM) were used to test for the direct (Objective 1) and indirect (Objective 3) effects of pathogen-induced tree mortality, the predicted increase of temperature (TEM, three levels) and the decrease of soil moisture (SWC, four levels), as well as all possible interactions among those factors on the different studied variables. Data for the two forest types were analyzed separately. To address whether tree health, warming, drought and their interactions impact on soil variables (Objective 1), we used data for the three health states of *Q. suber* (HEALTH, three levels). To explore the indirect effects of tree mortality as a consequence of species substitution (Objective 3), we used data for soils underneath healthy *Q. suber* and the different shrubs (SPE, three levels). Including interaction terms within the models allowed testing for deviation from the additive model and the identification of antagonistic and synergistic interactions among factors (i.e. interactive, Piggott et al. 2015). We fit GLMs using a gamma family with identity as the link function. We chose a gamma distribution because our response variables were not normally distributed and it provided a better fit than the normal or lognormal distribution. Backward model selection was applied starting from the saturated model that included all the factors and their interactions. We removed terms (starting by the interactions) until there was no further decrease in the Akaike Information Criterion (AIC,

Burnham & Anderson 2002). Two nested models were considered as equivalent in terms of fit when they did not differ in more than 2 AIC units. When significant interactions between factors were found, *post hoc* tests (Tukey test) were performed to determine which levels of the independent factor were significantly different (Crawley 2007). Model assumptions were verified by examination of residuals (McCullagh & Nelder 1989).

To evaluate how tree mortality affects the resistance of soil processes to predicted changes in temperature and soil moisture (Objective 2), we calculated a Resistance Index (RS, Orwin & Wardle 2004) for the studied variables (i.e. soil respiration, NH_4^+ and NO_3^- and P availability):

$$RS_i = 1 - \frac{2|D_i|}{(C_i + |D_i|)}$$

where D_i is the difference between the control (C_i) and the disturbed soil at the end of the disturbance for each soil sample (i). To calculate this index, we considered two scenarios of disturbance using data of soils incubated in two combinations of temperature and soil moisture: Scenario 2050 and Scenario 2100. For Scenario 2050 we considered the increase in temperature and decrease in soil water content predicted by climate models for 2050 (19°C and 32% of WHC), whereas in Scenario 2100 we used the predicted temperature and an extremely dry spring for 2100 (22°C and 15% of WHC, see scenarios above). We considered as reference the soils incubated at the mean spring temperature (16°C) and the average soil moisture in spring (40% of WHC). This index of resistance is bounded between -1 and +1, with a value of -1 showing less resistance and +1 maximal resistance. We fit GLMs using a Gaussian family with identity as the link function. Due to the bounded nature of the index, data were cube-root or fifth-root transformed when necessary to meet the assumptions of the models (Cox 2011). Model selection was based on a backward procedure using AIC as before. Multiple comparison using Tukey tests were performed to determine statistical differences among *Q. suber* health status and scenarios (Crawley 2007). All statistical analyses were performed using R 3.1.1 software (R Core Team 2014) and figures were created using Sigmaplot v. 12 (Systat Software 2011).

RESULTS

Direct effects of pathogen-driven tree mortality, warming and drought on soil respiration and nutrient availability

Our first objective was to address the effects of tree health, warming and drought on the studied soil variables. Our models showed that soil respiration was affected by the three studied factors, i.e. soil origin (i.e., soils collected under *Q. suber* trees with different health status), soil moisture and temperature (Table 1). However, soil moisture was the best predictor of soil respiration variability (i.e. the factor with the highest explained deviance in each model, Table 1). In the open woodland, the effects of *Q. suber* decline and the reduction in soil moisture on soil respiration were negative (Fig. 1a). We did not detect an effect of predicted warming on soil respiration (Table 1, Fig. 2a). Meanwhile, in the closed forests, we detected an interaction between soil origin and soil moisture. The reduction of soil respiration as soil moisture decreased from 80 to 32% of WHC was of higher magnitude in soils collected under dead trees (29%) than under healthy (19%) or defoliated trees (8%). A positive effect of *Q. suber* decline was detected when soil moisture decreased at 40 and 15% of WHC (Fig. 1e). The predicted warming led to an increase in soil respiration independently of the soil origin (Table 1, Fig 2e), but this positive effect on soil respiration was only significant at the higher soil moisture treatment (Table 1, see Fig S2 in the Supplementary Material). In both forest types, the simulation of an extremely dry spring (15% of WHC) strongly reduced soil respiration to similar values in all soil origins (Fig. 1a, e).

The availability of NH_4^+ and NO_3^- was strongly affected by soil origin and soil moisture in both forest types, whereas the effect of temperature was only detected for NH_4^+ (Table 1). Soil moisture was the factor that most explained the variance in N availability (Table 1). We detected significant interactions between the effects of soil origin and soil moisture in both forest types (Table 1). The reduction in N availability with decreasing soil moisture from 80 to 32% of WHC was generally of higher magnitude in soils collected under defoliated and dead trees than under healthy trees (Fig. 1b, c, f). We mostly detected a complete reduction in N

availability in the extreme moisture treatment (i.e. 15% of WHC, Fig 1b, c, f, g). We found a significant positive effect of predicted temperature on NH_4^+ in open woodlands, while in closed forests the positive effect of temperature was of higher magnitude in soils collected under dead trees (95%) than under defoliated (-8%) or healthy trees (32%) (Fig 2b, c, f, g). A significant interaction between temperature and soil moisture was found for NH_4^+ in closed forests. The positive effect of temperature was detected only for 32% of WHC (Table 1, see Fig S3 in the Supplementary Material).

Soil origin, soil moisture and temperature all contributed to explain PO_4^{3-} variability in both forest types (Table 1). We found that defoliated trees had lower PO_4^{3-} availability than healthy trees (Fig. 1d, h and 2d, h). However, in open woodlands, this effect disappeared as temperature increased (interactive effect between soil origin and temperature, Table 1, Fig. 2d). We also detected strong interactions between temperature and soil moisture on PO_4^{3-} availability in both forest types, with the negative effect of temperature disappearing as soil moisture decreased (Table 1, Fig. 3).

Resistance of soil respiration and nutrient availability to predicted future scenarios

We analyzed the resistance of soil respiration and nutrient availability in soils collected under *Q. suber* trees with different health status to changes in soil moisture and temperature in two future climatic scenarios (Objective 2, Table 2, Fig. 4). Resistance of soils to changes in soil respiration and nutrient availability tended to be lower for dead trees than for healthy and defoliated trees (Fig. 4). We detected significant differences between soil origins in their resistance to changes in soil respiration in the closed forest, and in NO_3^- and PO_4^{3-} availability in the open woodland (Fig. 4). We also found marginal significant differences between soil origins in the resistance of soils to changes in NH_4^+ availability in the open woodland and in PO_4^{3-} availability in the closed forest (Table 2, Fig. 4). Moreover, we detected a reduction in soil resistance in the extreme scenario (2100) compared to the conditions predicted for 2050 for soil respiration (WL and CF, Tukey test $\text{SC}_{2050} > \text{SC}_{2100}$, Tukey $p < 0.05$) and N availability (NH_4^+ in

WL, Tukey test $SC_{2050} > SC_{2100}$ $p < 0.05$; NO_3^- in WL and CF, Tukey test $SC_{2050} > SC_{2100}$ $p < 0.05$), but we did not detect a significant effect of scenarios for P availability (Table 2).

Indirect effects of pathogen-driven tree mortality, warming and drought on soil respiration and nutrient availability

When we analyzed the potential indirect effects of *Q. suber* mortality on soil respiration and nutrient availability (Objective 3), our models revealed significant interactive effects between soil origin (i.e. *Q. suber*, pioneer and late-successional shrubs) and soil moisture and temperature (Table 3). We found a negative effect of drought (from 80 to 32% of WHC) on soil respiration in soils under healthy *Q. suber* trees and late-successional shrubs (Fig. 5a,e), but a positive effect of temperature on soil respiration in all soil origins. We found complex interactive patterns on the effect of soil origin, soil moisture and temperature on N availability, showing a high level of context-dependency. In the open woodland, we found a negative effect of soil moisture (from 80 to 32% of WHC) on N availability for pioneer shrubs but no effect for healthy *Q. suber* trees. However, in closed forests, we detected a negative effect of drought (from 80 to 32% WHC) on N availability for healthy trees but not for pioneer shrubs (Fig. 5b, c, f, g). We detected a positive effect of predicted warming on NO_3^- (in open woodlands) and NH_4^+ (in closed forests) just for pioneer shrubs (Fig. 6b, c, f, g). In general, we detected lower PO_4^{3-} availability under pioneer shrubs than under healthy *Q. suber* and late-successional shrubs. We found a negative effect of predicted drought (from 80 to 32% of WHC) on PO_4^{3-} availability. In the closed forest this effect was of higher magnitude in soils collected under pioneer shrubs than under healthy *Q. suber* trees. The negative effect of temperature on PO_4^{3-} availability was of higher magnitude for shrubs than for *Q. suber* trees (SPExTEM, $p < 0.05$). In both forest types, the simulation of an extremely dry spring (15% of WHC) strongly reduced soil respiration and N availability to similar values in all soil origins (Fig. 5).

DISCUSSION

This study provides useful insights into how multiple global change drivers affect soil functioning in Mediterranean ecosystems. Our models demonstrated that *P. cinnamomi*-induced mortality and future warming and drought may interact to simultaneously affect biogeochemical cycles in *Q. suber* forest soils. Moreover, we found that in a context of pathogen-driven mortality, soils under affected trees had lower resistance to the warming and drought scenarios (predicted for 2050 and 2100) than soils under healthy trees, consistently in both forest types. Previous works have warned about the consequences of future climate conditions on soil functioning (Emmett et al. 2004; IPCC 2014a), but interactive effects of future climatic conditions and biotic disturbances have been rarely experimentally addressed so far. This study represents an effort to fill this gap by exploring how the effect of predicted climate change might differ in soils affected by biotic disturbances, using a manipulative lab experiment in ways that are not feasible to address in the field.

Direct effects of pathogen-tree mortality, warming and drought on soil variables

Our results indicate that the predicted drought would translate into a reduction in soil respiration and N availability in both forest types. However, the magnitude of such decrease varied strongly depending on tree health, as indicated by the interactive effects of soil origin and soil moisture in most of our best models. For example, in the open woodland the magnitude of the change in N availability as a consequence of increasing drought (from 80 to 32% of WHC) was much higher in soils collected under dead (-85% for NH_4^+ and -70% for NO_3^-) and defoliated trees (-73% for NH_4^+ and -59% for NO_3^-) than under healthy trees (+18% for NH_4^+ and -30% for NO_3^-). This result suggests synergistic interactions between stressors, since the cumulative effect of pathogen-induced mortality and drought was of higher magnitude than the additive sum of the effects of both factors. The interactions found here between the effects of *Q. suber* decline and reduced soil moisture exemplify how invasive pathogen species have a high potential for modifying the response of soil functioning to climatic stressors.

Extreme climatic events like droughts can drastically alter plant and microbial communities and soil functioning (Lloret et al. 2015; Wang et al. 2012). These extreme events

may be even more critical drivers of ecosystem functioning than changes in mean conditions (Heisler & Weltzin 2006; IPCC 2014a). In our study, we found a large reduction in soil respiration and N availability when soils experienced an extremely dry spring in both forest types. This high level of water stress reduced the differences in the studied variables between soils collected under *Q. suber* trees with different health status. When an important reduction in soil water availability occurs, it can decrease the movement of nutrients in the soil, reducing their availability for roots and microbes (Bradford & Hsiao 1982; Stark & Firestone 1995). Less water and nutrient availability may reduce microbial activity and therefore, microbial respiration and organic matter mineralization (Gerten et al. 2008; Knapp et al. 2008), independently of the pathogen-driven oak decline. In a context of predicted longer and extremely dry periods in Mediterranean regions (Beniston et al. 2007; IPCC 2007), our results suggest that a reduction in microbial functioning and nutrient turnover can be expected, which might translate into a general loss of ecosystem functions and services.

Our models detected mainly a positive effect of the predicted warming on soil respiration and NH_4^+ availability. Warming is expected to promote microbial activity and the decomposition of soil organic matter (MacDonald et al. 1995). As a result, higher soil respiration and N mineralization rates might be expected and, potentially, more inorganic nutrients could be released into the soil (Haugwitz et al. 2014). However, when we detected an effect of temperature, the magnitude of this effect on soil properties was much lower than the effect of soil moisture. These results are in line with previous work conducted in Mediterranean systems that has highlighted soil moisture, but not temperature, as the limiting factor of soil processes in a context of reduced soil water content (Emmett et al. 2004; Rey et al. 2002). We suggest that the large magnitude of the negative impact of drought on the C and N cycles might obscure the positive effect of temperature. Therefore, we could expect a decrease in both soil respiration and N availability along with climate change in Mediterranean forests, leading to more tight C and N cycles.

In general, we detected negative additive effects of pathogen-driven mortality and climate change on P availability, which is frequently considered the main limiting nutrient in

Mediterranean ecosystems (e.g. Morillas et al. 2012; Sardans et al. 2004). Our models showed a negative effect of pathogen-driven mortality on P availability, at least in terms of labile P after incubations. This result is in agreement with previous field studies where reductions in P were detected in areas affected by *P. cinnamomi* (Ávila et al. 2016; Shearer et al. 2009). This reduction may be explained by a decrease in the root activity in soils under defoliated and dead trees, due to the importance of root exudates in regulating P availability (Schneider et al. 2001). The decrease in tree root activity might have exerted a legacy effect in the soils under affected trees that might lead to lower P content (Ávila et al. 2016). Interestingly, we found an interactive effect of warming and drought on P availability, consistently in both forest types. Warming led to a decrease in P availability, but that effect diminished (in closed forest) or disappeared (in open woodland) with decreasing soil moisture, suggesting an antagonistic interaction. Warming has been shown to increase microbial activity and P immobilization, decreasing its availability for plants (Sardans et al. 2006; Van Meeteren et al. 2007). Drought, on the contrary, usually decreases microbial activity and therefore P sequestration in microbial biomass (Criquet et al. 2004; Matías et al. 2011), and can even cause a release of P derived from microbial cell lysis after drought stress (Sparling et al. 1985; Van Gestel et al. 1993). However, this P released under drought conditions might not be accessible for plants due to water limitation of nutrient diffusion, leading to an overall negative effect of climate change on P availability. Previous studies in Mediterranean systems have found that P limits tree growth, seedling performance or microbial biomass (Gallardo & Schlesinger 1994; Gómez-Aparicio et al. 2008; Sheriff et al. 1986). Therefore, a decrease in P availability as a consequence of global change would limit even further demographic and ecosystem processes in Mediterranean forests affected by pathogen-driven mortality.

Resistance of soil respiration and nutrient availability to predicted future scenarios

We found that in a context of pathogen-driven mortality, soils under affected trees had lower resistance to the warming and drought scenarios (predicted for 2050 and 2100) than soils under healthy trees. The higher resistance index found in non-affected soils may be a consequence of

the high stability of soils whose microorganisms could be adapted to the particular conditions of Mediterranean systems (Curiel Yuste et al. 2014). These systems are characterized by strong seasonal shifts in soil water availability as well as by high temperatures and extreme drought during summer, which might act as strong forces selecting communities dominated by drought-tolerant phenotypes (Barnard et al. 2013; Curiel Yuste et al. 2014). However, the process of *P. cinnamomi*-driven mortality could alter the abundance, diversity and/or functioning of the microbial soil community, as found by other studies conducted in forests affected by pest- or pathogen-induced mortality. For example, Stursova et al. (2014) found an increase in bacterial/fungal biomass ratio and changes in diversity of soil microbial community in coniferous forests affected by insect-induced mortality, and Cai et al. (2010) detected a decrease in soil bacterial functional diversity in plots affected by Eucalypt decline. The alteration of the soil microbiota may modify the resistance of soils to future climate conditions. Our results suggest that pathogen-driven tree mortality may contribute to the alteration of biogeochemical cycles in Mediterranean ecosystems, making their soils less resistant to the predicted warming and drought under the ongoing climatic change.

Indirect effects of pathogen-tree mortality, warming and drought on soil variables

P. cinnamomi-induced tree mortality can increase relative abundance of shrubs in woody community of invaded forests (Costa et al. 2011; Ibáñez et al. 2017), which might greatly impact soil functioning in the following decades. Most of our models showed interactive effects between soil origin (healthy *Q. suber* trees vs. shrubs) and the predicted reduction in soil moisture. For instance, we found that under certain circumstances predicted drought resulted into lower N and P availability under pioneer shrubs than under *Q. suber* trees. A previous climate change experiment conducted in Mediterranean forests also found soils under shrubs to show different response to predicted drought than soils under trees (Matías et al. 2012). Differences in terms of litter quality and production between trees and shrubs might lead to different soil characteristics such as soil fertility, which might modify the response to climate change of these soils (Gómez-Rey et al. 2013; Rolo et al. 2012). Our findings suggest that if the

process of tree mortality leads to shrub encroachment as foreseen by previous works, climate change impacts on soil functioning might be fundamentally different compared to healthy forests, being characterized in many situations by a persistent reduction in soil respiration and nutrient availability.

Our findings clearly suggest that pathogen-induced mortality and climate change might alter biogeochemical cycles in affected forests to a larger extent than predicted when considered separately. However, the results obtained also points towards a high degree of context-dependency in the exact direction and magnitude of these changes, in line with previous studies that have reported context-dependent impacts of global change drivers (e.g. Butenschoen et al. 2011; Parmesan et al. 2013). For instance, although we generally found a negative interacting effect of tree mortality and predicted drought on soil respiration and N availability, positive effects were also found under some specific combinations of soil moisture and forest type. These findings clearly exemplify the complexity inherent to predicting interactive effects among global change drivers, and support the need for further studies that allow separating main trends from context-specific idiosyncrasies.

Concluding remarks

This study represents a first experimental approach for a better understanding of the effects of predicted climate change on soil processes in Mediterranean forests affected by pathogen-induced tree mortality. Most of our models showed interactive effects between *P. cinnamomi*-induced tree mortality and drought on soil respiration and N availability. Moreover, we detected lower resistance to the warming and drought scenarios of soils under dead trees than under healthy trees. These findings clearly suggest that pathogen-induced mortality and climate change might alter biogeochemical cycles in affected forests to a much larger extent than predicted when considered separately. A previous field study conducted in the same study sites showed a decrease in soil respiration and nutrient availability under *Q. suber* trees affected by *P. cinnamomi* under current climatic conditions (Ávila et al. 2016). The experimental results shown here suggest that in many cases such decrease might be exacerbated under future climatic

conditions. If that is the case, the decrease in soil respiration and nutrient availability might lead to changes in ecosystem processes such as leaf litter quality and litter accumulation (Quétier et al. 2007), carbon sequestration capacity (Oren et al. 2001), plant mycorrhizal colonization (Smith & Read 2008) and plant soil feedbacks (Bennett et al. 2017), which will determine the future forest structure and functioning. Specifically, our results provide strong empirical support for interactions among global change stressors, in agreement with the conclusions of recent meta-analyses (Dieleman et al. 2012; Zhou et al. 2016). Because pest and pathogen-induced mortality is increasingly a concern in forests worldwide (Boyd et al. 2013; Garbelotto & Pautasso 2012), further research on its ecosystem impacts and interaction with other global change-stressors is very much needed for a better understanding of ecosystem functioning in a changing world.

Acknowledgments

We thank the director and technicians of Los Alcornocales Natural Park for facilities and support to carry out the field work. We specially thank to Ana Prado and Ana Pozuelos for invaluable field and laboratory assistance. This research was supported by the Ministerio de Ciencia e Innovación (MICINN) projects CGL2010-21381, CGL2011-26877 (RETROBOS) and CGL2014-56739-R (INTERCAPA). J.M.A. was supported by a FPU-MEC grant (AP2010-0229).

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Table 1 Analysis of deviance for the effects of soil origin (HEALTH: healthy, defoliated and dead tree), temperature (TEM: 16, 19 and 22°C) and soil moisture (SWC: 80, 40, 32 and 15% WHC) on soil respiration and nutrient availability in woodland (WL) and closed forest (CF). Values of degrees of freedom (d.f.), deviance (Dev.) and F statistic (F) are presented. Dash indicates a factor not included in the minimum adequate model.

Forest	Source	CO ₂			NH ₄ ⁺			NO ₃ ⁻			PO ₄ ³⁻		
		d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F
WL	NULL		18.86			825.93			352.82			91.32	
	HEALTH	2	0.55	8.85***	2	28.62	22.06***	2	3.02	3.42*	2	11.92	23.32***
	TEM		-		2	6.53	5.03**	2	1.81	2.05 ^{n.s.}	2	9.36	18.32***
	SWC	3	12.81	148.30***	3	465.83	239.39***	3	207.11	156.51***	3	9.98	13.02***
	HEALTHxTEM		-		4	3.51	1.35 ^{n.s.}		-		4	2.63	2.58*
	HEALTHxSWC		-		6	18.37	4.72***	6	70.15	26.51***	6	3.1	2.02 ^{n.s.}
	TEMxSWC		-			-			-		6	9.91	6.46***
	Residual	174	5.51		162	303.08		166	70.74		156	44.431	
CF	NULL		11.93			925.26			1421.7			766.99	
	HEALTH	2	0.31	6.35**	2	33.67	25.95***	2	56.16	21.88***	2	33.00	7.22***
	TEM	2	0.24	4.93**	2	4.58	3.53*	2	5.43	2.12 ^{n.s.}	2	190.82	41.77***
	SWC	3	6.50	89.22***	3	568.46	292.16***	3	604.07	156.87***	3	93.55	13.65***
	HEALTHxTEM		-		4	56.99	21.97***	4	11.94	2.33 ^{n.s.}		-	
	HEALTHxSWC	6	0.31	2.13*	6	26.42	6.79***	6	113.93	14.79***		-	
	TEMxSWC	6	0.59	4.07***	6	23.29	5.99***		-		6	70.42	5.14***
	Residual	160	3.97		156	211.85		162	640.15		166	379.21	

*** p<0.001, ** p<0.01, *p<0.05, n.s. non significant

Table 2 Analysis of variance for the effect of soil origin (HEALTH: healthy, defoliated and dead tree) and two different future scenarios (SC: 2050 and 2100) on the Resistance Index (RS) of soil respiration and nutrient availability in open woodland (WL) and closed forest (CF). Values of degrees of freedom (d.f.), mean square (MS) and F statistic (F) are presented. The results of the interaction between HEALTH and SC are not shown because it was not significant in any case. Dash indicates a factor not included in the minimum adequate model.

Forest	Source	CO ₂ RS			NH ₄ ⁺ RS			NO ₃ ⁻ RS			PO ₄ ³⁻ RS		
		d.f.	MS	F	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F
WL	HEALTH	-			2	0.596	2.519 •	2	0.092	5.29*	2	0.370	4.74*
	SC	1	0.853	47.03***	-			1	2.460	142.04***	-		
	Residual	28	0.508					26	0.017		27	1.014	
CF	HEALTH	2	0.053	4.52*	-			-			2	0.29	2.98 •
	SC	1	0.406	34.14***	1	2.496	61.14***	1	1.173	27.04***	-		
	Residual	26	0.012		28	0.041		28	0.064		27	0.098	

*** p<0.001, ** p<0.01, *p<0.05, •p<0.10, n.s. non significant

Table 3. Analysis of deviance for the effects of soil origin (SPE: *Q. suber*, pioneer shrub and late-successional shrub), temperature (TEM: 16, 19 and 22°C) and soil moisture (SWC: 80, 40, 32 and 15% WHC) on soil respiration and nutrient availability in woodland (WL) and closed forest (CF). Values of degrees of freedom (d.f.), deviance (Dev.) and F statistic (F) are presented. Dash indicates a factor not included in the minimum adequate model.

Forest	Source	CO ₂			NH ₄ ⁺			NO ₃ ⁻			PO ₄ ³⁻		
		d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F
WL	NULL		16.95			290.97			841.17			114.44	
	SPE	2	0.41	7.93***	2	14.53	15.29***	2	2.87	1.62 ^{n.s.}	2	14.98	23.68***
	TEM	2	0.18	3.51*	2	1.40	0.97 ^{n.s.}	2	11.28	6.39**	2	14.76	23.32***
	SWC	3	11.56	148.19***	3	77.87	35.81***	3	524.08	197.90***	3	9.62	10.13***
	SPExTEM	-	-	-	4	-	-	4	10.84	3.07*	4	2.83	2.23 ^{n.s.}
	SPExSWC	6	0.51	3.27**	3	12.53	2.88*	6	14.99	2.83*	6	3.47	1.83 ^{n.s.}
	TEMxSWC	-	-	-	-	-	-	6	12.71	2.40*	6	19.79	10.42***
	Residual	166	4.28		162	184.64		156	264.41		156	49.00	
CF	NULL		11.57			970.65			1681.78			146.26	
	SPE	2	1.07	18.85***	2	13.32	9.59***	2	147.88	62.20***	2	3.71	5.43**
	TEM	2	0.37	6.48**	2	22.7	16.33***	2	54.23	22.81***	2	28.83	42.21***
	SWC	3	4.47	52.40***	3	565.57	271.32***	3	262.98	73.74***	3	12.64	12.34***
	SPExTEM	-	-	-	6	51.94	18.69***	-	-	-	4	9.10	6.66***
	SPExSWC	6	0.47	2.78*	4	24.99	5.99***	6	445.59	62.47***	6	12.32	6.01***
	TEMxSWC	4	0.66	3.88**	-	-	-	6	117.39	16.46***	6	5.01	2.45*
	Residual	162	4.53		162	292.14		160	653.71		156	74.66	

*** p<0.001, ** p<0.01, *p<0.05, n.s. non significant

FIGURE CAPTIONS

Fig. 1 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean $\pm$ SE) for each soil origin (soils under healthy, defoliated and dead *Q. suber*) at each soil moisture level (n=15). Graphics in the first row represent soils from the woodland and graphics in the second row represents soils from the closed forest. The lower cases indicate significant differences ($p < 0.05$) in the post-hocs analyses: if interactive effects between soil origin and soil moisture were significant, symbols represent differences in the soil variables between soil origin within SWC levels and colored letters indicate significant differences in the soil variables between SWC within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and soil moisture were not significant, upper case letters indicate differences between levels of the main effects.

Fig. 2 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean $\pm$ SE) for each soil type at each temperature (n=20). Graphics in the first row represent soils from woodland and graphics in the second row represents soils from closed forests. The lower cases indicate significant differences ($p < 0.05$) in the post-hocs analyses: if interactive effects between soil origin and temperature were significant, symbols represent differences in the soil variables between soil origin within temperature levels and colored letters indicate significant differences in the soil variables between temperature within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and temperature were not significant, upper case letters indicate differences between levels of the main effects.

Fig. 3 PO_4^{3-} availability (mean $\pm$ SE) for each soil temperature at each soil moisture level (n=15) in a) woodland and b) closed forest. The lower case indicates significant differences in PO_4^{3-} availability between temperature levels within moisture levels ($p < 0.05$)

Fig. 4 Changes in the Resistance Index (RS) for microbial respiration (a), NH_4^+ (b), NO_3^- (c) and PO_4^{3-} (d) availability under predicted changes in climate induced by climate change for different soil types in woodland and closed forests. Data are means $\pm$ SE (n=10). Data were pooled among climate change scenarios because we did not detect a significant interaction between scenario and soil type. The lower case indicates significant differences in RS between health status of trees ($p < 0.05$)

Fig. 5 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean $\pm$ SE) for each soil origin (soils under healthy *Q. suber*, late-successional and pioneer shrubs) at each soil moisture level (n=15). Graphics in the first row represent soils from the woodland and graphics in the second row represents soils from the closed forest. The lower cases indicate significant differences ($p < 0.05$) in the

post-hocs analyses: if interactive effects between soil origin and soil moisture were significant, symbols represent differences in the soil variables between soil origin within SWC levels and colored letters indicate significant differences in the soil variables between SWC within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and soil moisture were not significant, upper case letters indicate differences between levels of the main effects.

Fig. 6 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean $\pm$ SE) for each soil origin (soils under healthy *Q. suber*, late-successional and pioneer shrubs) at each temperature (n=20). Graphics in the first row represent soils from the woodland and graphics in the second row represents soils from the closed forest. The lower cases indicate significant differences ($p < 0.05$) in the post-hocs analyses: if interactive effects between soil origin and temperature were significant, symbols represent differences in the soil variables between soil origin within temperature levels and colored letters indicate significant differences in the soil variables between temperature within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and temperature were not significant, upper case letters indicate differences between levels of the main effects.

SUPPLEMENTARY MATERIAL (DOCX 1146 kb):

[10533_2018_521_MOESM1_ESM.docx](#) (1.1 mb)

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

WOODLAND

CLOSED FOREST

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FIGURE 6

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