

RESEARCH ARTICLE

Evidence for antagonistic effects of climate change and exotic pathogens on regeneration of Mediterranean forests

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Abstract

1. Understanding the interactive effects of global change drivers on tree demography is fundamental for realistic predictions of future forest dynamics. Multiple studies have shown increasing drought and exotic pathogens to severely threaten forest persistence by increasing mortality and decreasing growth of adult trees. However, much less is known about their effects on regeneration, and how they might affect seedling performance in additive and non-additive (synergistic or antagonistic) ways.
2. Here we aimed to fill this gap by experimentally exploring the effects of increasing drought and soil-borne pathogens on tree regeneration in two types of mixed oak forests (*Quercus suber*-*Q. canariensis* and *Q. suber*-*Olea europaea*) invaded by the exotic soil-borne oomycete *Phytophthora cinnamomi*, one of the most aggressive plant pathogens on earth. We conducted a seed sowing experiment with oomycete-specific fungicide taking advantage of rainfall exclusion infrastructures that excluded 30% of the annual rainfall, simulating predictions of climate change models for Mediterranean systems. Seedling emergence, survival and growth of the three tree species were followed during 3 years.
3. We found that neutral or positive drought effects on regeneration dominated over negative effects in the tree community. Moreover, most positive drought effects on the dominant species (*Q. suber*) were not direct, but indirectly mediated by soil-borne pathogens. This was shown by the fact that positive drought effects disappeared with fungicide application.
4. **Synthesis:** Overall, our results suggest that rainfall reductions predicted by climate change models for the Mediterranean region might have minor direct negative effects on early regeneration of tree species, but could play a major indirect role by limiting the negative effects of exotic pathogens on highly susceptible tree species. These findings highlight that antagonisms among global change drivers should be recognized as important forces that might slow down the current loss of tree health.

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KEYWORDS

experimental drought, global change ecology, interactive effects, oomycetes, *Phytophthora cinnamomi*, rainout shelters, seedling performance, soil-borne pathogens

1 | INTRODUCTION

Global change is severely modifying tree demographic rates in forests world-wide (Allen et al., 2015; McDowell et al., 2020; Millar & Stephenson, 2015). In the last decades, multiple studies have aimed to disentangle how growth and survival of adult trees are being changed by higher CO₂ levels, a warmer climate, increasing drought or the attack by exotic pests and pathogens (Carnicer et al., 2011; Seidl et al., 2017; Sperry et al., 2019). Most of these studies have found that global change drivers are increasing mortality rates and decreasing growth of adult trees, and that this reduction in tree health might threaten the role of forest ecosystems as biodiversity reservoirs and carbon sinks (Anderegg et al., 2020; Brienen et al., 2015; Trumbore et al., 2015). However, understanding forest dynamics under global change requires not only evaluating the capacity of adult trees to grow and survive, but also to regenerate. Establishment of new individuals is a fundamental process for the maintenance of tree populations and their post-disturbance recovery (Batllori et al., 2020; Davis et al., 2019). Despite its relevance, the number of studies that have explored the impacts of global change drivers on tree regeneration is still relatively low compared to the many studies focused on adult trees (García de Jalón et al., 2020; Lloret et al., 2004; Matías et al., 2012).

Among forest ecosystems, Mediterranean forests are considered particularly threatened by global change (Peñuelas et al., 2017). On the one hand, the Mediterranean basin is a hot-spot of climate change, with a clear trend towards drier conditions due to the combination of higher temperatures and rainfall reduction (Giorgi & Lionello, 2008; IPCC, 2021). A drier climate is imposing a high stress level on a type of system where water is already a main limiting resource for plant species performance (Acácio et al., 2017; Peñuelas & Sardans, 2021). On the other hand, the Mediterranean basin is also a hot-spot of exotic species, due to its long history of human occupancy and high diversity of environments (Cherif et al., 2020). In Mediterranean countries, the number of invasive forest pathogens has increased exponentially in the last four decades influenced by an unprecedented growth of international travel and trade (Garbelotto & Pautasso, 2012; Santini et al., 2013). Among these new pathogens, oomycetes have shown the largest increase, and particularly those belonging to the genus *Phytophthora*, which represents one of the most important genera of plant pathogens on earth (Burgess et al., 2021; Jung et al., 2018; Scott et al., 2019). Exotic oomycetes have decimated populations of several Mediterranean woody species (Jung et al., 2018), and have been recently shown to be an important factor limiting forest regeneration (Domínguez-Begines et al., 2020, 2021). However, it is uncertain how regeneration dynamics of Mediterranean forests might be affected by the simultaneous, interactive effects of exotic pathogens and a drier climate.

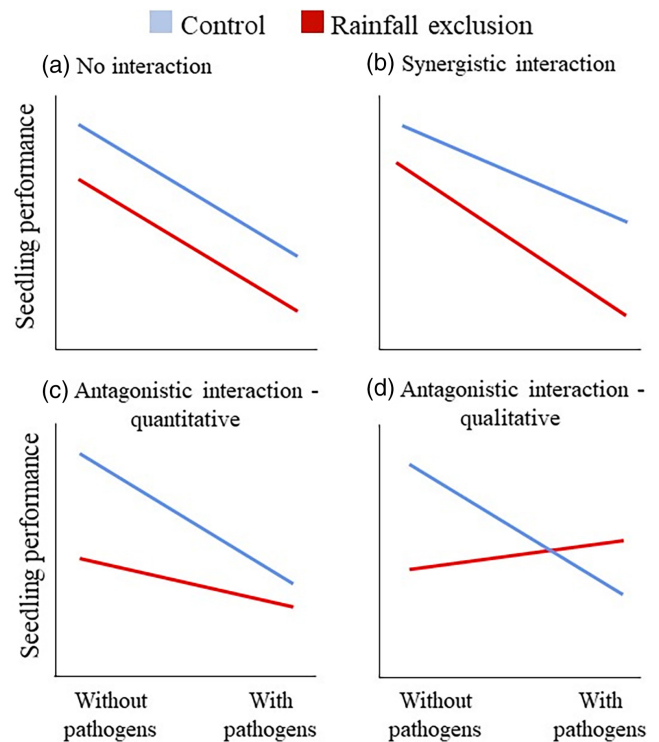


FIGURE 1 Theoretical scenarios of interaction among two global change drivers (increasing drought and exotic pathogens): (a) no interaction, where the negative effect of increasing drought on seedling performance is the same with or without pathogens; (b) synergistic interaction, where the negative effect of increasing drought is larger in the presence of pathogens (as shown by steeper slope of the red line); (c) antagonistic (qualitative) interaction, where the negative effect of increasing drought is smaller in the presence of pathogens (as shown by slower slope of the red line); (d) antagonistic (quantitative) interaction, where the effect of increasing drought on seedling performance changes of sign (from negative to positive) depending on the presence of pathogens (as shown by the crossing of the red and blue lines).

Conducting studies that simultaneously explore the effect of several global change drivers on tree demography is a complex and urgent challenge, since combined effects of global change drivers might not be always predicted from studies that explore their effects in isolation (Côté et al., 2016; Sage, 2020; Simler-Williamson et al., 2019; Tylianakis et al., 2008). From a theoretical perspective, three different scenarios of interaction among global change drivers might be expected: no interaction, synergistic interaction and antagonistic interaction (Figure 1). The lack of interaction among drivers would represent the simplest scenario, where the total effect of both drivers would be equal to the sum of their individual effects (Figure 1a). A synergistic interaction would happen when the combined effect of the two drivers is of larger magnitude

than the sum of their individual effects (Figure 1b). For example, in a forest affected by increasing drought and exotic pathogens, drought might amplify the negative effects of pathogens due to a weakening effect on trees that make them more vulnerable to biotic attack (Corcobado et al., 2014; Desprez-Loustau et al., 2006). Finally, an antagonistic interaction would happen when the combined effects of both drivers is of smaller magnitude than the sum of their individual effects (Figure 1c). In this case, negative effects of pathogens on tree performance could be smaller in a drier climate due to negative effects of drought on the population growth rate of pathogens, since most tree pathogens are fungi or oomycete that need water for the germination and dispersal of their spores (Erwin & Ribeiro, 1996; Homet et al., 2019). Antagonistic interactions could be not only quantitative, but also qualitative, if they involved a change of sign (from negative to positive, or vice versa) in the effect of one of the drivers (Figure 1d). This represents an extreme situation of antagonistic effects very rarely reported in the literature.

In this study, we aimed to experimentally explore the individual and interactive effects of increasing drought and exotic pathogens on tree regeneration dynamics in mixed Mediterranean oak forests invaded by the exotic soil-borne oomycete *Phytophthora cinnamomi*, one of the most aggressive plant pathogens on earth (Lowe et al., 2000). To this end, we conducted a seed sowing experiment with oomycete-specific fungicide taking advantage of rainfall exclusion infrastructures installed in the southernmost European oak forests, located in the mountain range north of the Strait of Gibraltar. This area contains the largest and best conserved European forests of *Quercus suber*, but currently suffers important problems of oak decline due to *P. cinnamomi* invasion (Ávila et al., 2017; Gómez-Aparicio et al., 2012). The experiment was replicated in two different types of mixed oak forests: a closed forest of *Q. suber* and *Q. canariensis*, and an open woodland of *Q. suber* and *Olea europaea*. The three species differ in their susceptibility to drought ($Q. canariensis > Q. suber > O. europaea$; Gómez-Aparicio, Pérez-Ramos, et al., 2008; Ibáñez et al., 2017) and *P. cinnamomi* attack ($Q. suber > O. europaea > Q. canariensis$; Gómez et al., 2020). The rainfall exclusion infrastructures excluded 30% of the annual rainfall, simulating predictions of climate change models for southern Europe (IPCC, 2021).

Seedling emergence, survival and growth of the three tree species were followed during 3 years to analyse their response to rainfall exclusion and the application of oomycete-specific fungicide. As in previous studies (Domínguez-Begines et al., 2020, 2021), we assumed that *P. cinnamomi* would be the main oomycete species driving fungicide effects due to its fundamental role as a driver of oak decline in southern Europe (Brasier, 1992; Camilo-Alves et al., 2013; Moricca et al., 2016) and its high abundance in the study area (Gómez-Aparicio et al., 2012; Serrano et al., 2022). This assumption is also supported by the fact that, although other *Phytophthora* species have been recently described in Spanish forests (e.g. *P. quercina*, *P. psychrophila*, *P. gonapodyides*, *P. cactorum*, Catalá et al., 2017; Mora-Sala et al., 2018; Ruiz-Gómez

et al., 2019), pathogenicity tests consistently find that they are much less aggressive than *P. cinnamomi* to oak species (Corcobado et al., 2017; Mora-Sala et al., 2019; Pérez-Sierra et al., 2013; Seddaiu et al., 2020). We hypothesized that the two experimental factors (drought and pathogens) would have negative effects on regeneration, with the magnitude of such effects varying among tree species according to their differential susceptibility to either abiotic or biotic stress. Moreover, based on previous studies conducted under controlled conditions (Homet et al., 2019), we expected drought and pathogens to have interactive (antagonistic) effects, particularly on *Q. suber*, the most susceptible species to *P. cinnamomi*. Finally, because in mixed forests tree species have species-specific footprints on the above- and below-ground environment that determine spatial patterns of regeneration (Gómez-Aparicio, Canham, et al., 2008; Ibáñez et al., 2017), we hypothesized that the impact of a given global change stressor on the success of seedling establishment might differ under different canopy species. Overall, the results of this study will contribute to improve our ecological understanding of the interactive effects of two main global change drivers—increasing drought and exotic pathogens—that, to our knowledge, have never been experimentally explored before under natural conditions.

2 | MATERIALS AND METHODS

2.1 | Study site and species

The study was conducted from 2017 to 2019 at Los Alcornocales Natural Park (Cádiz), in southern Spain (Figure S1). The climate is sub-humid Mediterranean; rainfall concentrates (95%) from October to May, ranging from 620 mm to 2100 mm (Junta de Andalucía, 2017). This variability in annual precipitation was well represented during the study: 2017 was an average year (980 mm), 2018 was a rainy year (1384 mm) and 2019 was a dry year (735 mm). Soils are sandy, acidic and nutrient poor, derived from bedrock dominated by Oligo-Miocene sandstones.

We selected two study sites located 4 km apart and representative of the main mixed *Q. suber* forests of the park: a closed forest where *Q. suber* coexists with the deciduous, shade-tolerant *Q. canariensis* (closed forest, hereafter) and an open woodland where *Q. suber* coexists with the evergreen and drought-tolerant *Olea europaea* var. *sylvestris* (open woodland, hereafter; Figure S1). Closed forests appear in wet areas on sandy soils, and usually have a sparse understorey dominated by tall shrubs, particularly *Erica arborea*. Open woodlands generally appear in the drier lowlands of the area on clayish soils prone to temporal waterlogging during the wet season, and have a dense and rich understorey composed by shrub species such as *Pistacia lentiscus*, *Genista* spp., *Ulex* spp., *Erica* spp. and *Cistus* spp. Soils at the two study sites were invaded by the oomycete soil-borne pathogen *P. cinnamomi* (Homet et al., 2021; Serrano et al., 2022), but only a few *Q. suber* trees showed above-ground symptoms of decline at the time of the experiment. Although *P.*

cinnamomi has the ability to infect the roots of the three tree species (at least during the seedling stage), *Q. suber* is considered more susceptible to the pathogen than *O. europaea* and *Q. canariensis* (Gómez et al., 2020; Moralejo et al., 2009).

2.2 | Rainfall exclusion general design

This study was conducted taking advantage of a rainfall exclusion infrastructure built to evaluate the combined effect of climate change and the exotic pathogen *P. cinnamomi* on the dynamics and functioning of Mediterranean mixed oak forests. Permission to build the infrastructure and conduct the fieldwork in private property was provided by the Jaime González-Gordon Foundation. At each of the two study sites (closed forest and open woodland), we established six plots of 20×15 m during early spring 2016. Plots were distributed in three pairs (hereafter, blocks), with a mean distance of 30 m among blocks. From each pair, one plot was assigned to a rainfall exclusion treatment, whereas the other plot served as a control (Figure S2). All plots were fenced to avoid the interference of large wild animals on the experiment. The rainfall exclusion treatment was designed to reduce in 30% the amount of rainfall, reproducing future climate change scenarios for southern Spain (model CMIP5 for the scenario RCP 8.5 for the period 2081–2100; Stocker, 2013). Rainfall was excluded using PVC gutters that covered 30% of the plot area in order to exclude a similar amount of natural precipitation. Gutters were placed at least 1 m height over the forest floor. In control plots, the same gutters were placed upside down to simulate potential secondary effects of the infrastructure on the forest microenvironment while maintaining the natural precipitation regime (Figure S2). Tree density, basal area and light levels were similar for rainfall exclusion and control plots (Table S1). A buffer area of 1 m was considered within each plot in order to avoid any possible edge effects. All trees within the plots and outside the buffer area were individually marked (three to four trees per plot and species), making a total of 21 *Q. suber* and 22 *Q. canariensis* in the closed forest, and 21 *Q. suber* and 22 *O. europaea* in the open woodland ($n=86$ trees in total). *P. cinnamomi* was isolated in the soil of all trees before the start of the experiment (Serrano et al., 2022, unpublished data).

The effect of the rainfall exclusion treatment on soil moisture was quantified at four depths (10, 20, 30 and 40 cm) with a PR2 Profile Probe, Delta-T Devices (UK). Measurements were conducted every 3–4 weeks at six points per plot where access tubes were permanently installed for easy probe insertion and removal. Access tubes were installed in November 2015, to have a record of soil moisture in the different plots before the start of the rainfall treatments in March 2016. Soil texture (0–20 cm) was measured next to each access tube because previous studies in the area have shown a large variability in the sand content at small spatial scales (Gómez-Aparicio et al., 2012), which might affect soil moisture measurements and therefore the quantification of the effects of the rainfall exclusion treatment.

2.3 | Seed sowing experiment

We conducted a sowing experiment using seeds of the two dominant species at each forest type: *Q. suber* and *Q. canariensis* in the closed forest, and *Q. suber* and *O. europaea* in the open woodland. In fall 2016, acorns of *Quercus* species were collected from at least 10 adult individuals per species in the study area. Due to the difficulties to collect and clean large quantities of *O. europaea* seeds, we bought them from a specialized supplier close to the study area (Semillas Silvestres S.L.). Seeds were stored in humid substrate at 4°C until sowing. Before sowing, healthy *Q. suber* acorns were selected by flotation (Gribko & Jones, 1995). To reduce differences in seedling performance due to acorn size, we selected only healthy acorns with a weight within the second and third quartile (25%–75%) of the whole acorn population (i.e. 5.77 ± 1.57 g for *Q. suber* and 5.16 ± 1.46 for *Q. canariensis*). Selected acorns were surface sterilized with a 10% bleach solution previous to sowing. *O. europaea* seeds were subjected to mechanical scarification and soaked in water for 5 h previous to sowing in order to break dormancy and improve germination (Baskin & Baskin, 1998; Costa & Sánchez, 2001).

In December 2016, two 25×25 cm sowing quadrats ('Control' and 'Fungicide') were established under each of the 86 study trees separated at least 30 cm. At each quadrat we established 16 sowing points separated 5 cm from each other. In the closed forest, one acorn of *Q. canariensis* or *Q. suber* was sown per point at 2 cm depth, making a total of 688 seeds per species (8 seeds×2 treatments×43 trees). In the open woodland, one acorn of *Q. suber* was also sown per point ($n=688$ acorns). However, we sowed a higher number of *O. europaea* seeds (17 seeds per point, 11,696 seeds in total) to compensate for the low germination rate of the species (see Domínguez-Begines et al., 2020 for a similar approach; Figure 2). Seeds were covered with a metal mesh to avoid seed predation by rodents. Seeds in the fungicide quadrats were treated monthly with an oomycete-specific fungicide (Armetil 25 WP, 25% metalaxyl; Industrias Químicas del Valles SA, Barcelona, Spain) applied as a spray (1.6 gr/L water solution) on seedlings and soil. Control seedlings were treated with an identical volume of water. Armetil is a metalaxyl-based, systemic fungicide that interferes with oomycete development through the inhibition of RNA (Schwinn & Staub, 1987; Sukul & Spiteller, 2000). Metalaxyl does not affect true fungi (Cohen & Coffey, 1986), so its use minimizes the likelihood of side effects on microbial biomass and nutrient dynamics associated with the application of broad-spectrum fungicides (Chen et al., 2001). Armetil has been previously used to successfully disentangle the role of oomycetes pathogens for tree seedling survival and growth under both natural (Domínguez-Begines et al., 2020) and controlled conditions (Domínguez-Begines et al., 2021).

Seedling emergence, survival and growth were followed monthly during 34 months. At each census, the state of each seed (emerged/not emerged) or seedling (alive/dead) was registered. In October 2019, all the living seedlings were harvested, including the root system to a standardized depth of 25 cm. Once in the laboratory, seedlings were divided into stem, leaves and roots. All plant material was dried at 70°C for a minimum of 48 h to estimate shoot and root

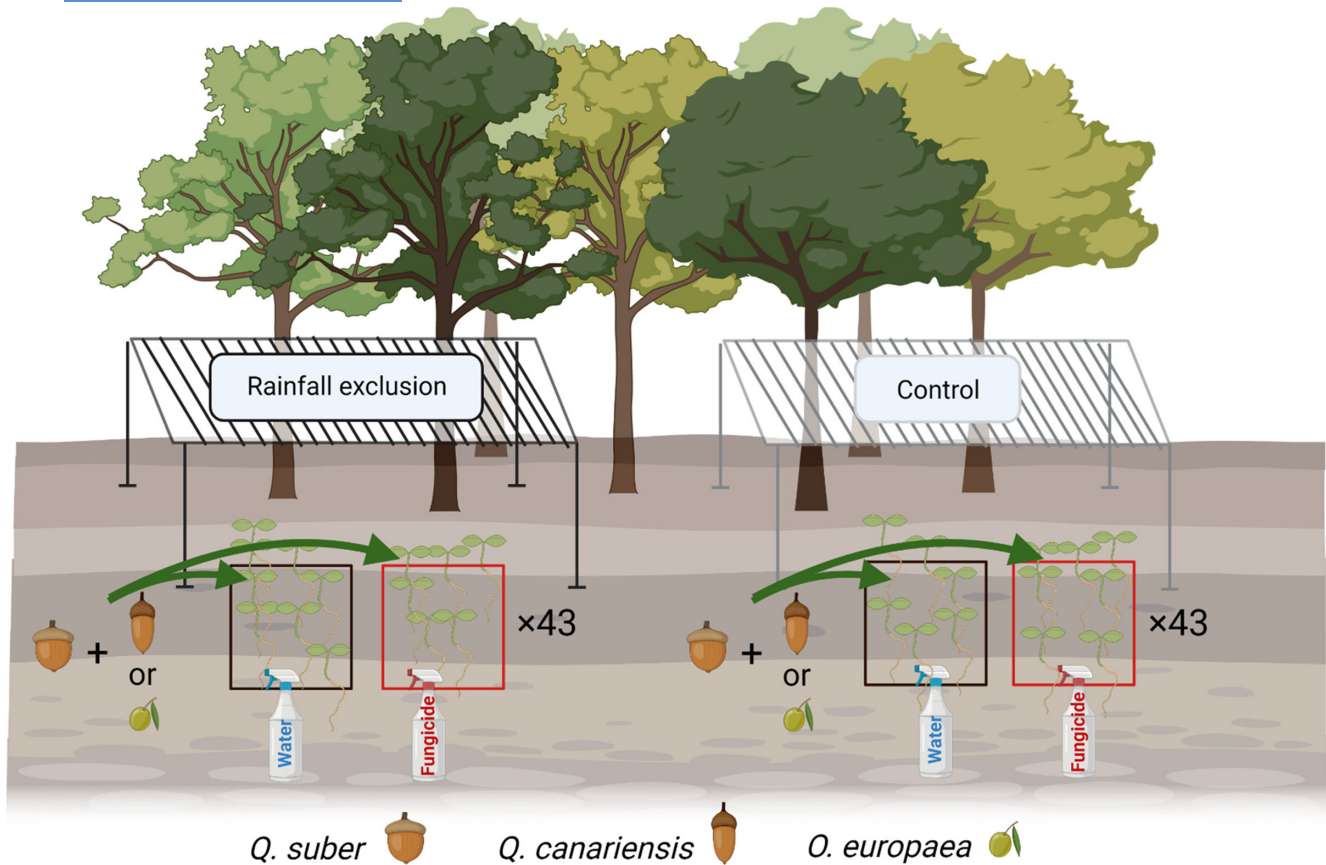


FIGURE 2 Design of the seed sowing experiment. At each of the two forest sites (closed forest and open woodland), seeds were sown in three control and three rainfall exclusion plots. Within each plot, sowing squares were installed under the canopy of each canopy tree (21 *Quercus suber* and 22 *Quercus canariensis* in the closed forest, 21 *Q. suber* and 22 *O. europaea* in the open woodland, 43 trees per site). Two 25 × 25 cm sowing quadrats ('Control' in brown, 'Fungicide' in red) were established under each tree, with 16 sowing points. In the closed forest, one acorn of *Q. canariensis* or *Q. suber* was sown per point (i.e. eight seeds per species within each quadrat). In the open woodland, one acorn of *Q. suber* was also sown per point, whereas a higher number of *O. europaea* seeds was sown per point (17 seeds) to compensate for the low germination rate of the species (i.e. 136 *Olea* seeds within each quadrat).

biomass. Because *Quercus* species develop a deep tap root that was not possible to recover in its totality in the field, only fine roots (i.e. roots <2 mm in diameter) were considered for further analyses.

2.4 | Data analysis

All analyses were conducted separately for each combination of forest type and seedling species. We used mixed effects Cox's proportional hazards models (Cox, 1972) to explore the effect of the experimental treatments on time to emergence along the first 5 months of the experiment (emergence rate), as well as on time to death along the whole duration of the experiment (survival rate). Rainfall exclusion, fungicide, canopy species and all double and triple interactions were included as fixed factors. Acorn weight was included as a covariate in all models of *Quercus* species to control for its large effect on seedling performance (Pérez-Ramos et al., 2013; Quero et al., 2007). To control for the nested structure of the experimental design, block was included as a fixed factor and the sowing quadrat as a random factor nested in tree. Survival analyses

were performed using packages 'survival' (Therneau, 2020a) and 'coxme' (Therneau, 2020b). Model fit was measured using root mean squared error (RMSE) and mean absolute error (MSA), with lower values being indicative of better fits.

Because of the long duration of the experiment, we also used GLMMs to evaluate treatment effects on survival at different time points: after the first summer (the period of usually largest seedling mortality in Mediterranean systems; Gómez-Aparicio, Canham, et al., 2008; Ibáñez et al., 2017), after the second summer (i.e. after 22 months) and at the end of the study (i.e. after three summers, 34 months). The structure of the full model was the same as for the Cox's proportional hazards models. The same full GLMM was applied for the analysis of the shoot and fine root biomass of the seedlings harvested at the end of the study. Emergence and survival were analysed using a binomial distribution, whereas shoot and fine root biomass were analysed using a normal distribution. In all cases, we followed a process of model comparison and simplification using AIC_c to find the best model (i.e. lowest AIC_c). GLMMs were performed using package 'lme4' (Bates et al., 2015) and compared with the MUMIN package (Barton, 2022). The MUMIN package was

also used to calculate the variance explained by the GLMM models (marginal and conditional R^2), according to the method proposed by Nakagawa and Schielzeth (2013). All statistical analyses were conducted with R software version 4.1.1 (R Core Team, 2021).

3 | RESULTS

3.1 | Effect of rainfall exclusion on soil moisture

Soil moisture levels showed some differences among drought and control plots before the installation of the rainfall exclusion infrastructure (Table S2). However, the rainfall exclusion treatment clearly magnified these differences across the different soil depths (Table S3). The largest differences occurred after large rainfall events, as those occurred during the spring of 2018, where soil moisture reached values 33% higher in control than drought plots (Figures S3 and S4).

3.2 | Seedling emergence

Emergence of *Quercus* species occurred progressively during the first 5 months of the experiment, reaching values up to 85% for *Q.*

suber (Figure 3a) and 75% for *Q. canariensis* (Figure 3b) in the closed forest, and 92% for *Q. suber* in the open woodland (Figure 3c). Emergence of *O. europaea* occurred during the first and second springs of the experiment, and was much lower than for *Quercus* species (10%) (Figure 3d). Best models of emergence included the effect of the rainfall exclusion treatment for the two *Quercus* species, but not for *O. europaea* (Tables 1 and 2). Effects of rainfall exclusion on oak emergence were species and site specific. In the closed forest, the rainfall exclusion effect on *Q. suber* emergence was strongly modulated by the fungicide (Rainfall exclusion \times Fungicide interaction, Table 1), changing from slightly negative with fungicide to very positive without fungicide (Figure 4a). At this same forest, rainfall exclusion negatively affected emergence of *Q. canariensis*, which emerged slower and had lower final emergence in rainfall exclusion plots (0.73) than under control conditions (0.80, Figure 3b). In the open woodland, rainfall exclusion had a positive effect on the emergence rate of *Q. suber* (Figures 2c and 3e).

Fungicide application increased the emergence rate in all species-site combinations, except for *Q. suber* in the open woodland (Tables 1 and 2, Figure 3). Among the three species, *O. europaea* showed the largest positive fungicide effects, particularly under conspecific *Olea* trees (Fungicide \times Canopy species interaction; Table 2), where emergence increased by 39% with the application of fungicide (Figure S5d).

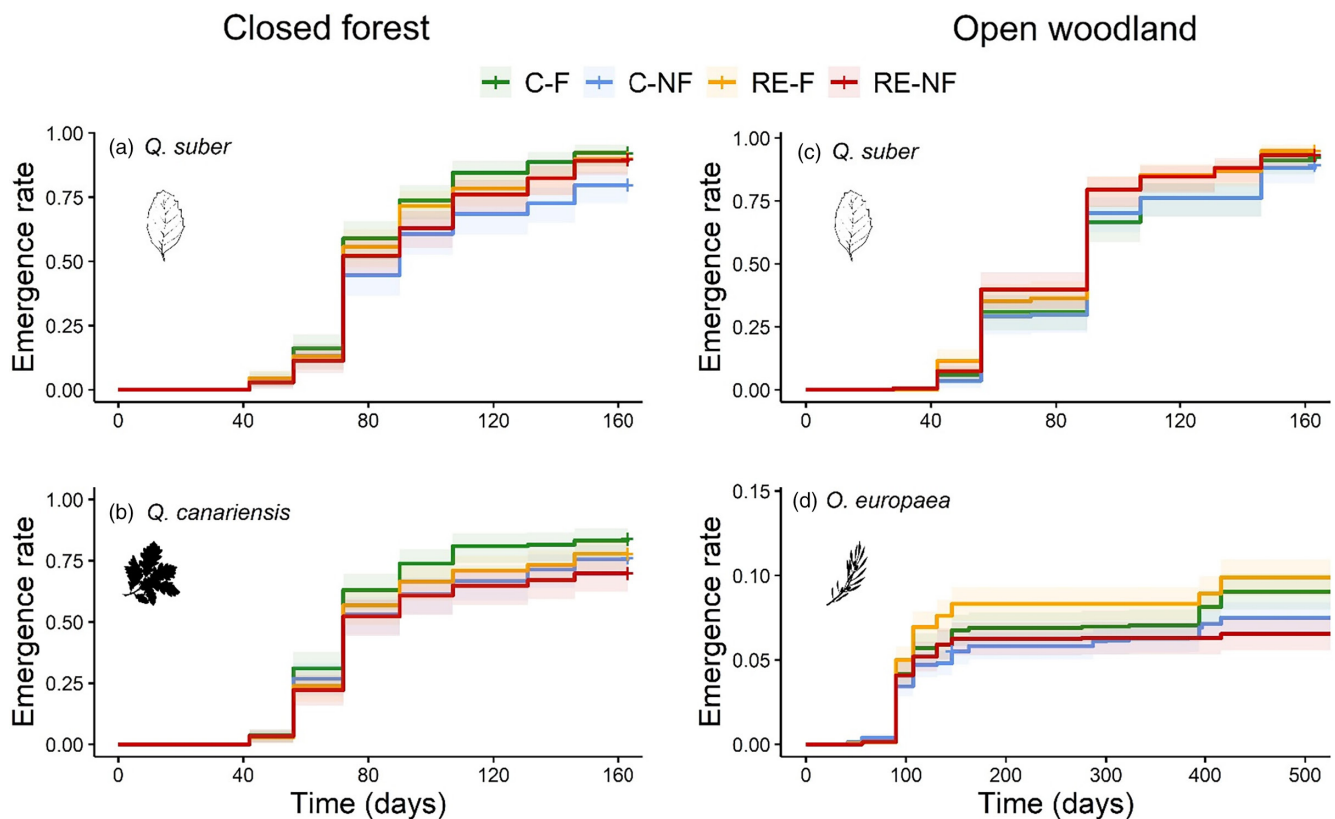


FIGURE 3 Emergence rate of *Quercus suber* (a) and *Quercus canariensis* (b) in the closed forest, and of *Quercus suber* (c) and *Olea europaea* (d) in the open woodland. Emergence is given separately for each of the four experimental treatment combinations (RE, rainfall exclusion; C, control; F, with fungicide; NF, no fungicide). Germination of the two *Quercus* species took place during the first spring of the experiment, whereas germination of *Olea europaea* took place during the first and second spring.

TABLE 1 Best models analysing the effect of rainfall exclusion (RE), fungicide (F) and canopy species (SP) on emergence and survival rates along the study period, survival at different time points (first, second and third year), and final shoot and fine-root biomass of experimental seedlings in the closed forest. Full models also included seed weight (SW) as a covariate and block (B) to control for the spatial structure of the experimental design. Δ AICc represents the differences between the AICc (Akaike information criterion corrected for small sample sizes) of the best and null models. The total number of parameters in the best model ('Par') is also given. Conditional R^2 (R_c^2) reflects the variance explained by all factors (fixed and random) in the GLMMs, whereas marginal R^2 (R_m^2) reflects the variances explained only by fixed effects.

Seedling species	Variable	Best model	N	Par	Δ AICc	R_c^2	R_m^2
<i>Quercus suber</i>	Emergence rate	$y \sim \text{SW} + \text{RE} + \text{F} + \text{RE} \times \text{F}$	688	5	18.4		
	Survival rate	$y \sim \text{SW} + \text{RE}$	581	3	11.9		
	Survival—first year	$y \sim \text{SW} + \text{RE} + \text{F} + \text{SP} + \text{RE} \times \text{F} + \text{RE} \times \text{SP} + \text{F} \times \text{SP} + \text{RE} \times \text{F} \times \text{SP}$	606	9	6.1	0.23	0.20
	Survival—second year	$y \sim \text{SW} + \text{RE} + \text{F} + \text{RE} \times \text{F}$	581	5	11.8	0.29	0.17
	Survival—third year	$y \sim \text{SW} + \text{RE}$	581	3	9.9	0.25	0.13
	Shoot biomass	$y \sim \text{SW} + \text{RE} + \text{SP} + \text{RE} \times \text{SP}$	273	5	10.1	0.25	0.17
	Fine root biomass	$y \sim \text{SW} + \text{RE} + \text{F} + \text{RE} \times \text{F}$	169	5	9.7	0.33	0.16
<i>Quercus canariensis</i>	Emergence rate	$y \sim \text{SW} + \text{RE} + \text{F}$	688	4	19.1		
	Survival rate	$y \sim \text{RE}$	504	2	10.5		
	Survival—first year	$y \sim \text{F} + \text{SP} + \text{F} \times \text{SP}$	529	3	1.1	0.24	0.10
	Survival—second year	$y \sim \text{RE} + \text{F}$	504	3	8.2	0.19	0.11
	Survival—third year	$y \sim \text{RE}$	504	2	6.1	0.21	0.14
	Shoot biomass	$y \sim \text{SW} + \text{SP}$	287	3	10.3	0.22	0.15
	Fine root biomass	$y \sim \text{RE} + \text{SP}$	256	3	3.1	0.17	0.12

Note: Models are mixed-effects Cox' proportional hazards models for emergence and survival rate, and GLMMs for the other variables. Model fit of Cox proportional hazards models was measured using root mean squared error (RMSE) and mean absolute error (MSA): *Q. suber* emergence: 0.49 and 0.49; *Q. suber* survival: 0.59 and 0.52; *Q. canariensis* emergence: 0.48 and 0.47; *Q. canariensis* survival: 0.58 and 0.55.

TABLE 2 Best models analysing the effect of rainfall exclusion (RE), fungicide (F) and canopy species (SP) on emergence and survival rates along the study period, survival at different time points (first, second and third years), and final shoot and fine root biomass of experimental seedlings in the open woodland. Full models also included seed weight (SW) as a covariate and block (B) to control for the spatial structure of the experimental design. Δ AICc represents the differences between the AICc (Akaike information criterion corrected for small sample sizes) of the best and null models. The total number of parameters in the best model ('Par') is also given. Conditional R^2 (R_c^2) reflects the variance explained by all factors (fixed and random) in the GLMMs, whereas marginal R^2 (R_m^2) reflects the variances explained only by fixed effects.

Seedling species	Variable	Best model	N	Par	Δ AICc	R_c^2	R_m^2
<i>Quercus suber</i>	Emergence rate	$y \sim \text{SW} + \text{RE}$	688	3	33.6		
	Survival rate	$y \sim \text{B} + \text{SW} + \text{SP}$	636	4	4.9		
	Survival—first year	$y \sim \text{RE} + \text{SP} + \text{RE} \times \text{SP}$	636	4	3.4	0.51	0.12
	Survival—second year	$y \sim \text{B} + \text{SW} + \text{RE} + \text{SP} + \text{RE} \times \text{SP}$	636	6	12.2	0.44	0.24
	Survival—third year	$y \sim \text{B} + \text{SW} + \text{RE} + \text{F} + \text{SP}$	636	6	12.7	0.42	0.20
	Shoot biomass	$y \sim \text{SW} + \text{RE} + \text{F} + \text{SP} + \text{RE} \times \text{F} + \text{RE} \times \text{SP}$	239	7	7.1	0.31	0.22
	Fine root biomass	$y \sim \text{SW} + \text{RE} + \text{F} + \text{SP} + \text{RE} \times \text{F} + \text{F} \times \text{SP}$	146	7	11.8	0.17	0.16
<i>Olea europaea</i>	Emergence rate	$y \sim \text{F} + \text{SP} + \text{F} \times \text{SP}$	11,668	5	12.5		
	Survival rate	$y \sim \text{F}$	1015	2	5.1		
	Survival—first year	$y \sim \text{SP} + \text{F}$	808	3	3.1	0.33	0.13
	Survival—second year	$y \sim \text{SP} + \text{F}$	636	3	12.3	0.44	0.23
	Survival—third year	$y \sim \text{F}$	1015	2	3.2	0.31	0.20
	Shoot biomass	$y \sim \text{B} + \text{SP}$	146	3	17.2	0.34	0.29
	Fine root biomass	$y \sim \text{B} + \text{SP}$	142	3	13.9	0.35	0.26

Note: Models are mixed-effects Cox proportional hazards models for emergence and survival rate, and GLMMs for the other variables. Model fit of Cox proportional hazards models was measured using root mean squared error (RMSE) and mean absolute error (MSA): *Q. suber* emergence: 0.50 and 0.50; *Q. suber* survival: 0.56 and 0.53; *O. europaea* emergence: 0.55 and 0.54; *O. europaea* survival: 0.46 and 0.43.

3.3 | Seedling survival

Survival at the end of the experiment varied from 56% in *Q. canariensis* to 20% in *O. europaea*, with *Q. suber* showing intermediate values (47% in the closed forest, 37% in the open woodland). Most mortality occurred after the first summer, particularly at the open woodland (Figure 5). Best models of survival included the effect of rainfall exclusion for the two *Quercus* species, but not for *O. europaea* (Tables 1 and 2). Effects of rainfall exclusion on survival of *Q. canariensis* were consistently positive along the study period, final survival being 20% higher under rainfall exclusion than in control plots (0.64 vs. 0.51, Figure 5b). Effects on *Q. suber* were, however, more complex and modulated by both the fungicide and the tree canopy treatment at the two sites (i.e. best models included Rainfall exclusion × Fungicide and Rainfall exclusion × Canopy species interactions; Tables 1 and 2). At the closed forest, rainfall exclusion effects on first- and second-year survival of *Q. suber* varied from slightly negative with fungicide to largely positive without the fungicide (Figure 4b), particularly under conspecific canopies (Figure 6a). Larger benefits of drought without fungicide (i.e. with natural pathogen populations) than with fungicide remained along most of the study (Figure 5a). In the open woodland, rainfall exclusion effects on *Q. suber* survival were also modulated by the identity of the canopy tree (Table 2), being positive only under heterospecific *O. europaea* canopies (Figure 6c).

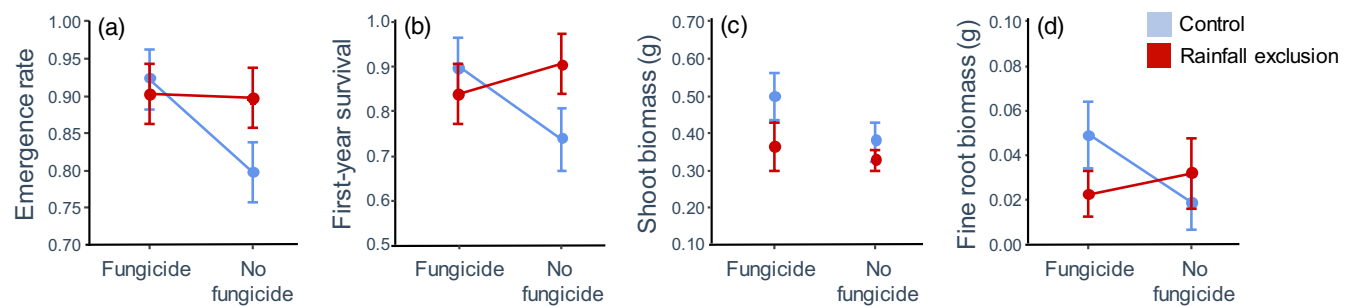
The application of oomycete-specific fungicide had positive effects on survival in the four species-site combinations (Tables 1 and

2, Figure 5). Fungicide was included in 50% of all best survival models and increased final survival of the four species, particularly *O. europaea* (32% increase, Figure 5d). Positive fungicide effects were modulated by the identity of the canopy tree in the closed forest (Fungicide × Canopy species interactions, Table 1), being particularly large under *Q. suber* trees (Figure S5a,b).

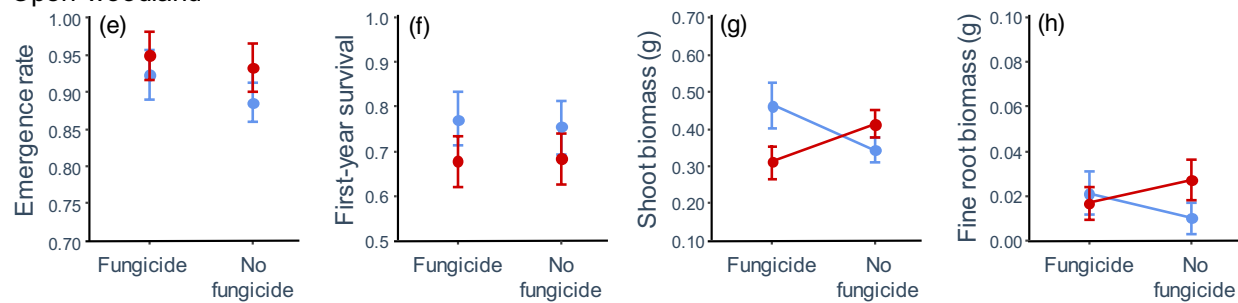
3.4 | Shoot and root biomass

Best biomass models of the two oak species included the effect of rainfall exclusion, whereas only best models of *Q. suber* biomass included the effect of fungicide (Tables 1 and 2). Biomass of *O. europaea* seedlings was not affected but any of the two experimental treatments (Table 2). As for survival, effects of rainfall exclusion on biomass were more complex for *Q. suber* than for *Q. canariensis*. Rainfall exclusion increased the fine root biomass of *Q. canariensis* seedlings by 10% (0.076 ± 0.001 g vs. 0.068 ± 0.001 g), but it had no effect on shoot biomass (Table 1). Rainfall exclusion effects on *Q. suber* biomass were strongly modulated by both the fungicide treatment and the canopy species (Tables 1 and 2). Rainfall exclusion effects on shoot and root biomass of *Q. suber* seedlings varied from mostly negative with fungicide to positive without fungicide at the two sites (Rainfall exclusion × Fungicide interaction; Figure 4d,g,h). Rainfall exclusion effects on *Q. suber* shoot biomass also varied from negative to positive among coexisting canopy species (Figure 6b,d).

Closed forest



Open woodland



Fungicide treatment

FIGURE 4 Effects of rainfall exclusion and fungicide application on emergence rate (a, e), first-year survival (b, f), shoot biomass (c, g) and fine root biomass (d, h) of *Quercus suber* seedlings at the two study sites. Values represent mean \pm SE. Lines linking fungicide levels are shown only for variables for which best models included the interaction between rainfall exclusion and fungicide application (see Tables 1 and 2).

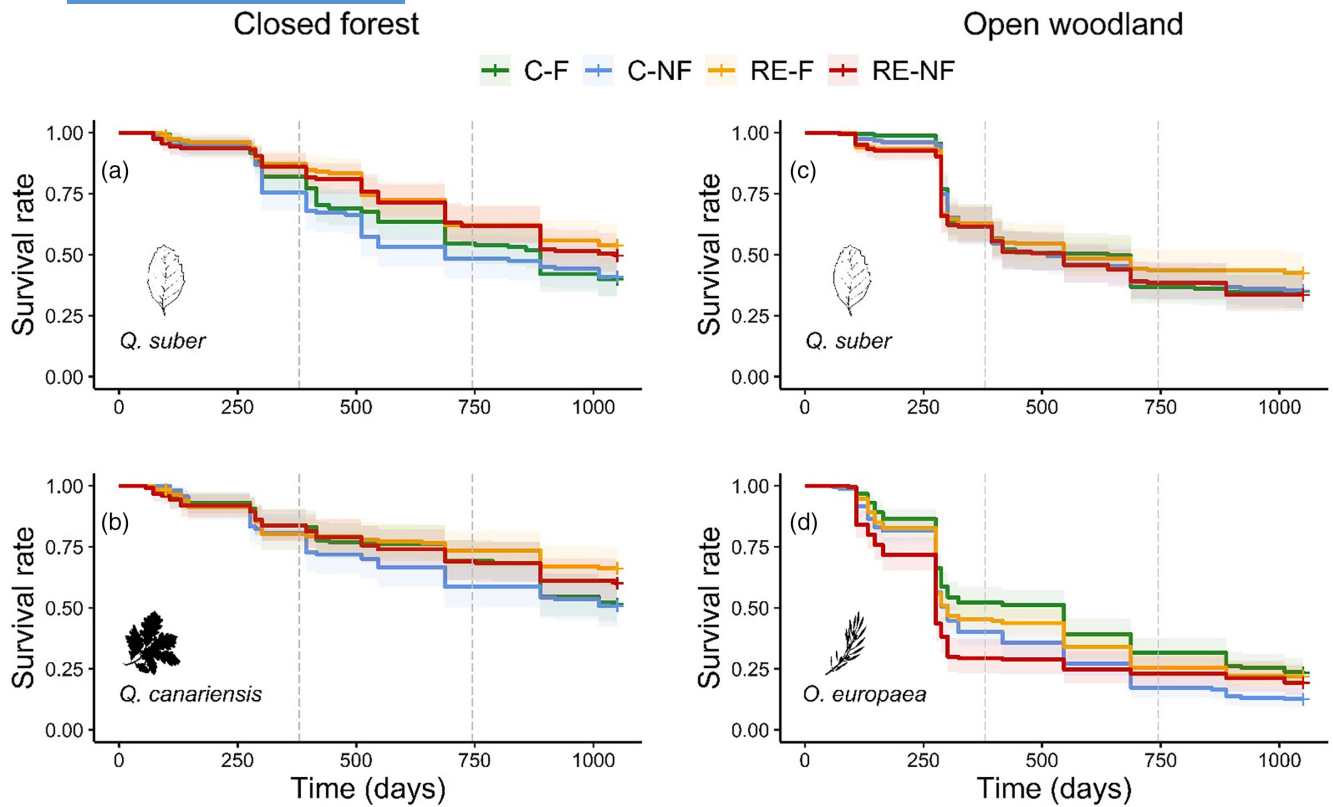


FIGURE 5 Survival curves of *Quercus suber* (a) and *Quercus canariensis* (b) in the closed forest, and of *Quercus suber* (c) and *Olea europaea* (d) in the open woodland. Survival is given separately for each of the four experimental treatment combinations (RE, rainfall exclusion; C, control; F, with fungicide; NF, without fungicide). Dashed lines separate the 3 years of study.

Closed forest

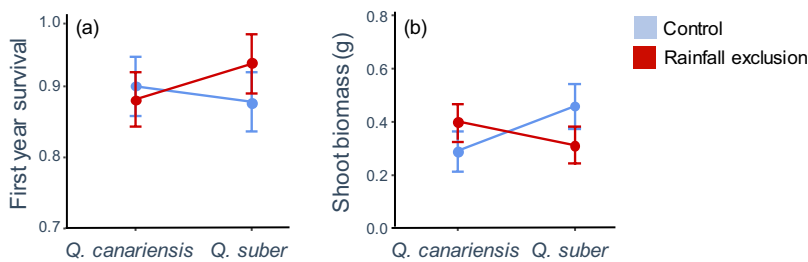
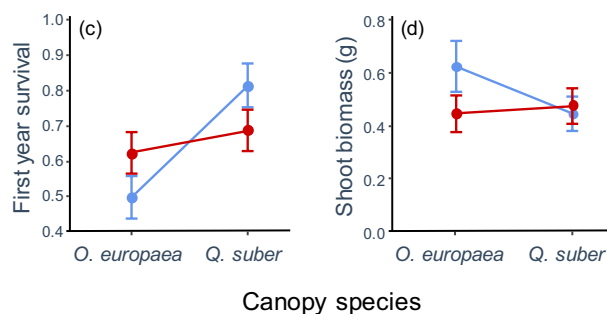


FIGURE 6 Interactive effects of rainfall exclusion and canopy species on first-year survival and shoot biomass of *Q. suber*. *Q. suber* seedlings in the closed forest (a, b) and the open woodland (c, d). Emergence and fine-root biomass are not shown because no interactions were found (see Tables 1 and 2). Values represent mean \pm SE.

Open woodland



4 | DISCUSSION

In the last decade, rainfall exclusion experiments have become a very popular method to experimentally address climate change impacts on plant communities under natural conditions (Hoover

et al., 2018). Despite the many rainout shelters established throughout the world, the number of studies that have analysed their impact on regeneration dynamics is still very limited, and only a handful have explored the interacting effects of increasing drought with other global change drivers (Classen et al., 2010; Lloret et al., 2009).

Here we aimed to fill this gap by providing new evidence from a rainfall exclusion experiment that allowed exploring not only direct impacts of climate change on regeneration, but also indirect impacts mediated by soil-borne pathogens. Interestingly, our results suggest that rainfall reductions predicted by climate change models for the Mediterranean region might have minor direct negative effects on early regeneration of tree species, but that could play a major indirect role by limiting the negative effects of soil-borne pathogens on susceptible tree species.

4.1 | Individual and interactive effects of drought and soil-borne pathogens on tree regeneration

We predicted negative effects of rainfall exclusion on regeneration, since seedling establishment in Mediterranean-type ecosystems is severely limited by summer drought (Gómez-Aparicio, 2008; Mendoza et al., 2009). However, we found poor empirical support for this prediction, with positive drought effects dominating over negative effects in the tree community. Moreover, a main result of this study is that most effects of a drier climate on the dominant species (*Q. suber*) were not direct, but indirectly mediated by soil-borne pathogens. This conclusion is supported by the many interactive effects of rainfall exclusion and fungicide application found consistently for the emergence, survival and growth (both above- and below-ground) of *Q. suber* seedlings in the two forest types. Thus, whereas in plots treated with fungicide rainfall exclusion had mostly (minor) negative effects on seedling performance, these effects became largely positive under natural conditions of pathogen abundance in the soil. These results matched a scenario of qualitative antagonistic effects (Figure 1d) where, in a forest invaded by *P. cinnamomi*, seedlings of tree species highly susceptible to the pathogen (as *Q. suber*) would perform better under drought. This is because the benefits of a lower pathogen abundance would overcompensate any potential direct negative effect of lower soil moisture on seedling performance. In fact, it has been proven that the rainfall exclusion treatment caused reductions of *P. cinnamomi* abundance in the soil as large as 30%, likely due to negative effects of lower soil moisture on the dispersal and infective capacity of the pathogen (Serrano et al., 2022). Antagonistic effects of climate change and exotic pathogens have been suggested before under controlled conditions (Homet et al., 2019), but rarely proved under natural field conditions.

Our findings also suggest that interactive effects of increasing drought and exotic pathogens might be more relevant for species with at least moderate susceptibility to both stressors (as *Q. suber*), than for species relatively resistant to one of them (either to exotic pathogens as *Q. canariensis*, or to drought as *O. europaea*). The relatively low susceptibility of *Q. canariensis* to *P. cinnamomi* agrees with the modest positive fungicide effects detected for this species. However, it was the only species for which consistent negative drought effects were found, specifically on seedling emergence,

likely due to its requirement of high moisture levels for successful seed germination (Pérez-Ramos & Marañón, 2009). On the other hand, *O. europaea* was the only species unaffected by the experimental drought, supporting previous studies that have shown species of the *Oleaceae* family to be more tolerant of rainfall reductions than coexisting *Quercus* species (Liu et al., 2018). However, *O. europaea* showed a large increase in emergence (39%) and survival (32%) in response to the fungicide treatment, corroborating the susceptibility of the species during early ontogenetic stages not only to *P. cinnamomi* (Gómez et al., 2020; Serrano et al., 2021), but also to other oomycete pathogens (Dominguez-Begines et al., 2020). Overall, our results suggest that both additive and non-additive (antagonistic) effects of abiotic and biotic global change drivers can coexist in a tree community composed by species differing in their relative sensitivity to these drivers.

4.2 | Variation among canopy species in the effect of drought and soil-borne pathogens on tree regeneration

In this study we explored whether the impact of rainfall exclusion and soil-borne pathogens on tree regeneration differed under the canopy of coexisting tree species. If so, then some tree species could dampen the impacts of global change stressors on regeneration in mixed forests. This is a relevant question for our understanding of the multiple mechanisms that make mixed forests more resistant and resilient to droughts and pests than monospecific stands (Bravo-Oviedo et al., 2018; Pardos et al., 2021). Our results provide some support for our hypothesis of differential impacts among canopy species, as shown by the several best models that included the interaction among experimental treatments (rainfall exclusion or fungicide application) and the canopy tree species. However, these interactions were mostly found for *Q. suber* seedlings, and the patterns and potential mechanisms implied were quite site specific. In the closed forest, the benefits of both rainfall exclusion and fungicide application on survival of *Q. suber* seedlings were larger under conspecific adults, probably reflecting a major role of soil-borne pathogens for establishment limitation under conspecifics, in agreement with the Janzen–Connell hypothesis (Connell, 1971; Janzen, 1970). In the open woodland, on the contrary, positive rainfall reduction effects on *Q. suber* survival occurred mostly under heterospecific *O. europaea* canopies. Because soils of *O. europaea* trees have much lower abundance of *P. cinnamomi* than soils of *Q. suber* trees (Gómez-Aparicio et al., 2012), it is unlikely that the benefits of drought in this site were also mediated by pathogens. Instead, we propose that drought benefits under *O. europaea* were due to a lower probability of waterlogging, since *O. europaea* trees are frequently associated with clayey soil patches with poor drainage. In these soils, temporal waterlogging might reduce *Q. suber* emergence and survival by limiting oxygen supply and radicle development (Gómez-Aparicio, Pérez-Ramos, et al., 2008; Urbietta et al., 2008). Overall, and independently of

the underlying mechanisms, our results suggest that the impacts of global change drivers on regeneration can differ substantially at a local scale among coexisting canopy species. This finding implies not only that the spatial pattern of seedling establishment in the forest understorey might be altered under global change, but also that mixed forests might offer a larger range of possible safe-sites for recruitment under changing environmental conditions than monospecific forests.

4.3 | Implications for tree regeneration dynamics under global change

Overall, our results suggest that in Mediterranean forests invaded by exotic soil-borne pathogens, pathogens represent a more relevant threat for tree regeneration than moderate rainfall reductions due to climate change. However, it is important to recognize that the minor negative effects of the experimental drought applied in this study might be related to the fact that it mainly affected soil moisture during the wet season. During the study years, the dry season without any rain lasted as much as 5 months (June–October), which implies that all seedlings were exposed to similar low soil moisture levels during the critical hot summers. We suggest that other components of climate change, such as a higher frequency of extreme climatic events, might have larger impacts on regeneration of Mediterranean systems than a moderate rainfall reduction (Matías et al., 2012). Further regeneration studies that take advantage of the long-term vocation of rainfall exclusion infrastructures should be conducted, since they might provide valuable insights into the combined effects of both experimental moderate droughts and natural extreme droughts (Liu et al., 2015).

Beyond regeneration, our findings have relevant general implications for our understanding of tree demography under global change. On the one hand, they imply that, at least in some situations, forests could be less susceptible to the combined effects of global change drivers than to their individual effects. This type of antagonistic interactions among drivers are virtually absent from the global change literature, where mostly additive or synergistic effects are reported (Ma et al., 2020; Ogle et al., 2021; Song et al., 2019). On the other hand, results from this study are relevant for the hot debate of how increasing drought and biotic disturbances might interact to affect forest health. A frequently repeated message in the literature is that increasing drought will amplify insect and pathogen epidemics that cause tree mortality (Anderegg et al., 2015; McDowell et al., 2020). However, our results illustrate that dampening effects can also be important, particularly in systems dominated by species highly susceptible to aggressive soil-borne pathogens as those belonging to the genus *Phytophthora*. We therefore support the view that synergies among ecosystem stressors might be over-emphasized in the ecological literature (Côté et al., 2016), and highlight that antagonisms should be recognized as important forces that might slow down the loss of tree health under global change.

AUTHOR CONTRIBUTIONS

Lorena Gómez-Aparicio conceived the ideas and designed the methodology. Pablo Homet led the field work, with the help of all coauthors. Data analysis was conducted by Pablo Homet, assisted closely by Lorena Gómez-Aparicio. Pablo Homet and Lorena Gómez-Aparicio led the writing of the manuscript, and Luis Matías and Oscar Godoy substantially contributed to the subsequent drafts.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have a conflict of interest related to the work and information presented in this manuscript. Lorena Gómez-Aparicio is an Associate Editor of Journal of Ecology, but took no part in the peer review and decision-making processes for this paper.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14227>.

DATA AVAILABILITY STATEMENT

Data used for statistical analyses are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.wstqjq2t3> (Gómez-Aparicio et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Average values (mean ± SE) and results of the linear models testing differences between control and rainfall exclusion plots

before the start of the exclusion treatment in tree density (ind/ha), basal area (m²/ha), tree diameter (dbh, in cm), tree height (m) and light availability in the understorey (Global Site Factor).

Table S2. Results of the linear mixed models that test the differences in soil moisture between the control and rainfall exclusion plots before the installation of the rainfall reduction structure at both sites. Block was included as fixed factor to control for spatial autocorrelation in the experimental design, whereas month was included as a random factor to control for temporal autocorrelation. Texture was included as a covariable in all models. Df show the degrees of freedom of the numerator and denominator.

Table S3. Results of the linear mixed models testing the effect of the rainfall exclusion treatment on soil moisture at the two study sites. Spatial and temporal autocorrelation was controlled by including block and year as fixed factors (due to its reduced number of levels) and month as a random factor. Texture was included as a covariable in all models. Df show the degrees of freedom of the numerator and denominator.

Figure S1. (A) Location of Los Alcornocales Natural Park (red rectangle) in the south of the Iberian Peninsula. (B) Location of the two study sites within the natural park (1: closed forest of *Quercus suber* and *Quercus canariensis*, 2: open woodland of *Quercus suber* and *Olea europaea*).

Figure S2. Experimental design of the rainfall exclusion infrastructure installed at each of the two forest sites. Photographs below the figure show control (left) and rainfall exclusion plots (right).

Figure S3. (A) Daily precipitation at the closed forest site during the study period; (B) Soil water content (SWC) at different soil depths (10, 20, 30 and 40 cm) in rainfall exclusion and control plots (mean ± SE) at the closed forest. Vertical grey lines separate different years. The rainfall exclusion infrastructure was built and start working on March 2016.

Figure S4. (A) Daily precipitation at the open woodland during the study period; (B) Soil water content (SWC) at different soil depths (10, 20, 30 and 40 cm) in rainfall exclusion and control plots (mean ± SE) at the open woodland. Vertical grey lines separate different years. The rainfall exclusion infrastructure was built and start working on March 2016.

Figure S5. Interactive effects between fungicide application and canopy species on first-year survival of (a) *Quercus suber* (b) and *Q. canariensis* seedlings in the closed forest, and of (c) fine root biomass of *Q. suber* seedlings and (d) emergence rate of *O. europaea* seedlings in the open woodland. Only performance variables for which the Fungicide × Canopy species interaction was included in the best model (Tables 1 and 2) are represented.

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