Efectos de la interacción del cambio climático y los patógenos exóticos sobre las comunidades bióticas y los procesos ecosistémicos de bosques mediterráneos

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Tesis Doctoral

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A mis padres, a mi hermana,

a María del Mar.

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Resumen general

Los bosques mediterráneos son conocidos por albergar una elevada biodiversidad además de tener una gran importancia ecológica, económica y social. Desafortunadamente, están siendo afectados severamente por distintos motores de cambio global como son el cambio climático o las especies invasoras. Entre las especies invasoras que afectan a estos bosques, los patógenos de raíz cada vez tienen más importancia, produciendo importantes cambios en su funcionamiento. Históricamente, el impacto de los distintos motores de cambio global se ha estudiado de manera aislada. Sin embargo, éstos generalmente actúan de manera simultánea pudiendo producir unos efectos distintos a los que podrían generar cada uno de ellos de manera aislada.

Uno de los patógenos exóticos que causan más daños a los bosques mediterráneos es *Phytophthora cinnamomi* Rands. Este patógeno se ha extendido en las últimas décadas por la región Mediterránea, y junto con las sequías recurrentes, cada vez más frecuentes en la región, está afectando dramáticamente a los bosques y dehesas atacando principalmente a los árboles del género *Quercus*. *P. cinnamomi* es muy dependiente de la humedad del suelo para su propagación e infección, por lo que el descenso de las precipitaciones inducido por el cambio climático podría alterar el impacto de este organismo sobre los ecosistemas forestales. El impacto combinado de *P. cinnamomi* y el descenso de las precipitaciones sobre el arbolado se podría presuponer que será aditivo (la fuerza de ambos factores se suma para debilitar al árbol) o sinérgica (los daños sobre los árboles se multiplican al entrar en juego ambos factores), aunque no se puede descartar un efecto negativo de la reducción de precipitaciones sobre los patógenos debido a su dependencia de la humedad.

Con el desarrollo de esta tesis hemos tratado de conocer los efectos combinados del descenso de las precipitaciones y la infección de *P. cinnamomi* sobre una serie de variables clave en los bosques relacionadas con la demografía y regeneración de la comunidad de especies arbóreas (capítulos 1 y 2), y los organismos edáficos y la dinámica del ciclo de nutrientes (capítulos 3 y 4). Para ello utilizamos distintas metodologías experimentales tanto en invernadero (capítulo1) como en el campo, utilizando estructuras de exclusión de lluvia (capítulos 2,3 y 4).

En el **primer capítulo** desarrollamos un experimento de invernadero con plántulas de alcornoque en el que cruzamos 4 niveles de humedad del suelo y 4 niveles de densidad de *P. cinnamomi* asociados a escenarios reales de cambio climático e invasión del patógeno. De esta manera se exploró el efecto interactivo del cambio climático y *P. cinnamomi* sobre el crecimiento y la fisiología de las plántulas, analizando el efecto combinado de distintos niveles de sequía y de infección del patógeno. Los resultados mostraron cierta sensibilidad del patógeno a la sequía, reduciéndose la capacidad infectiva de éste ante las reducciones previstas en la humedad del suelo, haciendo necesaria una infección muy severa por parte del patógeno para causar síntomas graves bajo escenarios de reducción moderada de la humedad.

En el **capítulo 2**, se exploró como afecta la acción combinada del patógeno y el cambio climático a la regeneración del bosque, proceso fundamental para su conservación. Para ello llevamos a cabo un experimento de siembra bajo la estructura de exclusión de lluvia en el Parque Natural de Los Alcornocales (Cádiz). Periódicamente se añadió un tratamiento de fungicida específico para oomicetos a la mitad de las semillas (bellotas y semillas de acebuche) para determinar cómo afectan estos organismos a la regeneración del bosque en un escenario de reducción de precipitaciones. En línea con lo demostrado en el capítulo anterior, se comprobó que en determinados casos la reducción de la precipitación mejoró la emergencia y supervivencia de igual o mayor modo que la aplicación del fungicida. De esta manera podemos confirmar un efecto indirecto positivo de la reducción de la precipitación sobre la regeneración, mediado por su relación antagónica con el patógeno.

En el **capítulo 3**, se realizó un experimento de descomposición de hojarasca bajo la estructura de exclusión de lluvia poniendo el foco principalmente en la mesofauna descomponedora (ácaros y colémbolos) y cómo estos organismos modulan los efectos del cambio climático. Los resultados mostraron una importante función de estos organismos en la descomposición de la hojarasca, siendo capaces de compensar los efectos negativos que provocó la disminución de la humedad asociado al descenso en

las precipitaciones. En este caso se volvió a poner de manifiesto la importancia de los efectos indirectos del cambio climático, pero esta vez a través de su influencia en la fauna edáfica.

En el **capítulo 4** examinamos los efectos de la sequía sobre las redes tróficas edáficas usando la comunidad de nematodos como modelo. En este trabajo, se tomaron muestras en la estructura de exclusión de lluvia del P.N de Los Alcornocales, así como de otra localizada en sur de Francia (Puechabon), que lleva 13 años con el mismo tratamiento experimental utilizado en nuestras parcelas de estudio. De esta manera, además de evaluar la respuesta a corto plazo a la reducción de las precipitaciones que pudieron tener estos organismos, pudimos evaluar si esos cambios fueron consistentes a largo plazo en otros bosques. En este caso, encontramos que la sequía es capaz de producir efectos muy rápidos en la comunidad de nematodos (vistos a los dos años de aplicar el tratamiento), los cuales también se detectaro a largo plazo afectando a la estructura de las redes tróficas del suelo y su papel en el funcionamiento del mismo.

La presente tesis aporta una explicación mecanicista sobre cómo el descenso de las precipitaciones asociado al cambio climático puede influir en los procesos ecológicos que ocurren en los bosques mediterráneos, tanto directa como indirectamente. Estos estudios nos muestran cómo se producen efectos indirectos de la reducción de la precipitación sobre la regeneración y salud de las plántulas a través de su interacción con el *P. cinnamomi*. También observamos cómo el cambio climático puede actuar sobre la fauna del suelo, afectando con ello de manera indirecta el funcionamiento del ecosistema. Por tanto, esta tesis pone de manifiesto la importancia del estudio de los efectos indirectos del cambio climático que, si bien pueden ser más difíciles de cuantificar que los directos, tienen tanta o más importancia en el funcionamiento de los ecosistemas.

Introducción general

Los bosques mediterráneos frente al cambio climático.

El cambio climático ha dejado de ser una amenaza futura para pasar a ser una preocupante realidad en las últimas décadas que está alterando numerosos ecosistemas a lo largo de todo el planeta (Stocker et al., 2013). Este proceso se debe, aunque no exclusivamente, principalmente al incremento de emisiones de gases de efecto invernadero, siendo el hombre sin duda su principal responsable (Christidis et al., 2011; Fischer & Knutti, 2015). Las sucesivas llamadas de alerta de la comunidad científica y los intentos de las instituciones por llegar a acuerdos para mitigar las emisiones de gases no han evitado que a día de hoy la temperatura del planeta se haya incrementado en aproximadamente 1.5 ºC desde finales de siglo XIX. Lo más preocupante de la situación es que a pesar de todos los esfuerzos, esta tendencia sigue al alza, ya que el principal incremento de la temperatura se ha producido en los últimos 40 años (Jia et al., 2019).

Además del incremento en la temperatura, el cambio climático está produciendo cambios en los patrones de precipitación a escala global. Sin embargo, estos cambios en los patrones de precipitaciones son variables a lo largo del planeta: mientras que en ciertas regiones la tendencia es a aumentar las precipitaciones (p. ej. las precipitaciones asociadas a monzones tropicales), en las regiones subtropicales y templadas la tendencia es hacia un descenso claro en las precipitaciones (Jia et al., 2019). La frecuencia de eventos extremos (lluvias torrenciales y sequías, así como olas de calor) también está aumentando (Rahmstorf & Coumou, 2011; Sippel & Otto, 2014). Por tanto, estamos ante un proceso con una elevada heterogeneidad espacial y temporal (Lindner et al., 2010; Stocker et al., 2013; Fischer & Knutti, 2015).

La magnitud que tenga el cambio climático en una zona concreta dependerá de una serie de características específicas de esa región, como su localización y accidentes geográficos o el clima preexistente. La cuenca Mediterránea se caracteriza por ser una zona de transición entre el clima árido norteafricano y el clima templado centroeuropeo. Debido a ello, pequeños cambios en la circulación atmosférica pueden suponer cambios sustanciales en su clima (Giorgi & Lionello, 2008). Sabemos que esta región ha sufrido importantes cambios en su clima en el pasado (Luterbacher et al., 2006) y actualmente está considerada como uno de los “puntos calientes” en las predicciones de cambio climático (Diffenbaugh & Giorgi, 2012). En esta región, aunque se prevén cambios durante todo el año, las alteraciones climáticas más importantes se espera que estén concentradas durante los meses de verano (IPCC, 2021). El aumento de la temperatura esperado durante estos meses llega a los 4.6 ºC en el período 2081-2100, acompañado de una reducción de la precipitación del 28% en el mismo periodo con respecto al promedio 1961-1990 (Giorgi & Lionello, 2008). Esta reducción va a afectar no solo a la cantidad de lluvia caída sino también a la cantidad de días con precipitaciones (Polade et al., 2014). Debido a la característica aridez estival del clima mediterráneo, este incremento en la intensidad y duración de la sequía estival puede suponer un grave problema para los organismos que habitan en esta región (Guiot & Cramer, 2016), considerada como un “punto caliente” también de biodiversidad (Cuttelod et al., 2008).

El clima es un factor determinante de la distribución y ecología de los organismos (Thomas, 2010). Teniendo en cuenta que los ecosistemas mediterráneos son bastante vulnerables al cambio climático por las características descritas anteriormente, y que muchas de las especies que en ella habitan se encuentran en el límite árido de su margen de distribución, es lógico pensar que el cambio climático va a tener un fuerte impacto en la biodiversidad mediterránea. En particular, la vegetación de esta región está limitada mayoritariamente por la disponibilidad de agua, sobre todo los bosques y formaciones forestales (Archibold, 1995), y el incremento de la aridez que trae consigo el cambio climático reduce esta disponibilidad poniendo en peligro la permanencia de estos ecosistemas (Myers et al., 2000; Cramer et al., 2018; Peñuelas et al., 2018). Por tanto, los bosques mediterráneos son uno de los ecosistemas más amenazados de una de las regiones más amenazadas por el cambio climático. Debido a la gran diversidad que albergan, su importancia tanto ecológica como social y su potencial vulnerabilidad al cambio climático, el estudio de la respuesta de los ecosistemas forestales mediterráneos a los cambios en el clima constituye una línea de investigación de especial relevancia.

Patógenos exóticos. El caso de *Phytophthora cinnamomi* y su papel en los bosques mediterráneos.

A pesar de su gran importancia, el cambio climático es solo uno de los motores de cambio global que afectan a la región mediterránea y a sus bosques. La región mediterránea es además especialmente vulnerable a las especies invasoras, y su impacto sobre los ecosistemas terrestres va en aumento (Vilá et al., 2008). El contexto histórico de la zona, la milenaria presencia humana en la región y el tránsito e intercambio comercial han favorecido las invasiones biológicas en el pasado y el actual proceso de globalización está acrecentando esta problemática (Garbelotto & Pautasso, 2012).

Dentro de los organismos invasores, en las últimas décadas se está incrementando la importancia de los patógenos como agentes causantes de enfermedades emergentes en bosques de todo el mundo (Santini et al., 2013). De hecho, los patógenos invasores se están convirtiendo en factores fundamentales para muchas especies vegetales en zonas donde hasta ahora apenas tenían importancia. En la cuenca mediterránea, el estrés hídrico siempre ha sido el factor fundamental que controla el crecimiento y la supervivencia de las plantas. Sin embargo, la proliferación de patógenos exóticos está ganando importancia frente al estrés hídrico en las dinámicas poblacionales de las especies arbóreas, provocando fenómenos de decaimiento forestal que afectan a numerosas especies (Garbelotto & Pautasso, 2012; Camilo-Alves et al., 2013).

Uno de los patógenos exóticos que está alterando drásticamente los bosques mediterráneos es el oomiceto edáfico *Phytophthora cinnamomi* Rhands. Perteneciente a un género que incluye a algunos de los patógenos exóticos más agresivos en plantas (Jung et al., 2016, 2018), ataca las raíces de sus hospedadores reduciendo la capacidad de absorción de agua y nutrientes y provocando efectos similares a los producidos por las sequías (Hardham & Blackman, 2018). De hecho, está considerado como una de las 100 peores especies invasoras del mundo (Burgess et al., 2017). Afecta a más de 5000 especies vegetales por todo el planeta (Hardham & Blackman, 2018), siendo un auténtico problema en algunos casos para la conservación de los espacios naturales. Por ejemplo, en Australia, donde gran parte de su vegetación es susceptible, este patógeno ha estado a punto de provocar la extinción de al menos una especie de eucalipto (*Eucalyptus marginata*) (Shearer et al., 2007) y es una grave amenaza para su flora, por lo que el gobierno del país ha desarrollado un plan nacional para el control de esta enfermedad. En la cuenca mediterránea, este oomiceto patógeno está presente al menos desde los años 90 (Brasier, 1992, 2001), asociándose con el decaimiento forestal, principalmente de especies del género *Quercus* (Fig. 1).

Figura 1. Ejemplares de *Quercus suber* mostrando síntomas de decaimiento. Fotografía: Lorena Gómez Aparicio.

El decaimiento forestal es un proceso complejo y global por el cual las masas forestales ven reducida su densidad y vigor, conduciendo a la muerte de ejemplares, que puede llegar a ser masiva, y produciendo grandes cambios en la dinámica de los bosques. En los bosques y dehesas del centro y sur de la Península Ibérica, este decaimiento lo sufren principalmente *Quercus ilex* L. y *Q. suber* L. (Brasier, 1992; Sánchez et al., 2002; Camilo-Alves et al., 2013;) dos especies de hoja perenne altamente adaptadas al clima mediterráneo. Este proceso, conocido comúnmente como “la seca”, ha estado históricamente ligado a otros factores como el cambio climático y plagas de insectos, pero en los años 90 ya se detectó que *P. cinnamomi* podía estar implicada en él (Brasier, 1992) y actualmente se considera su principal causante (Tuset & Sánchez, 2004; Sánchez et al., 2006). El decaimiento forestal está modificando el paisaje mediterráneo drásticamente (Camilo-Alves et al., 2013), alterando la funcionalidad de los bosques dominados por estas especies de *Quercus*, pudiendo afectar a procesos fundamentales como la regeneración (Ibáñez et al.,2015a, 2017; Domínguez-Begines et al., 2020), el ciclo de nutrientes (Ávila et al., 2016, 2021), y la biodiversidad y estructura trófica del suelo (Ibáñez et al., 2015b; Domínguez-Begines et al., 2019; Gómez-Aparicio et al., 2022), por lo que su capacidad de alterar la funcionalidad de los ecosistemas está más que demostrada.

***Phytophthora cinnamomi* y cambio climático. Posibles interacciones entre dos motores de cambio global.**

Cuando hablamos de cambio climático y especies invasoras, solo estamos hablando de partes individuales de un todo, el cambio global. El concepto de cambio global se refiere al conjunto de cambios en el medio producidos principalmente por la actuación del hombre a escala global y del que forman parte, entre otros, los cambios de usos del suelo, las invasiones biológicas, la nitrificación, el incremento de la polución, la acidificación de los océanos y los cambios en los ciclos de nutrientes (Vitousek et al., 1997). En muchos casos, varios de estos motores de cambio global pueden afectar simultáneamente a un mismo ecosistema, y su capacidad de interactuar puede provocar unos efectos difíciles de predecir si los estudiamos de manera aislada (Sala et al., 2000; Matesanz et al., 2009). Sin embargo, estas interacciones pueden resultar complejas, implicando numerosos efectos directos e indirectos sobre el escosistema.

En el caso concreto del patógeno exótico *P. cinnamomi*, su capacidad de multiplicación, propagación e infección está altamente vinculada a la humedad del suelo (Erwin & Ribeiro, 1996). Por lo tanto, su relación con el cambio climático es de gran interés, y cabría esperar que éste influyera en gran medida sobre su efecto sobre la vegetación. La dependencia de *P. cinnamomi* de la humedad del suelo permite predecir un efecto directo negativo de la disminución de las precipitaciones sobre el oomiceto. Esta disminución de las precipitaciones se traducirá en una reducción de la humedad del suelo provocando unas condiciones subóptimas para el patógeno, que al reducir su capacidad para propagarse reducirá también su capacidad para infectar y afectar a la salud de los árboles, ya que su efecto es muy dependiente de su abundancia (Gómez-Aparicio et al., 2012). Sin embargo, los efectos indirectos que puede producir el cambio climático sobre *P. cinnamomi*, y el modo en que pueden interactuar ambos factores sobre la salud de los árboles, no es tan fácil de predecir al estar mucho menos estudiados. Por tanto, en función de cómo se produzca esta interacción vamos a hablar de 3 posibles escenarios de efectos sobre la vegetación (Fig. 2):

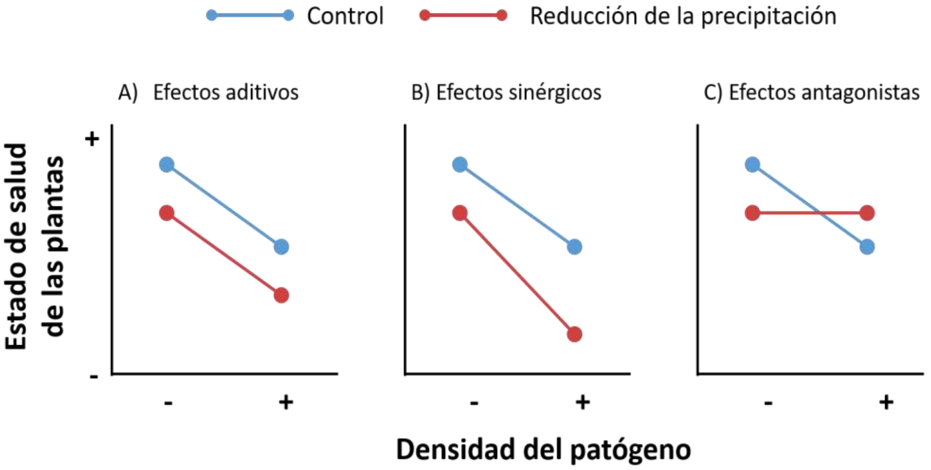

Figura 2. Posibles escenarios de interacción entre los patógenos exóticos y la reducción de lluvia debida al cambio climático sobre el estado de salud de las plantas.

1. Efectos aditivos: Tanto la sequía como *P. cinnamomi* son dos factores de estrés que van a producir efectos negativos sobre la salud de los árboles, provocando un efecto aditivo entre ellos (los efectos negativos de uno se suman a los efectos negativos del otro) sin que se produzca interacción entre ambos motores de cambio.
2. Efectos sinérgicos: En este escenario, el efecto combinado de ambos motores de cambio sería mayor a la suma de los efectos individuales al estar el patógeno actuando sobre individuos debilitados por la sequía (Roubtsova & Bostock, 2009; Corcobado et al., 2014; Peñuelas et al., 2017).
3. Efectos antagónicos: El efecto combinado de ambos motores de cambio es menor a la suma de los efectos individuales. En este

caso, el daño del patógeno *P. cinnamomi* sobre las plantas sería menor en condiciones climáticas más secas debido a los efectos negativos de la sequía sobre el patógeno.

Es muy difícil de prever ante cuál de estos escenarios es más factible que nos encontremos en el futuro. Por tanto, se revela como urgente llevar a cabo estudios detallados que nos permitan conocer mejor los efectos interactivos de estos dos motores de cambio global, cambio climático y patógenos exóticos, sobre ecosistemas únicos como son los bosques mediterráneos.

Efecto del cambio climático y los patógenos exóticos en la regeneración del bosque y la salud de las plántulas.

La regeneración es un proceso clave en el funcionamiento del bosque por su importancia para el mantenimiento del mismo y su recuperación frente a las perturbaciones (Batllori et al., 2020). Además, se trata de un proceso durante el cual la planta atraviesa etapas de una gran vulnerabilidad. Esto se debe tanto a la mayor dependencia de las condiciones ambientales de los primeros estadios vitales (Houle, 1994; Hampe & Arroyo, 2002), como a la mayor vulnerabilidad de las plántulas a factores bióticos como los patógenos o los herbívoros (Barton & Hanley, 2013) (Fig.3). Está demostrado que tanto las futuras variaciones climáticas como los patógenos exóticos van a afectar al proceso de regeneración de los bosques Mediterráneos (Gómez-Aparicio et al., 2008; Pérez-Ramos et al., 2013; Domínguez-Begines et al., 2020; Serrano et al., 2021). Además, se ha comprobado que la manera en la que el cambio climático afectará a la regeneración variará en función de las especies vegetales y sus rasgos funcionales (Nardini et al., 2014), favoreciendo a aquellas más adaptadas a las nuevas condiciones (Lloret et al., 2004; Matías et al., 2012). Por otro lado, se ha demostrado que la susceptibilidad de las plántulas a los patógenos también varía entre las distintas especies (Moralejo et al., 2009; Jung et al., 2018) e incluso entre distintas fases vitales de una misma especie. Por ejemplo, estudios recientes han demostrado que *P. cinnamomi* puede infectar y afectar negativamente a la regeneración de especies de árboles cuyos ejemplares adultos parecen ser tolerantes a la infección del patógeno (Domínguez-Begines et al., 2020; Serrano et al., 2021). La variabilidad tanto inter-específica como intraespecífica en la susceptibilidad a los patógenos podría constituir por tanto un importante motor de cambio de la estructura y composición específica del bosque a largo plazo, favoreciendo a las especies menos susceptibles.

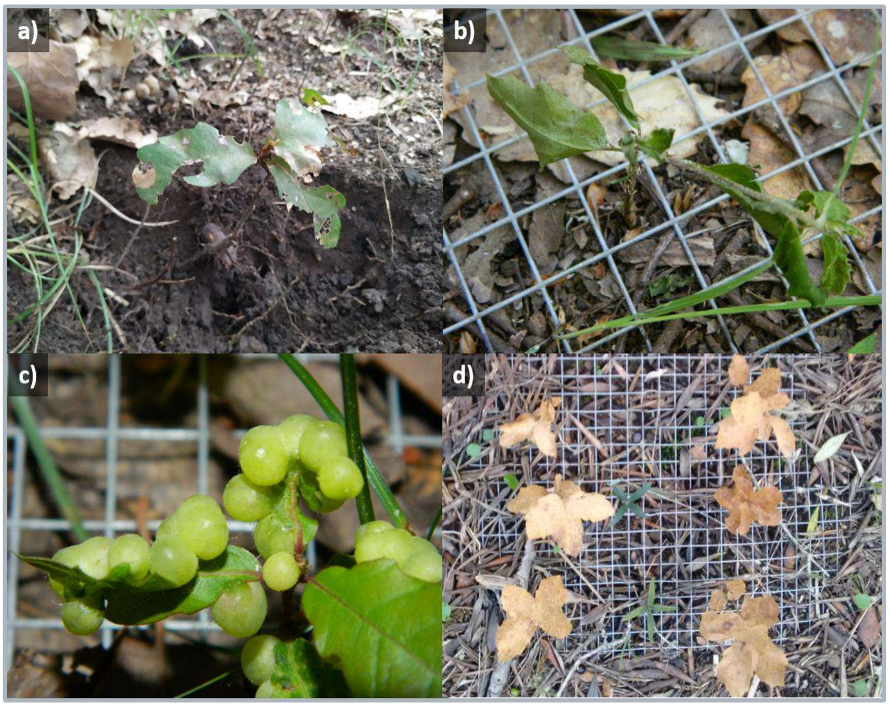

Figura 3. Ejemplos de distintos daños bióticos y abióticos sufridos por plántulas de *Quercus:* a) daños por herbívoros foliares, b) marchitamiento (damping-off) debido al efecto de patógenos edáficos, c) agallas por el ataque de un parasitoide, d) plántulas secas en verano por falta de agua. Fotografías: Pablo Homet Gutiérrez.

A pesar de su importancia en la dinámica de población de plantas, se han hecho pocos estudios que aborden el efecto combinado del cambio climático y los patógenos en la regeneración del bosque. Las posibles interacciones descritas anteriormente entre estos dos motores de cambio global (Fig. 2) podrían alterar sus efectos individuales sobre las plántulas, y tener por tanto un importante papel en la regeneración de las distintas especies determinando la estructura futura del bosque y su funcionamiento. Por tanto, creemos que es indispensable el estudio simultáneo de los efectos de estos dos motores de cambio global sobre la regeneración y demografía del bosque para tratar de conocer cuál puede ser su futuro.

Efecto de la reducción de las precipitaciones en las comunidades bióticas del suelo y los ciclos de nutrientes.

La biodiversidad del suelo, aunque suele ser menos estudiada que la macroscópica, es fundamental en el mantenimiento y funcionamiento de los ecosistemas (Bardgett & Van Der Putten, 2014). Sabemos que la respuesta de los organismos edáficos a los cambios ambientales va a depender mucho del tipo de ecosistema y el clima preexistente (Blankinship et al., 2011). Sin embargo, el estudio de los efectos del cambio climático sobre las comunidades edáficas es escaso en muchos ecosistemas (Blankinship et al., 2011; Orgiazzi et al., 2016), en especial en aquellos donde el agua es un factor limitante (Guerra et al., 2020) como son los bosques mediterráneos. En estos sistemas, los posibles efectos negativos de la reducción de las precipitaciones sobre los organismos de suelo pueden parecer evidentes, pero los efectos indirectos mediados por otros organismos de suelo o por la vegetación (Prescott & Grayston, 2013; Franco et al., 2019) pueden provocar resultados inesperados. La presencia de *P. cinnamomi* en estos bosques también podría afectar indirectamente a los organismos del suelo, aunque ante una fase temprana de la infección que apenas afecta a los árboles adultos consideramos más complicado detectar sus efectos. Por tanto, consideramos que el estudio de los cambios inducidos en la biodiversidad del suelo en respuesta a los distintos motores de cambio global en los bosques mediterráneos es muy necesario en la actualidad.

Generalmente el estudio de los efectos de cambio climático y otros motores de cambio global sobre el suelo se ha centrado en microorganismos, más concretamente en hongos y bacterias (Maestre et al., 2015; Guerra et al., 2021). Sin embargo, la biodiversidad del suelo está formada por una gran variedad de organismos entre los que se encuentran además nematodos, ácaros, colémbolos, tardígrados, amebas, etc., interactuando entre sí formando complejas redes tróficas. Estas redes tróficas pueden ser estudiadas de manera simplificada poniendo el foco en un grupo muy diverso de organismos que representa las principales funciones tróficas, como son los nematodos. Estos organismos son unos muy buenos bioindicadores del estado del suelo (Bongers & Ferris, 1999; Ferris et al., 2001; Neher, 2001). Se trata de los animales más abundantes del mundo, se encuentran prácticamente en cualquier parte del planeta (Wilson & Kakouli-Duarte, 2009), ocupan todos los niveles de la cadena trófica desde herbívoros y detritívoros hasta depredadores (Fig.4) (Yeates et al., 1993) y tienen una gran variedad de estrategias vitales que les confieren distinta sensibilidad o resistencia a cambios ambientales y perturbaciones (Ferris et al., 2001). Además, estos organismos tienen un papel fundamental en procesos de funcionamiento del suelo como los ciclos de nutrientes o la producción primaria (Ruess & Ferris, 2003; Yeates, 2003; Neher & Weicht, 2013), por lo que son un gran modelo para estudiar el efecto del cambio global en el suelo, su biodiversidad y las repercusiones en su funcionamiento. A pesar de esto, existen muy pocos estudios que muestren la respuesta de los nematodos a las condiciones climáticas predichas, y generalmente estos estudios están localizados en praderas de zonas templadas (Holmstrup et al., 2017; Franco et al., 2019; Ankrom et al., 2020), existiendo un gran vacío de conocimiento sobre la respuesta de estos organismos al cambio climático en bosques mediterráneos. Estudiar los efectos del cambio climático sobre los nematodos contribuiría mucho a la comprensión del efecto que tendrán las futuras condiciones climáticas sobre las comunidades y los procesos ecológicos que se dan en los suelos de estos bosques.

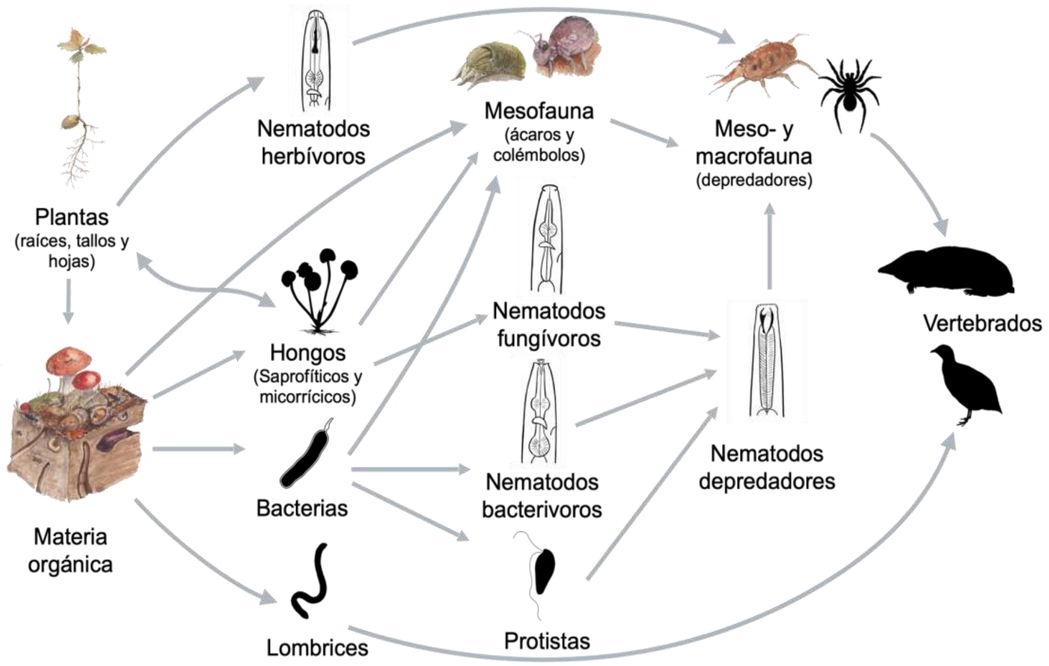

Figura 4. Esquema simplificado de una red trófica del suelo. Las flechas representan direccionalidad en las relaciones (Basado en Global Soil Biodiversity Atlas, 2019).

Uno de los procesos más importantes que se da en los suelos de los bosques es la descomposición de la hojarasca, ya que su gran aportación a la liberación de nutrientes la hace fundamental para el mantenimiento de estos ecosistemas (García-Palacios et al., 2017; Zhou et al., 2018). Además, se trata del proceso que libera mayor cantidad de carbono a la atmósfera proveniente de los ecosistemas terrestres (Prentice et al., 2001), por lo que tiene una gran importancia sobre la regulación del clima y puede inducir eventos de retroalimentación con el cambio climático. En la descomposición de la hojarasca intervienen principalmente tres factores: organismos descomponedores (García-Palacios et al., 2013; Santonja et al., 2017, 2018), características de la hojarasca (Facelli & Pickett, 1991) y condiciones ambientales (Djukic et al., 2018; Suseela & Tharayil, 2018; Qiu et al., 2020). Estos tres elementos van a tener efectos directos sobre la descomposición, pero sobre todo van a ejercer una serie de efectos indirectos unos sobre otros formando una red de interacciones complejas (Hättenschwiler & Gasser, 2005; Fanin et al., 2019; Prieto et al., 2019). El cambio climático, al modificar drásticamente las condiciones ambientales de los ecosistemas, va a alterar el proceso de descomposición y las relaciones que se dan entre los distintos componentes. Mientras varios estudios han demostrado la importancia de los efectos directos de la sequía reduciendo las tasas de descomposición (Salamanca et al., 2003; Vogel et al., 2013), los estudios que han tratado de descifrar sus efectos indirectos mediados por los organismos descomponedores o por cambios en las características de la hojarasca muestran resultados contradictorios (García-Palacios et al., 2016a, 2016b; Santonja et al., 2017; De Smedt et al., 2018; Zhou et al., 2018; Ashton et al., 2019). A pesar de las diferencias registradas hasta la fecha, los efectos indirectos del cambio climático sobre los procesos de descomposición mediados por los organismos descomponedores han demostrado tener una gran importancia, por lo que es fundamental incrementar su estudio para mejorar nuestra comprensión de estos procesos.

Manipulación climática. El valor de los experimentos de exclusión de lluvia para el estudio del cambio climático.

El estudio del cambio climático y otros factores de cambio global se puede llevar a cabo mediante diversas aproximaciones, cómo estudios de modelización, experimentos en condiciones controladas (mesocosmos e invernaderos) o estudios en condiciones naturales. Entre estos últimos encontramos trabajos observacionales a lo largo de gradientes climáticos o experimentos manipulativos del clima en los cuales se alteran algunas de las variables abióticas en un contexto lo más natural posible (Beier et al., 2012; Michelsen et al., 2012). Cada metodología tiene sus ventajas y desventajas. Por ejemplo, los estudios de modelización nos permiten dar un punto de generalidad y predecir respuestas al cambio climático de grandes áreas, perdiendo resolución en escalas espaciales más reducidas y presentando dificultades para reflejar las interacciones bióticas y abióticas que se dan en los ecosistemas. Los estudios bajo condiciones completamente controladas nos permiten manipular la intensidad de uno o varios factores al detalle aislando sus efectos, aunque sin embargo este tipo de estudios simplifican enormemente nuestra percepción de los ecosistemas.

Para reflejar mejor la complejidad biológica y mostrar una visión más real del funcionamiento de los ecosistemas son necesarios los estudios de campo. El estudio del cambio climático a través de la observación de gradientes ambientales es muy útil para conocer respuestas a largo plazo (Yuan et al., 2017), sin embargo, no es tan útil a la hora de conocer la evolución de los organismos de una zona determinada bajo unas condiciones climáticas futuras, ya que no tienen en cuenta los procesos de adaptación local. En cambio, los estudios de manipulación experimental en condiciones naturales nos permiten identificar y explicar mucho mejor los mecanismos subyacentes a la respuesta de los organismos a dichas variables (Montgomery, 2008), permitiendo conocer al detalle los efectos indirectos y directos del cambio global sobre los procesos ecológicos (Tilman, 1989; Chapin & Shaver, 1996). Estos experimentos aúnan el control de las variables abióticas con la aproximación a las condiciones naturales, permitiéndonos someter la vegetación a distintos escenarios climáticos futuros (Fay et al., 2000; Hoover et al., 2018) y conocer su respuesta en distintos sistemas, ya que se trata de una metodología fácilmente reproducible.

Los experimentos de manipulación climática son cada vez más frecuentes en los estudios del cambio climático en ecosistemas terrestres. Sin embargo, la mayor parte de estudios están basados fundamentalmente en sistemas agrícolas, herbáceos o de matorral (Beier et al., 2012), siendo escasos en bosques. Además, la mayoría de estos estudios están localizados en zonas templadas, por lo que los bosques Mediterráneos han sido escasamente estudiados con esta metodología (i.e: DroughtNet, http://www.drought-net.org) a pesar de su vulnerabilidad al cambio climático. Recientemente se está poniendo de manifiesto el interés de testar los distintos componentes de cambio global en conjunto, y no de manera aislada. Una ventaja que tienen estos experimentos de manipulación climática es que se pueden establecer en ecosistemas donde está actuando otro motor de cambio global y de esta manera testar el efecto conjunto de ambas fuentes de estrés en condiciones naturales. Por ejemplo, en sistemas invadidos por patógenos exóticos, las manipulaciones climáticas permiten estudiar la interacción entre dos componentes de cambio global en las condiciones más realistas posible.

Objetivos e hipótesis de la tesis doctoral

El objetivo principal de esta tesis doctoral es evaluar el efecto interactivo de dos importantes motores de cambio global, el cambio climático y los patógenos exóticos, sobre procesos fundamentales de los ecosistemas forestales y las comunidades bióticas que participan en ellos. Concretamente, se ha evaluado el impacto de estas dos fuentes de estrés sobre la salud de las plántulas y la regeneración de la comunidad de especies arbóreas existentes, y sobre los organismos edáficos y la descomposición de la hojarasca. Estos objetivos han sido abordados a través de una serie de estudios específicos que se desarrollan en los distintos capítulos de la tesis, cada uno encaminado a responder una serie de preguntas más concretas (Fig.5). A continuación, se detallan los objetivos específicos de los cuatro capítulos principales:

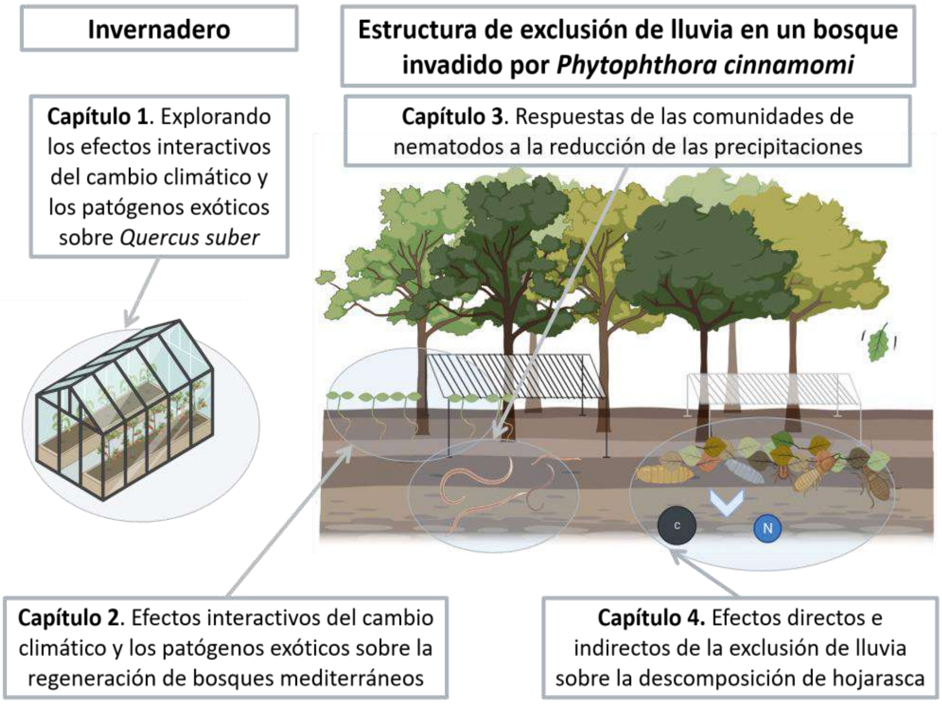

Figura 5. Esquema de los trabajos que componen esta tesis. Creado en BioRender.com

En el **capítulo 1** se analiza el efecto interactivo de distintos niveles de humedad del suelo y densidad del patógeno *P. cinnamomi* sobre plántulas de *Q. suber* en condiciones controladas. Con ello pretendemos conocer cómo las reducciones en la humedad relacionadas con el cambio climático pueden afectar al patógeno y su efecto sobre las plántulas. Para ello llevamos a cabo un experimento de invernadero cruzando 4 niveles de humedad del suelo, relacionados con distintos escenarios de cambio climático, con 4 niveles realistas de abundancia del patógeno. Nuestra hipótesis de partida es que los dos factores de estrés (baja humedad del suelo y patógenos) perjudicarán la salud de las plantas. Sin embargo, debido a la importancia de la humedad del suelo para *P. cinnamomi*, podría producirse un efecto interactivo antagonista entre ambos motores de cambio global. En este caso, la disminución de la humedad del suelo perjudicaría la capacidad infectiva del patógeno, disminuyendo sus daños sobre las plantas.

En el **capítulo 2** realizamos un experimento de siembra bajo la estructura de exclusión de lluvia en el P.N. Los Alcornocales con el objetivo de analizar el efecto interactivo del cambio climático y los patógenos exóticos en condiciones naturales de campo. La mitad de las semillas se sembraron bajo condiciones de exclusión de lluvia y la otra mitad bajo condiciones control. Sembramos semillas de las 3 especies de árboles dominantes en la vegetación de la zona (*Q. suber*, *Q. canariensis* y *Olea europaea*). A la mitad de las semillas sembradas bajo ambos tratamientos climáticos se les aplicó periódicamente un tratamiento con fungicida específico para oomicetos. Nuestra hipótesis de partida es que *P. cinnamomi* reducirá la emergencia de plántulas y aumentará su mortalidad. También prevemos un efecto negativo de la sequía en las etapas tempranas de la regeneración. Sin embargo, estimamos que debido a los requerimientos de humedad del suelo de *P. cinnamomi*, es posible que se den efectos interactivos antagónicos con la exclusión de lluvia. En este caso, la sequía experimental podría atenuar el efecto negativo del patógeno en la germinación y supervivencia de las plántulas, especialmente en las especies más susceptibles al patógeno.

El **capítulo 3** muestra los resultados de un experimento de descomposición de hojarasca en la estructura de exclusión de lluvia, cuyo objetivo es entender los efectos directos e indirectos de la reducción de la precipitación en las dinámicas de descomposición de la hojarasca en suelos infectados por *P. cinnamomi*, con particular énfasis en el papel de la mesofauna (ácaros y colémbolos). Nuestra hipótesis de partida es que la exclusión de lluvia ralentizará directamente las dinámicas de carbono y nitrógeno en la hojarasca, a través de la reducción en la humedad del suelo. Sin embargo, creemos que habrá importantes efectos indirectos de la reducción en la humedad mediados por la mesofauna y la calidad de la hojarasca, lo que influirá en la pérdida de carbono y las dinámicas del nitrógeno. El conocimiento previo nos hace prever que la abundancia de *P. cinnamomi* podría también influir en la calidad inicial de la hojarasca, alterando de esta manera el proceso de descomposición. Además, esperamos que el efecto de la reducción de lluvia sobre los distintos factores que intervienen en la descomposición varíe a lo largo del tiempo. En concreto, esperamos que las condiciones ambientales como la humedad del suelo influyan a lo largo de todo el proceso, mientras que la importancia de la calidad inicial de la hojarasca y la mesofauna sea mayor en las etapas tempranas de la descomposición.

Por último, en el **capítulo 4** se analizan los efectos de la reducción de la precipitación en la estructura trófica del suelo, centrándonos en el estudio de las comunidades de nematodos. Para este capítulo, además de tomar muestras en la estructura de exclusión de lluvia del P.N. Los Alcornocales, se muestreó en otra estructura similar instalada hace 15 años en el sur de Francia. El replicar el estudio en dos infraestructuras de distinta duración permite explorar efectos de la sequía tanto a corto como largo plazo. Nuestra hipótesis de partida es que la reducción de la precipitación tendrá un efecto negativo sobre la abundancia general de nematodos y su diversidad, con un impacto especial en los niveles tróficos más altos (Franco et al., 2019; Siebert et al., 2020). Esperamos por tanto que la reducción de la precipitación se traduzca en una red trófica del suelo simplificada y menos estructurada. Asimismo, esperamos encontrar efectos de mayor magnitud a corto que a largo plazo, asumiendo cierta capacidad de adaptación al estrés hídrico en las comunidades de nematodos a lo largo plazo.

Localización del estudio y metodologías utilizadas.

Área de estudio

Todos los trabajos de este estudio realizados en campo se llevaron a cabo dentro del Parque Natural Los Alcornocales (Cádiz), a excepción de parte del capítulo 4 para el que además de Los Alcornocales se utilizó un experimento de exclusión de lluvia situado en el sur de Francia (Puechabon – Montpellier). El P.N. Los Alcornocales contiene los bosques de alcornoque (*Q. suber*) más grandes y mejor conservados de Europa (Urbieta et al., 2008). Tiene un clima sub-húmedo Mediterráneo, con el 95% de las precipitaciones concentradas entre octubre y mayo, la precipitación anual es de unos 620mm, mientras que la temperatura media está en torno a los 15,7ºC (Junta de Andalucía, 2017). Los suelos son generalmente arenosos, ácidos y pobres en nutrientes, formados a partir de areniscas del Oligo-Mioceno, intercalados con suelos ricos en arcilla derivados de capas de sedimentos de marga. Nuestro estudio se realizó en dos tipos de bosque distintos distanciados por 4 km y que varían en diferentes condiciones ambientales y especies dominantes (Fig. 6).

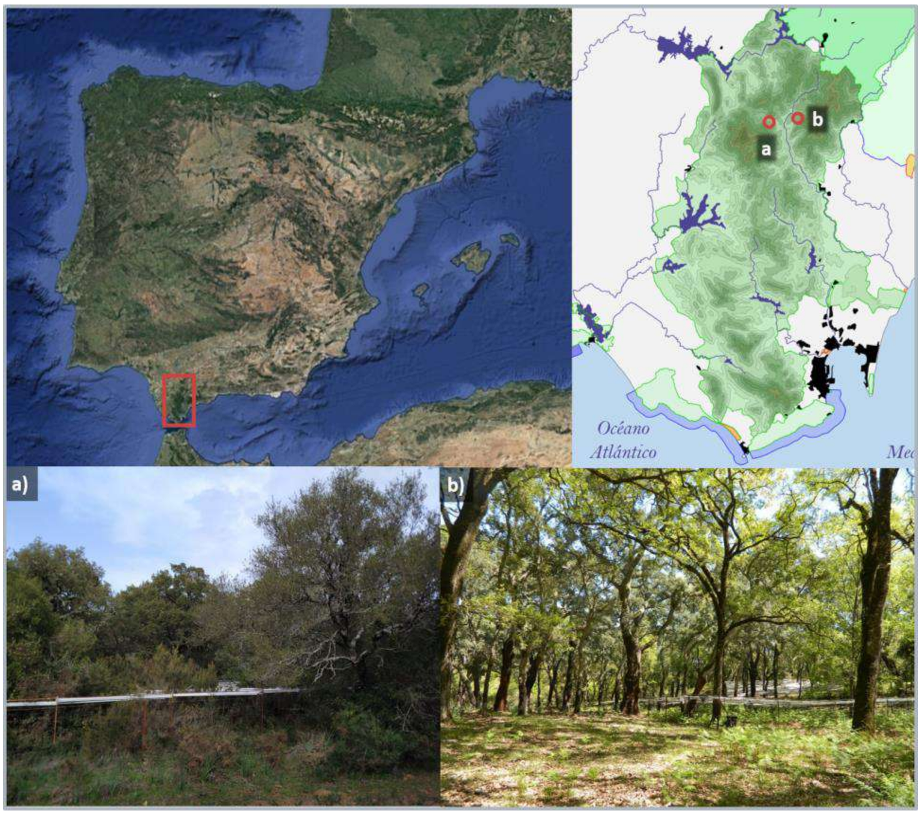

Figura 6. *Arriba izquierda*: Localización del Parque Natural de los Alcornocales dentro de la Península Ibérica. *Arriba derecha*: Localización de las parcelas de estudio dentro del parque natural e imágenes *(abajo)* de una de nuestras parcelas de exclusión de lluvia en cada tipo de bosque. a) bosque abierto, b) bosque cerrado. Fotografías: Pablo Homet Gutiérrez.

1- Un bosque abierto, que es más común en las zonas más áridas del parque, con suelos más arcillosos, donde *Q. suber* aparece acompañado de *Olea europaea*, un árbol perenne intolerante a la sombra. En este bosque hay formaciones de matorral denso dominado por especies como *Genista monspessulana, Crataegus monogyna, Erica arborea, Teucrium fruticans, Pistacia lentiscus,* y numerosas especies de *Salvia y Cistus*.

2- Un bosque cerrado en las partes más húmedas del parque en las que *Q. suber* aparece acompañado de *Q. canariensis*. En estas zonas los suelos son más arenosos y apenas hay vegetación arbustiva, limitada a unos pocos ejemplares aislados de *Erica arborea.*

Quercus suber es una especie perenne nativa del oeste de la cuenca mediterránea. Su corteza, el corcho, está compuesta por un tejido formado por capas de células suberizadas compactas, con paredes altamente impermeables, que puede tener un grosor de hasta 20 cm y que funcionan como aislante térmico y protección contra los incendios (Aronson et al., 2009). Su uso comercial está ampliamente extendido tanto como aislante para la construcción, como materia prima para artesanía o para el más que común tapón de botellas (Aronson et al., 2009). Debido a ello, el problema del decaimiento de esta especie va a suponer un gran problema no solo ecológico, sino también a escala social, ya que los ecosistemas ligados a esta especie proveen de servicios ecosistémicos, culturales y económicos muy valiosos, además de albergar una enorme biodiversidad (Marañón et al., 2012).

En el P.N Los Alcornocales, *Q. suber* ha mostrado síntomas del decaimiento provocado por *P. cinnamomi* (Fig.1). En algunas zonas encontramos más de un 60% de árboles de esta especie afectados (Avila et al., 2017). Sin embargo, aunque se ha demostrado que en condiciones óptimas de invernadero este patógeno es capaz de infectar a las otras especies arbóreas acompañantes (Serrano et al., 2021), *Q. suber* es la única especie que muestra claros síntomas de decaimiento en los bosques de estudio. El hecho de replicar el experimento en dos tipos de bosque va a permitir entender la posible variación de los efectos de la reducción de la precipitación y la presencia del patógeno en función de la vegetación y características del suelo de cada bosque.

Estructuras de exclusión de lluvia

Las infraestructuras de exclusión de lluvia utilizadas en el desarrollo de esta tesis fueron construidas a partir del diseño de las utilizadas en otros países bajo el proyecto europeo MIND (EVK2-CT-2002-000158). En cada tipo de bosque se establecieron 6 parcelas experimentales de 20 x 15 m, tres de exclusión de lluvia y tres controles, distribuidas en tres bloques separados entre sí un mínimo de 30 m. Cada parcela de exclusión lleva asociada una parcela control adyacente para minimizar variaciones edáficas entre los dos tratamientos de lluvia, fundamentalmente en lo relativo a abundancia de *P. cinnamomi*, la cual suele presentar una distribución agregada en manchas

(Gómez-Aparicio et al., 2012). Cada parcela incluye al menos 3 individuos adultos de cada una de las 2 especies arbóreas dominantes en cada tipo de bosque. Previamente a la instalación del experimento de exclusión de lluvia se comprobó la ausencia de diferencias significativas entre el tratamiento exclusión y control en parámetros estructurales y ambientales: densidad arbórea, diámetro medio, área basal, porcentaje de defoliación, luz incidente y humedad del suelo (ver apéndices capítulos 2,3 y 4). La exclusión de lluvia se llevó a cabo mediante la colocación de canalones de PVC blanco de 12,5 cm de anchura cubriendo aproximadamente el 30% de la superficie del suelo forestal y reduciendo por tanto la precipitación en un porcentaje similar (Fig.7). De esta manera tratamos de simular los cambios en los valores medios de precipitación predichos por el modelo CMIP5 para el escenario RCP 8.5 en el periodo 2081-2100 (respecto al promedio de 1986-2005) en el sur de la Península Ibérica (IPCC, 2013). Los canalones de exclusión se colocaron a una altura mínima de 1 m para minimizar su efecto microclimático sobre el suelo. En las parcelas control se colocó la misma estructura, pero con los canalones invertidos, de manera que se reproduzcan los posibles efectos microclimáticos de la infraestructura, pero sin interceptar el agua de lluvia. Para evitar efecto borde, no se realizó ninguna medida en el último metro de cada parcela. Para controlar la efectividad del tratamiento de exclusión, se midió la humedad del suelo cada 3-4 semanas (además de después de cada evento de lluvia) mediante el establecimiento de 6 puntos fijos de medición por parcela donde se instalaron tubos de acceso permanente de 40 cm de profundidad para inserción de la sonda de humedad PR2 (Delta-T Devices), permitiéndonos conocer la humedad del suelo a distintas profundidades. Todas las parcelas fueron valladas para evitar pisoteo y destrozos por la abundante fauna salvaje del entorno.

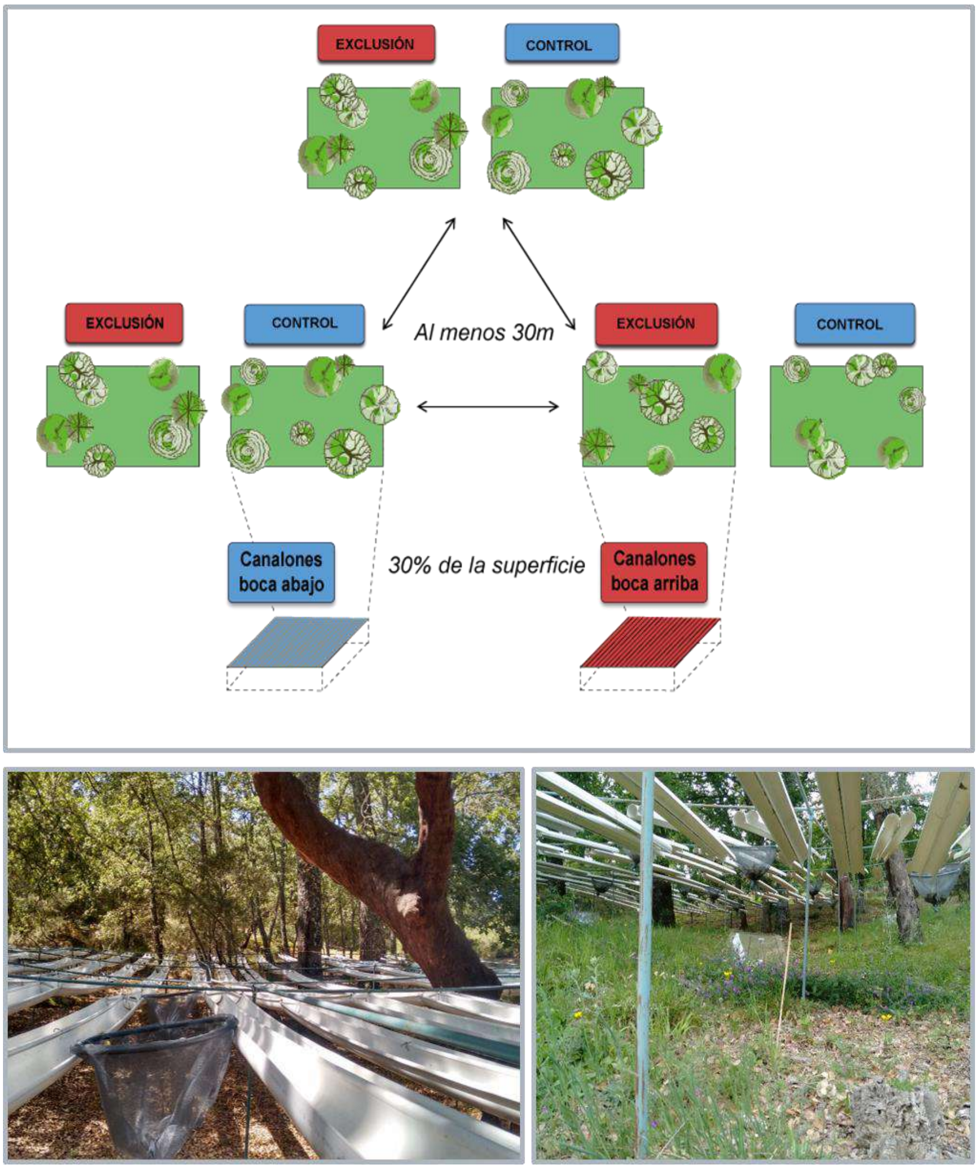

Figura 7. Esquema de las parcelas de exclusión de lluvia y las parcelas de control asociadas. En la parte inferior, se muestran dos fotografías de las estructuras de exclusión de lluvia (izquierda) y control (derecha) situadas en el bosque cerrado. Fotografías: Pablo Homet Gutiérrez.

Publicaciones derivadas de la tesis doctoral

Los diferentes capítulos de la tesis han dado lugar a diversas publicaciones científicas. A continuación, se detalla la información bibliográfica de cada una de ellas.

Capítulo 1**:** Homet P., González M., Matías L., Godoy O., Pérez-Ramos I.M., García L.V. & Gómez–Aparicio L. (2019). Exploring interactive effects of climate change and exotic pathogens on *Quercus suber* performance: Damage caused by *Phytophthora cinnamomi* varies across contrasting scenarios of soil moisture. *Agricultural and Forest Meteorology*, **276–277**: 107605.

Capítulo 2**:** Homet P., J., Matías L., Godoy O. & Gómez-Aparicio, L. How bad is increasing drought for tree regeneration? Evidence for additive and antagonistic effects of climate change and exotic pathogens on regeneration of Mediterranean forests. (Submitted to *Global Change Biology*).

Capítulo 3**:** Homet P., J., Matías L., Gómez-Aparicio L. & Godoy O. (2021). Soil fauna modulates the effect of experimental drought on litter decomposition in forests invaded by an exotic pathogen. *Journal of Ecology*, **109**: 2963-2980.

Capítulo 4**:** Homet P., Ourcival J.M., Domínguez-Begines J., Matías L., Godoy O., Gómez-Aparicio L. (2022). Short- and long-term responses of nematode communities to predicted rainfall reduction in Mediterranean forests (Submitted to *Soil Biology and Biochemistry*).

Otras publicaciones

Jiménez-Chacón A., Homet P., Matías L., Gómez-Aparicio L. & Godoy O. (2018). Fine scale determinants of soil litter fauna on a Mediterranean mixed oak forest invaded by the exotic soil-borne pathogen *Phytophthora cinnamomi*. *Forests*, **9**: 218.

Serrano M.S, Romero M.A., Homet P & Gómez-Aparicio L. (2022) Climate change impact on the population dynamics of exotic pathogens: the case of the worldwide pathogen *Phytophthora cinnamomi*. (Under review in *Agricultural and Forest Meteorology*).

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Capítulo 1

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

Homet, P., González, M., Matías, L., Godoy, O., Pérez-Ramos, I.M., García, L.V., Gómez–Aparicio, L. (2019). *Agricultural and Forest Meteorology*. 276–277: 107605.

Resumen

El cambio climático y las plagas y patógenos exóticos están provocando un alarmante decaimiento forestal en todo el mundo. Sin embargo, aún no comprendemos completamente cómo los daños causados por plagas y patógenos exóticos pueden variar en los diferentes escenarios de disponibilidad de agua influenciados por el cambio climático, particularmente en bosques de zonas donde el agua es un factor limitante como en la región mediterránea. En este artículo, nuestro objetivo fue analizar experimentalmente los efectos interactivos del patógeno exótico *Phytophthora cinnamomi* y las reducciones en la humedad del suelo relacionadas con el cambio climático sobre el rendimiento de las plántulas de *Quercus suber*. Para ello realizamos un experimento de invernadero factorial completo donde se midió la fisiología y el crecimiento de las plántulas de *Q*. *suber* en suelos con diferentes combinaciones de densidad de inóculo de *P. cinnamomi* (0, 30, 60 y 120 unidades formadoras de colonias por gramo de suelo seco) y de humedad (15%, 40%, 50% y 100% de capacidad de retención de agua del suelo) simulando diferentes escenarios de invasión y cambio climático. Encontramos efectos aditivos de *P. cinnamomi* y la sequía en el rendimiento de *Q. suber*, aunque estos efectos no siempre fueron negativos. De hecho, las plántulas mostraron una respuesta fisiológica compensatoria a la infección por *P. cinnamomi* al aumentar sus tasas fotosintéticas netas. Nuestros resultados también respaldaron importantes efectos interactivos de los patógenos y la humedad del suelo sobre las raíces de las plántulas. Así pudimos demostrar que la densidad del inóculo en el suelo requerida para causar un daño significativo a las raíces en las plántulas disminuyó a medida que aumentaba la humedad del suelo. Desde la perspectiva del cambio climático, estos resultados sugieren que un clima promedio más seco podría implicar condiciones subóptimas para las infecciones por *P. cinnamomi,* lo que permitiría un avance más lento de la enfermedad en las áreas invadidas. Sin embargo, este efecto será modulado por los eventos climáticos extremos más frecuentes también previstos. Una mayor frecuencia de lluvias extremas que saturen el suelo podría ser particularmente beneficiosa para *P. cinnamomi*, aumentando su densidad en el suelo más allá de cualquier posible capacidad de respuesta de los huéspedes susceptibles.

Abstract

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

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

Introduction

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

The Mediterranean Basin is considered one of the most susceptible regions to global change around the world (Schröter et al., 2005; Doblas-Miranda et al., 2017). On the one hand, climatic models predict for this region a 2-5ºC increase in mean annual temperature by the end of the 21st century, as well as a 30% annual rainfall reduction and a higher frequency of extreme climatic events such as droughts and floodings (Giorgi & Lionello, 2008; Lindner et al., 2010; IPCC, 2013). On the other hand, exotic pathogenic microbes are causing devastating epidemics throughout the Mediterranean Basin, particularly those belonging to the genus *Phytophthora* (Hansen, 2015; Jung et al., 2018). This oomycete genus includes some of the most aggressive pathogens of woody species on earth, as *Phytophthora cinnamomi* Rands. This pathogen has a likely origin in Papua New Guinea (Arentz & Simpson, 1986), but it is already introduced in forests worldwide (Sena et al., 2018). *Phytophthora cinnamomi* is a soil-borne pathogen that destroys the fine roots of its hosts, impeding nutrient and water uptake, and leading to defoliation, loss of vigour, and eventual death of infected trees. In the Mediterranean Basin, *P. cinnamomi* is decimating natural populations of dominant evergreen oaks such as *Quercus ilex* and *Quercus suber* (Brasier, 1992; Sánchez et al., 2002; Camilo Alves et al., 2013; Ávila et al., 2017). This high mortality rate represents a problem of paramount ecological and social importance, since evergreen oaks are major structural elements in Mediterranean forests and savannah-like ecosystems (“dehesas” and “montados”) that provide extremely valuable biodiversity, economic and cultural services (Marañón et al., 2012). Predicting the future viability of Mediterranean oak forests will undoubtedly require a comprehensive understanding of how the severity of the disease caused by *P. cinnamomi* might vary under the different scenarios of water availability (the main limiting resource in these forests) imposed by a changing climate.

Understanding the interactive effects of exotic soil-borne pathogens and climate change on tree health in water-limited forests is not an easy task, since it will depend on complex responses of host resistance and pathogen growth to decreasing levels of soil moisture. Low values of soil moisture associated with lower precipitation and extreme droughts cause a direct abiotic stress on tree species that strongly reduces their performance (Peñuelas et al., 2013, 2017) and that might make them more susceptible to pathogen attack (Marçais et al.,1993; Corcobado et al., 2014). However, most pathogenic soil-borne fungi and oomycetes require high soil moisture for the germination and dispersal of their spores (Lacey & Harper 1986; Desprez-Loustau et al.,2006). Moreover, low water potential of the host cortical tissues has been shown to have a negative direct effect on pathogen growth within the host (Tippett et al.,1987; Marçais et al.,1993). Therefore, it could happen that the severity of the damages caused by exotic soil-borne pathogens to susceptible hosts in a climate change scenario were lower than expected due to direct negative effects of lower soil moisture on pathogen population growth. Since the damage caused by soil-borne pathogens (and by *P. cinnamomi* in particular) on tree species is strongly dependent on soil inoculum density (Gómez-Aparicio et al., 2012; Serrano et al., 2015), its maintenance in the soil under the minimum threshold required for disease expression might indirectly favour performance of seedlings and adults of susceptible species.

In this paper we aimed to experimentally analyse the interactive effects of the exotic pathogen *P. cinnamomi* and climate change-related reductions in soil moisture on seedling performance of the highly susceptible host *Q. suber*. To achieve this aim, we conducted a full-factorial greenhouse experiment where the growth and physiology of *Q. suber* seedlings was measured in soils with different combinations of *P. cinnamomi* density and soil moisture. We simulated a range of increasing pathogen densities from zero inoculum to large inoculum densities (120 colony forming units per gram of dry soil, CFU/g) known to produce root symptoms in *Q. suber* seedlings under controlled conditions (Serrano et al., 2015). Such large densities, and even much larger, can be found in declining *Q. suber* forests of the Mediterranean Basin (Gómez-Aparicio et al., 2012). We then reproduced different scenarios of soil moisture expected under contrasting scenarios of climate change, from very low levels (15% of soil water holding capacity) typical of springs in extremely dry years to saturated soils typical of wet years in Mediterranean forests. We tested three specific hypotheses: 1) pathogen infection and drought will act as multiple stressors of *Q. suber*, both having negative effects on seedling performance; 2) *Q. suber* seedlings will show physiological and morphological adaptive responses to the two stress factors, with the aim of increasing water use efficiency and compensating the root damages caused by *P. cinnamomi* (i.e. lower photosynthetic rate and stomata conductance, higher biomass allocation belowground); and 3) pathogens and drought will have interactive effects on performance of *Q. suber* seedlings, the severity of the damages caused by *P. cinnamomi* increasing with soil moisture. The results of this experiment will greatly contribute to achieving a better understanding of the future of Mediterranean oak forests under interactive effects of global change drivers of yet unclear consequences.

Material and Methods

Experimental design

The experiment was conducted from January to April 2016 in a greenhouse at the University of Córdoba (Córdoba, Spain, 37°51′N, 4°48′ W). Average air temperature and moisture during the experiment were 12.3 ± 3.1°C and 77 ± 13.2% (respectively), varying from 10.6± 2.9°C and 89.3±7.2% in January to

15.7±2.4ºC and 66.5±11.3% at the end of the experiment. On January 13th, one-year old seedlings of *Q. suber* provided by a local nursery (Viveros San Jerónimo, Junta de Andalucía) were individually planted in 3L pots filled with a mixture of sand, silt, clay, peat and soil from a natural *Q. suber* forest free of *P. cinnamomi* (Marismillas de Doñana, Huelva, Spain) in proportions 55:20:10:10:5 v/v. With this substrate we aimed to reproduce the sandy texture and acidic pH typical of *Q. suber* forest soils (Gómez-Aparicio et al.,2012), as well as its native microbiota. The absence of *P. cinnamomi* in the soil was tested following Romero et al., (2007). Soil samples (10 g) from three different trees in the area were air dried, sieved and suspended in 100 ml 0.2% sterilized water-agar. Then, 1 ml aliquots were plated on Petri dishes containing NARPH selective medium and incubated. No *P. cinnamomi* colonies were detected under the inverted microscope.

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

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

Physiological and morphological seedling measurements

To evaluate the effect of the different treatments on *Q. suber* seedlings, physiological traits were measured during the length of the experiment. Net photosynthetic rate (A_{max}) and stomatal conductance (g_s) were measured three times along the experiment (after 4, 9 and 14 weeks of the experimental treatments) using a portable infrared CO_2 gas analyzer (Li6400XT, Li-Cor, Inc., Lincoln, NE, USA) fitted with a 6-cm^2 cuvette. Environmental conditions were fixed during measurements at 400 ppm of ambient CO_2, air flow of 400 cm^3/min, saturating light conditions of 1000 $\mu mol/m^2s$, and leaf temperature of 20 ºC. All measurements were conducted between 11:00 am and 13:30 am (GMT). Physiological measurements were made in a subset of four seedlings per treatment (n = 4 seedlings × 16 treatments = 64 seedlings).

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

Data analysis

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

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

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

Results

Effects of pathogen density and soil moisture on physiological traits

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

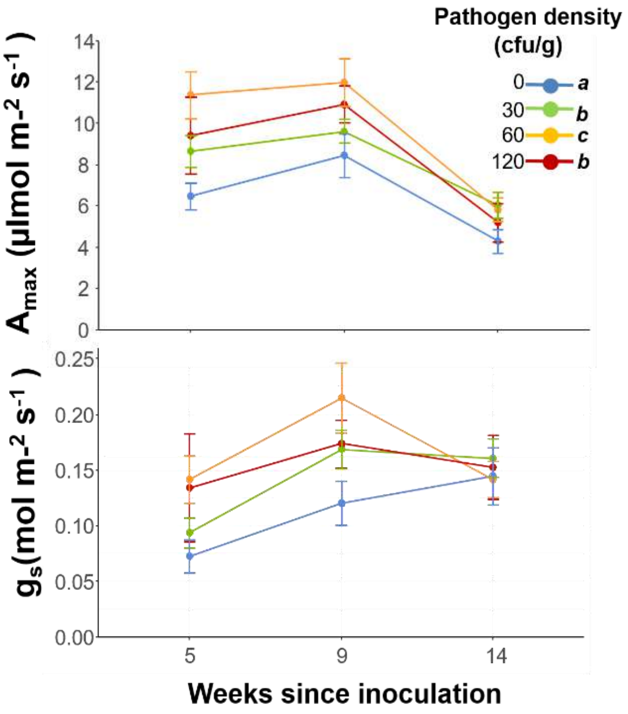

Figure 1. Net photosynthetic rate (A_{max}) and stomata conductance (g_s) of *Quercus suber* seedlings under the four different treatments of pathogen density (measured as number of colony forming units per gram of dry soil, CFU/g) along the course of the experiment.

Different letters in the legend indicate significant differences among treatments after Tukey tests. The effect of pathogen density on stomata conductance was only marginally significant (Table 1). Bars represent means±SE (n=6).

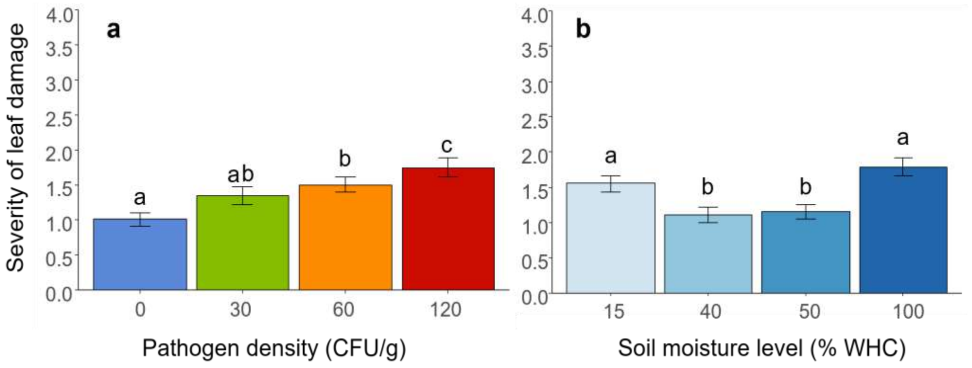

Figure 2. Effects of pathogen density (measured as number of colony forming units per gram of dry soil, CFU/g) and soil moisture (measured as % of water holding capacity, WHC) on the severity of leaf damage of *Quercus suber* seedlings. Different letters show significant differences after Tukey tests.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

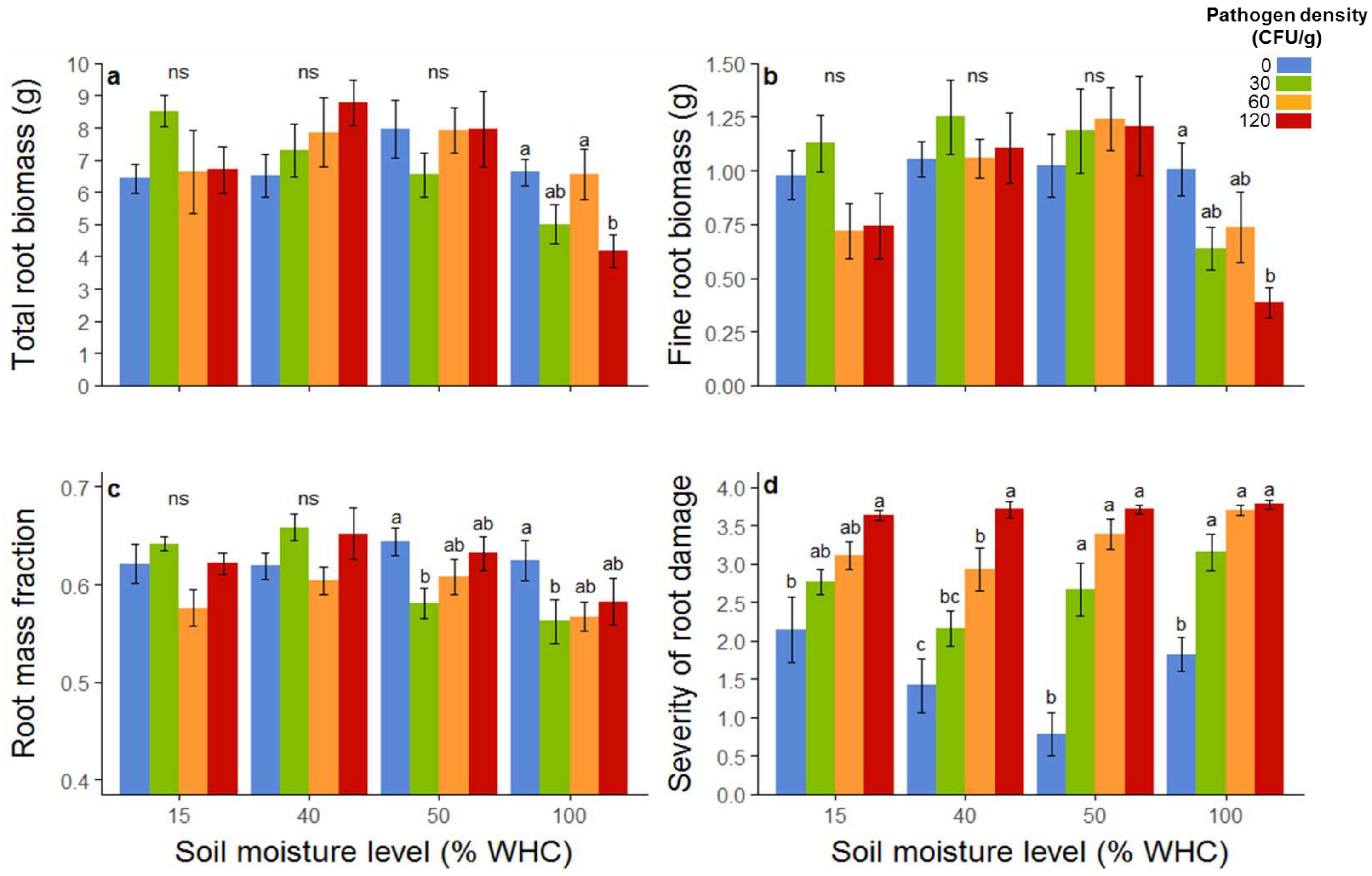

Figure 3. Interactive effects of pathogen density (measured as number of colony forming units per gram of dry soil, CFU/g) and soil moisture (measured as % of water holding capacity, WHC) on belowground morphological traits of *Quercus suber* seedlings. Different letters show significant differences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

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

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

Effects of pathogen density and soil moisture on aboveground morphological traits

Shoot biomass was not affected by either pathogen density or soil moisture (Table 2). However, the two experimental factors have additive effects on the severity of leaf damage (Table 2). Leaf damage increased with the density of *P. cinnamomi* in the soil, being significantly higher at 60 CFU/g and 120 CFU/g than in control seedlings (Fig. 2a). Leaf damage was higher at the two extreme soil moisture levels (15% and 100% WHC) than at the two intermediate levels (40% and 50%, Fig. 2b).

Effects of pathogen density and soil moisture on belowground morphological traits

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

Discussion

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

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

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

We did not find significant effects of soil moisture on the physiological performance of *Q. suber* seedlings, despite the fact that we reproduced four contrasted soil moisture scenarios ranging from a very low soil water content (15% WHC) to a saturated soil (100% WHC). This result likely reflects that *Q. suber* is a drought-tolerant Mediterranean species evolutionary adapted to cope with low levels of soil water availability under long periods of time (Ramírez-Valiente et al., 2009; Gil-Pelegrín et al., 2017). Soil moisture did however affect the severity of leaf damage shown by the seedlings at the end of the experiment, although the severity of the damage did not show a linear response to the decrease in soil moisture. On the contrary, leaf damage was maximal at the two extremes of the soil moisture gradient, showing how the lack of water can be as detrimental as its excess (Gómez-Aparicio et al., 2008; Pérez-Ramos & Marañón, 2009). Soil saturation can generate hypoxia conditions that translate into a poor root functioning and consequent wilting of some part of the foliage (Sairam et al., 2008). Overall, our results demonstrated that *P. cinnamomi* and soil moisture had additive and non-linear effects on aboveground seedling performance that varied in sign and magnitude depending on the trait considered. However, these effects were in general of much lower magnitude than those detected belowground, likely due to a delay between pathogen infection and aboveground symptoms (Robin et al.,1998; Moreira et al., 2000; Ruiz-Gómez et al., 2018), which suggests that understanding the interactive effects of global change drivers on plant performance requires a close look belowground.

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

A main result of our study is that pathogen density and soil moisture had interactive effects on the belowground performance of *Q. suber* seedlings, in agreement with our third hypothesis. Thus, the severity of the root damage caused by the aggressive soil-borne pathogen *P. cinnamomi* to *Q. suber* seedlings differed substantially among the four soil moisture levels used as surrogates of different climate change scenarios. As predicted, the largest levels of root damage occurred in general at the highest levels of soil moisture (50% and 100% WHC), translating into a somewhat lower RMF in infected seedlings compared to control seedlings. Pathogen effects on total and fine root biomass were detected only at the highest soil moisture level (100% WHC), where highly infected seedlings had on average 22% and 61% lower total and fine root biomass (respectively) than control seedlings (Fig. 3a,b). Because of the poor ability of *P. cinnamomi* to degrade the lignocellulose complex of mature roots (Cahill & McComb, 1992; Nicoski, 1996), it is likely that our estimation of root biomass included damaged root tissue not yet totally decomposed, therefore representing a more conservative estimate of the belowground effects of *P. cinnamomi* than the direct visual estimation of necrotic root tissue.

The fact that the largest negative effects of *P. cinnamomi* on root biomass occurred under soil water saturation conditions is consistent with previous knowledge that shows high soil water levels to favor the infective inoculum build-up of *P. cinnamomi*. High soil moisture and free water favors sporangial production and zoospore release, and increases zoospore mobility through the soil to infect roots, allowing for rapid secondary cycles and multiple infections on the host roots (see Camilo-Alves et al., 2013 and references therein). However, our results also suggest that the pathogen capacity to cause root damage can be sensitive to moderate reductions in soil moisture, in agreement with previous studies that have shown water restriction to reduce root damage induced by *P. cinnamomi* (Tippett et al.,1987, 1989; Maurel et al., 2001). Thus, whereas in the 100% and 50% WHC scenarios a low density of *P. cinnamomi* (30 CFU/g) was enough to cause significant higher root damage in infected than control *Q. suber* seedlings, in the 40% WHC scenario only seedlings infected with moderate and high densities (>60 CFU/g) differed from control seedlings. This implies that even a slight decrease of soil moisture simulating a 30% reduction in annual precipitation was enough to trigger differences in root damage caused by *P. cinnamomi*. Further decreasing the soil water content to 15% WHC increased even further (>120 CFU/g) the minimum threshold required to cause significant damage to the root system. This negative relationship between soil moisture and the inoculum threshold required to cause disease is consistent with the more acute decline of *Quercus* species observed in fine textured soils with high water retention compared to well-drained sandy soils (Gómez-Aparicio et al., 2012; Corcobado et al., 2013).

From a global change perspective, these results imply that disease trajectories of forests already invaded by *P. cinnamomi* will strongly depend on rainfall regimes and associated variations in soil moisture that might exert a significant control on the growth rates of *P. cinnamomi* populations.

Conclusions

Understanding how climate change might affect plant disease epidemics is a current challenge, since the different components of climate change (i.e. changes in average temperature and precipitation, increase of extreme events) can have contrasting effects on the dynamics of pathogen populations. To date, the most frequently recognized consequence of climate change on plant diseases has been the positive effect of a warmer climate due to enhance reproductive capacity and survival of pathogenic species and their expansion towards cold areas (Trumbore et al., 2015; Burgess et al., 2017). Therefore, it is often assumed that plant disease epidemics might become more frequent as climate changes (Sturrock et al., 2011). However, in systems like southern European forests where the most severe abiotic stress is undoubtedly drought, changes in precipitation and associated levels of soil moisture might be the most relevant factors driving alterations in the rate and magnitude of tree diseases, with yet unforeseen consequences. To our knowledge, this study addressed for the first time the consequences of different realistic scenarios of soil moisture on the damages caused by the aggressive exotic pathogen *P. cinnamomi* at different levels of soil inoculum that might represent different stages of invasion (from non-invaded soils to highly invaded areas). Although results involving experimental seedlings under controlled conditions need to be carefully extrapolated to adult trees under natural conditions, our results strongly suggest that the capacity of *P. cinnamomi* to cause disease is modulated by even small variations in soil moisture, and that a drier climate might imply sub-optimal conditions for root infections, lengthening the time required for disease expression in susceptible hosts. This finding, together with the physiological plasticity shown by *Q. suber* to counteract pathogen effects under moderate inoculum abundance, might allow for a slower advance of the root rot caused by *P. cinnamomi* in a drier future.

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

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Capítulo 2

How bad is increasing drought for tree regeneration? Evidence for additive and antagonistic effects of climate change and exotic pathogens on regeneration of Mediterranean forests

Homet, P., J., Matías, L., Godoy, O., Gómez-Aparicio, L. (2022).

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Resumen

Comprender los efectos interactivos de los motores del cambio global sobre la demografía de los árboles es fundamental para poder hacer predicciones sobre la dinámica de los bosques en el futuro. Múltiples estudios han demostrado que el incremento de la sequía y los patógenos exóticos amenaza gravemente la persistencia de los bosques al aumentar la mortalidad y disminuir el crecimiento de los árboles adultos. Sin embargo, se sabe mucho menos sobre sus efectos en la regeneración de los árboles, y cómo estos factores pueden afectar al rendimiento de las plántulas de forma aditiva y no aditiva (sinérgica o antagónica). En este caso, hemos querido llenar este vacío explorando experimentalmente los efectos del aumento de la sequía y de los patógenos sobre la regeneración de los árboles en dos tipos de bosques mixtos de robles (*Quercus suber*-*Quercus canariensis* y *Quercus suber*-*Olea europaea*) invadidos por el oomiceto exótico *Phytophthora cinnamomi*, uno de los patógenos de árboles más agresivos del planeta. Para ello llevamos a cabo un experimento de siembra de semillas con fungicida específico para el oomiceto aprovechando infraestructuras de exclusión de lluvias que excluían el 30% de las precipitaciones anuales, simulando las predicciones de los modelos de cambio climático para sistemas mediterráneos. Se siguió la emergencia de las plántulas, la supervivencia y el crecimiento de las tres especies de árboles durante 34 meses. Encontramos que, en contra de nuestra hipótesis, los efectos neutros o positivos de la sequía sobre la regeneración dominaron sobre los efectos negativos en la comunidad de árboles. Además, la mayoría de los efectos positivos de la sequía sobre la especie dominante (*Q*. *suber*) no fueron directos, sino indirectamente mediados por los patógenos del suelo, como muestra el hecho de que los efectos positivos de la sequía desaparecieron con la aplicación del fungicida. En general, nuestros resultados sugieren que las reducciones moderadas de las precipitaciones, como las previstas por los modelos climáticos para la región mediterránea, podrían tener efectos negativos directos menores sobre la regeneración temprana de las especies arbóreas, pero que podrían desempeñar un papel indirecto importante al limitar los efectos negativos de los patógenos exóticos sobre las especies arbóreas altamente susceptibles. En términos más generales, nuestros resultados ponen de manifiesto que los antagonismos entre los impulsores del cambio global deberían reconocerse como fuerzas importantes que podrían frenar la actual pérdida de salud de los árboles.

Abstract

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 tree regeneration, and how these drivers might affect seedling performance in additive and non-additive (synergistic or antagonistic) ways. 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*-*Quercus canariensis* and *Quercus suber*-*Olea europaea*) invaded by the exotic soil-borne oomycete *Phytophthora cinnamomi,* one of 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 34 months. We found that, contrary to our hypothesis, 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, by indirectly mediated by soil-borne pathogens, as shown by the fact that positive drought effects disappeared with fungicide application. Overall, our results suggest that moderate rainfall reductions as those predicted by climate 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 exotic pathogens on highly susceptible tree species. More generally, our results highlight that antagonisms among global change drivers should be recognize as important forces that might slow down the current loss of tree health.

Keywords: experimental drought, interactive effects, Mediterranean forests, oomycetes, *Phytophthora cinnamomi*, *Quercus suber,* rainout shelters, seedling performance, soil-borne pathogens.

Introduction

Global change is severely modifying tree demographic rates in forests worldwide (Millar & Stephenson, 2015, Allen et al., 2015, McDowell et al., 2020). In the last decades, multiple studies have aimed to disentangle how growth and survival of adult trees are being changed by higher CO_2 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 (Brienen et al., 2015, Trumbore et al., 2015, Anderegg et al., 2020). However, understanding forest dynamics under global change require 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 (Davis et al., 2019, Batllori et al., 2020). Despite its relevance, the number of studies that have explored the impacts of global change drivers on tree regeneration is still very limited, particularly when compared to the many studies focused on adult trees.

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 in a type of system where water is already a main limiting resource for plant species performance (Acacio et al., 2017, Peñuelas & Sardans, 2021). On the other hand, the Mediterranean basin is also a hot-spot of exotic species in general, and of exotic pathogens in particular. In Mediterranean countries, the number of invasive forest pathogens has increased exponentially in the last four decades influenced by high international trade, but also by their enormous heterogeneity of environments and diversity of potential hosts (Garbelotto & Pautasso, 2012, Santini et al., 2013). Among these new pathogens, oomycete is the group that has shown the largest increase, and particularly those belonging to the genus *Phytophthora*, which represents one of the most important genus of plant pathogens on earth (Jung et al., 2018, Scott et al., 2019, Burgess et al., 2021). Exotic oomycetes have decimated populations of several Mediterranean woody species, 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.

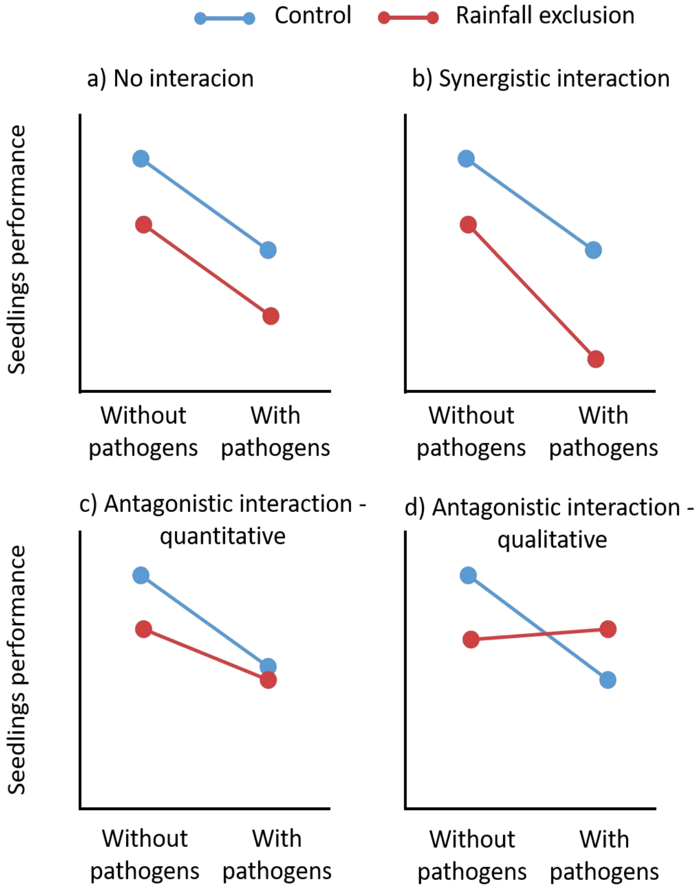

Figure 1. Theoretical scenarios of interaction among two global change drivers (increasing drought and exotic pathogens): 1) no interaction, where the negative effects of increasing drought on seedling performance is the same with or without pathogens; 2) synergistic effect, where the negative effects of increasing drought are larger in the presence of pathogens (as shown by steeper slope of the red line); 3) antagonistic (qualitative) interaction, where the negative effects of increasing drought are smaller in the presence of pathogens (as shown by slower slope of the red line); 4) 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 (Sala et al., 2000; Tylianakis et al., 2008; Côte et al., 2016; Sage, 2020). From a theoretical perspective, three different scenarios of interaction among global change drivers might be expected: no interaction, synergistic interaction and antagonistic interaction (Fig. 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 (Fig. 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 (Fig. 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 (Desprez-Loustau et al., 2006, Corcobado et al., 2014). Finally, an antagonistic interaction would happen when the combined effects of both drivers would be smaller than the sum of their individual effects (Fig. 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 in the effect of one of the drivers (Fig. 1d). This represents quite an extreme situation of antagonist effects that has almost never been 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 mountains 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 (Gómez-Aparicio et al., 2012; Avila et al., 2017). The experiment was replicated in two different types of mixed oak forests: a closed forest of *Quercus suber* and *Quercus 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 et al., 2008a, 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 34 months 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; Morica et al., 2016) and its high abundance in the study area (Gómez-Aparicio et al., 2012). However, this assumption does not discard that other oomycete pathogens of yet unknown relevance might be also involved in fungicide effects (Seddaiu et al., 2020). We hypothesized that the two experimental factors 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 stresses. 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 et al., 2008b, Ibañez 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 – climate change and exotic pathogens – that, to our knowledge, have never been experimentally explored before under natural conditions.

Methods

Study site and species

The study was conducted from 2017 to 2019 at Los Alcornocales Natural Park (Cádiz), in southern Spain. The climate is subhumid 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* (woodland, hereafter). Closed forests appear in wet areas on sandy soils, and usually have a sparse understory 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*, *Crataegus monogyna*, *Teline* sp., *Genista* sp., *Ulex* sp., *Erica* sp. and *Cistus* sp. Soils at the two study sites were invaded by the oomycete soil-borne pathogen *P. cinnamomi* (Serrano et al., submitted) but only a few *Q. suber* trees showed aboveground 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* (Moralejo et al., 2009, Gómez et al., 2020).

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. At each of the two study sites (closed forest and 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. 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. 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 (3-4 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 woodland (n = 86 trees in total). *P. cinnamomi* was isolated in the soil of all trees before the start of the experiment, and showed larger abundance in the closed forest than in the woodland along the study period (Serrano et al., *submitted*).

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.

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 woodland. In fall 2017, acorns of *Quercus* species were collected from at least 10 adult individuals per species in the study area, whereas *O. europaea* seeds were bought from a specialized supplier close to the study site using local genetic pools (Semillas Silvestres S.L.). Seeds were stored in humid substrate at 4 ºC 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 first 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 (Costa & Sánchez, 2001; Baskin & Baskin, 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 woodland, one acorn of *Q. suber* was also sown per point (n = 688 acorns). However, we sown a higher number of *O. europaea* seeds per point (17 seeds per point, 11696 seeds in total) to compensate for the low germination rate of the species (see Domínguez-Begines et al., 2020 for a similar approach) (Fig. S1). 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 to the application of broad-spectrum fungicides (Chen et al., 2001). Armetil has been previously used to successfully disentangle the role of oomycetes pathogens for 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 lab, seedlings were divided into stem, leaves and roots. All plant material was dried at 70 ºC for a minimum of 48 h to estimate shoot and root 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 in the statistical analyses.

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 emergence rates along the first five months of the experiment, as well as on survival rates along the whole duration of the experiment (34 months). 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 as included as a fixed factor and the sowing quadrat as a random factor nested in tree.

Because of the long duration of the experiment, we also used Generalized Linear Mixed Models (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 et al., 2008b, Ibañez 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 analyzed 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 using AIC_c to find the simplest model that was not a worse fit than any other more complex model (i.e. $\Delta AIC_c < 2$). Survival analyses were performed using packages "survival" (Therneau, 2020a) and "coxme" (Therneau, 2020b). GLMMs were performed using package "lme4" (Bates et al., 2015) and compared with the MUMIN package (Barton, 2009). The MUMIN package was also used to calculate the variance explained by the models (marginal and conditional R^2), according to the method proposed by Nakagawa & Schielzeth (2013). All statistical analyses were conducted with R software version 4.1.1 (R Core Team, 2021).

Results

Effects of the rainfall exclusion experiment 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 (Fig. S2, S3).

Seedling emergence

Emergence of *Quercus* species occurred progressively during the first five months of the experiment, reaching values up to 85% for *Q. suber* (Fig. 2a) and 75% for *Q. canariensis* (Fig. 2b) in the closed forest, and 92% for *Q. suber* in the woodland (Fig. 2c). Emergence of *O. europaea* occurred during the first and second springs of the experiment, and was much lower than for *Quercus* species (10%) (Fig. 2d). Best models of emergence rate included the effect of the rainfall exclusion treatment for the two *Quercus* species, but not for *O. europaea* (Table 1). 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 × Fungicide interaction, Table 1), changing from slightly negative with fungicide to very positive without fungicide (Fig. 3a). 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, Fig. 2c). In the woodland, rainfall exclusion had a positive effect on the emergence rate of *Q. suber* (Fig. 2b, 3e).

Fungicide application increased the emergence rate in all species-site combinations, except for *Q. suber* in the woodland (Table 1; Fig. 2). Among the three species, *O. europaea* showed the largest positive fungicide effects, particularly under conspecific *Olea* trees (Fungicide × Canopy species interaction*;* Table 1), where emergence increased by 39% with the application of fungicide (Fig. S4).

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 woodland). Most mortality occurred after the first summer, particularly at the woodland (Fig. 4). Best models of survival included the effect of rainfall exclusion for the two *Quercus* species, but not for *O. europaea* (Table 1). Effects of rainfall exclusion on survival of *Q. canariensis* were consistently positive along the study period, final survival being 20% larger under rainfall exclusion than in control plots (0.64 vs. 0.51, Fig. 4b). Effects on *Q. suber* were, however, more complex and modulated by both the fungicide and the tree canopy treatment at the two sites (best models included Rainfall exclusion × Fungicide and Rainfall exclusion × Canopy species interactions; Table 1). 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 (Fig. 3b), particularly under conspecific canopies (Fig. 5a). Larger benefits of drought without fungicide (i.e. with natural pathogen populations) than with fungicide remained along most of the study (Fig. 4a). In the woodland, rainfall exclusion effects on *Q. suber* survival were also modulated by the identity of the canopy tree (Table 1), being positive only under heterospecific *O. europaea* canopies (Fig. 5c).

The application of oomycete-specific fungicide had positive effects on survival in the four species-site combinations (Table 1, Fig. 4). Although the magnitude of the positive fungicide effects varied along the study period, fungicide was included in 50% of all best survival models and increased final survival of the four species, particularly *O. europaea* (32% increase, Fig. 4d). 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 (Fig. S4).

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* included the effect of fungicide (Table 1). Growth of *O. europaea* seedlings was not affected but any of the two experimental treatments (Table 1). 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 not 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 (Table 1). 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; Fig. 3 d,g,h). Rainfall exclusion effects on *Q. suber* shoot biomass also varied from negative to positive among coexisting canopy species (Fig. 5 b,d).

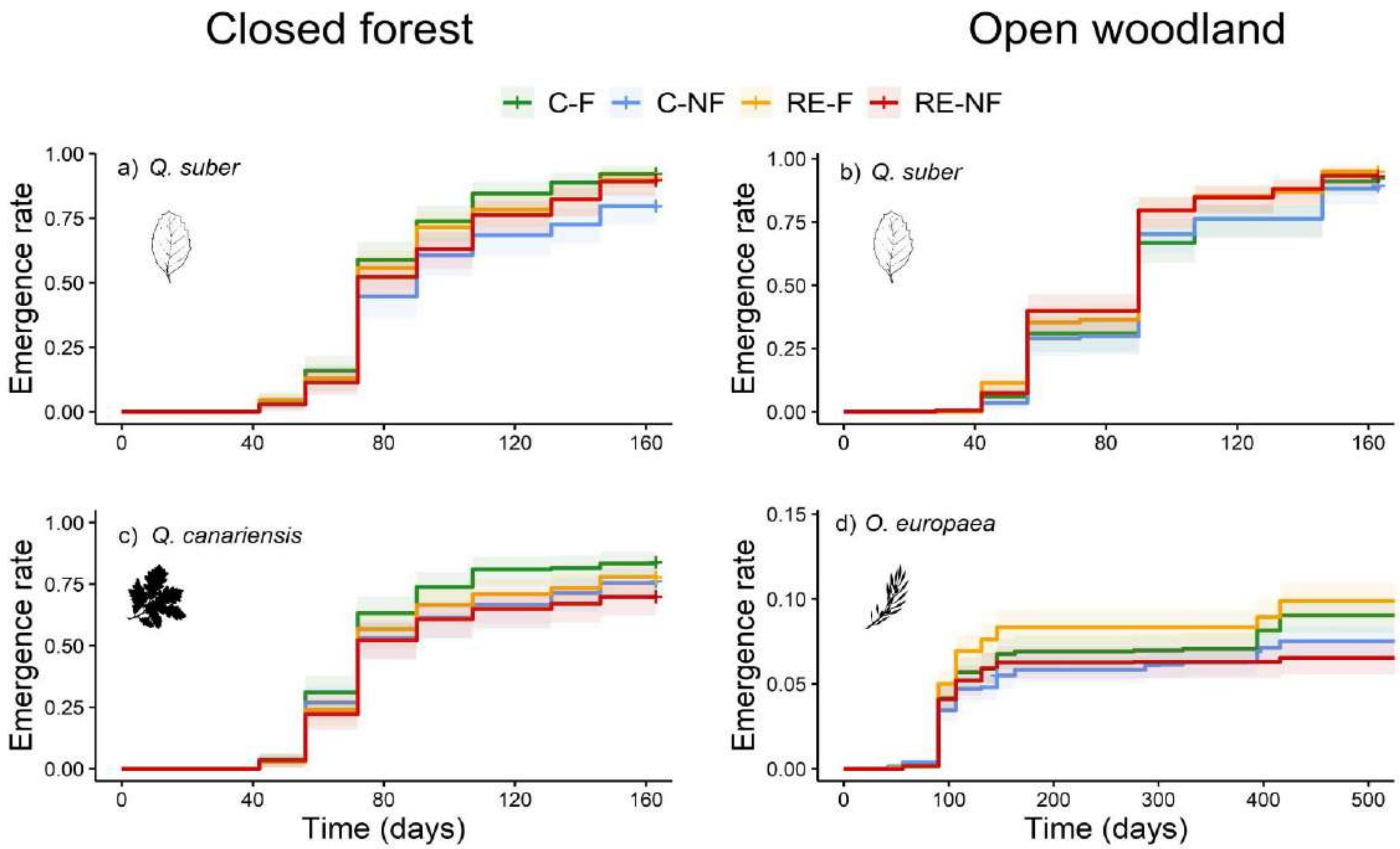

Figure 2. Emergence rate of tree species in the four experimental treatment combinations (RE, rainfall exclusion; C, control; F, with fungicide; NF, no fungicide). Germination of Quercus species took place during the first spring of the experiment, whereas germination of Olea europaea took place during the first and second springs.

Table 1 Best models analyzing 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 study sites. Full models also included seed weight (SW) as a covariate, and block (B) to control for the spatial structure of the experimental design. ∆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") and the goodness of fit (R^2) are also given. Conditional R^2 (R_c^2) reflects the variance explained by all factors (fixed and random) in the model, whereas marginal R^2 (R_m^2) reflects the variances explained only by fixed effects. Note: Models are mixed-effects Cox proportional hazards models for emergence and survival rate, and Generalized Linear Mixed Models (GLMMs) for the other variables. Goodness-of-fit estimates (R^2) are only given for GLMMs.

Seedling species	Variable	Best model	N	Par	∆AICc	R^2_c	R^2_m
Closed forest							
Quercus suber	Emergence rate	y ~ SW + RE + F + RE × F	688	5	18.4		
	Survival rate	y ~ SW + RE	581	3	11.9		
	Survival – 1st summer	y ~ SW + RE + F + SP + RE × F + RE × SP + F × SP + RE × F × SP	606	9	6.1	0.23	0.20
	Survival – 2nd summer	y ~ SW + RE + F + RE x F	581	5	11.8	0.29	0.17
	Survival – 3rd summer	y ~ SW + RE	581	3	9.9	0.25	0.13
	Shoot biomass	y ~ SW + RE + SP + RE × SP	273	5	10.1	0.25	0.17
	Fine root biomass	y ~ RE + F + RE × F	169	4	2.0	0.33	0.15
Quercus canariensis	Emergence rate	y ~ SW + RE + F	688	4	19.1		
	Survival rate	y ~ RE	504	2	10.5		
	Survival – 1st summer	y ~ F + F × SP	529	3	1.1	0.24	0.10
	Survival – 2nd summer	y ~ RE + F	504	3	8.2	0.19	0.11
	Survival – 3rd summer	y ~ RE	504	2	6.1	0.21	0.14
	Shoot biomass	y ~ SW + SP	287	3	10.3	0.22	0.15
	Fine root biomass	y ~ RE + SP	256	3	3.1	0.17	0.12
Woodland							
Quercus suber	Emergence rate	y ~ SW + RE	688	3	33.6		
	Survival rate	y ~ B + SW + SP	636	4	4.9		
	Survival – 1st summer	y ~ RE + SP + RE × SP	636	4	3.4	0.51	0.12
	Survival – 2nd summer	y ~ B + SW + RE + SP + RE × SP	636	6	12.2	0.44	0.24
	Survival – 3rd summer	y ~ B + SW + RE + F + SP	636	6	12.7	0.42	0.20
	Shoot biomass	y ~ SW + RE + F + SP + RE × F + RE × SP	239	7	25.8	0.31	0.22
	Fine root biomass	y ~ SW + RE + F + SP + RE × F + F × SP	146	7	11.8	0.17	0.16
Olea europaea	Emergence rate	y ~ F + SP + F × SP	11668	5	12.5		
	Survival rate	y ~ F	1015	2	5.1		
	Survival – 1st summer	y ~ SP + F	808	3	3.1	0.33	0.13
	Survival – 2nd summer	y ~ B + SP	636	3	12.3	0.44	0.23
	Survival – 3rd summer	y ~ F	1015	2	2.2	0.31	0.20
	Shoot biomass	y ~ B + SP	146	3	17.2	0.34	0.29
	Fine root biomass	y ~ B + SP	142	3	13.9	0.35	0.26

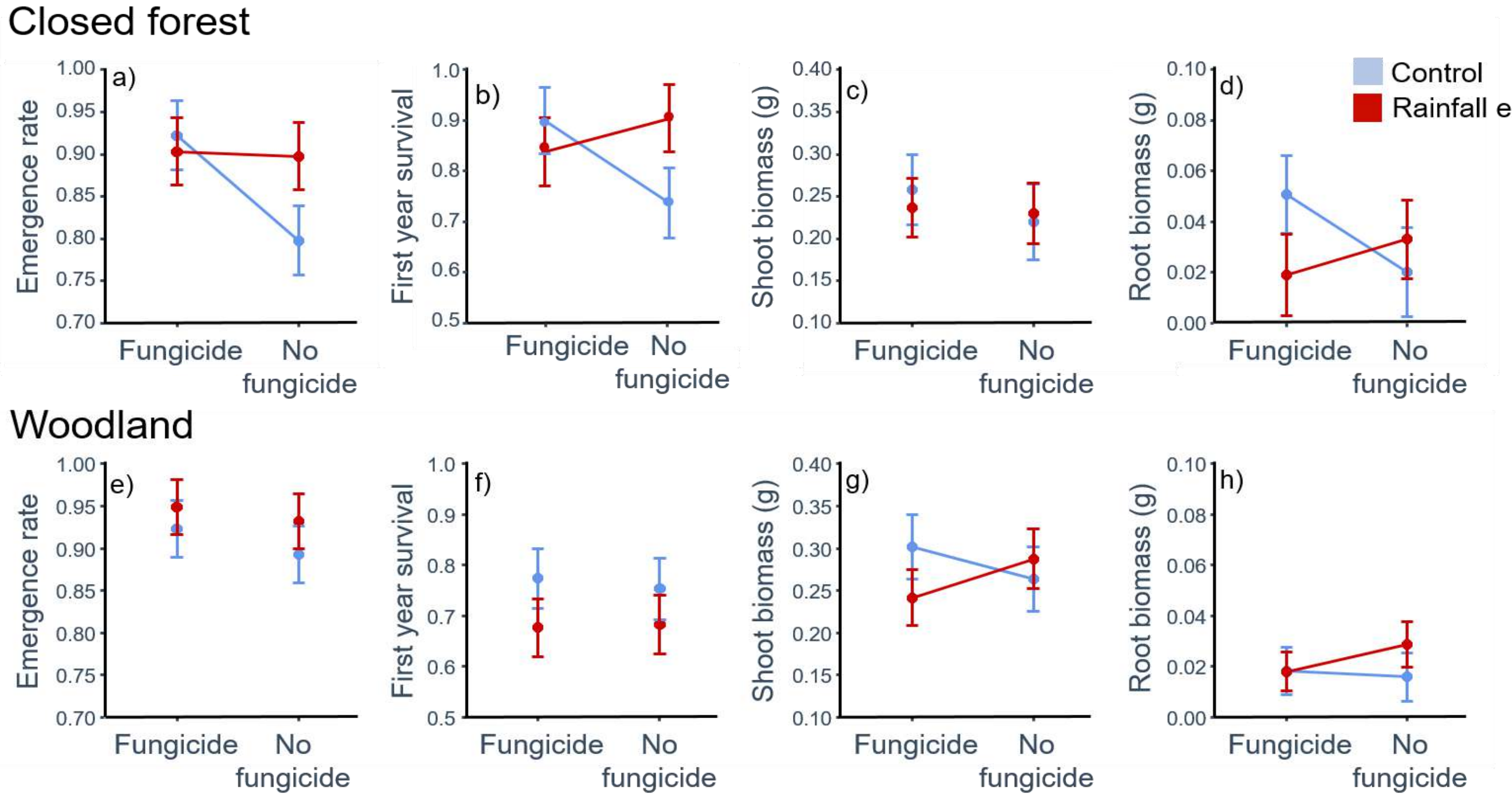

Figure 3. Effects of rainfall exclusion and fungicide application on emergence rate, first year survival, shoot biomass and fine root biomass of Quercus suber seedlings at the two study sites. Values represent mean ± SE. Lines linking fungicide levels are shown only for variables for which best models included the interaction between rainfall exclusion and fungicide application (see Table 1)

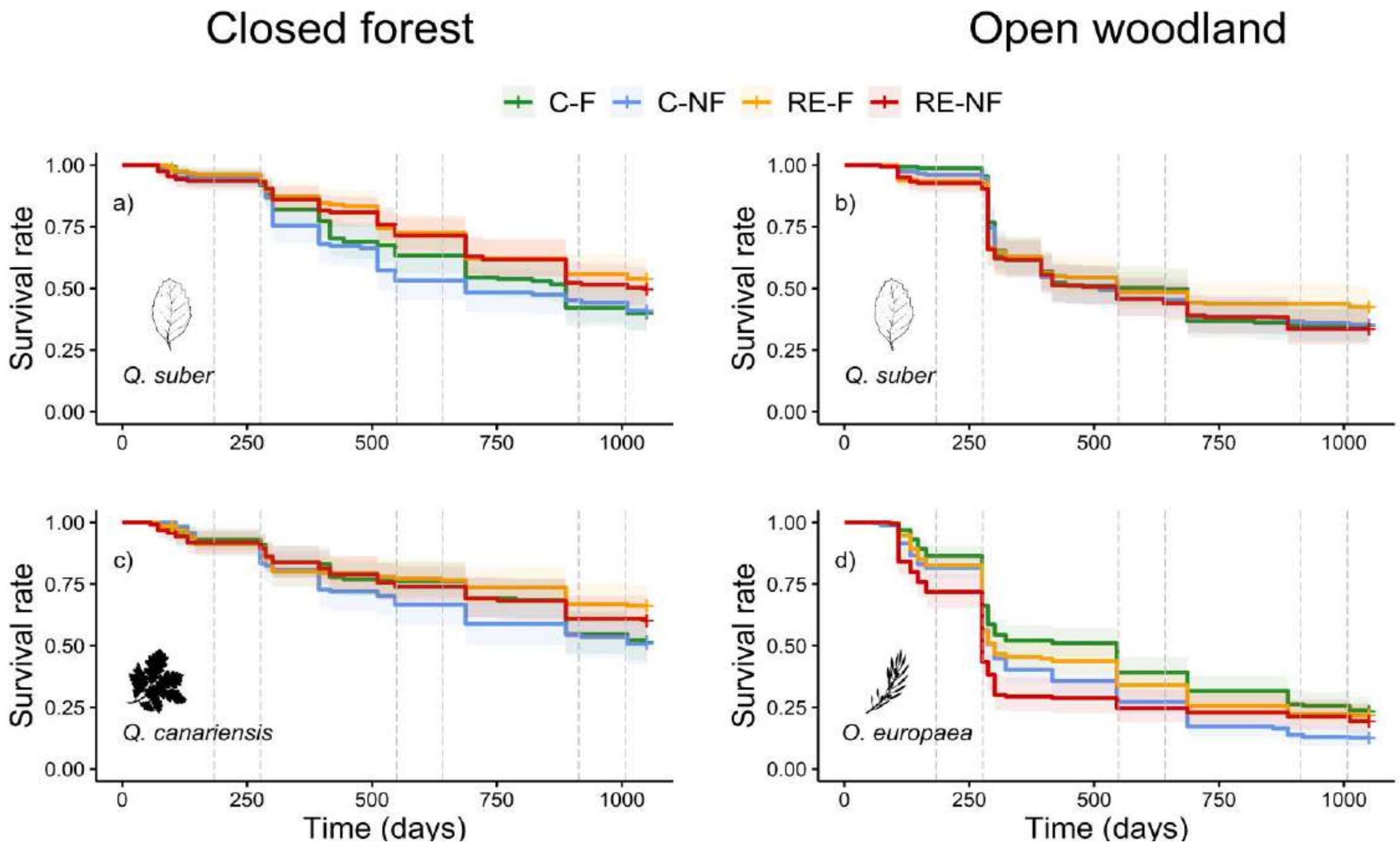

Figure 4. Survival curves of tree seedlings in the four experimental treatment combinations (RE, rainfall exclusion; C, control; F, with fungicide; NF, without fungicide). Dashed lines reflects the beginning and the end of each summer during the experiment.

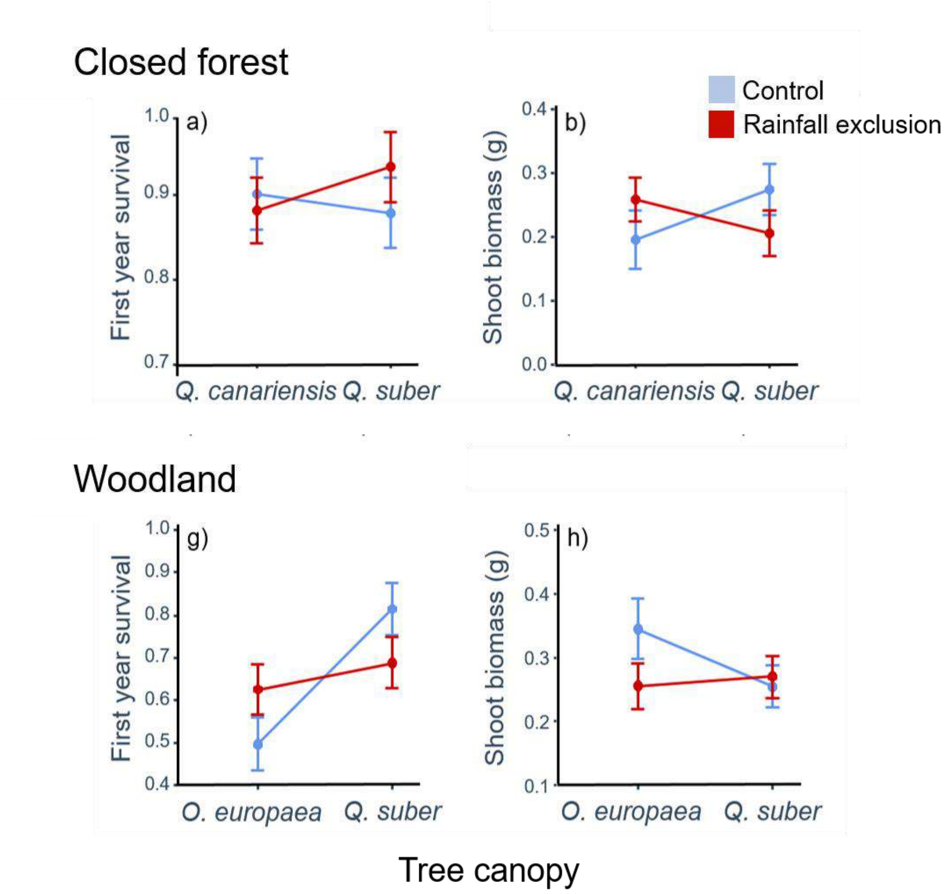

Figure 5. Interactive effects of rainfall exclusion and canopy tree species on first year survival and shoot biomass of *Q. suber* seedlings at the two study sites. Emergence and fine root biomass are not shown because no interactions were found (see Table 1). Values represent mean ± SE.

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 explored their impact on regeneration dynamics is still extremely scarce (Lloret et al., 2004, 2009, Classen et al., 2010, Matías et al., 2011, 2012, Pérez-Ramos et al., 2013, García de Jalón et al., 2020). 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 moderate rainfall reductions as those predicted by climate 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 exotic pathogens on susceptible tree species.

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

We predicted rainfall exclusion effects to be largely of negative sign, since the establishment of woody species in Mediterranean-type ecosystems is severely limited by summer drought (Gómez-Aparicio, 2008). However, we found poor empirical support for this prediction, 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, by 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 belowground) of *Q. suber* seedlings in the two forest sites. 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 *P. cinnamomi* abundance in the soil. These results matched a scenario of qualitative antagonistic effects (Fig. 1c) where, in a forest invaded by *P. cinnamomi*, seedlings of tree species highly susceptible to the pathogen (as those of *Q. suber*) would perform better under drought because the benefits of a lower pathogen abundance would overcompensate any potential direct negative effect of lower soil moisture on seedling performance. In fact, 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., *submitted*). Antagonistic effects of climate change and exotic pathogens had been suggested before under controlled conditions (Homet et al., 2019), but never 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 pathogens as *Q. canariensis*, or to drought as *O. europaea*). Thus, the relatively low susceptibility of *Q. canariensis* seedlings 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 on seedling performance were found, specifically on seedling emergence. This result agrees with previous studies that have shown *Q. canariensis* to require high moisture levels for successful seed germination and to be highly tolerant to soil waterlogging, particularly in comparison to its congener *Q. suber* (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 *Oleacea* family to be more tolerant of rainfall reductions than coexisting *Quercus* species (Liu et al., 2018). However, it showed large increases in its 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 (Domínguez-Begines et al., 2020). Overall, our results support the coexistence of both additive and non-additive (antagonistic) effects of abiotic and biotic global change factors within a tree community composed by species differing in their relative sensitivity to these factors.

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

Here we asked whether the impact of rainfall exclusion and soil-borne pathogens on tree regeneration differed among coexisting canopy 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 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 seedlings of *Q. suber*, 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 (Janzen, 1970; Connell, 1971). In the 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 *Olea* were due to a lower probability of waterlogging, since *Olea* trees are frequently associated to 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 (Urbieta et al., 2008, Gomez-Aparicio 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 alter 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.

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. This conclusion supports previous studies that claim a role for soil-borne oomycete pathogens as a relevant factor limiting regeneration of Mediterranean woody communities (Domínguez-Begines et al., 2020). 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 five 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., 2011). 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 (Song et al., 2019, Ma et al., 2020, Ogle et al., 2021). 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. The most repeated message in the literature is that increasing drought will amplify insect and pathogen epidemics that cause tree mortality (Anderegg et al., 2015). 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 recognize as important forces that might slow down the loss of tree health under global change.

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Apenddix

Table S1. Average values (mean ± SE) and results of the linear mixed models testing differences between control and rainfall exclusion plots in tree density (ind/ha), basal area (m^2/ha), tree diameter (dbh, in cm), tree height (m) and light availability in the understory (Global Site Factor). Plot was included as a random factor for all variables, except for tree density and basal area, for which analyses were conducted at the plot level (n = 3) instead of at the tree level (n = 43).

		Control (average ± SE)	Rainfall exclusion (average ± SE)	F-value	P-value
Closed forest	Tree density	277.78±44.44	333.33±33.33	1	0.38
	Basal area	31.34±5.92	35.83±2.27	0.50	0.52
	Tree diameter	37.54±3.05	35.07±3.14	0.32	0.58
	Tree height	11.59±0.61	11.63±0.62	<0.01	0.96
	Light (GSF)	0.24±0.01	0.25±0.01	1.08	0.3
Woodland	Tree density	288.89±11.11	311.11±40.06	0.29	0.62
	Basal area	19.20±2.56	17.31±0.38	0.53	0.51
	Tree diameter	22.02±2.92	24.84±2.65	0.51	0.48
	Tree height	7.62±0.42	7.93±0.44	0.51	0.61
	Light (GSF)	0.19±0.01	0.19±0.01	<0.01	0.98

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 (closed forest and woodland). 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, in that order.

		Closed forest			Woodland		
		Df	F-value	p-value	df	F-value	p-value
10 cm	Block	2, 111	18.37	**<0.0001**	2, 109	4.91	**0.009**
	Texture	1, 111	23.73	**<0.0001**	1, 109	1.82	0.18
	Rainfall exclusion	1, 111	0.28	0.59	1, 109	2.67	0.105
20 cm	Block	2, 111	4.22	**0.017**	2, 108	8.66	**0.0003**
	Texture	1, 111	2.29	0.133	1, 108	6.1	**0.015**
	Rainfall exclusion	1, 111	4.14	**0.044**	1, 108	0.56	0.454
30 cm	Block	2, 111	0.61	0.545	2, 109	6.68	**0.002**
	Texture	1, 111	6.51	**0.012**	1, 109	20.73	**<0.0001**
	Rainfall exclusion	1, 111	6.42	**0.013**	1, 109	4.29	**0.041**
40 cm	Block	2, 111	0.99	0.374	2, 109	2.18	0.117
	Texture	1, 111	11.15	**0.001**	1, 109	57.36	**<0.0001**
	Rainfall exclusion	1, 111	5.85	**0.017**	1, 109	10.86	**0.001**

Table S3. Results of the linear mixed models that test the effect of the rainfall exclusion treatment on soil moisture at the two study sites (closed forest and woodland). 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, in that order.

		Closed forest			Woodland		
10 cm		Df	F-value	p-value	Df	F-value	p-value
	Block	2,1026	7.6	**<0.0001**	2,1069	6.58	**0.001**
	Year	2,1027	12.41	**<0.0001**	2,1070	20.84	**<0.0001**
	Texture	1,1026	10.57	**0.001**	1,1069	0.13	0.72
	Rainfall exclusion	1,1026	13.09	**0.0003**	1.1069	34.25	**<0.0001**
20 cm	Block	2,993	35.28	**<0.0001**	2,1036	13.7	**<0.0001**
	Year	2,994	17.67	**<0.0001**	2,1037	14.19	**<0.0001**
	Texture	1,993	4.05	**0.045**	1,1036	49.02	**<0.0001**
	Rainfall exclusion	1,993	61.75	**<0.0001**	1,1036	17.77	**<0.0001**
30 cm	Block	2,1027	38.21	**<0.0001**	2,1068	9.36	**<0.0001**
	Year	2,1028	33.01	**<0.0001**	2,1070	18.51	**<0.0001**
	Texture	1,1027	4.46	**0.035**	1,1068	197.93	**<0.0001**
	Rainfall exclusion	1,1027	146.73	**<0.0001**	1,1068	78.63	**<0.0001**
40 cm	Block	2,1025	41.93	**<0.0001**	2,1060	5.04	**0.006**
	Year	2,1026	32.01	**<0.0001**	2,1061	40.78	**<0.0001**
	Texture	1,1025	18.53	**<0.0001**	1,1060	202.92	**<0.0001**
	Rainfall exclusion	1,10250	155.93	**<0.0001**	1,1060	70.76	**<0.0001**

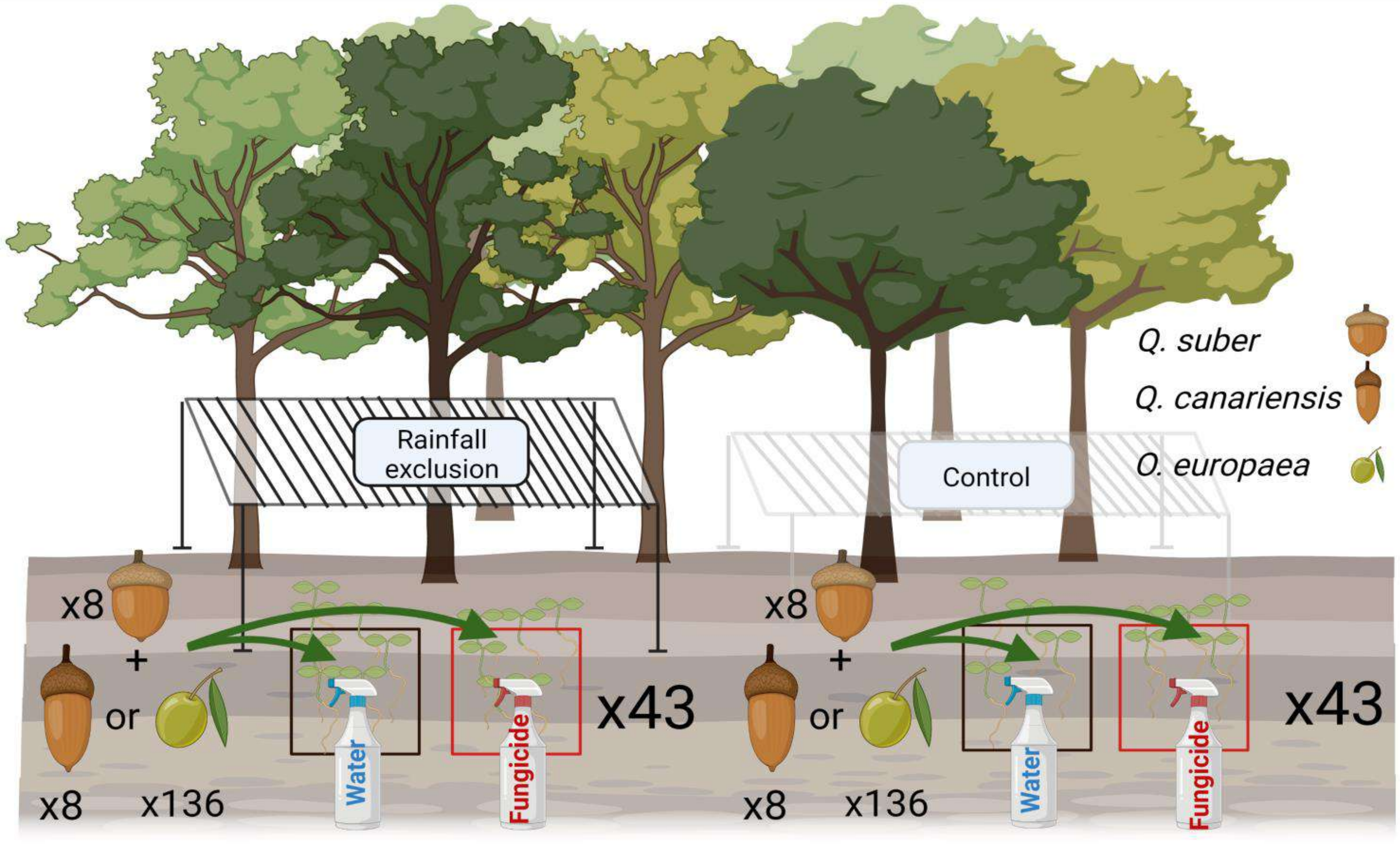

Figure S1. Design of the seed sowing experiment. At each of the two forest sites, seeds were sown in 3 control and 3 rainfall exclusion plots. Within each plot, sowing squares were installed under the canopy of each canopy tree (21 *Quercus suber* and 22 *Quercus canariensis* in the closed forest, 21 *Q. suber* and 22 *O. europaea* in the woodland, 43 trees per site). Two 25 × 25 cm sowing quadrats ("Control" in brown, and "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. 8 seeds per species within each quadrat). In the 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). Figure created in BioRender.com.

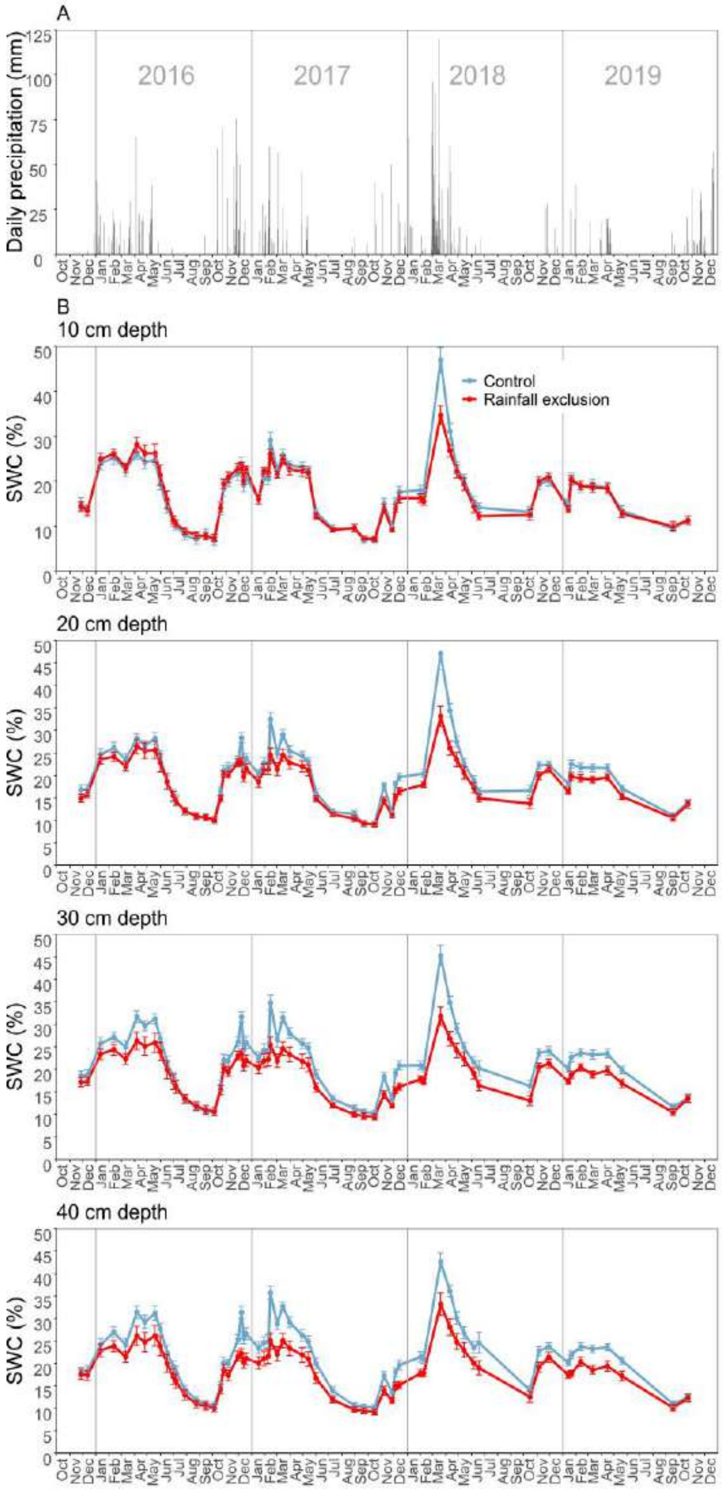

Figure S2. A) Daily precipitation at the experimental site during the study period B) Soil water content (SWC) at different soil depths (10, 20, 30 and 40 cm) in rainfall exclusion and control plots (mean ± SE) at the closed forest. Vertical grey lines separate different years. The rainfall exclusion infrastructure was built and start working on March 2016**.**

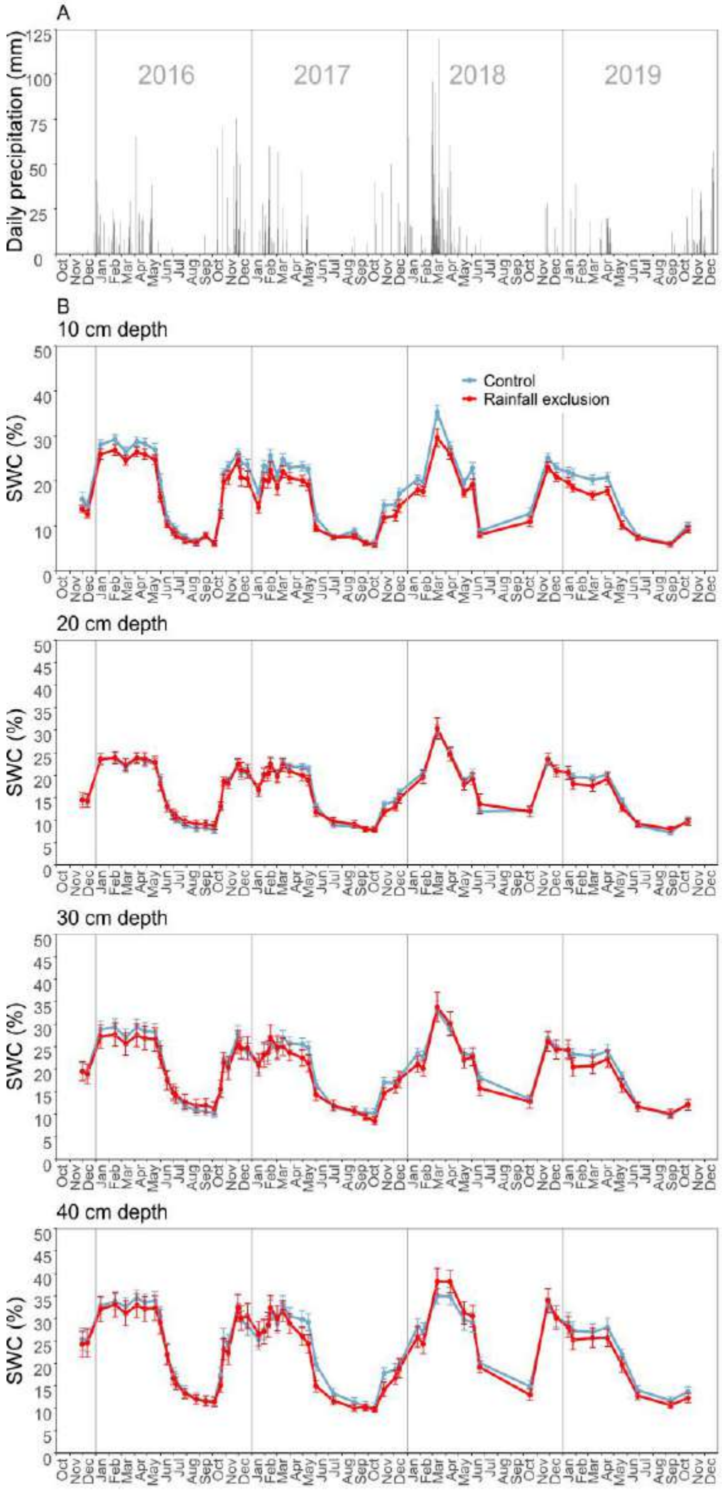

Figure S3. A) Daily precipitation at the experimental site during the study periid B) Soil water content (SWC) at different soil depths (10, 20, 30 and 40 cm) in rainfall exclusion and control plots (mean ± SE) at the woodland. Vertical grey lines separate different years. The rainfall exclusion infrastructure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

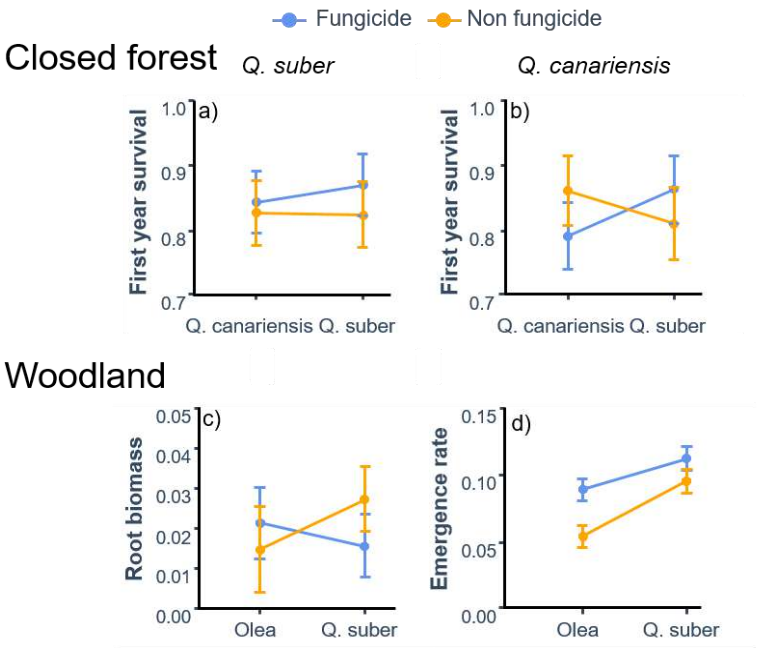

Figure S4. Interactive effect between fungicide and canopy species on first-year survival of (a) *Quercus suber* (b) and *Q. canariensis* seedlings in the closed forest, and of (c) fine-root biomass of *Q. suber* seedlings and (d) emergence rate of *O. europaea* seedlings in the woodland. Only performance variables for which the Fungicide × Canopy species interaction was included in the best model (Table 1,2) are represented.

Capítulo 3

Soil fauna modulates the effect of experimental drought on litter decomposition in forests invaded by an exotic pathogen

Homet, P., J., Matías, L., Gómez-Aparicio, L., Godoy, O. (2021). *Journal of Ecology,* 109, 2963-2980.

Resumen

La descomposición de la hojarasca es un proceso fundamental para el ciclo de nutrientes y los flujos de C entre los ecosistemas terrestres y la atmósfera. Se sabe que en este proceso ecológico influyen directa e indirectamente múltiples factores que interaccionan entre sí, como las condiciones ambientales, la calidad de la hojarasca y los organismos descomponedores. Sin embargo, se desconoce hasta qué punto este intrincado conjunto de interacciones complejas puede verse afectado por diversos factores del cambio global, como el cambio climático y las especies invasoras. En este estudio presentamos los principales hallazgos de un experimento de descomposición de hojarasca realizado en dos bosques mixtos mediterráneos invadidos por *Phytophthora cinnamomi*, un oomiceto exótico conocido por causar enfermedades de los árboles y decaimiento forestal. En primer lugar, construimos una infraestructura de exclusión de lluvia para reducir el 30% de las precipitaciones naturales. A continuación, estudiamos la variación en el contenido de C y N de la hojarasca, así como la abundancia de *P. cinnamomi* durante 18 meses bajo condiciones de control y exclusión de lluvia. Utilizamos bolsas de hojarasca con diferentes tamaños de malla para evaluar en profundidad el papel de la mesofauna en la dinámica temporal del C y N de la hojarasca. La reducción de la humedad del suelo producida por la exclusión experimental de lluvia afectó directamente incrementando la pérdida de C de la hojarasca con el tiempo, e indirectamente a través de un aumento en la abundancia general de la mesofauna y cambios en la proporción entre depredadores y descomponedores. Por el contrario, la dinámica del N fue modulada principalmente por la calidad inicial de la hojarasca. Sorprendentemente, la abundancia de *P. cinnamomi* no se correlacionó con la variación en la calidad inicial de la hojarasca, aunque el oomiceto exótico se correlacionó negativamente con la abundancia de descomponedores y depredadores. Estos resultados sugieren que *P. cinnamomi* podría participar indirectamente en la dinámica de descomposición de la hojarasca con efectos sobre el componente biótico del suelo.

Al contrario de lo esperado, nuestros resultados muestran que una reducción moderada de las precipitaciones puede acelerar el proceso de descomposición de la hojarasca. También señalan a la mesofauna como un componente clave que modula los impactos indirectos de los motores del cambio global en la descomposición de la hojarasca.

Abstract

Litter decomposition is a fundamental process for nutrient cycling and C fluxes between terrestrial ecosystems and the atmosphere. Multiple factors such as environmental conditions, litter quality, and decomposer organisms are known to influence this ecological process in direct and indirect ways. However, it remains poorly understood to what extent this entangled bank of complex interactions can be disrupted by several global change drivers such as climate change and invasive species. Here we report main findings from a litter decomposition experiment conducted in a natural mixed forest invaded by *Phytophthora cinnamomi,* an exotic oomycete well-known for causing tree disease and forest decline. In two Mediterranean forest types (namely woodland and closed forest), we first built a rainfall exclusion infrastructure to reduce natural precipitation by 30 %. Then, we followed the spatial variation in C and N leaf litter dynamics as well as the abundance of the exotic oomycete *P. cinnamomi* during 18 months under both control and rainfall exclusion conditions using a litter bag methodology with different mesh sizes to further evaluate the indirect role of mesofauna abundance and diversity in litter C and N temporal dynamics. Significant reductions in soil moisture produced by the experimental rainfall exclusion increased directly C litter loss with time, and indirectly via increases in overall mesofauna abundance and changes in the ratio between predators and decomposers. N litter dynamics were in contrast modulated mainly by initial litter quality. Surprisingly, *P. cinnamomi* abundance did not correlate with variation in initial litter quality as previously suggested, but we found that the exotic oomycete was negatively correlated with both decomposer and predator abundances. These results suggest it might participate indirectly in litter decomposition dynamics through the biotic soil component. Contrary to expected, our results show that a moderate but realistic rainfall reduction can accelerate the litter decomposition process. It also points out to litter fauna as a key component modulating the indirect impacts of global change drivers on litter decomposition.

Key words: Carbon, Nitrogen, Global change, Litter fauna, *Phytophthora cinnamomi*, litter decompo

Introduction

Litter decomposition is considered a main driver of nutrient cycling, producing inputs up to 90 gigatons of biomass in terrestrial ecosystems yearly (Gessner et al., 2010). Unveiling the mechanisms controlling this ecosystem process is important under current global change for two main reasons. First, it represents the biggest single flux of C to the atmosphere in terrestrial ecosystems (Prentice et al., 2001), impacting climate regulation and the possibilities of positive feedbacks with climate change (Davidson & Janssens, 2006; Ochoa-Hueso et al., 2019). Second, it is also fundamental for the maintenance of community structure and ecosystem resilience by controlling the release of N and other nutrients involved in regulating soil fauna diversity and plant nutrition (García-Palacios et al., 2017; Zhou et al., 2018).

Prior work has extensively shown that litter decomposition is a complex process involving three main drivers, namely, environmental conditions (Delgado-Baquerizo et al., 2015; Djukic et al., 2018; Suseela & Tharayil, 2018), litter quality (Facelli & Picket, 1991; Cornwell et al., 2008; Godoy et al., 2010; García-Palacios et al., 2016a; Djukic et al., 2018; Zhou et al., 2020), and biotic interactions through decomposing organisms (García-Palacios et al., 2013, Santonja et al., 2017a; Zhou et al., 2020). All of them have direct effects on litter decomposition as well as indirect or interactive effects entangled in complex ways. For instance, the effect of climate on litter decomposition has been shown to be modulated by soil organisms and litter quality (Hättenschwiler & Gasser, 2005; Fanin et al., 2019), which in turn depend on plant diversity (Hättenschwiler et al., 2005). At the same time, climate and litter traits indirectly alter litter decomposition via changes in decomposers abundance (Luan et al., 2020), and microbial composition and activity (Zhou et al., 2018, 2020; Prieto et al., 2019).

Changes in rainfall patterns are a main factor of climate change, which is resulting in widespread reductions of precipitation on many regions of the planet. In water-limited ecosystems, such as the Mediterranean region, this reduction could have outsize consequences on ecosystem processes. Making specific predictions on how climate change might impact litter decomposition dynamics is currently difficult, partly because of the described complexity, but also because there is little agreement on previous findings. On one hand, some studies have shown that rainfall reduction and artificially prolonged drought directly reduce litter mass and C loss and leaching (Salamanca et al., 2003; Vogel et al., 2013); and indirectly alter this process via changes in microbial communities (Salamanca et al., 2003; García-Palacios et al., 2016a; Santonja et al., 2017b; Zhou et al., 2018), soil fauna activity (Walter et al., 2013; Santonja et al., 2015) and soil fauna composition (Santonja et al., 2017a; De Smedt et al., 2018). On the other hand, rainfall reduction has been shown to not modify significantly litter mass loss despite producing indirect modifications in several key drivers of litter decomposition such as litter quality and decomposers activity (García-Palacios et al., 2016b) and decomposers abundance (Ashton et al., 2019). This lack of clarity also holds for litter N dynamics. Some studies have shown that rainfall reduction produces higher N immobilization (Zheng et al., 2017; Prieto et al., 2019) and lower N release rates (Santonja et al., 2018), while others have documented that the effects of rainfall reduction on litter N dynamics are minor compared to plant diversity and litter chemistry (Wieder et al., 2009; Zheng et al., 2017).

This lack of consistency of previous findings calls for further studies aiming to better understand how climate change affects litter decomposition dynamics through direct and indirect pathways involving several abiotic and biotic properties of ecological communities. Here we report results from a study conducted in mixed Mediterranean oak forests taking advantage of a rainfall exclusion infrastructure built to understand climate change effects on the dynamics of oak forests in Southern Europe. Obtaining insights into these forest systems is of critical importance because the Mediterranean basin is predicted to be among the most affected areas by climate change (Schröter et al., 2005). In addition, the exotic soil-borne oomycete *Phytophthora cinnamomi* is present in many parts of these forest systems (Serrano et al., 2012; Burgess et al., 2017). This exotic pathogen is considered one of the most aggressive invasive species in the world, destroying the fine root of the trees, producing abrupt changes in soil nutrient dynamics (Ávila et al., 2016, 2019), and leading to massive forest declines (Lowe et al., 2000). *P. cinnamomi* might indirectly affect litter decomposition dynamics via changes in initial litter quality (Pazianoto et al., 2019), or via changes in resource availability such as increases in root and litter quantity, which in turn increases the activity and abundance of soil fauna associated with litter decomposition (Postle et al., 1986). Yet, the magnitude of this modification will likely depend on how severely forests are affected by the invasive pathogen and how its abundance is modulated by climate change. Most notably, climate change-related drought is expected to negatively affect pathogen abundance (Homet et al., 2019), which could in turn offset the indirect effect of *P. cinnamomi* on altering litter quality (Pazianoto et al., 2019) and decomposer fauna (Cobb & Rizzo, 2016).

In this study, we put special emphasis on investigating separately the drivers of litter C and N dynamics since these are two of the most important elements involved in litter decomposition and usually show contrasting temporal patterns. Litter C content usually decreases with time, which is directly affected by soil moisture, whereas N generally shows a less defined pattern depending on temporal changes in litter quality and decomposers activity (Parton et al., 2007; García-Palacios et al., 2016c; Prieto et al., 2019). Here we experimentally modified such decomposer activity using different mesh sizes in a litter bag experiment that allowed or excluded the activity of mesofauna. This procedure allowed us to gain a more complete understanding of the role of biotic activity on litter decomposition, which has been traditionally most focused on microorganism activity (García-Palacios et al., 2013, 2016c, but see Taylor et al., 2004; Santonja et al., 2017a). With this overall experimental set-up, we specifically aim to answer the following four questions: 1) What is the direct effect of rainfall reduction and litter mesofauna on litter decomposition dynamics? 2) How experimental drought indirectly modifies litter decomposition dynamics through changes in mesofauna community and litter quality? 3) How these direct and indirect effects, combined with the natural variation in the abundance of the invasive pathogen, modulate litter decomposition? and 4) Does the overall structure of the entangled bank of direct and indirect interactions vary along litter decomposition stages?

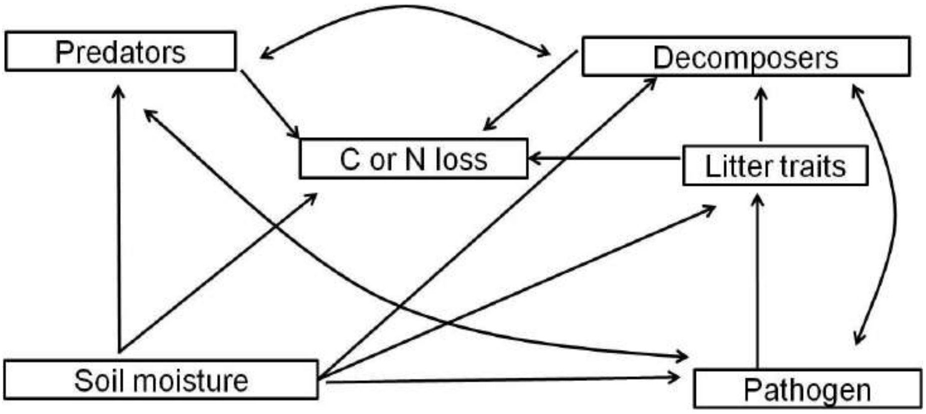

Figure 1. A priori causal relationships between C or N loss and their drivers. Single headed arrows indicate influence of one variable upon another. Double-headed arrows indicate mutual influence (correlation). Decomposers and predators were included in the model as abundance. Soil moisture was included as average values during the period between litter harvests. C/N ratio represents the initial litter traits.

Given these questions, we built a theoretical scheme including direct and indirect pathways of litter decomposition (Fig. 1), in which we overall hypothesized that rainfall reduction will directly slow down C and N loss through reductions in soil moisture (Santonja et al., 2017a; Zhou et al., 2018), but mesofauna, both decomposers and associated predators will in contrast accelerate litter decomposition rates (Handa et al., 2014) (Question 1). We also expected that rainfall reduction will reduce mesofauna abundance and diversity (Santonja et al., 2017a), and modify initial litter quality (Barba et al., 2016; Suseela & Tharayil, 2018; Prieto et al., 2019), which indirectly can modulate litter C loss and litter N dynamics (Question 2). Following previous studies (Cobb & Rizzo, 2016, Pazianoto et al., 2019), we expected infection by exotic pathogens to indirectly affect litter decomposition rates through alterations of the litter quality of their hosts (Question 3). Finally, we hypothesize that the drivers determining litter decomposition will vary their importance over litter decomposition stages (García-Palacios et al., 2016c). In particular, we expected environmental conditions such as soil humidity to be important across time, while initial litter quality and mesofauna might have a significant effect mostly at earlier decomposition stages (Question 4).

Methods

Study site

The study was conducted from 2017 to 2019 in Los Alcornocales Natural Park (Cádiz, Spain, 36.568749, -5.535640), where the largest and best-conserved natural *Quercus suber* forests in Europe are found (Urbieta et al., 2008). The climate is subhumid Mediterranean; rainfall is concentrated (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 (only 735 mm). Soils are sandy, acidic and nutrient poor, derived from bedrock dominated by Oligo-Miocene sandstones. Our study was performed in two forest sites located 4 km apart and characterized by different environmental conditions and dominant species: an open mixed woodland common in the drier parts of the park with clayish soils, where *Quercus suber* appears with the evergreen and shade-intolerant *Olea europaea* var. *sylvestris* (*woodland*, hereafter); and a closed forest in wetter areas with more sandy soils, where *Q. suber* coexists with the deciduous shade-tolerant *Q. canariensis* (*closed forest*, hereafter). Both sites differ in the cover and diversity of the understory vegetation. While the woodland site has a dense and rich shrub vegetation dominated by species such as *Teline monspessulana, Crataegus monogyna, Erica arborea, Teucrium fruticans, Pistacia lentiscus,* and several *Salvia* and *Cystus* species, the understory vegetation of the closed forest is scarce and limited to large isolated individuals of *E. arborea*. The invasive soil-borne pathogen *Phytophthora cinnamomi* is a generalist pathogen with thousands of hosts that strongly differ in their susceptibility to the pathogen attack (Hardham & Blackman, 2018). It was first isolated in the natural park in the early 1990s (Brasier, 1992), and since then it has been identified as a main driver of *Quercus suber* decline in the park and more generally in southern Europe (Gómez-Aparicio et al., 2012; Camilo-Alves et al., 2013; Matías et al. 2019). Coexistent species can serve as hosts. Although *P. cinnamomi* is present in Los Alcornocales Natural Park, its abundance generally shows a variable spatio-temporal pattern (Gómez-Aparicio et al., 2012), which we explicitly quantified in detail for this experiment.

Rainfall exclusion general design

This study was conducted taking advantage of a rainfall exclusion (RE) infrastructure built to evaluate the combined effect of climate change and the exotic soil-borne pathogen *P. cinnamomi* on the dynamics and functioning of Mediterranean mixed oak forests. In particular, at each of the two study sites (woodland and closed forest) 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 reduction treatment, whereas the other plot served as control. All plots were fenced to avoid the interference of large wild animals in the experiment. The rainfall reduction 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. Total basal area, tree density and light levels were similar for the two treatments at the two study sites prior to build the infrastructure (Supporting Information 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 (3-4 trees per plot and species), making a total of 21 *Q. suber* and 22 *O. europaea* in the woodland, and 21 *Q. suber* and 22 *Q. canariensis* in the closed forest. Litter traps (0.5 m in diameter, 2-3 traps per tree depending on tree size) were installed under the canopy of each tree for the collection of the litterfall used in the litter bag experiment.

The effect of the rainfall exclusion treatment on soil moisture was quantified at 4 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 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.

Litter bag experiment

We conducted a litter bag experiment using two types of mesh sizes that allowed us to explore the role of litter fauna in litter decomposition: a mesh size of 100 x 100 µm that avoided the entrance of meso and macrofauna, and a mesh size of 0.6 x 0.6 mm in the bottom side and 0.5 x 0.8 cm in the top side that allowed meso and macrofauna to colonize and feed on the litter. The mesh size of these two litter bag types obeys to previous work indicating that mesofauna body sizes usually ranges from 2 mm to 100 µm (Swift et al., 1979; Verhoef & Brussaard, 1990). Litter bags (18 x 18 cm) were filled with 10-gr of oven-dried (60 ºC) litterfall collected in the litter traps of the 86 target trees (see detailed numbers for the different tree species below). For the two *Quercus* species, the litterfall of each individual tree was used to fill the litter bags installed under its canopy in order to keep the intra-population variability in litterfall chemistry. In the case of *O. europaea*, due to the low quantity of litterfall collected under the canopy of several trees (foliar longevity of *O. europaea* is higher than for *Quercus* species), litterfall from all trees was mixed in a unique pool that was used to fill all the litterbags of the species. This methodological issue limited our capacity to test the effect of pathogen abundance or rainfall reduction on the initial litter quality of this species. Although it has been recently shown that *P. cinnamomi* can infect the roots of *O. europaea* seedlings under controlled conditions (Gómez et al., 2020), adult individuals seem to be resistant to the pathogen (Moralejo et al., 2009). In fact, soils associated with adult *O. europaea* trees show on average low pathogen abundance in our forest system (Gómez-Aparicio et al., 2012). Likewise, *O. europaea* is in our study the species with higher tolerance to drought (Blanco et al., 1996). Therefore, we could expect a much lower impact of pathogen abundance and climate change on the initial litter quality of *O. europaea* than of the two *Quercus* species. Litter bags were installed in 2017 on the forest floor following the phenology of litterfall of each tree species: summer (early July) for the evergreens *Q. suber* and *O. europaea*, and late winter (early March) for the deciduous *Q. canariensis*. To account for the temporal dynamics of litter decomposition, we harvested the litterbags of *Q. suber* and *Q. canariensis* at three different times (after 3, 9 and 18 months) and of *O. europaea* at two times (9 and 18 months) due to the limited amount of litterfall available. This experimental design resulted in a total of 472 litterbags (64 *Quercus* trees × 2 mesh types × 3 harvesting times and 22 *Olea* trees × 2 mesh types × 2 harvesting times).

Characterization of litter quality, microsite conditions and pathogen abundances

Before preparing the litter bags, we measured several physical and chemical properties of the litter samples. We selected 10 leaves per sample, which were used to quantify leaf litter thickness, litter water content, specific leaf litter area (SLA), and initial total C and N content (%). Leaf thickness was measured with a calliper, taking between 5 and 10 measurements per leaf depending on its size. To estimate SLA, leaves were first scanned and their area measured using ImageJ software. Then, leaves were weighed, oven dried at 60 ºC for 72 h and weighed again to measure litter water content. For analysis of C and N, leaves were ground to fine powder and their concentration determined by dry combustion with a Leco TruSpec CN elemental analyzer (LECO, 2008). C and N values were used for the calculation of the C/N ratio used as a proxy of litter quality (Innangi et al., 2018).

We additionally measured several abiotic (soil moisture, light availability, litter depth) and biotic (tree size, tree defoliation, *P. cinnamomi* abundance) variables in the microsites used for the installation of the litterbags in order to explore their relevance as drivers of the decomposition process. Soil moisture was periodically measured during the study period at 10 cm using a PR2 Profile probe. Light availability was quantified by hemispherical photography (Valladares & Guzmán, 2006). Photographs were taken at the beginning of the experiment at ground level using a horizontally levelled digital camera (Nikon, Tokyo, Japan) with a fish-eye lens of 180º field of view aimed at the zenith (FCE8, Nikon). The images were analysed using the Hemiview software (1999, delta-T Devices, Cambridge, UK) to calculate the global site factor (GSF), which integrate the total amount of light over the whole year, ranging from 0 (light absence) to 1 (100 % light availability). Tree defoliation was visually characterized every year using a semi-quantitative scale ranging from 0 (no defoliation) to 3 (high defoliation). Average litter depth was measured with a ruler from the soil mineral layer to the top off the litter layer before placing the litterbags in the field and after each harvest.

The abundance of the soil-borne pathogen *P. cinnamomi* was measured in soil aliquots of 10 g as described in Romero et al., (2007) during the spring (March) of both 2017 and 2018. We selected spring because it is the season of the year with the highest soil pathogen abundance (Serrano et al., unpublished), due to the combination of high soil water availability and mild temperatures (Weste & Rappin, 1977). Briefly, we took three to four soil replicates at each sampling point (i.e. each tree where litterbags were placed) using a soil auger (Ø 3 cm × 0-20 cm depth), previous removal of the litter layer. Soil replicates were combined to form a unique sample per tree, which was stored in a plastic bag at 4°C until processing. In the lab, soil suspensions were prepared in 100 ml of water–agar 0.2 %. Aliquots of 1 ml taken from the soil suspensions were then plated onto NARPH Petri dishes (20 dishes per sample). Colonies growing on the plates were morphologically identified and counted. As soil samples had been dried previously, it was assumed that each colony obtained resulted from the germination of at least one resistant spore (oospore or chlamydospore). Results were expressed as colony-forming units per gram of dry soil (cfu g^{-1}). Overall, we found a high variation in pathogen abundance that changed over time and ranged from 0 to approx. 700 cfu/g (112.2 ± 4.0; mean ± SE). This average abundance is above the threshold for expression of disease in *Q. suber* detected under controlled conditions (Serrano et al., 2015). Comparing across springs of 2017 and 2018, we observed variability in pathogen abundance across sites and plots. Overall, *P. cinnamomi* abundances were greater in the closed forest than in the woodland, but plots within sites showed more idiosyncratic patterns. While some plots in the woodland showed a high spatial correlation in *P. cinnamomi* abundances between the two temporal points of collection, other plots showed some much more variable dynamics (see Supporting information S2). This natural variation found across sampling sites and time in the abundance of *P. cinnamomi* allow us testing its indirect influence on litter decomposition at the beginning of the experiment (via correlation with changes in initial litter quality) and during the experiment (via correlation with changes in mesofauna abundances).

Collection and identification of litter fauna invertebrates

At each collection time, litterbags were stored in hermetic plastic bags and put in a cooler for transportation to the lab. Once in the lab, all bags with the bigger mesh size were put in Tullgren funnels during ten days for extraction of mesofauna (Berlese, 1905). To check that the smaller mesh size did not allow the entrance of mesofauna, we also placed six random samples at each harvest in the Tullgren funnels, with no evidence of mesofauna presence in any case. Six litter bags of fauna exclusion were damaged during the field experiment and were discarded. It is worth noting that our methodology using litterbags does not give instantaneous information across time of the mesofauna present during the litter decomposition process. Yet, this is currently the most common method used to study the temporal cumulative effect of soil and litter fauna on litter decomposition (e.g. Santonja et al., 2017a; Liu et al., 2019). Solving this problem would involve preparing more litterbags and harvesting them more frequently, which was not done in this study due to logistic limitations.

Arthropods collected were stored in 70 % ethanol and separated into different groups with a binocular scope. We focused on the most important groups of mesofauna found in forest litter (Lavelle, 1997.), *i.e.*, *Acari* (separated in three different suborders: *Oribatida*, *Mesostigmata* and *Prostigmata*), *Collembola* (separated in four different morphological groups: *Symphypleona*, *Entomobryomorpha*, *Poduromorpha* and *Neelipleona*), *Araneae*, *Pseudoscorpionida*, *Isopoda*, *Thysanoptera*, *Myriapoda* (*Chilopoda* and *Diplopoda*), *Lepidoptera*, *Psocoptera*, and *Diptera*. In the last step of the analyses, we assigned each group to a trophic role, either detritivores (*Oribatida, Collembola, Isopoda, Thysanoptera, Psocoptera* and *Diplopoda*) or predators (*Mesostigmata, Prostigmata, Araneae, Pseudoscorpionida* and *Chilopoda*), according to previous literature (Santonja et al., 2017a; Jiménez-Chacón et al., 2018). We did not distinguish between adult and juvenile stages because our interest was to obtain metrics of soil fauna community composition and diversity rather than to evaluate the effect of different mesofauna life stages on litter decomposition. For each litter bag, we calculated the relative and total abundances of each trophic group, the species richness, and the Shannon diversity Index.

Litter mass loss, C and N losses and C/N ratios

After faunal collection, the litter material from each bag was cleaned, oven-dried at 60 °C during three days, and weighed to estimate litter water content and loss of litter mass at each sampling time. Total C and N content was analyzed in a sample from each bag with the above-described method. Then, we calculated the C and N loss (%), using the initial and after-harvesting litter mass and the C and N concentrations following (Handa et al., 2014) as:

$$CN_{loss} = \frac{(M_i \times CN_i)(M_f \times CN_f)}{(M_i \times CN_i)}$$

where M_i and M_f are the initial and final litter dry mass, respectively, and CN_i and CN_f are the initial and final C or N concentration (in % of litter dry mass), respectively. C loss values were highly correlated with mass loss values and with the C/N ratio (r Pearson = 0.96 and - 0.76, respectively), thus only C and N loss dynamics were further analyzed.

Data analysis.

We first tested the effect of the rainfall exclusion infrastructure on soil moisture using Linear Mixed Models separately for each soil depth (10 cm, 20 cm, 30 cm and 40 cm). Models were run for measurements conducted before the installation of the exclusion structure (November 2015 - March 2016) and after its installation (April 2016 - March 2018). To account for the spatiotemporal structure of our analyses, we compared models that considered different combinations of spatial (block, plot, tree) and temporal (year, month) factors using the Akaike Information Criterion (AIC). Although in essence all these factors might be considered random (*i.e.,* they have uninformative levels so their relevance is related to their effects on the variance and not the mean of the dependent variable, (see Crawley (2005) for details), block and year were included as fixed factors in the models due to their low replication (< 4 levels, Ieno & Zuur, 2015), whereas plot, tree and month were included as random factors. Once the best spatiotemporal structure was selected, we run models of soil moisture with the rainfall exclusion treatment as fixed factor and soil texture as a covariable.

We also used Linear Mixed Models to test the effect of the experimental treatments on litter C and N dynamics over time (Question 1). As for soil moisture data, we first compared models using different combinations of spatial factors (block, plot, tree) to control for the nested structure of the experimental design. Best models (*i.e.,* lowest AIC) were always those that considered the plot and the tree nested in plot in their random structure. We then analyzed the fixed structure of the models in which rainfall exclusion, mesh size, tree species, time and their interactions were included as predictors. Where significant differences were found, we performed post-hoc Tukey´s test to determine differences for factors with more than two levels. Following the same statistical approach, we also tested the effect of rainfall exclusion and tree species on litter quality as well as the effect of rainfall exclusion, tree species and time on the abundance and diversity of mesofauna communities (Question 2). Mesofauna richness was also calculated but it was not further discussed since it was highly correlated with Shannon diversity index (Pearson r=0.82) at the woodland site.

To explore the indirect effects of rainfall exclusion on the determinants of mesofauna composition (*i.e.,* litter quality and microsite conditions, Question 2), we performed non-metric multidimensional scaling (NMDS) analyses. These analyses were separately done for each forest site and rainfall exclusion treatment as we were interested in the effect of rainfall exclusion on the composition of soil fauna communities. For conducting NMDS analyses, the matrix containing abundances for the different taxonomic groups identified across all sites was squared-root transformed and then standardized using a Wisconsin double standardization following previous standard procedures (Santonja et al., 2017a). Then, we calculated distances between groups using the Bray-Curtis distance index, which ranges between 0 and 1 (Bray & Curtis, 1957). To test for statistical differences on mesofauna composition across tree species we performed a PERMANOVA analysis with 999 randomizations. We used a threshold of stress level ≤ 0.2 to validate the goodness of fit of NMDS analyses.

Finally, to understand the direct and indirect mechanisms that determine C and N loss during the process of litter decomposition (Question 3), we used structural equation models (SEM). Prior to SEM analyses, we performed pairwise correlations to make sure that the environmental predictors selected were not highly correlated (Pearson r > 0.8). We also transformed several variables to reduce variance. Specifically, we log-transformed soil moisture, litter C and N, and *P. cinnamomi* abundance. Mesofauna abundances were also squared-root transformed and standardized using a Wisconsin double transformation. Departing from the scheme presented in Fig. 1, several initial path diagrams were independently tested for C and N dynamics (data not shown) until we reached consensus in a model that provided at the same time a good statistical fit following several indices (root mean square error of approximation (RMSEA), comparative fit index (CFI) and standardized root mean square residual (SRMR)) (Kline, 2015) In this step, we included species as a dummy variable because litter information for two different species was included together into the SEM analyses. However, such procedure did not improve the statistical fit according to the indices selected, accordingly, we dropped the inclusion of the dummy variable. To introduce the effect of soil moisture in these analyses, we calculated the average soil moisture at 10 cm depth at each microsite during the time range of each decomposition stage (*i.e.,* between two litterbag harvests). To test the effect of pathogen abundance on this process, we used the data from the closest pathogen sampling date to each litter bags harvest. With this model selected, we then tested whether the importance of these directs and indirect effects changed over time (Question 4). For this approach, we used multigroup comparison within the overall SEM model. Following methods described in Garcia-Palacios et al., (2016c), we first rounded mass loss values for each litterbag to the next 10% (i.e. creating discrete groups of 10% mass loss intervals), allowing to determine the consecutive stages (*i.e.,* multigroups) along the litter decomposition process. Such smoothing approach required in SEM multigroup comparison was achieved separately with different ranges for each forest site due to the higher decomposition rates at the woodland. In the closed forest, we selected a 20 %

mass loss smoothing for five different groups (0-20, 10-30, 20–40, 30–50, 40–60 % mass loss) while in woodland the smoothing was done by 30 % (0-30,10-40, 20-50, 30-60, 40-70 % mass loss). Differences between multigroup selection across forests obey to observed differences in mass loss. This division on litter decomposition stages has been proved useful to address variation in litter decomposition processes for relatively short time frames (García-Palacios et al., 2016c; Parsons et al., 2014). To validate the multigroup approach, we finally compared this time-lag model with a constrained model with the same path structure but without considering the different decomposition groups. In both cases, the time-lag model performed significantly better suggesting that the importance of abiotic and biotic drivers for litter decomposition varied over time (Supporting information S6).

All statistical analyses were conducted with R software (R version 3.3.2, 2017-10-31). Linear Mixed Models were performed using package “nlme” (Pinheiro et al., 2019), NMDS analyses were performed using package “vegan” (Oksanen et al., 2013) which contains a version of Clarke and Ainsworth BIOENV analysis (Clarke & Ainsworth, 1993), and structural equation models (SEM) were conducted using package “lavaan” (Rosseel, 2011).

Results

Effects of the rainfall exclusion experiment on soil moisture

Soils from the two forest sites showed a very large variability in their texture, the sand content varying between 40-74% in the woodland and between 53-82% in the closed forest. The sand content was significantly lower ($p<0.05$) in drought (57.4± 3.6%) than control plots (66.9±1.4%) in the woodland, but it did not differ among treatments in the closed forest (72.5±2.0% and 72.0±2.3% in drought and control plots, respectively). At both forest sites, texture had a very significant effect on soil moisture. Soil moisture measurements conducted before the installation of the rainfall exclusion infrastructure showed the existence of some previous differences in moisture levels among treatments after controlling for texture effects, mainly at the largest depths. However, the rainfall exclusion infrastructure clearly magnified these differences across the different soil depths. 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. All tables of results and graphs of soil moisture differences are available at Supporting information S3.

Effects of rainfall and mesofauna exclusion on C loss and N dynamics

The amount of litter C loss was higher at the woodland than at the closed forest site (Fig. 2), but in both sites the two experimental factors (rainfall and mesofauna exclusion) as well as tree species had significant effects on litter C loss (Table 1). Rainfall exclusion increased C loss in *Q. canariensis and O. europaea,* and in *Q. suber* at woodland (Fig.2). Mesofauna exclusion diminished significantly C loss in all cases (average loss decrease of 18 %) except for *Q. suber* in woodland. The effect of the exclusion treatments on litter decomposition varied with time (significant RE x T and ME x T interactions, Table 1), appearing mainly in the last stage of the decomposition process. Finally, tree species differed in C loss, being *O. europaea* and *Q. canariensis* the species with higher and lower C losses, respectively (Table 1, Fig. 2). Tree species interacted with the two experimental treatments in the closed forest site, indicating a higher C loss under the combination of rainfall exclusion and mesofauna presence, but only for *Q. canariensis* litter.

Litter N dynamics followed a much more complex temporal pattern when compared to the negative decay observed in C litter. Overall, litter N content increased during the initial steps of the litter decomposition process followed by a linear decay (Fig. 3). Tree species identity was a major predictor of litter N loss in both closed forest and woodland, although in this latter forest site rainfall and particularly mesofauna exclusion also significantly influenced N loss (Table 1, Fig. 3). Moreover, the effect of mesofauna exclusion varied with time and species (significant ME x T and ME x T x SP interactions, Table 1), promoting N immobilization in *Q. suber* litter during early decomposition and N release in the litter of *Q. canariensis* and *O. europaea* during the late stages of the decomposition process (Fig. 3).

		Woodland			Closed forest		
		df	F-value	p-value	df	F-value	p-value
C loss	Rainfall exclusion (RE)	2,4	299.43	**<0.0001**	2,4	4341.92	**<0.0001**
	Mesofauna exclusion	1,116	55.69	**<0.0001**	1,185	23.78	**<0.0001**
	Tree species (SP)	1,36	42.12	**<0.0001**	1,36	6.81	**0.011**
	Time (T)	1,116	238.93	**<0.0001**	2,185	615.65	**<0.0001**
	RE × ME	1,116	0.02	0.892	1,185	0.78	0.381
	RE × SP	1,36	0.66	0.423	1,36	0.25	0.623
	RE × T	1,116	4.76	**0.031**	2,185	0.09	0.992
	ME × SP	1,116	32.55	**<0.0001**	1,185	0.64	0.422
	ME × T	1,116	0.099	0.753	2,185	3.78	**0.025**
	SP × T	1,116	1.48	0.226	2,185	50.50	**<0.0001**
	RE × ME × SP	1,116	0.27	0.612	1,185	4.07	**0.045**
	RE × ME × T	1,116	0.07	0.791	2,185	2.06	0.133
	RE × SP × T	1,116	0.026	0.873	2,185	2.36	*0.097*
	ME × SP × T	1,116	2.96	*0.087*	2,185	1.31	0.272
	RE × ME × SP × T	1,116	0.52	0.471	2,185	0.61	0.541
N loss	Rainfall exclusion (RE)	2,4	10.91	**0.024**	2,4	5.68	*0.068*
	Mesofauna exclusion	1,116	14.56	**0.0002**	1,185	0.23	0.634
	Tree species (SP)	1,36	9.03	**0.005**	1,36	9.18	**0.004**
	Time (T)	1,116	17.28	**0.0001**	2,185	20.25	**<0.0001**
	RE × ME	1,116	0.27	0.604	2,185	1.16	0.283
	RE × SP	1,36	0.48	0.494	2,36	0.69	0.412
	RE × T	1,116	0.72	0.397	2,185	0.92	0.401
	ME × SP	1,116	16.61	**<0.0001**	2,185	0.04	0.852
	ME × T	1,116	5.98	**0.016**	2,185	10.29	**0.0001**
	SP × T	1,116	1.08	0.291	2,185	1.22	0.296
	RE × ME × SP	1,116	0.49	0.481	2,185	0.65	0.421
	RE × ME × T	1,116	2.43	0.121	2,185	1.25	0.292
	RE × SP × T	1,116	0.21	0.651	2,185	0.29	0.751
	ME × SP × T	1,116	4.72	**0.032**	2,185	2.82	*0.063*
	RE × ME × SP × T	1,116	1.21	0.273	2,185	0.33	0.744

Table 1: Results of the LMMs evaluating the effect of rainfall exclusion (RE), mesofauna exclusion (ME), tree species (SP) and time (T) on C and N loss at the two study forests (woodland and closed forest). Df shows the degrees of freedom of the numerator and denominator. Significant effects (p<0.05) are shown in bold, whereas marginally significant effects (p<0.1) are shown in cursive.

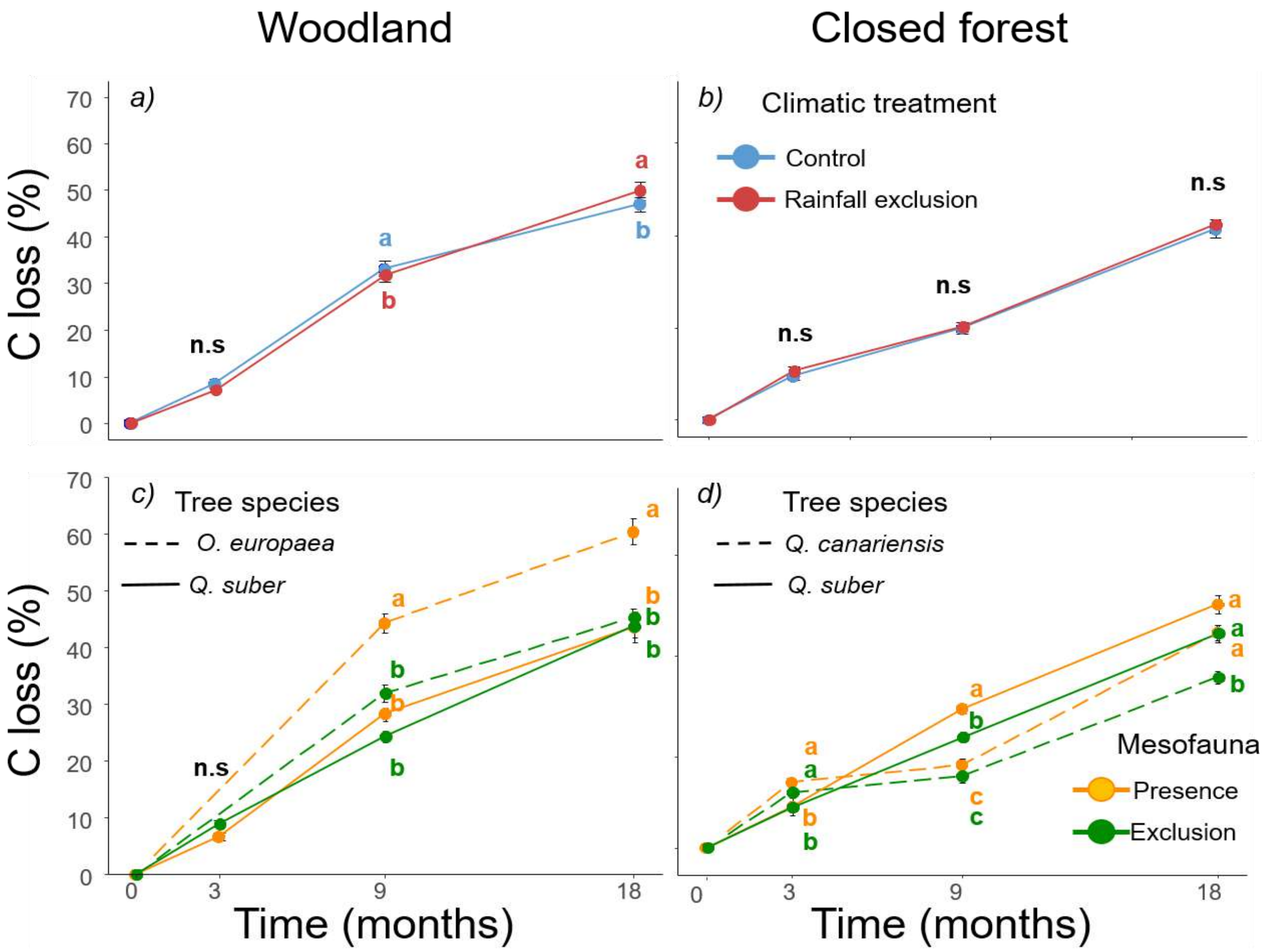

Figure 2: Leaf litter C dynamics of the three study species (*Quercus suber*, *Olea europaea* and *Quercus canariensis*) in response to rainfall exclusion (upper graphs; a, b) and mesofauna exclusion (lower graphs; c, d) at the two study sites (a and c woodland, b and d closed forest) across time. Significant differences among levels for each time ($p < 0.05$) are indicated with different letters as result of post-hoc analyses. Error bars represent SE.

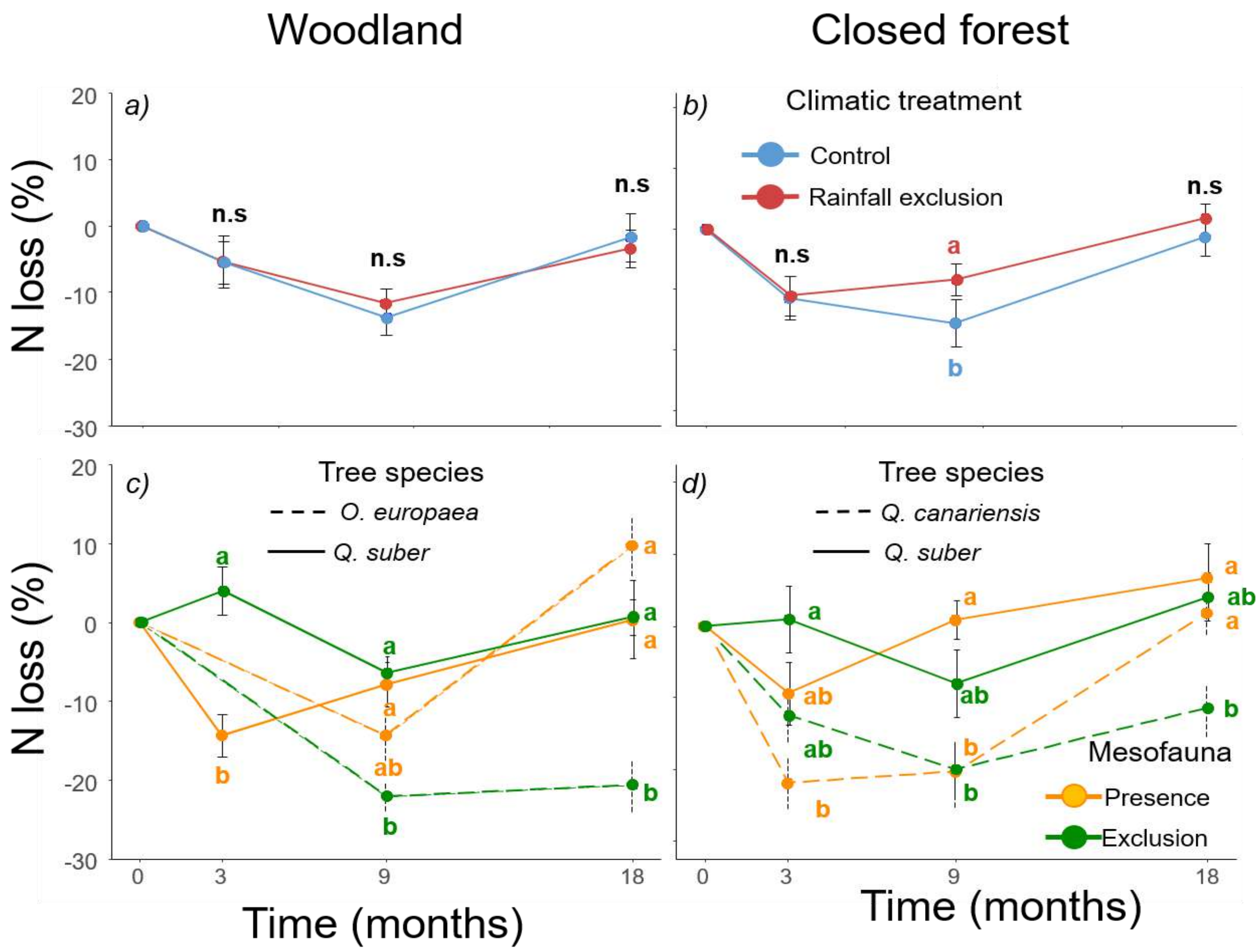

Figure 3: Leaf litter N dynamics in response to rainfall exclusion (upper graphs; a, b) and mesofauna exclusion (lower graphs; c, d) at the two study sites (a and c woodland, and b and d closed forest) across time. In panels (c) and (d), the different species were represented with different line types and significant differences among levels for each time ($p < 0.05$) are indicated with different letters as result of post-hoc analyses. Error bars represent SE.

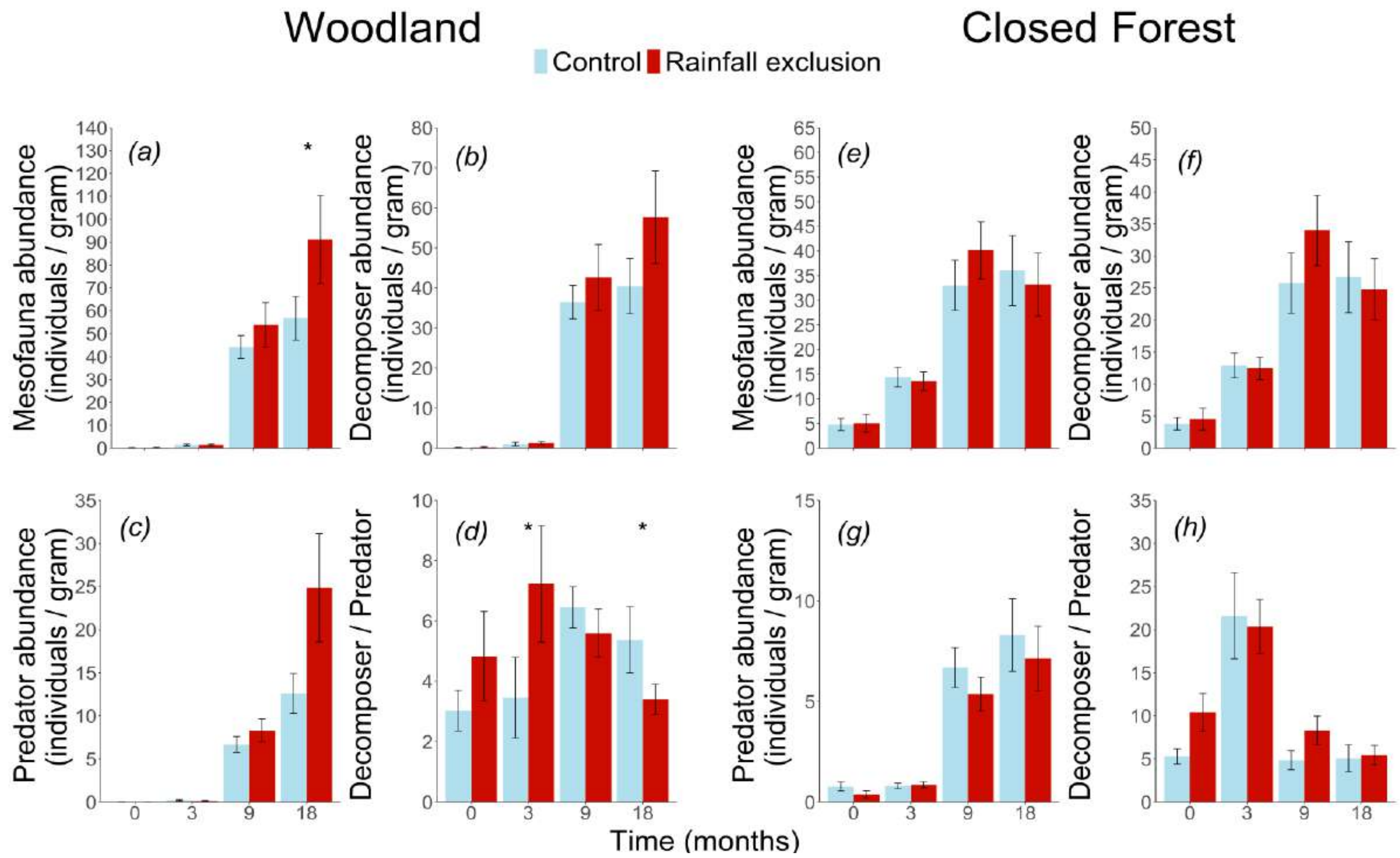

Figure 4: Effects of rainfall exclusion at the different harvest times of litterbags at woodland (a, b, c and d) and at closed forest (e, f, g and h) on mesofauna abundance (a, e), decomposer abundance (b, f), predator abundance (c, g) and the ratio between decomposers and predators (d, h). Abundance is expressed as the number of individuals per gram of litter. Error bars represent ±SE. We considered the groups Oribatida, Collembola, Isopoda, Thysanoptera, Psocoptera and Diplopoda as *detritivores* and Mesostigmata, Prostigmata, Araneae, Pseudoscorpionida and Chilopoda as *predators*.

Effects of rainfall exclusion on litter decomposition drivers and mesofauna characteristics

Initial tree litter quality (measured in terms of the litter C/N ratio) differed among control and rainfall exclusion plots, but only in the closed forest and for one of the two tree species (significant Rainfall exclusion x Species interaction, Supporting information S4). Thus, in the closed forest, *Q. suber* litter had a higher C/N ratio in the rainfall exclusion than in the control plots, whereas the C/N ratio of *Q. canariensis* litter did not differ among treatments (Supporting information S4). The rainfall exclusion experiment had a significant positive effect on mesofauna abundance in the woodland site, but not in the closed forest (Fig. 4). Moreover, these differences appeared mainly during the latest stages of the decomposition process and were driven mainly by a disproportionately increase in predators abundance compared to that of decomposers (Table S5.1, Fig. 4). However, no effects of the rainfall exclusion treatment were detected at lower taxonomic levels for the main arthropod groups (Supporting information S5, Table S5.2, Figs. S5.2-S5.3).

Besides the direct effects of rainfall exclusion on mesofauna abundance, we explored the variation in the importance of main drivers of mesofauna composition between control climate conditions and the experimental rainfall exclusion (Supporting information, Figs. S5.4-S5.5). In woodland, C/N ratio, N content, litter water content, and soil moisture were the main determinants of the different litter faunal groups. For instance, humid conditions favoured the presence of different groups of decomposers as collembolla (*Entomobryomorpha*, *Poduromorpha*, and *Symphypleona*) and acari (*Oribatida*). In the closed forest, NMDS analyses revealed that litter depth and litter water content were the main determinants explaining the different faunal taxonomic groups across climatic conditions. Mesofauna composition in the closed forest differed between tree species in control plots, but such differences were reduced under rainfall exclusion. By contrast, the pattern was the opposite at woodland, with differences in mesofauna composition between tree species observed only in rainfall exclusion plots.

Direct and indirect drivers of litter decomposition and their variation with decomposition stage.

SEM analyses revealed soil moisture as the main direct driver of C loss at both forest sites, followed by mesofauna abundance and initial litter C/N ratio (Figs. 5-6; Supporting information, Table S6.1). Soil moisture at earlier decomposition stages accounted for 50% to 70% of the variability in litter decomposition at both forest types, while its importance decreased as decomposition advanced. The abundance of decomposers and predators significantly affected C dynamics, but the sign of their effects varied over time. Predators showed at both sites early positive effects on C loss, shifting to negative ones at later stages. Decomposers had negative effects during the last decomposition stages at both forest types; however, this trophic group also produced positive effects on C loss at the earliest stage in the woodland but not in the closed forest. Interestingly, there were some differences when mesofauna diversity data instead of abundance was included as predictor in the SEM analysis (discussed in Supporting information S6, Table S6.2, Figs. S6.1-S6.2). With respect to N dynamics, SEM multigroup analyses revealed that N loss was mainly influenced by the initial litter C/N ratio (Figs. 5-6). The negative effect of the initial litter C/N ratio on N loss was consistent across decomposition stages accounting for 33% to 55% of total effects. Surprisingly, soil moisture did not play an important role on N loss as showed with C loss. Finally, predators but not decomposers abundance positively influenced N loss at later decomposition stages in the closed forest. Results were very similar when including mesofauna diversity instead of abundance.

When focusing on the indirect effects of the different drivers on C and N litter loss with the SEM multigroup approach, we observed a wide diversity of interesting results, mainly mediated by changes in mesofauna abundance and diversity (Figs. 5-6; Supporting information, Table S6.1). Effects of soil moisture on mesofauna communities varied between sites and trophic groups. In the woodland, the effect of soil moisture on mesofauna abundance was restricted to the first decomposition stages, being negative for decomposers and positive for predators. However, in the closed forest soil moisture effects were restricted to soil predators and ranged from negative to positive depending on the decomposition stage. As expected, both functional trophic groups were strongly and negatively correlated. Mesofauna diversity instead of abundance slightly changed observed relationships included in the SEM model (discussed in Supporting information S6).

Contrary to expectations, natural variation in the spatial abundance of the pathogen did not indirectly influence litter decomposition through its correlation with changes in initial litter quality, but it showed significant correlations with mesofauna abundance (Figs. 5-6; Supporting information, Table S6.1). These correlations varied in magnitude between decomposers and predators, and sites. Stronger correlations were found in the woodland than in the closed forest, and in predator than in decomposer abundances. Surprisingly, soil moisture only affected pathogen abundance in the closed forest and the effect was restricted to intermediate decomposition stages. Finally, the initial litter C/N ratio had a weaker but significant effect on litter decomposition, enhancing decomposers abundance at the last stages on both sites.

Discussion

Understanding the effects of global change drivers on litter decomposition is critical to determine the alterations to the correct functioning of this ecosystem process, key for nutrients dynamics in terrestrial ecosystems and climate regulation (Bonan, 2008). In this study, we took the challenge to mechanistically explore numerous direct and indirect drivers of litter decomposition associated with two of the most important global change drivers in Mediterranean-type regions, as are climate change and invasive species. For this, we used a rainfall exclusion infrastructure to simulate future climate-change scenarios for the study area (Stocker, 2013) in a Mediterranean forest invaded by the exotic pathogen *P. cinnamomi*. Our main findings suggest that climate change significantly affects C and N losses during litter decomposition in complex ways via direct changes in soil moisture and indirectly by alterations in mesofauna abundance and composition, but that *P. cinnamomi* does not correlate with litter decomposition via changes in initial litter quality (i.e., initial C/N ratio) as previously suggested (Pazianoto et al., 2019). We found, however, a significant positive correlation of *P. cinnamomi* abundance with both decomposers and predators abundance and diversity. Overall, because both climate change and invasive species interact with mesofauna characteristics at several levels (*i.e.,* changes in abundance, taxonomic composition, and relative proportion of decomposers and predators), our study points out to mesofauna as a key component to evaluate the importance of global change drivers for litter decomposition.

Relative importance of drought and mesofauna on litter decomposition dynamics.

Our experimental climate-change drought produced a strong direct effect on litter decomposition dynamics, but contrary to previous work (Santonja et al., 2017a, 2018; Zhou et al., 2018; Prieto et al., 2019), the reduction in soil moisture boosted litter C loss, an effect that was intensified at final decomposition stages (9 to 18 months after the beginning of the experiment) (Fig. 2). This increasing importance of the effect of abiotic drivers at final decomposition stages has been previously documented (García-Palacios et al., 2016b), but again the direction reported was to slow down C litter loss. In our study, significant increases in C loss under the rainfall exclusion treatment can be explained by microenvironmental changes in litter conditions which increased in turn mesofauna abundance (Fig. 4, Supporting information Tables S5.4, S5.5). These results therefore suggest that in our study mesofauna activity on fragmenting and consuming litter (Frouz et al., 2008) was not water-limited, and indicate that this biotic compartment is key to increase C mineralization rates under future drier conditions.

Litter N dynamics instead did not follow the negative exponential decay common for litter mass and C. We observed an initial increase in N content, likely due to the colonization of microbial organisms that immobilize N at the first stages of decomposition (Berg & Laskowski, 2005; García-Palacios et al., 2017). Interestingly, we observed that both rainfall exclusion and litter mesofauna modulate the amplitude of changes in N litter during the decomposition processes. Slight modifications of rainfall exclusion on N litter dynamics has been previously documented (Santonja et al., 2017a) but not the effect of litter mesofauna, which has important implications for N releases (*i.e.,* difference in N content between the beginning and end of the experiment). Mesofauna presence mainly reduced the timing of the inflexion point between

N immobilization and N release (3 months versus 9 months in the absence of mesofauna), yet it increased the amplitude of the cycles between N immobilization and N release (Fig, 4). These two results are of critical importance because N is a frequently limiting resource in Mediterranean forests (Sardans & Peñuelas, 2013), and our results suggest that future drier conditions can increase soil N availability thanks to the action of mesofauna on litter decomposition.

Impacts of climate change on mesofauna abundance, diversity and composition.

The observed key role of litter mesofauna on litter decomposition deserve investigating how environmental variation influence several mesofauna community characteristics. Environmental stress such as rainfall exclusion is expected to change mesofauna diversity and the relative abundance of higher trophic levels (Pires et al., 2018; Maaroufi & De Long, 2020). However, these predictions were not consistently observed in any of our forest sites (Supporting information S5), suggesting that the context dependency of climate change effects on litter fauna is stronger than previously assumed. For instance, similar to Santonja et al., (2017a), we found higher predation pressure under rainfall exclusion but only at the woodland and not at closed forest. Discrepancies between findings can be related to the fact that our study simulated realistic rainfall exclusion conditions in two different types of forests with contrasted soil characteristics and forest physiognomy. Previous findings were observed in forest sites under extreme or longer rainfall exclusion conditions (Santonja et al., 2017a; Pires et al., 2018), likely resulting in very strong changes in the invertebrate communities, which favour those organisms highly adapted to drought (Maaroufi & De Long, 2020).

When looking more in depth at the different taxonomic groups of litter mesofauna and their physiological requirements, our results suggest that drought-tolerant groups such as acari are likely influencing the positive responses of mesofauna abundance to drought (Walter & Proctor, 1999; Wise & Lensing, 2019) because they were the most abundant group in both decomposers and predators in the woodland (Supporting information Fig. S5.2). Acari shows in general better adaptations to drought conditions than other mesofauna groups as Collembola (Xu et al., 2012), probably due to their physical adaptations (sclerotized and dorsoventrally flattened body) that allow them to cope with a high range of moisture conditions (Walter & Proctor, 1999). We also observed that more functionally-diverse faunal communities also increased their abundance in the woodland, which evidence the critical importance of biodiversity for maintaining forest functionality. Eventually, all these changes in taxonomic groups will depend on their specific responses to microenvironmental conditions. Here we explored some of the most important drivers we thought were involved in modifying litter fauna communities. Some of these drivers vary at the scale of cm such as light availability or litter depth, yet most of our fauna predictors vary at the scale of meters and kilometres. This means that there might be other microhabitat differences not evaluated here such as soil characteristics, litter decomposition degree, and litter species composition (availability of different resources) (Hättenschwiler et al., 2005; Jiménez-Chacón et al., 2018) that might be playing a role in determining changes in abundance, richness and composition of litter mesofauna. Identifying the plethora of drivers modulating litter fauna characteristics is important, as our results show they are a key component canalising the indirect contribution of global changes drivers to litter decomposition.

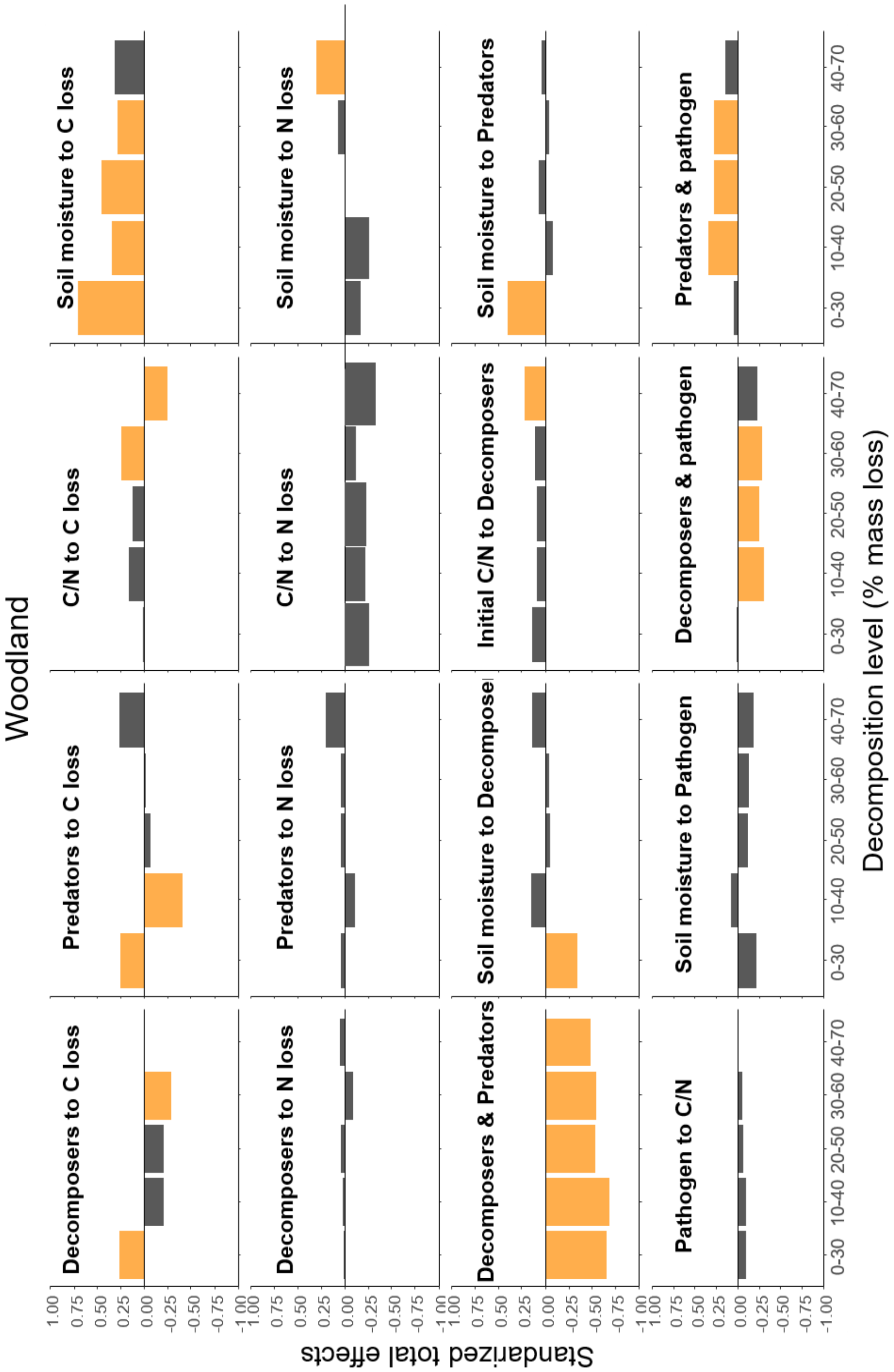

Figure 5. Standardized total effects along the decomposition process of the direct and indirect drivers of litter C and N loss in woodland. Mesofauna was included in the models using abundance data. Path coefficients that were statistically significant are represented in orange and their values are included in Table S6.1 of Supporting information. Goodness of fit tests of the multigroup comparison were: CFI=1, RMSEA=0, SRMR=0.038, p-value=0.731 for C loss and CFI=1, RMSEA=0, SRMR=0.036, p-value=0.751 for N loss. Both multigroup SEM differed significantly from the constrained model (p-value <0.0001 for C and p-value = 0.037 for N)

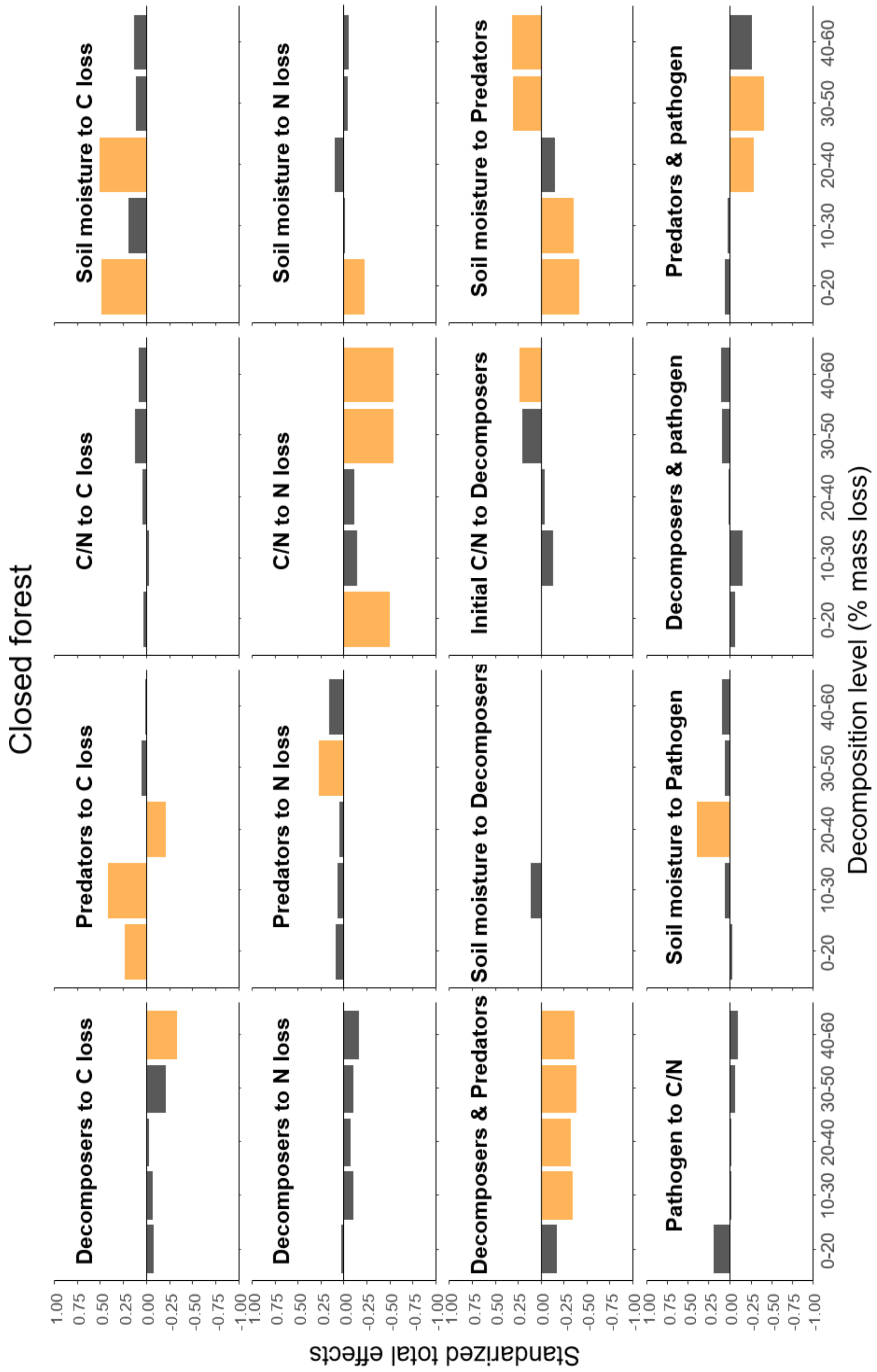

Figure 6. Standardized total effects over time of the indirect and direct effects of different variables on C and N loss in closed forest using abundance as variable of fauna. Path coefficients that were statistically significant are represented in orange and their values are included in Table S6.1 of Supporting information. Goodness of fit tests of the multigroup comparison were: CFI =1, RMSEA =0, SRMR =0.044, p-value=0.578 for C loss and CFI =1, RMSEA =0 SRMR =0.046, p-value=0.469 for N loss. Both multigroup SEM differ from the constrained model (p-value < 2.2e-16 for C and p-value <0.0001 for N).

Temporal variation in the relative importance of direct and indirect drivers of litter decomposition.

Our results clearly showed that the entangled bank of complex direct and indirect relationships determining litter decomposition varied along litter decomposition stages. Drivers involved in the loss of C litter illustrate these temporal dynamics. Soil moisture was the most important driver determining C loss, but its importance decreased over time to such point that the C/N ratio became the best predictor at late decomposition stages. This turnover is due to the importance that C/N ratios have on decomposers activity (García-Palacios et al., 2016a). The effect of soil moisture on litter mesofauna abundance and diversity varied also between trophic groups and forest types, surely influenced by the negative relationship between decomposers and predators and by the different tolerances to drought of the different taxa. However, the sign of the effect of decomposers activity on C loss varied depending on the parameter considered. While the diversity of both groups (decomposers and predators) always produced positive effects on C loss, mesofauna abundance changed from negative to positive effects depending on the decomposition stage and trophic group. Specifically, the control of predators on herbivores indirectly resulted on a positive effect over C loss during the first decomposition stages, which turned to negative at successive decomposition stages (Setälä et al., 1996; Lawrence & Wise, 2004). This latter positive effect suggests that predators could affect more intensively some decomposers taxa with smaller or negative influence in litter decomposition dynamics (Lawrence & Wise, 2004; Zhou et al., 2010), favouring therefore some groups that accelerate litter decomposition dynamics directly or through changes on microbial activity (Hättenschwiler et al., 2005; Frouz, 2018). Our results therefore provide direct evidence about the importance of trophic interactions in litter decomposition (Lawrence & Wise, 2004; Santonja et al., 2017a; Aupic-Samain et al., 2019) as a top down mechanism regulating C loss.

Litter quality was the more important and constant predictor of N litter dynamics. Patterns of N immobilization and release depend fundamentally on initial N concentration (García-Palacios et al., 2017). Lower N concentration promotes N immobilization due to high N microbial demand (Innangi et al., 2018), but N release occurs when a determined C/N mass ratio is reached (Zhou et al., 2018). In our case, the presence of mesofauna diminished this immobilization period showing therefore an important influence of litter decomposers on microbial activity. Soil moisture instead had a more complex interpretation. Although soil moisture affected N dynamics negatively at first decomposition stages but positively at late decomposition stages, this result can be interpreted as soil moisture acting as a speeding up driver of N dynamics. This is because soil moisture accelerated first N immobilization (Zhou et al., 2018) and later N losses (Santonja et al., 2018; Zhou et al., 2018). Our results therefore reinforce the fact that the net effect of reduced precipitation slows down N litter decomposition (Zheng et al., 2017).

Finding support for changes in the temporal importance of litter decomposition drivers make us hypothesize that observed relationships might be modified if future scenarios of climate change severity and invasive species change in magnitude (e.g., stronger reduction in rainfall, higher pathogen abundance, and/or a combination of both), although it seems too speculative to hypothesize the direction of the change. In particular, it deserves further attention to explore changes in the mesofauna importance according to 1) how moisture is reduced depending on the severity of future climate change, 2) how litter depth increases by the pathogen action on producing defoliation and sudden death (Brasier & Buck, 2001), and 3) how the correlation between the exotic pathogen and mesofauna abundance changes over time. Regarding this third point, it might deserve as well further exploration of to what extent such correlation is due to a trophic interaction. To our knowledge there is only one study that has discussed the existence of a trophic relationship between mesofauna and oomycetes. Specifically, Proctor et al., (1997) found evidence for a trophic interaction between detritivore acari and a parasitic oomycete of aquatic animals. Although we consider this scenario unlikely in our forest system because the mycelium of *P. cinnamomi* does not develop in the soil, but within host roots (Ruiz-Gómez et al., 2012) (i.e. it is not easily accessible to decomposers), we still think this is something worth exploring in more detail.

Conclusions

Our study provides strong evidence that the simulated reduction of precipitation, through direct changes in soil moisture and indirect changes in litter mesofauna abundance and diversity, affect both C and N litter dynamics. Contrary to previous studies, our moderate but realistic rainfall exclusion suggests an acceleration in C loss and less variation in the temporal dynamics of litter N. Further studies need to evaluate the consequences for forest dynamics of these higher nutrient inputs coming from litter decomposition processes. Contrary to expected, *P. cinnamomi* infection was not correlated with changes in initial litter quality (i.e. we did not observe a significant correlation between increases in *P. cinnamomi* abundance and changes in initial litter quality for the two *Quercus* species studied, *Q. suber* and *Q. canariensis*), but its temporal variation during our litter decomposition experiment was correlated with changes in litter mesofauna abundance. This intriguing result is novel and deserves further investigation to separate potential indirect interactions between the pathogen and the mesofauna via changes in resource availability (e.g. higher root and leaf litter availability) or in trophic interactions from just co-occurrence due to similar environmental requirements. As expected, our results picture a complex situation of direct and indirect drivers of litter decomposition whose importance change over time, yet it supports the fact that most of these effects can be summarized through changes in litter mesofauna characteristics. Therefore, our results show the importance of studying litter mesofauna to understand the effect of multiple global change drivers on litter decomposition and nutrient cycling.

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Author contributions

P.H, L.G.A., L.M and O.G conceived the ideas and designed the study; P.H. conducted field work and mesofauna extraction and identification; P.H. and O.G analyzed the data; P.H. and O.G. led the writing of the manuscript with substantial contributions from L.G.A. and L. M. All authors gave final approval for publication.

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Appendix

Supporting information S1- Design of the rainfall exclusion experiment

Table S1.1: Average values and results of the LMMs performed to test the differences on tree density (number of individuals), basal area (m^2/ha) and total light (GSF) between the experimental plots before the installation of the rainfall exclusion structure at both sites (woodland and closed forest). Plot was included as a random factor. We can observe no differences exists between the plots of both treatments for these parameters.

		Control (average ± SE)	Rainfall exclusion (average ± SE)	F-value	P-value
Woodland	Tree density	8.33±0.88	8.33±0.88	0.000	1
	Basal area	19.20±2.56	17.31±0.38	0.53	0.51
	Light (GSF)	0.30±0.03	0.36±0.04	0.83	0.41
Closed forest	Tree density	8.67±1.67	10.67±0.88	1.125	0.35
	Basal area	31.34±5.92	35.83±2.27	0.50	0.52
	Light (GSF)	0.33±0.01	0.35±0.03	0.45	0.54

Supporting information S2 – Pathogen abundance at the study site

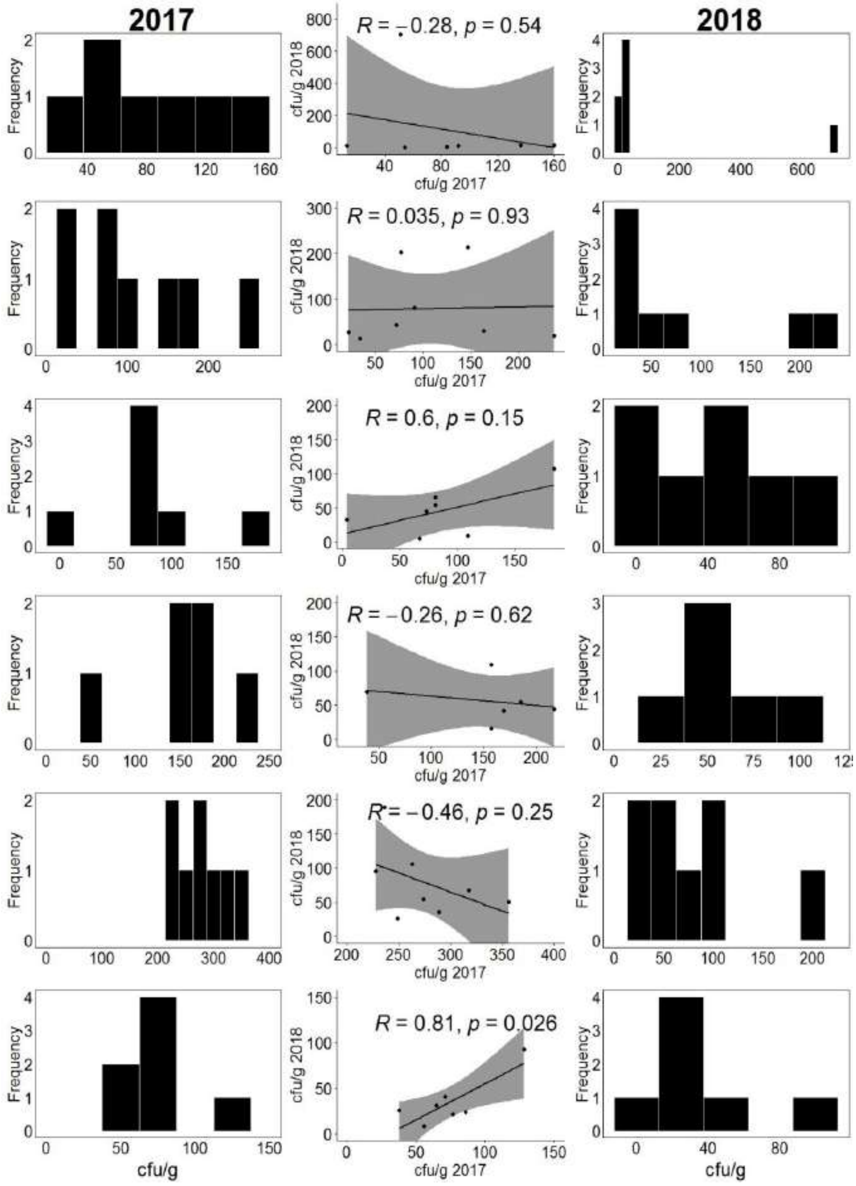


Figure S2.1. Diagram representing the variance of *P. cinnamomi* abundances during the experiment (measured each spring) in each plot of the woodland (in order from top to bottom). Abundances are expressed as colonies forming units per gram (cfu/g). Side graphs are histograms of frequency distribution of *P. cinnamomi* abundances (left panels from 2017 and right panels from 2018). Center panels represent the spatio-temporal correlation of *P. cinnamomi* abundances

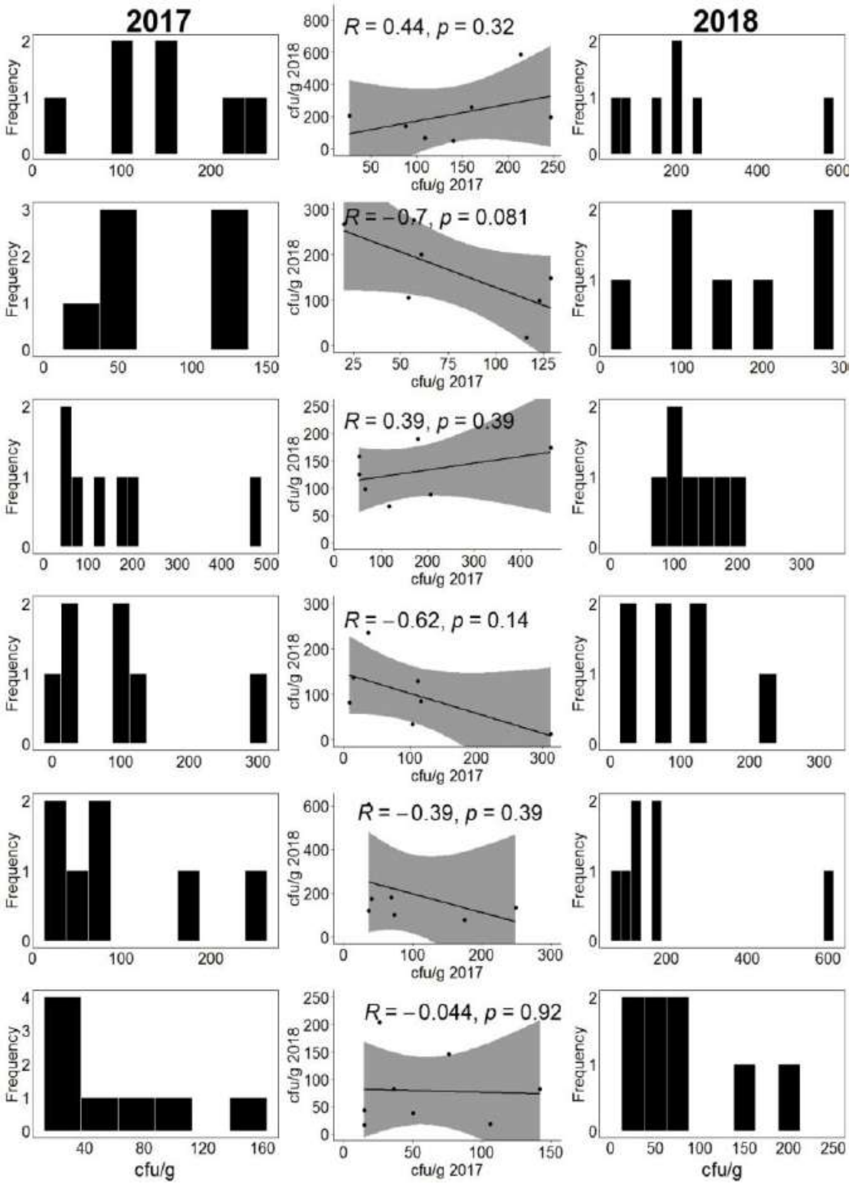


Figure S2.2. Diagram representing the variance of *P. cinnamomi* abundances during the experiment (measured each spring) in each plot of the closed forest (in order from top to bottom). Abundances are expressed as colonies forming units per gram (cfu/g). Side graphs are histograms of frequency distribution of *P. cinnamomi* abundances (left panels from 2017 and right panels from 2018). Center panels represent the spatio-temporal correlation of *P. cinnamomi* abundances.

Supporting information S3 - Soil moisture differences

Table S3.1: Results of the LMMs performed to test the differences in soil moisture between the control and rainfall exclusion plots before the installation of the rainfall reduction structure at both sites (woodland and closed forest). Month was included as a random factor. Df show the degrees of freedom of the numerator and denominator, in that order.

		Woodland			Closed forest		
		df	F-value	p-value	df	F-value	p-value
10 cm	Block	2, 109	4.91	**0.009**	2, 111	18.37	**<0.0001**
	Texture	1, 109	1.82	0.18	1, 111	23.73	**<0.0001**
	Rainfall exclusion	1, 109	2.67	0.105	1, 111	0.28	0.59
20 cm	Block	2, 108	8.66	**0.0003**	2, 111	4.22	**0.017**
	Texture	1, 108	6.1	**0.015**	1, 111	2.29	0.133
	Rainfall exclusion	1, 108	0.56	0.454	1, 111	4.14	**0.044**
30 cm	Block	2, 109	6.68	**0.002**	2, 111	0.61	0.545
	Texture	1, 109	20.73	**<0.0001**	1, 111	6.51	**0.012**
	Rainfall exclusion	1, 109	4.29	**0.041**	1, 111	6.42	**0.013**
40 cm	Block	2, 109	2.18	0.117	2, 111	0.99	0.374
	Texture	1, 109	57.36	**<0.0001**	1, 111	11.15	**0.001**
	Rainfall exclusion	1, 109	10.86	**0.001**	1, 111	5.85	**0.017**

Table S3.2: Results of the LMMs performed to test the effect on soil moisture of the rainfall exclusion (RE) at both sites (woodland and closed forest). Block and year were included as fixed factors due to the limited number of levels, to eliminate the spatial and temporal differences between plots and years on rainfall exclusion effect. Texture was included as covariate to avoid the effect of the differences in geological composition between plots. Df show the degrees of freedom of the numerator and denominator, in that order.

		Woodland			Closed forest		
10 cm		df	F-value	p-	Df	F-value	p-value
	Block	2,106	6.58	**0.001**	2,1026	7.6	**0.0005**
	Year	2,107	20.84	**$1.32e^{-9}$**	2,1027	12.41	**$4.71\ e^{-6}$**
	Texture	1,106	0.13	0.72	1,1026	10.57	**0.001**
	Rainfall exclusion	1.106	34.25	**$6.42\ e^{-}$**	1,1026	13.09	**0.0003**
20 cm	Block	2,103	13.7	**$1.34\ e^{-}$**	2,993	35.28	**$1.57\ e^{-15}$**
	Year	2,103	14.19	**$8.35\ e^{-}$**	2,994	17.67	**$2.88e^{-8}$**
	Texture	1,103	49.02	**$4.56e^{-}$**	1,993	4.05	**0.045**
	Rainfall exclusion	1,103	17.77	**$2.72\ e^{-}$**	1,993	61.75	**$1.01\ e^{-14}$**
30 cm	Block	2,106	9.36	**$9.37\ e^{-}$**	2,1027	38.21	**$<2.2\ e^{-16}$**
	Year	2,107	18.51	**$1.25\ e^{-}$**	2,1028	33.01	**$1.27\ e^{-14}$**
	Texture	1,106	197.93	**$<2.2\ e^{-}$**	1,1027	4.46	**0.035**
	Rainfall exclusion	1,106	78.63	**$<2.2\ e^{-}$**	1,1027	146.73	**$<2.2\ e^{-16}$**
	Block	2,106	5.04	**0.006**	2,1025	41.93	**$<2.2\ e^{-16}$**
40	Year	2,106	40.78	**$<2.2\ e^{-}$**	2,1026	32.01	**$3.26\ e^{-14}$**
	Texture	1,106	202.92	**$<2.2\ e^{-}$**	1,1025	18.53	**$1.83\ e^{-5}$**
	Rainfall exclusion	1,106	70.76	**$<2.2\ e^{-}$**	1,10250	155.93	**$<2.2\ e^{-16}$**

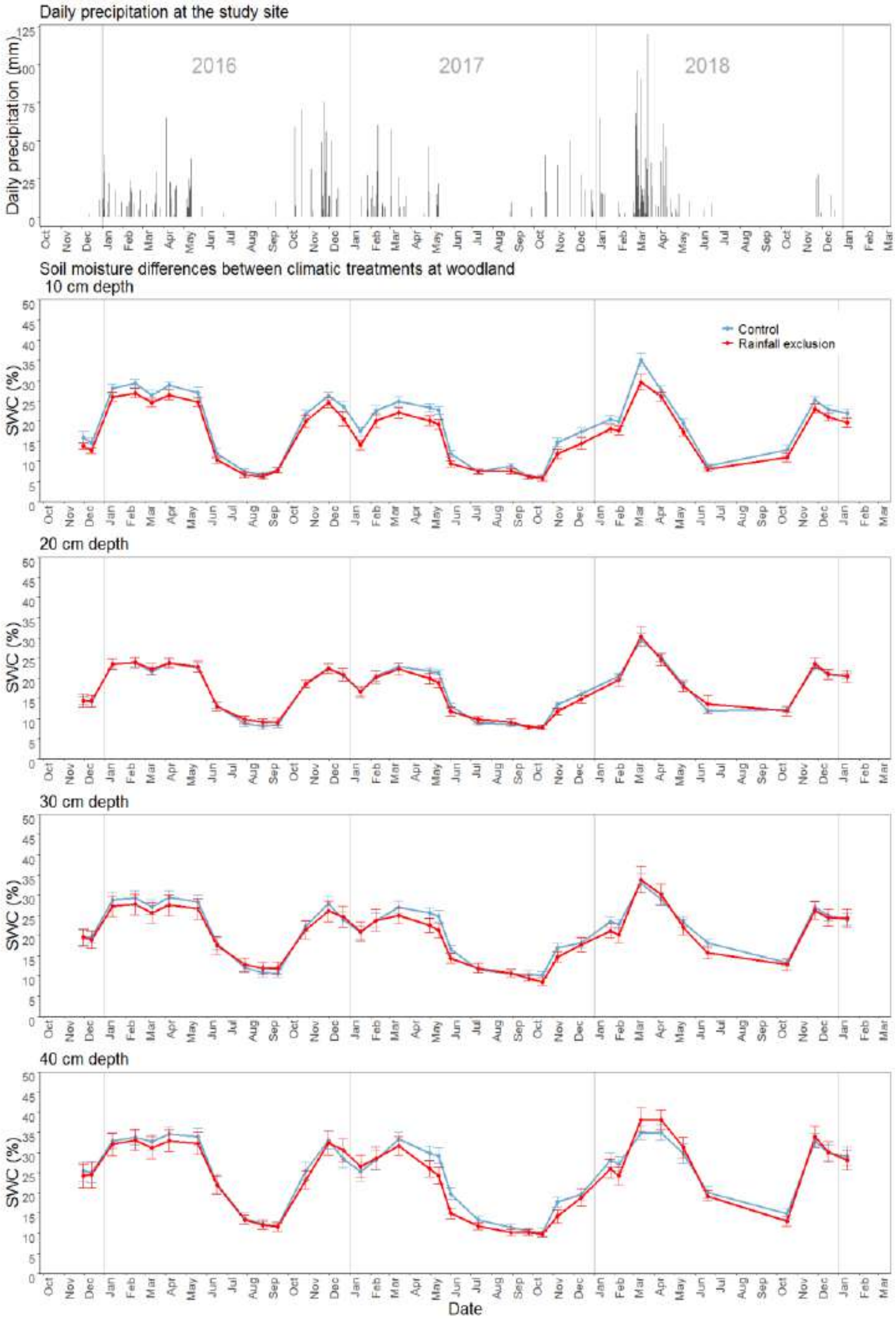
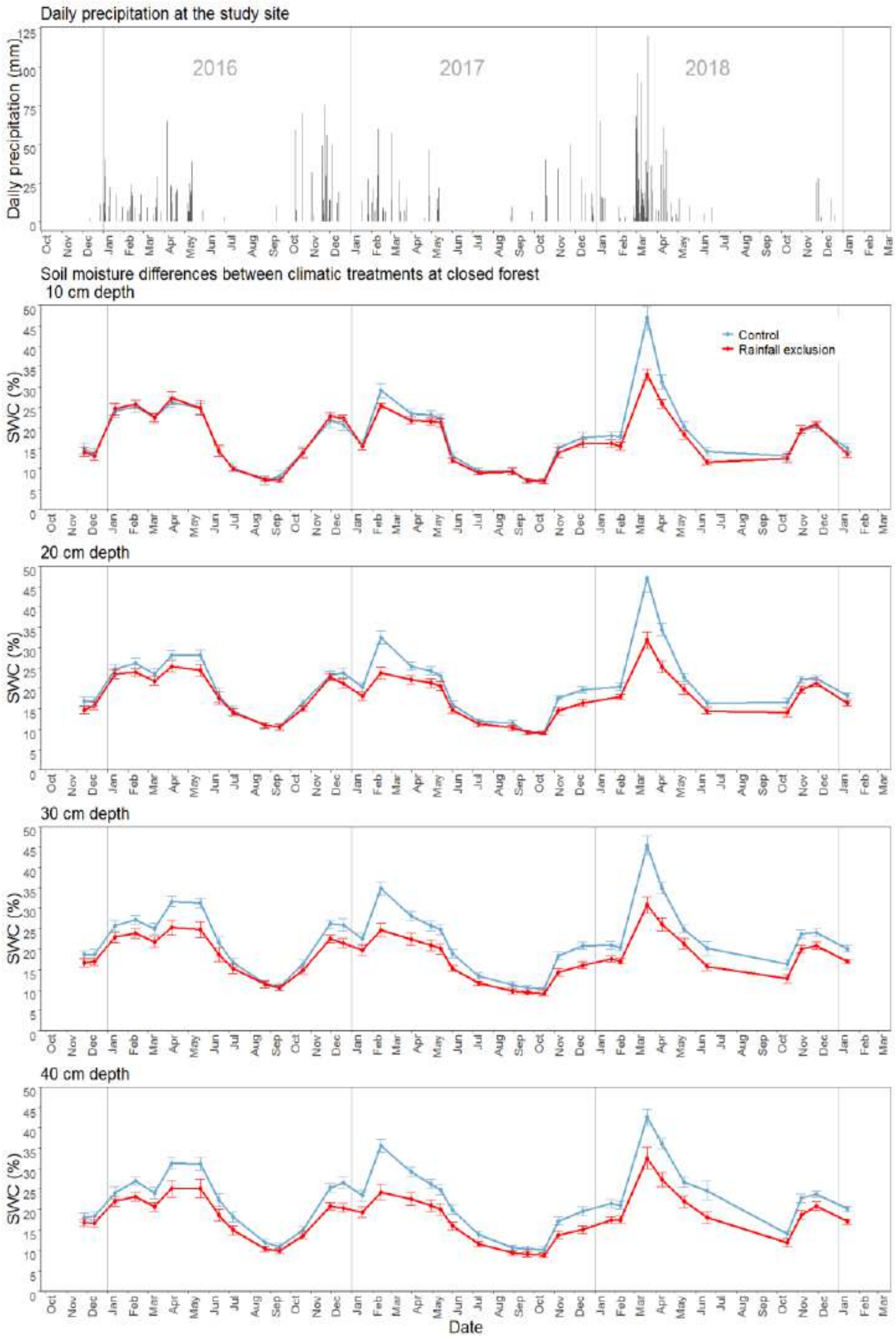


Figure S3.1. Average soil moisture values at different soil depths (10, 20, 30 and 40 cm) according to climatic experimental treatment at the woodland since rainfall exclusion structure installation. Upper graph shows total rainfall precipitation, vertical grey lines separate different years. Error bars represent ±SE.


Daily precipitation at the study site

Soil moisture differences between climatic treatments at closed forest

10 cm depth

20 cm depth

30 cm depth

40 cm depth

Figure S3.2. Average soil moisture values at different soil depths (10, 20, 30 and 40 cm) according to climatic experimental treatment at closed forest since rainfall exclusion structure installation. Upper graph shows total rainfall precipitation, vertical grey lines separate different years. Error bars represent ±SE.

Supporting information S4 - Litter quality

Table S4.1: Results of the GLMM analyses testing effects of rainfall exclusion, tree species and their interaction in the initial litter C:N ratio at the two forest types. Litter from different *O. europaea* trees was mixed to form one single litter pool due to the low quantify of fresh litter available, and therefore this species was not included in the analysis. Df show the degrees of freedom of the numerator and denominator, in that order.

		DF	*F-value*	*p-value*
Woodland	Rainfall exclusion (RE)	1,4	0.6747	0.4576
Closed forest	RE	1,4	0.57	0.4937
	Tree species (TE)	1,35	0.68	0.4142
	RE x TE	1,4	5.35	**0.0265**

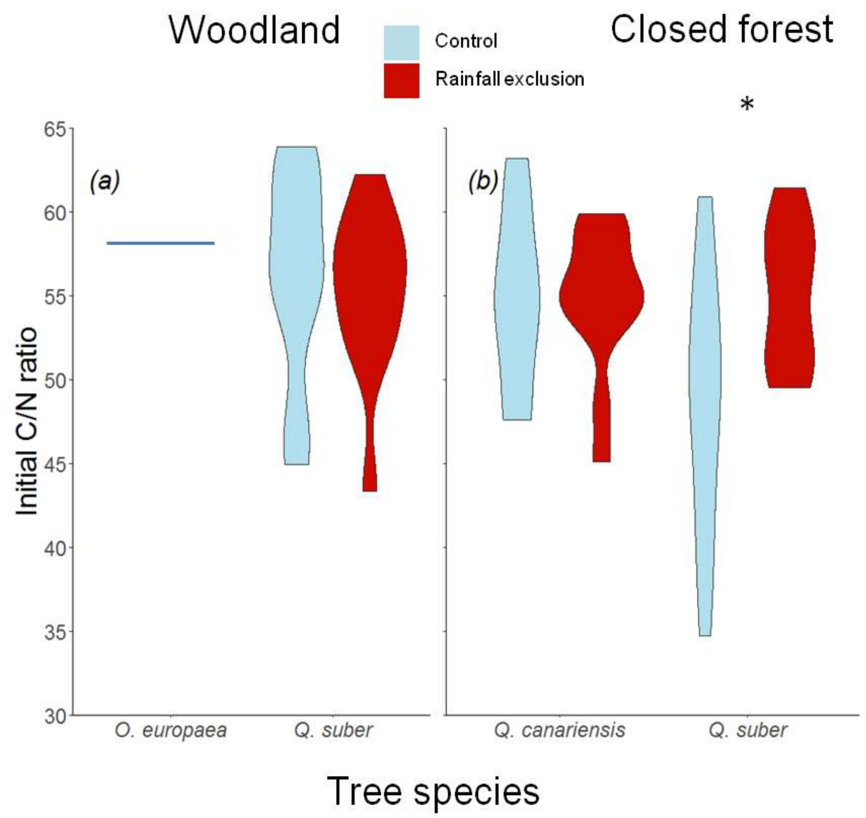


Figure S4.1. Initial C/N ratio of litter of focal species at each studied forest (woodland, closed forest) under the different climatic treatments. Asterisks denote significant differences between climatic treatments. Error bars show ±SE. We only show one bar for *O. europaea* due to the initial pool made with its litter to perform the experiment.

Supporting information S5 - Litter fauna

		Total mesofauna abundance			Decomposers abundance			Predators abundance			Decomposers/predators			Mesofauna diversity		
		df	F-value	p-value	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Woodland	RE	1,4	0.02	0.87	1,4	0.06	0.81	1,4	0.14	0.72	1,4	0.35	0.58	1,4	0.0007	0.98
	SP	1,35	0.19	0.66	1,35	0.03	0.84	1,35	1.04	0.31	1,35	2.74	0.1	1,35	2.84	0.1
	time(t)	2,78	126.46	**<0.0001**	2,78	115.58	**<0.001**	2,78	145.16	**<0.0001**	2,78	6.35	**0.004**	2,78	128.05	**<0.0001**
	RE x SP	1,35	0.27	0.61	1,35	0.03	0.87	1,35	0.06	0.8	1,35	2.70	0.11	1,35	0.16	0.69
	RE x T	2,78	3.88	**0.02***	2,78	2.19	0.11	2,78	2.6	*0.08*	2,78	5.34	**0.008**	2,78	4.71	**0.01**
	SP x T	2,78	2.08	0.13	2,78	1.72	0.18	2,78	0.88	0.41	2,78	0.84	0.43	2,78	2.81	*0.06*
	RE x SP x T	2,78	1.07	0.35	2,78	0.31	0.73	2,78	0.99	0.37	2,78	0.322	0.72	2,78	2.15	0.12
Closed forest	RE	1,4	0.02	0.9	1,4	0.15	0.71	1,4	0.17	0.7	1,4	0.88	0.4	1,4	0.81	0.42
	SP	1,35	6.16	**0.01**	1,35	6.57	**0.014**	1,35	4.17	0.05	1,35	4.08	*0.05*	1,35	14.31	**0.0006**
	time(t)	3,117	55.71	**<0.0001**	3,117	45.59	**<0.0001**	3,117	84.01	**<0.0001**	3,100	25.41	**<0.001**	3,117	5.59	**0.0013**
	RE x SP	1,35	0.93	0.34	1,35	0.98	0.33	1,35	0.24	0.62	1,35	0.35	0.56	1,35	0.13	0.73
	RE x T	3,117	0.32	0.81	3,117	0.54	0.67	3,117	0.39	0.75	3,100	0.69	0.56	3,117	0.55	0.71
	SP x T	3,117	10.09	**<0.0001**	3,117	10.97	**<0.0001**	3,117	6.34	**0.0005**	3,100	4.59	**0.005**	3,117	9.61	**<0.0001**
	RE x SP x T	3,117	0.55	0.65	3,117	0.29	0.83	3,117	0.69	0.56	3,100	0.97	0.41	3,117	0.69	0.56

Table S5.1 : Results of the GLMMs performed to test the effect of the rainfall exclusion (RE) , tree species (SP), time (T) and their interactions on total mesofauna abundance, decomposers abundance, predators abundance, decomposers/predators ratio and Mesofauna diversity. Df shows the degrees of freedom of the numerator and denominator. Significant effects (p<0.05) are shown in bold, whereas marginally significant effects (p<0.1) are shown in cursive

Table S5.2: Results of the GLMM analyses testing effects of rainfall exclusion, tree species and their interaction on the abundance of specific taxa of litter mesofauna. Df show the degrees of freedom of the numerator and denominator, in that order.

		Woodland			Closed forest		
		DF	F-value	p-value	DF	F-value	p-value
Oribatida	Rainfall exclusion (RE)	1,4	0	0.9568	1,4	0.62	0.4758
	Time (t)	2,78	119.64	**<.0001**	3,117	41.5	**<.0001**
	Tree specie (SP)	1,35	0	0.9784	1,35	0.6	0.445
	RE × T	2,78	0.74	0.4794	3,117	0.7	0.5522
	RE × SP	1,35	0.07	0.7926	1,35	2.58	0.1175
	SP × T	2,78	1.4	0.2523	3,117	18.82	**<.0001**
	RE×SP x T	2,78	0.67	0.5138	3,117	0.13	0.9405
Prostigmata	RE	1,4	0.4	0.5629	1,4	0.35	0.5836
	T	2,78	85.55	**<.0001**	3,117	83.72	**<.0001**
	SP	1,35	1.06	0.3113	1,35	7.52	0.0096
	RE x t	2,78	0.95	0.3907	3,117	0.28	0.8394
	RE x SP	1,35	0.03	0.8673	1,35	1.33	0.2563
	SP x t	2,78	1.62	0.205	3,117	0.5	0.6831
	RE x SP x t	2,78	0.48	0.6189	3,117	0.39	0.7587
Mesostigmata	RE	1,4	0.39	0.564	1,4	0	0.9597
	t	2,78	126.16	**<.0001**	3,117	25.23	**<.0001**
	sp	1,35	0.32	0.5777	1,35	1.93	0.1738
	RE x t	2,78	2.11	0.1277	3,117	0.54	0.6559
	RE x sp	1,35	0.14	0.7091	1,35	1.54	0.223
	Sp x t	2,78	0.24	0.788	3,117	19.82	**<.0001**
	RE x sp x t	2,78	0.73	0.4868	3,117	1.49	0.2213
Entomobryomorpha	RE	1,4	0.14	0.7291	1,4	0.02	0.8954
	T	2,78	71.3	**<.0001**	3,117	29.57	**<.0001**
	Sp	1,35	0.05	0.8211	1,35	4.15	**0.0492**
	RE x t	2,78	0.13	0.8778	3,117	0.53	0.6606
	RE x sp	1,35	0	0.9714	1,35	3.55	0.0681
	Sp x t	2,78	1.2	0.3069	3,117	21.69	**<.0001**
	RE x sp x t	2,78	1.18	0.312	3,117	3.27	0.0237
Poduromorpha	RE	1,4	0.04	0.8572	1,4	0.06	0.8171
	t	2,78	66.92	**<.0001**	3,117	14.05	**<.0001**
	sp	1,35	0.39	0.5376	1,35	11.12	**0.002**
	RE x t	2,78	0.8	0.4509	3,117	0.3	0.8234
	RE x sp	1,35	0.05	0.8267	1,35	0.88	0.355
	Sp x t	2,78	1.15	0.3207	3,117	1.45	0.2315
	RE x sp x t	2,78	1.16	0.3185	3,117	2.01	0.1162

		Woodland			Closed forest		
		DF	F-value	p-value	DF	F-value	p-value
Symphypleona	RE	1,4	0.51	0.5157	1,4	0.09	0.7779
	t	2,78	37.58	**<.0001**	3,117	34.77	**<.0001**
	sp	1,35	0.01	0.927	1,35	7.92	0.008
	RE x t	2,78	0.38	0.6867	3,117	0.61	0.6103
	RE x sp	1,35	0.16	0.6931	1,35	0.49	0.4884
	Sp x t	2,78	1.47	0.2365	3,117	5.19	**0.0021**
	RE x sp x t	2,78	0.4	0.6726	3,117	0.61	0.6104
Neelipleona	RE	1,4	0.68	0.4572	1,4	2.78	0.1707
	t	2,78	5.78	**0.0045**	3,117	1.85	0.1423
	sp	1,35	0.43	0.5181	1,35	1.12	0.2962
	RE x t	2,78	2.32	0.1053	3,117	2.54	0.0595
	RE x sp	1,35	1.32	0.2589	1,35	1.73	0.1967
	Sp x t	2,78	2.93	0.0593	3,117	3.08	**0.03**
	RE x sp x t	2,78	1.69	0.1907	3,117	1.08	0.3618
Psocoptera	RE	1,4	0.02	0.8872	1,4	0.1	0.7659
	t	2,78	3.98	0.0226	3,117	10.2	<.0001
	sp	1,35	0.1	0.7527	1,35	81.48	<.0001
	RE x t	2,78	1.25	0.292	3,117	0.35	0.7914
	RE x sp	1,35	1.09	0.3031	1,35	0.01	0.9256
	Sp x t	2,78	3.23	0.045	3,117	19.55	<.0001
	RE x sp x t	2,78	1.62	0.2039	3,117	2.77	0.0448
Thysanoptera	RE	1,4	5.87	0.0725	1,4	0.1	0.7659
	t	2,78	1.02	0.3645	3,117	10.2	**<.0001**
	sp	1,35	3.94	0.0549	1,35	81.48	**<.0001**
	RE x t	2,78	1.55	0.2182	3,117	0.35	0.7914
	RE x sp	1,35	0.39	0.5362	1,35	0.01	0.9256
	Sp x t	2,78	1.91	0.155	3,117	19.55	**<.0001**
	RE x sp x t	2,78	1.23	0.2988	3,117	2.77	0.0448
Diplopoda	RE	1,4	0.24	0.6524	1,4	2.5	0.189
	T	2,78	4.93	**0.0096**	3,117	8.38	**<.0001**
	Sp	1,35	0.06	0.8041	1,35	5.31	**0.0273**
	RE x t	2,78	2.25	0.112	3,117	0.05	0.983
	RE x sp	1,35	2.53	0.1207	1,35	0.38	0.5413
	Sp x t	2,78	0.29	0.7527	3,117	3.65	**0.0147**
	RE x sp x t	2,78	0.47	0.6286	3,117	0.44	0.7247

		Woodland			Closed forest		
		DF	F-value	p-value	DF	F-value	p-value
Chilopoda	RE	1,4	0.15	0.7201	1,4	0.15	0.7207
	T	2,78	3.05	0.0532	3,117	2.68	0.0501
	Sp	1,35	1.57	0.2187	1,35	0.7	0.4077
	RE x t	2,78	0.06	0.9406	3,117	0.7	0.5563
	RE x sp	1,35	0.03	0.868	1,35	1.76	0.193
	Sp x t	2,78	0.67	0.5134	3,117	4.02	**0.0093**
	RE x sp x t	2,78	0.53	0.5881	3,117	0.57	0.6342
Pseudoscorpionida	RE	1,4	0.06	0.8182	1,4	0	0.9584
	T	2,78	23.59	**<.0001**	3,117	3.52	**0.0173**
	Sp	1,35	4.48	0.0415	1,35	0.12	0.731
	RE x t	2,78	0.12	0.8896	3,117	1.23	0.3004
	RE x sp	1,35	0.09	0.7616	1,35	1.41	0.2431
	Sp x t	2,78	0.91	0.4065	3,117	0.94	0.425
	RE x sp x t	2,78	1.54	0.2215	3,117	0.94	0.4256

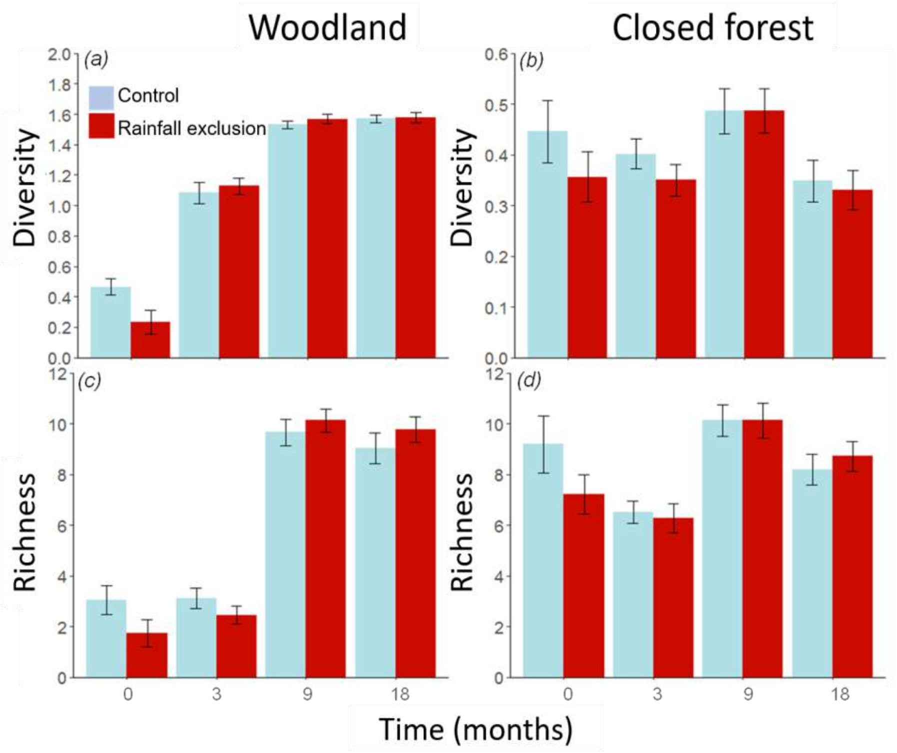


Figure S5.1: Mesofauna diversity and richness changes over time depending on the climatic treatment at both forest sites. Diversity was calculated as the Shannon Index. Error bars represent ±SE.

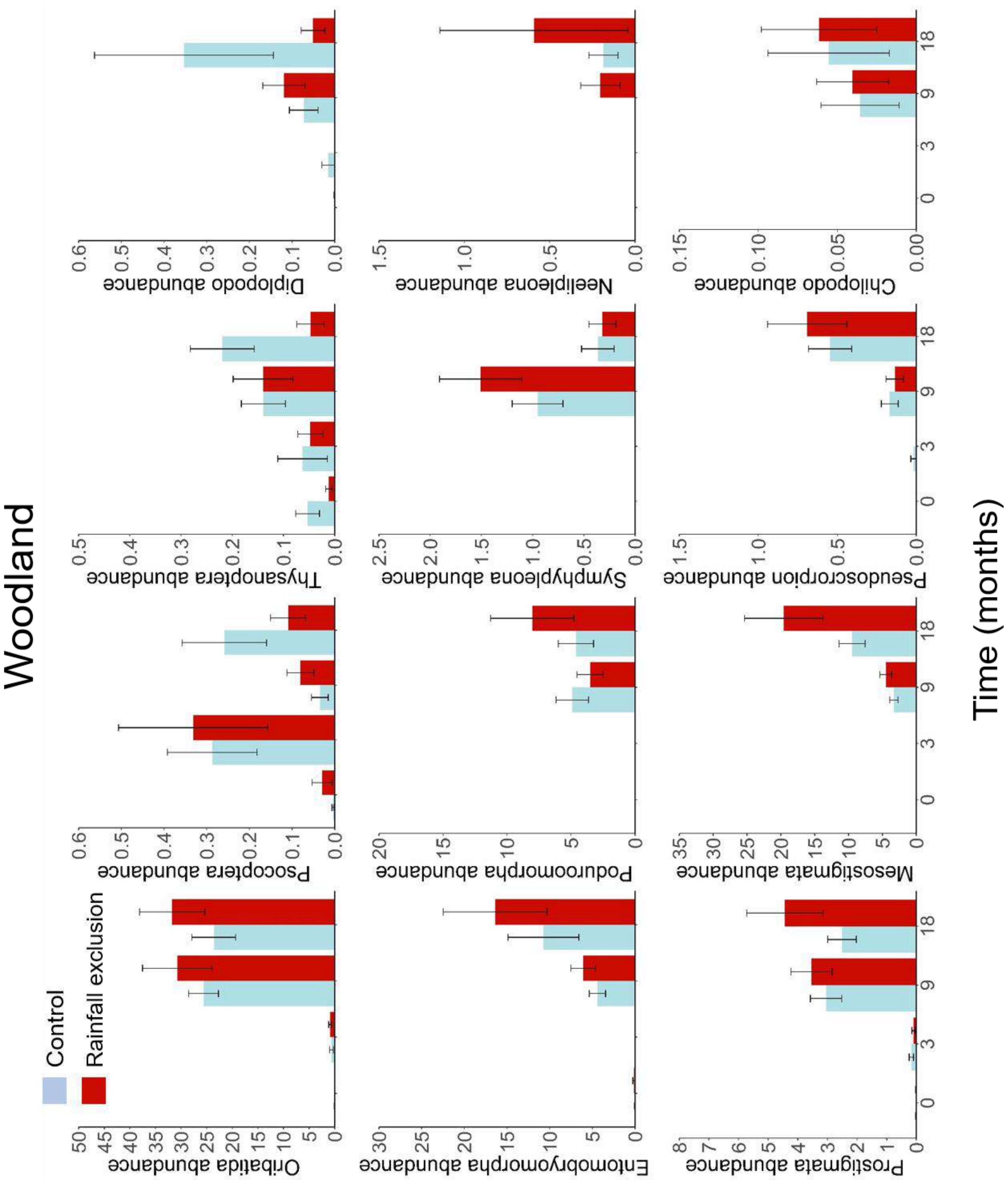


Figure S5.2. Abundances of different taxonomic groups of mesofauna depending on the climatic experimental treatment at the woodland. Abundance is expressed as number of individuals per gram of litter. Error bars represent ±SE.

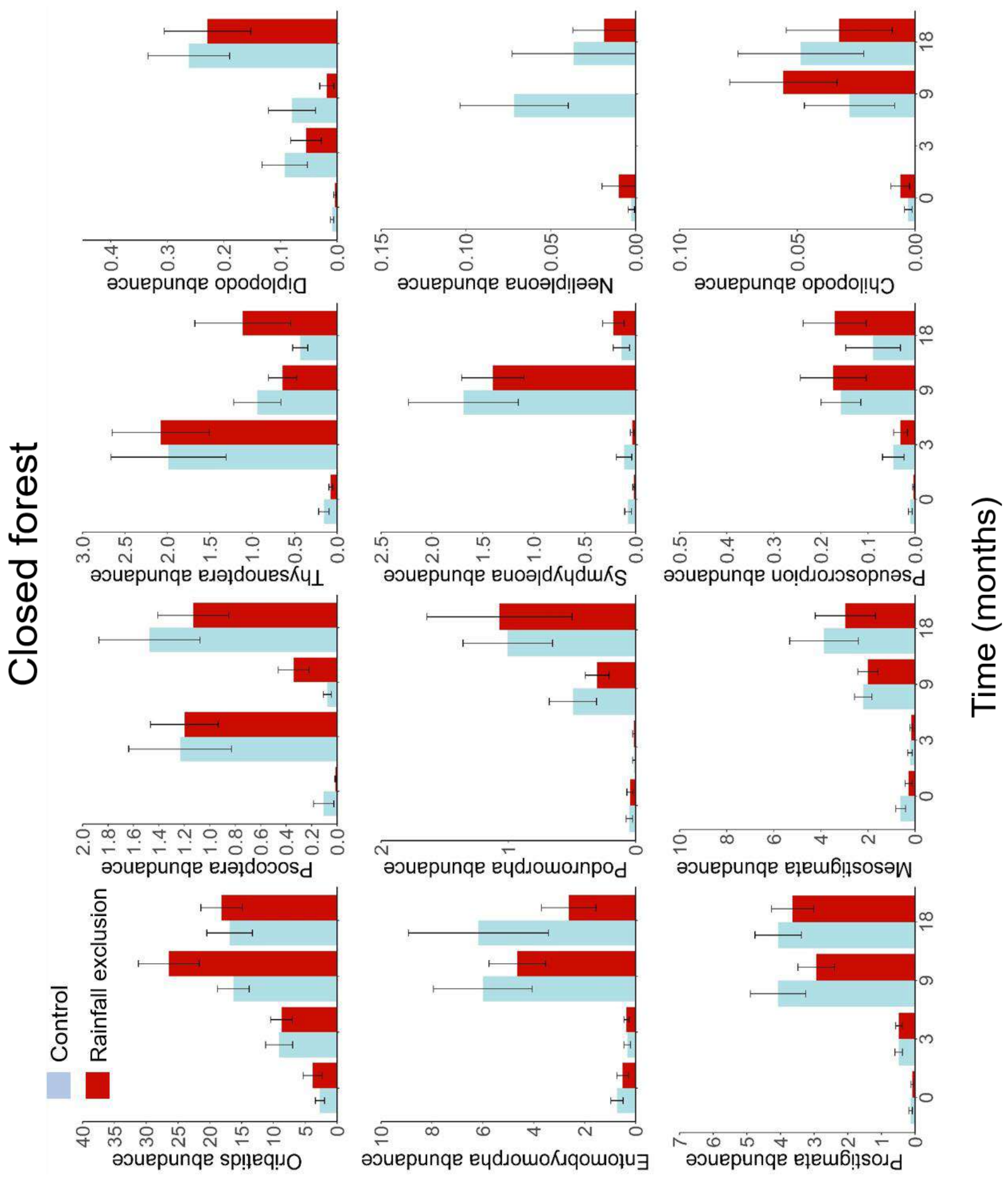


Figure S5.3. Abundances of different taxonomic groups of mesofauna depending on the climatic experimental treatment at the closed forest. Abundance is expressed as number of individuals per gram of litter. Error bars represent ±SE.

Table S5.3. Results of the Permanova test performed to analyze the effect of the tree species in the ordination of mesofauna species abundance on the NMDS. The analysis was performed separately for each site and climatic treatment.

			DF	Sums	MeanSqs	F-	R^2	P-value
Woodland	Rainfall exclusion	Tree	1	0.49	0.9	2.09	0.033	0.034*
		Residuals	62	14.51	0.23		0.97	
		Total	63	15.0005			1	
	Control	Tree	1	0.25	0.25	0.98	0.014	0.42
		Residuals	68	17.39	0.26		0.99	
		Total	69	17065			1	
Closed forest	Rainfall exclusion	Tree	1	0.32	0.32	1.67	0.02	0.084
		Residuals	86	16.37	0.19		0.98	
		Total	87	16.69			1	
	Control	Tree	1	0.48	0.48	2.64	0.03	0.011*
		Residuals	80	14.64	0.18		0.97	
		Total	81	15.12			1	

Table S5.4. NMDS axis scores for rainfall exclusion and control plots in the woodland site.

Rainfall Exclusion						Control					
Environmental variables						***Environmental variables***					
	NMDS1	NMDS2	R^2	Pr(>r)			NMDS1	NMDS2	R^2	Pr(>r)	
Soil moisture	0.972	0.236	0.145	0.005	***	Soil moisture	-0.996	-0.093	0.134	0.012	*
Water content	0.995	0.097	0.096	0.049	*	Water content	-0.942	-0.335	0.117	0.018	*
Litter depth	-1.000	0.026	0.025	0.475		Litter depth	0.350	0.937	0.068	0.089	.
Light	0.834	-0.552	0.021	0.513		Light	-0.972	-0.234	0.029	0.361	
Thickness	-0.373	0.928	0.020	0.552		Thickness	-0.508	0.861	0.082	0.054	.
SLA	-0.797	-0.604	0.025	0.485		SLA	-0.542	0.841	0.029	0.379	
Nitrogen %	0.928	0.373	0.075	0.081		Nitrogen %	-0.987	0.159	0.173	0.001	***
Carbon %	-0.765	-0.644	0.053	0.160		Carbon %	0.870	-0.493	0.011	0.645	
Initial N%	0.959	-0.284	0.033	0.316		Initial N%	-0.847	0.532	0.039	0.261	
Initial C %	0.012	-1.000	0.004	0.885		Initial C %	-0.023	-1.000	0.019	0.534	
Initial C/N	-0.950	0.311	0.041	0.265		Initial C/N	0.881	-0.474	0.035	0.296	
C/N	-0.889	-0.457	0.102	0.033	*	C/N	0.946	-0.325	0.251	0.001	***
Pathogen	-0.102	0.995	0.040	0.297		Pathogen	-0.884	-0.468	0.013	0.538	
Defoliation	-0.939	0.343	0.026	0.455		Defoliation	-0.494	0.869	0.014	0.612	
Taxa						***Taxa***					
Oribatida	0.964	-0.268	0.374	0.002	**	Oribatida	-0.990	0.139	0.292	0.001	***
Mesostigmata	0.960	0.281	0.098	0.051	.	Mesostigmata	-0.996	-0.089	0.102	0.029	*
Prostigmata	0.369	0.929	0.307	0.004	**	Prostigmata	-0.881	0.472	0.251	0.001	***
Araneae	-0.999	0.032	0.033	0.346		Araneae	0.069	-0.998	0.412	0.001	***
Pseudoscorpionida	0.771	0.637	0.048	0.221		Pseudoscorpionida	-0.582	-0.813	0.108	0.044	*
Isopoda	0.436	-0.900	0.002	0.913		Isopoda	-0.918	-0.397	0.003	0.761	
Entomobryomorpha	0.884	0.468	0.073	0.123		Entomobryomorpha	-0.840	-0.542	0.169	0.004	**
Neelipleona	0.972	0.234	0.016	0.481		Neelipleona	-0.984	0.176	0.011	0.635	
Poduromorpha	0.969	0.247	0.060	0.143		Poduromorpha	-0.790	-0.613	0.134	0.013	*
Symphypleona	0.992	-0.123	0.063	0.142		Symphypleona	-0.866	-0.499	0.036	0.328	
Thysanoptera	-0.502	-0.865	0.753	0.001	***	Thysanoptera	1.000	-0.018	0.874	0.001	***
Diplopodo	0.919	-0.394	0.023	0.432		Diplopodo	-0.119	0.993	0.126	0.044	*
Chilopodo	0.856	0.517	0.012	0.609		Chilopodo	-0.556	-0.831	0.009	0.674	
Psocoptera	-0.864	0.503	0.816	0.001	***	Psocoptera	0.064	0.998	0.556	0.001	***

Table S5.5. NMDS axis scores for rainfall exclusion and control plots in the closed forest**.**

Rainfall Exclusion						Control					
Environmental variables						***Environmental variables***					
	NMDS1	NMDS2	r2	Pr(>r)			NMDS1	NMDS2	R^2	Pr(>r)	
Soil moisture	-0.810	0.586	0.037	0.182		Soil moisture	0.572	-0.820	0.054	0.106	
Water content	-0.990	0.140	0.227	0.001	***	Water content	-0.407	-0.914	0.284	0.001	***
Litter depth	-0.892	0.453	0.120	0.006	**	Litter depth	0.439	-0.898	0.130	0.005	**
Light	-0.033	0.999	0.015	0.542		Light	-0.379	-0.925	0.031	0.283	
Thickness	0.418	-0.908	0.013	0.575		Thickness	-0.542	0.840	0.020	0.447	
SLA	0.024	1.000	0.008	0.705		SLA	0.940	-0.340	0.017	0.526	
Nitrogen %	-0.960	0.282	0.002	0.899		Nitrogen %	-0.309	-0.951	0.040	0.197	
Carbon %	0.962	0.272	0.011	0.601		Carbon %	-0.021	1.000	0.033	0.223	
Initial N%	-0.148	-0.989	0.016	0.495		Initial N%	0.244	-0.970	0.013	0.61	
Initial C %	0.325	-0.946	0.004	0.826		Initial C %	-0.999	-0.043	0.019	0.515	
Initial C/N	0.246	0.969	0.009	0.668		Initial C/N	-0.548	0.837	0.012	0.662	
C/N	0.905	0.425	0.004	0.83		C/N	0.117	0.993	0.047	0.159	
Pathogen	-0.423	-0.906	0.004	0.839		Pathogen	-0.744	-0.668	0.023	0.394	
Defoliation	-0.560	0.829	0.005	0.826		Defoliation	-0.494	0.869	0.014	0.612	
Taxa						***Taxa***					
Oribatida	-0.559	-0.829	0.582	0.001	***	Oribatida	-0.914	0.406	0.444	0.001	***
Mesostigmata	-0.946	-0.324	0.368	0.001	***	Mesostigmata	-0.938	-0.347	0.327	0.001	***
Prostigmata	0.807	0.590	0.047	0.132		Prostigmata	0.003	-1.000	0.018	0.45	
Araneae	0.224	-0.975	0.126	0.005	**	Araneae	0.930	-0.368	0.402	0.001	***
Pseudoscorpionida	-0.091	0.996	0.086	0.029	*	Pseudoscorpionida	-0.188	-0.982	0.286	0.001	***
Isopoda	-0.548	0.836	0.072	0.06	.	Isopoda	-0.529	-0.849	0.074	0.073	.
Entomobryomorpha	-0.879	-0.477	0.176	0.001	***	Entomobryomorpha	-0.951	-0.309	0.150	0.004	**
Neelipleona	-0.682	0.732	0.024	0.29		Neelipleona	-0.350	-0.937	0.022	0.384	
Poduromorpha	-0.910	0.415	0.123	0.009	**	Poduromorpha	0.159	-0.987	0.064	0.085	.
Symphypleona	-0.937	0.348	0.079	0.031	*	Symphypleona	-0.956	-0.294	0.066	0.08	.
Thysanoptera	0.968	0.250	0.658	0.001	***	Thysanoptera	0.399	0.917	0.735	0.001	***
Diplopoda	0.448	0.894	0.059	0.083	.	Diplopoda	0.998	-0.065	0.235	0.001	***
Chilopoda	-0.796	0.606	0.195	0.002	**	Chilopoda	0.050	-0.999	0.047	0.136	
Psocoptera	0.549	-0.836	0.793	0.001	***	Psocoptera	0.814	0.581	0.011	0.669	

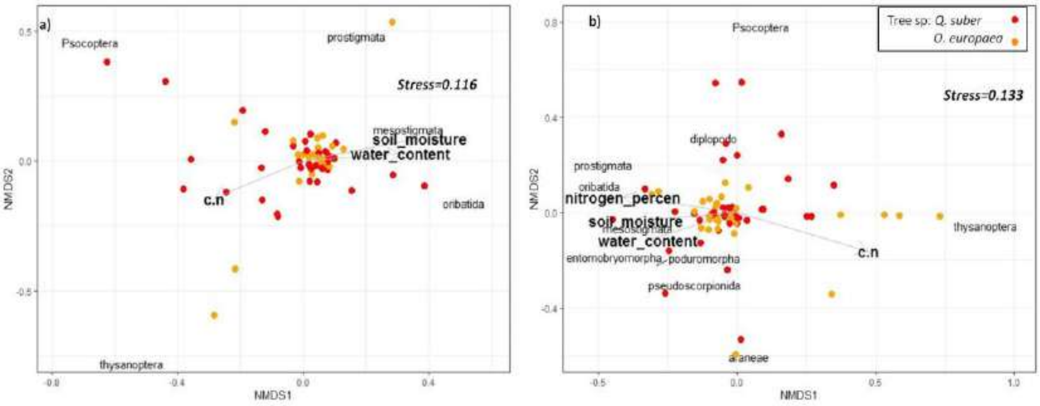


Figure S5.4. NMDS representing the ordination of mesofauna species in the woodland under rainfall exclusion (a) and control conditions (b). The different tree species present in this site *(Q. suber and O. europaea*) are represented with different colors. The stress of the models is shown in the graph.

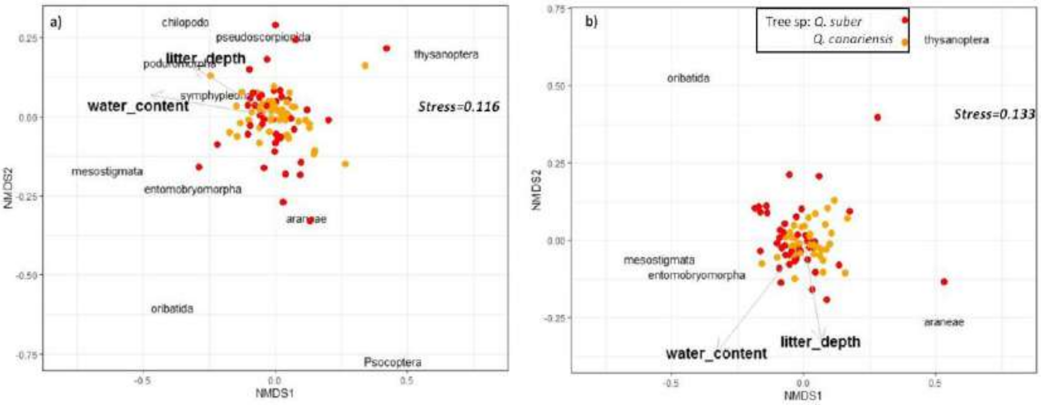


Figure S5.5. NMDS representing the ordination of mesofauna species on closed forest under rainfall exclusion (a), and control conditions (b). We explored the effect of the different tree species present in this site *(Q. suber and Q. canariensis*) represented in this graph with different colors. The stress of the models is shown in the graph.

Supporting information S6 - Direct and indirect drivers of litter decomposition and its variation with decomposition stage. (SEM)

Results of the SEM shown in the paper, performed with abundance mesofauna data. First of all, we compared the multigroup model with a constrained one, detecting significant differences in all the cases (p-values were < 2.2e-16 for C and 0.0367 for N in woodland and p-values < 2.2e-16 for C and <0.0001 for N in closed forest). In consequence, we demonstrate that our approximation to litter decomposition analyzing separately the different decomposition stages was correct. As shown in Table S6.1, the relations between the variables measured and its direct and indirect effects on C and N loss were changing across the different decomposition stages.

Table S6.1. Path coefficients from the C and N loss Structural Equation Models used to perform the multigroup comparisons between the five decomposition stages at the two study sites. Significant paths are showed in bold.

	% Mass loss	***0-30***	***10-40***	***20-50***	***30-60***	***40-70***
Woodland	Decomposers to C loss	**0.259**	-0.209	-0.211	**-0.251**	-0.017
	Predators to C loss	**0.25**	**-0.411**	-0.065	-0.023	0.265
	Initial C:N to C loss	0.017	0.164	0.125	**0.242**	**-0.257**
	Soil moisture to C loss	**0.709**	**0.349**	**0.45**	**0.288**	0.308
	Decomposers to N loss	0.006	0.017	0.033	-0.089	0.052
	Predators to N loss	0.043	-0.108	0.044	0.046	0.191
	Initial C:N to N loss	**-0.268**	-0.219	**-0.233**	-0.125	**-0.331**
	Soil moisture to N loss	-0.175	**-0.267**	0.001	0.067	**0.3**
	Decomposers vs predators	**-0.651**	**-0.69**	**-0.541**	**-0.543**	**-0.484**
	Soil moisture to decomposers	**-0.344**	0.145	-0.057	-0.041	0.13
	Initial C:N to decomposers	0.14	0.086	0.084	0.107	**0.222**
	Soil moisture to predators	**0.399**	-0.085	0.065	-0.05	0.038
	Pathogen to initial C:N	-0.102	-0.1	-0.065	-0.049	-0.004
	Soil moisture to pathogen	-0.225	0.08	-0.124	-0.136	-0.186
	Pathogen vs decomposers	0.018	**-0.305**	**-0.254**	**-0.293**	-0.231
	Pathogen vs predators	0.043	**0.336**	**0.269**	**0.28**	0.147
	% Mass loss	***0-20***	***10-30***	***20-40***	***30-50***	***40-60***
Closed Forest	Decomposers to C loss	-0.075	-0.069	-0.031	-0.216	**-0.34**
	Predators to C loss	**0.233**	**0.42**	**-0.209**	0.055	0.01
	Initial C:N to C loss	0.03	-0.031	0.039	0.12	0.08
	Soil moisture to C loss	**0.487**	0.196	**0.51**	0.11	0.128
	Decomposers to N loss	0.025	-0.107	-0.074	-0.105	-0.17
	Predators to N loss	0.084	0.069	0.044	**0.262**	0.157
	Initial C:N to N loss	**-0.504**	-0.153	-0.113	**-0.551**	**-0.55**
	Soil moisture to N loss	**-0.231**	-0.019	0.091	-0.048	-0.057
	Decomposers vs predators	-0.169	**-0.346**	**-0.321**	**-0.389**	**-0.37**
	Soil moisture to decomposers	0.006	0.11	0.001	0.007	-0.014
	Initial C:N to decomposers	-0.011	-0.132	-0.036	0.201	**0.236**
	Soil moisture to predators	**-0.423**	**-0.351**	-0.155	**0.305**	**0.314**
	Pathogen to initial C:N	0.189	-0.028	-0.025	-0.069	-0.107
	Soil moisture to pathogen	-0.034	0.054	**0.391**	0.058	0.082
	Pathogen vs decomposers	-0.073	-0.154	0.015	0.082	0.101
	Pathogen vs predators	0.06	0.019	**-0.291**	**-0.411**	**-0.271**

Discussion of SEM multrigroup results including as variable mesofauna diversity instead of mesofauna abundance

We detected some changes in the direct and indirect effects of the different drivers involved in C loss when models included mesofauna diversity instead of mesofauna abundance. At both sites, we observed that predators and decomposers diversity only had positive effects on C loss concentrated in the first stages of the process. However, at the woodland, the negative effects of both trophic groups on C loss disappeared, whereas at the closed forest decomposers diversity was positively associated with C loss. The differences of soil moisture effects on mesofauna abundance and diversity varied between trophic groups. The effects of soil moisture on predator diversity and abundance were similar, while the effect on decomposers abundance and diversity were completely different. In this case, soil moisture increased diversity of both groups during the first decomposition stages at the woodland, but this increase declined over time. Meanwhile, in the closed forest, soil moisture negatively affected the diversity of predators and decomposers at different litter decomposition stages. Unlike abundances, the relationship between the diversity of both trophic groups was positive in both sites. This relationship remained similar across time at the closed forest, while it decreased in importance over time at the woodland.

Contrary to decomposers abundance, litter quality had a positive effect on decomposers diversity at the first decomposition stage in the closed forest, but not at woodland. However, the effect of initial litter quality (C/N ratio) was less clear over decomposition stages, with a negative significant effect only at the last decomposition stages at the woodland. Mesofauna abundance had stronger relations with pathogens abundance than mesofauna diversity for almost every case. However, decomposers diversity and decomposer abundances showed similar relations with pathogen at woodland. Looking into the N dynamics, mesofauna diversity did not produce much better insights than mesofauna abundance.

Table S6.2. Path coefficients from the C and N loss Structural Equation Models used to perform the multigroup comparisons between the five decomposition stages sites with mesofauna diversity data at the two study. Marked in bold are the paths that showed significant effects. We compared the multigroup model with a constrained model, detecting significant differences in all cases (p-values <0.001 in all cases).

	% Mass loss	***0-30***	***10-40***	***20-50***	***30-60***	***40-70***
Woodland	Decomposers to C loss	**0.245**	0.189	0.071	0.032	-0.01
	Predators to C loss	**0.459**	-0.009	-0.044	0.073	0.2
	Initial C:N to C loss	-0.003	0.143	0.116	**0.225**	**-0.258**
	Soil moisture to C loss	**0.245**	**0.321**	**0.46**	**0.312**	**0.393**
	Decomposers to N loss	**-0.304**	-0.128	-0.131	-0.087	-0.026
	Predators to N loss	0.15	0.114	-0.12	0.016	0.151
	Initial C:N to N loss	**-0.259**	**-0.251**	**-0.245**	**-0.142**	**-0.319**
	Soil moisture to N loss	-0.095	-0.216	-0.009	0.059	**0.32**
	Decomposers vs predators	**0.609**	0.265	0.071	0.096	0.11
	Soil moisture to decomposers	**0.57**	0.17	-0.046	-0.13	-0.179
	Initial C:N to decomposers	-0.051	-0.234	-0.128	-0.142	0.08
	Soil moisture to predators	**0.724**	0.114	-0.041	-0.125	-0.237
	Pathogen to initial C:N	-0.102	-0.1	-0.067	-0.049	0.03
	Soil moisture to pathogen	-0.225	0.08	-0.124	-0.136	-0.186
	Pathogen vs decomposers	**-0.345**	**-0.458**	**-0.32**	**-0.245**	-0.08
	Pathogen vs predators	-0.217	0.009	0.072	0.117	0.075
	% Mass loss	***0-20***	***10-30***	***20-40***	***30-50***	***40-60***
Closed Forest	Decomposers to C loss	**0.304**	**-0.374**	0.211	-0.008	-0.196
	Predators to C loss	0.218	**0.464**	-0.172	0.115	0.074
	Initial C:N to C loss	-0.067	-0.036	0.067	0.08	0.002
	Soil moisture to C loss	**0.419**	0.189	**0.496**	0.121	0.075
	Decomposers to N loss	0.061	-0.126	-0.057	-0.11	-0.149
	Predators to N loss	0.045	0.087	0.02	0.101	0.058
	Initial C:N to N loss	**-0.523**	-0.133	-0.116	**-0.579**	**-0.588**
	Soil moisture to N loss	**-0.259**	-0.034	0.091	-0.003	-0.052
	Decomposers vs predators	**0.502**	**0.407**	**0.451**	**0.303**	**0.32**
	Soil moisture to decomposers	0.105	-0.126	0.064	**-0.29**	**-0.3**
	Initial C:N to decomposers	0.206	0.155	-0.018	-0.08	0.012
	Soil moisture to predators	**-0.305**	**-0.45**	-0.203	0.026	0.03
	Pathogen to initial C:N	0.189	-0.028	-0.025	-0.069	-0.107
	Soil moisture to pathogen	-0.034	0.054	**0.391**	0.058	0.082
	Pathogen vs decomposers	-0.02	0.123	0.16	0.005	0.045
	Pathogen vs predators	0.062	0.021	-0.131	0.031	0.106

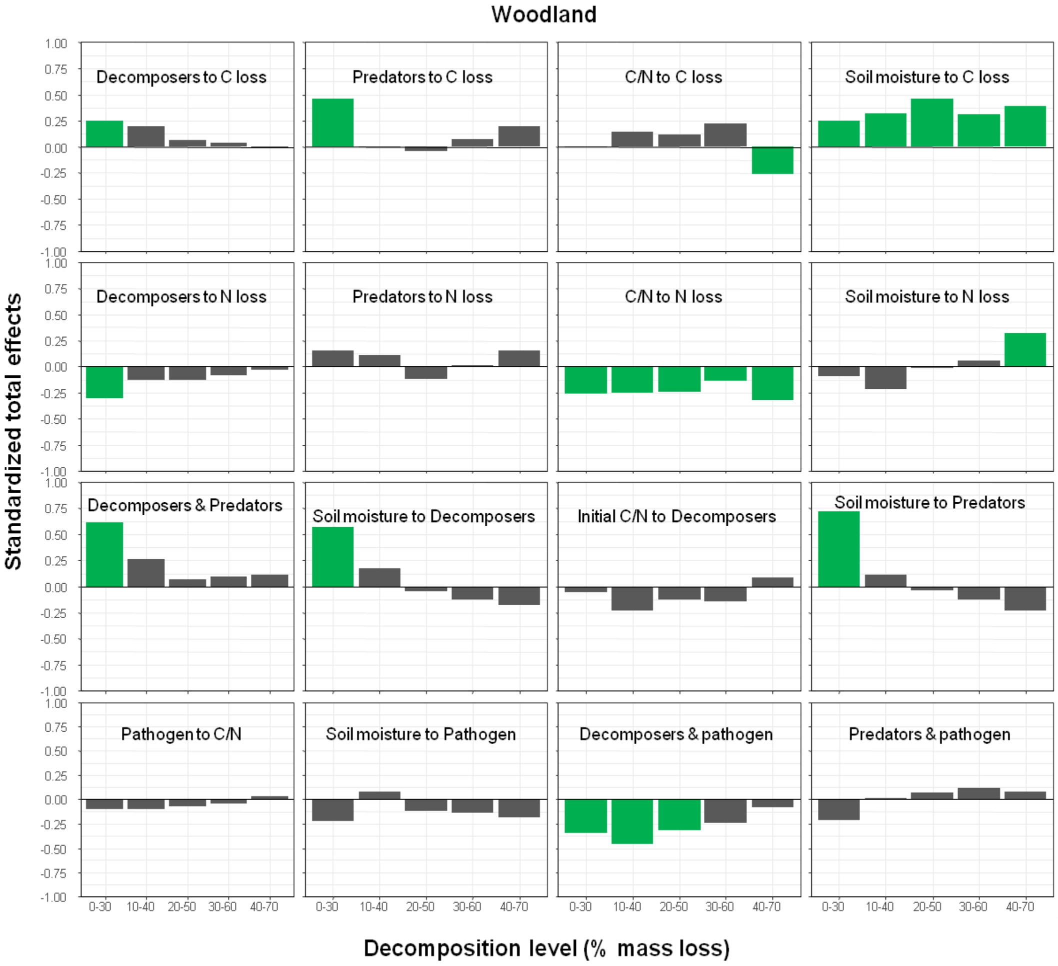


Figure S6.1. Standardized effects over time of the indirect and direct effects of different variables on C and N in the Woodland using diversity as variable of mesofauna. In this site we use 30% mass loss intervals to compare the five decomposition stages. Significant effects between paths could be found in Table S5.2 and are colored in green in the figure. Goodness of fit tests of the multigroup comparison were: CFI=1, RMSEA=0, SRMR=0.017, p-value=0.994 for C loss and CFI=1, RMSEA=0, SRMR=0.017, p-value=0.999 for N loss. Both multigroup SEM differed from the constrained model (p-values < 2.2e-16 for C and 0.0367 for N).

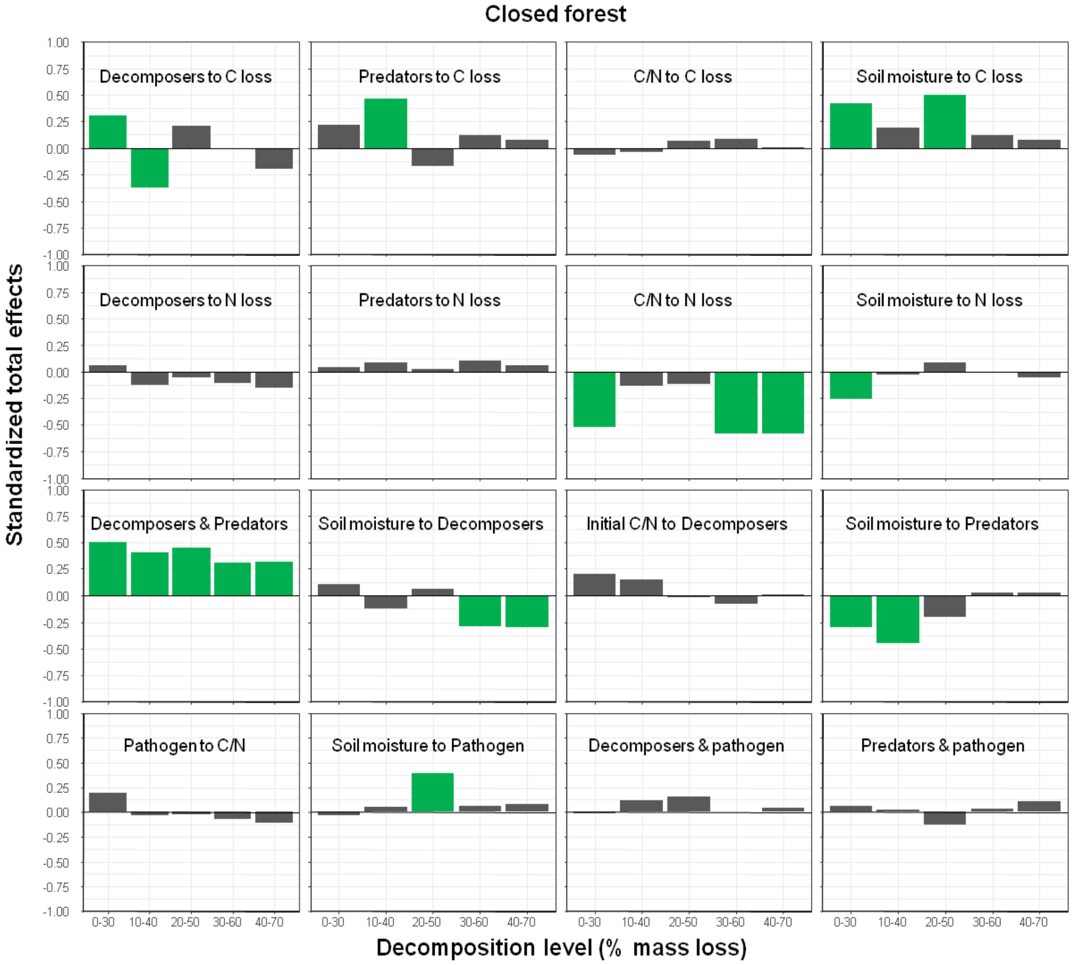


Figure S6.2. Standardized total effects over time of the indirect and direct effects of different variables on C and N in the Closed Forest using diversity as variable of fauna. In this site we use 20% mass loss intervals to compare the five decomposition stages with this method. Significant effects between paths could be found in table 2 of this appendix and are colored in green in the figure. Goodness of fit tests of the multigroup comparison were: cfi=1, rmsea=0, srmr=0.049, p-value=0.508 for C loss and cfi=0.972, rmsea=0.062, srmr=0.053, p-value=0.274 for N loss. Both multigroup SEM differ from the constrained model (P-values <0.0001).

Capítulo 4

Short- and long-term responses of nematode communities to predicted rainfall reduction in Mediterranean forests

Homet, P., Ourcival, J.M., Domínguez-Begines, J., Matías, L., Godoy, O., Gómez-Aparicio, L. (2022). (Submitted to *Soil Bioloogy and Biochemistry*).

Resumen

El cambio climático es un proceso fundamental que afecta a los ecosistemas terrestres. Sin embargo, hay relativamente poco conocimiento sobre sus impactos en las comunidades del suelo, con un alto grado de incertidumbre con respecto a su resistencia y resiliencia a las alteraciones previstas en la temperatura y, en particular, las precipitaciones. Además, la mayoría de los estudios que exploran la respuesta de la biota del suelo a la reducción prevista de las precipitaciones se han centrado en los entornos mésicos y los microbios del suelo, limitando nuestra capacidad para encontrar patrones generales entre ecosistemas y organismos del suelo. En este estudio, analizamos el impacto de la reducción de las precipitaciones pronosticadas debido al cambio climático en las redes tróficas del suelo de los bosques mediterráneos utilizando nematodos como bioindicadores. Aprovechamos las infraestructuras de exclusión de lluvia replicadas establecidas en los bosques de *Quercus* del sur de España en 2016 (exclusión de 2 años) y del sur de Francia en 2003 (exclusión de 15 años) para explorar la sensibilidad de la red trófica del suelo a las reducciones previstas en la precipitación (30%) tanto a corto como a largo plazo. La reducción de las precipitaciones tuvo grandes efectos negativos a corto plazo sobre la abundancia de nematodos, particularmente de los grupos tróficos inferiores (bacterívoros y fungívoros). La reducción de las precipitaciones también tuvo impactos consistentes tanto a corto como a largo plazo en la composición de la comunidad de nematodos (disminución de fungívoros, aumento marginal de omnívoros) y en los índices basados en nematodos indicativos de la estructura de la red trófica del suelo (mayor índice de madurez y estructura, menor ratio presa: depredador). Estos resultados muestran la existencia de respuestas rápidas en la red trófica del suelo de los bosques mediterráneos a un clima más seco y su persistencia a lo largo del tiempo, lo que indica una baja resistencia y resiliencia a las reducciones moderadas de precipitaciones previstas por el cambio climático. En general, nuestros hallazgos demuestran la sensibilidad de los bosques con agua limitada a mayores reducciones en la disponibilidad de agua del suelo, lo que podría alterar sustancialmente sus comunidades de suelo y probablemente afectar los muchos procesos del ecosistema que controlan.

Abstract

Climate change is a fundamental process affecting terrestrial ecosystems. However, there is relatively little knowledge about its impacts on soil communities, with a large degree of uncertainty regarding their resistance and resilience to predicted alterations in temperature and, particularly, precipitation. Moreover, most studies exploring the response of soil biota to predicted rainfall reduction have focused on mesic environments and soil microbes, which limit our ability to find general patterns across ecosystems and soil organisms. In this study, we analysed the impact of predicted climate change scenarios of rainfall reduction on soil food webs of Mediterranean water-limited forests using nematodes as bioindicators. We took advantage of replicated rainfall exclusion infrastructures established in *Quercus* forests of southern Spain in 2016 (2-year exclusion) and of southern France in 2003 (15-year exclusion) to explore the sensibility of the soil food web to predicted reductions in precipitation (30%) at both short- and long-term scales. Rainfall reduction had large negative short-term effects on nematode abundance, particularly of lower trophic groups (bacterivores and fungivores). Rainfall reduction had also consistent impacts at both short and long temporal scales in the composition of the nematode community (decrease of fungivores, marginal increase of omnivores) and in nematode-based indices indicative of soil food web structure (higher maturity and structure index, lower prey:predator ratio). These results show the existence of fast responses in the soil food web of Mediterranean forests to a drier climate and their persistence through time, indicative of both low resistance and resilience to moderate rainfall reductions predicted by climate change. Overall, our findings demonstrate the sensibility of water-limited forests to further reductions in soil water availability, which might substantially alter their soil communities and likely affect the many ecosystem processes that they control.

Key-words: Climate change, experimental drought, precipitation, rainfall exclusion, resilience, resistance, soil fauna, soil food web

Introduction

In the last two decades, multiple studies have shown the large capacity of recent and future changes in temperature and precipitation to modify terrestrial ecosystems (IPCC, 2014). As a result, we have today ample knowledge regarding the consequences of climate change for the performance of plant species, as well as the implications of such changes for the organization of plant communities and ecosystem functioning (Bjorkman et al., 2018; Peñuelas et al., 2018; Harrison et al., 2020). On the contrary, there is relatively little knowledge about the impacts of climate change on soil communities, with a large degree of uncertainty regarding their resistance (i.e. ability to remain unchanged when subjected to a disturbance) and resilience (i.e. ability to return to the pre-disturbed state) to predicted alterations in temperature and precipitation (Blankinship et al., 2011; de Vries & Griffiths, 2018). Studies on the effects of altered precipitation are particularly scarce since they have traditionally received lower attention than the effects of warming (García-Palacios et al., 2015; Nielsen & Ball 2015; de Vries & Griffiths, 2018). However, the proliferation in recent years of rainfall exclusion infrastructures in the field has strongly contributed to improve this knowledge, providing empirical support for the large capacity of climate change-related drought to alter the abundance and composition of soil communities, but also evidencing the strong variability of these impacts through spatio-temporal gradients and among taxonomic groups (Blankinship et al., 2011; Ren et al., 2018).

Climate change impacts on soil organisms can vary strongly in space and time. Across spatial climatic gradients, the response of soil communities to increasing aridity is likely non-linear (Xiong et al., 2020). In fact, available evidence suggests that a given rainfall reduction can cause larger impacts on soil communities in mesic systems than in arid systems (Liu et al., 2016; Ren et al., 2018; Franco et al., 2019). A mechanistic explanation for the high inherent resistance of communities in arid systems is that they are dominated by specialized species selected over time for their high drought tolerance (Vandegehuchte et al., 2015; Curiel-Yuste et al., 2014). However, the number of studies that have explored the response of soil biota to rainfall reduction in water-limited ecosystems is still relatively low compared to mesic systems, limiting our ability to find general patterns (Nielsen & Ball 2015; Ren et al., 2018). Across time, it has been suggested that short-term impacts of climate change on soil organisms might be of larger magnitude than long-term impacts (Holmstrup et al., 2017; Ren et al., 2018), due to acclimation and evolutionary adaptation of soil populations to the new environmental conditions (Manzoni et al., 2012; Romero-Olivares et al., 2017; Yin et al., 2019). However, we lack comparative short- versus long-term studies investigating the resilience over time of soil biota to climate change. A better understanding of the heterogeneity in soil biota responses to climate change would strongly benefit from an increase in the number of standardized studies with similar methodology that allows direct comparison of results across space and time (Halbritter et al., 2020).

Current knowledge of climate change impacts on soil organisms, and of rainfall reduction effects in particular, is very much based on the study of the two main kingdoms of soil microorganisms (fungi and bacteria), whereas soil animals have been comparatively unexplored (García-Palacios et al., 2015; Ren et al., 2018; Nielsen, 2019). Within soil fauna, nematodes represent the most abundant group (Wilson & Kakouli-Duarte, 2009) and can serve as a unique model taxon for the study of rainfall reduction effects on the soil compartment. They are aquatic organisms that inhabit the water films in the surface of soil particles, and therefore their movement and activity might be particularly vulnerable to the direct effects of drought (Freckman et al., 1987; Coleman et al., 2004). Moreover, they cover a variety of life-history and feeding strategies, including bacterivores, fungivores, herbivores, omnivores and predators (Yeates et al., 1993). This variety of life histories and trophic habits have allowed the development of nematode-based indices that reflect the overall state of the soil food web, such as its maturity and complexity (maturity and structure index), the availability of resources (enrichment index) or the predominant decomposition pathway (fungivores:bacterivores ratio; Bongers & Ferris, 1999; Ferris et al., 2001; Neher 2001). Despite their potential interest, experimental studies of the response of soil nematodes to changes in precipitation are extremely scarce, and mainly restricted to temperate grasslands (Eisenhauer et al., 2012; Sylvain et al., 2014; Cesarz et al., 2015; Ankrom et al., 2020). These studies have revealed a variety of complex responses of the nematode community to rainfall reduction, largely dependent on the nematode trophic habit, but mostly indicative of negative drought impacts on the structure and complexity of soil food webs (Franco et al., 2019; Siebert et al., 2020). However, because the effect of altered precipitation on soil biota is highly dependent on ecosystem type (Blankinship et al., 2011), it is uncertain how these results might be extrapolated to systems dominated by woody species such as forests.

In this study, we analysed the impact of predicted climate change scenarios of rainfall reduction on soil food webs of Mediterranean forests using nematodes as bioindicators. We took advantage of replicated rainfall exclusion infrastructures established in *Quercus* forests of southern Spain in 2016 (2-year exclusion, short-term site hereafter) and of southern France in 2003 (15-year exclusion, long-term site hereafter) to explore the sensibility of the soil food web to predicted reductions in precipitation (30%) at both short- and long-term scales. We aimed to answer four main questions: i) How does experimental rainfall reduction affect the abundance, diversity and composition of nematode communities in water-limited Mediterranean forests? Based on the ecology of nematodes, we hypothesised that rainfall reduction would reduce overall abundance of nematode species and their diversity. Moreover, we expected this negative impact to be particularly large on higher trophic levels (omnivores and predators), since their life-history traits (i.e. long generation time, large body size, low fecundity, permeable cuticle) make them more vulnerable to disturbances than nematodes in lower trophic levels; ii) What do changes in nematode indices tell us about the impact of rainfall reduction on soil food webs of Mediterranean forests? Based on previous knowledge, we expected rainfall reduction to translate into a simplified and less structured soil food web (i.e. lower values of the maturity and structure index), a change towards the slow fungal decomposition channel (i.e. higher fungivores:bacterivores ratio), and a lower top-down pressure on the soil food web (i.e. higher prey:predator ratio); iii) Are impacts of rainfall exclusion on soil food webs consistent at both short- and long-term temporal scales? We expected impacts to be restricted mainly to the short-term due to the potential for the evolutionary adaption of soil nematodes over time to water stress. Finally, we conducted a detailed mechanistic study at the short-term site that allowed us to answer a fourth question: iv) Which abiotic and biotic factors are driving rainfall exclusion effects on nematode communities? We used structural equation models (SEMs) to explore the relative importance of changes in soil abiotic properties (soil moisture, soil organic matter), biotic microbial (soil microbial biomass C and N) and plant (root chemistry) properties, and trophic relationships as drivers of the impact of rainfall reduction on the abundance of main nematode trophic groups (Fig. S1, Table S1). By answering these questions, we aim to contribute to advance current knowledge about the short- and long-term implications of increasing drought on soil food webs. In fact, to our knowledge, this is the first study that explores the response of soil nematode communities to realistic changes in rainfall abundance under field conditions in water-limited forests.

Methods

Study sites

The study was conducted at two different locations in southern Europe: Los Alcornocales Natural Park in Southern Spain and the Puéchabon National Forest in Southern France (Fig. S2). Both areas are occupied by Mediterranean forests dominated by evergreen *Quercus* species (*Q. suber* in Spain, *Q. ilex* in Puéchabon). In Los Alcornocales, *Q. suber* coexists with the deciduous *Q. canariensis* and a sparse understory dominated by *Erica arborea*, whereas in Puéchabon *Q. ilex* is accompanied by a more diverse and abundant (c. 25% cover) understory dominated by *Phyllirea latifolia, Buxus sempervirens*, *Pistacia terebinthus* and *Juniperus oxycedrus.* The climate at both sites is typical Mediterranean, with mild winters and long, dry summers. Mean annual precipitation is 890 mm at Los Alcornocales and 910 mm at Puéchabon (mean for the series 1984–2017), although most precipitation concentrates between October and April. Soils in Los Alcornocales derive from a bedrock dominated by Oligo-Miocene sandstones and have a sandy loam texture, whereas in Puéchabon soils derive from a bedrock of Jurassic limestone and have a silty clay loam texture (Table S2).

Rainfall exclusion general design

We took advantage of rainfall exclusion infrastructures installed at each of the two study sites in different years (2016 in Spain and 2003 in France) but following a similar design. Six plots (20 × 15 m in Los Alcornocales, and 14 ×

10 m in Puéchabon) were established at each site 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 control. The rainfall exclusion treatment was designed to reduce ca. 30% the amount of rainfall, reproducing future climate change scenarios for the Mediterranean region (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 each plot area in order to exclude a similar amount of natural precipitation. Gutters were placed at least 1 m height over the forest floor supported by a metal structure. 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. Total basal area and tree density were similar for the two treatments at the two study sites prior to the construction of the infrastructure (Limousin et al., 2009; Homet et al., 2021; Table S2). The effect of the rainfall exclusion treatment on soil moisture was continuously quantified at both sites: in Spain soil moisture was measured at four depths (10, 20, 30 and 40 cm) using a FDR sensor (PR2 Profile Probe, Delta-T Devices, Cambridge, UK), whereas in France it was measured at 0-15 and 0-30 cm depth using water content reflectometers (CS615, Campbell Scientific Ltd., Loughborough, UK). Rainfall reduction translated into significant reductions of soil moisture at both experimental sites (Limousin et al., 2008; Homet el al., 2021) (Table S3, S4; Fig S3, S4).

Nematode sampling, identification, and indices

In spring 2018, soil samples were collected at both study sites within 15 days of difference. Therefore, at the time of the sampling, rainfall exclusion had been applied during 2 years in Spain and during 15 years in France. Soil samples were collected under the canopy of 6 trees per plot (three replicates per tree, 0-20 cm depth, previous removal of the litter layer) and stored in plastic bags at 4 ºC until processing. The three replicates per tree were combined and mixed by hand, and soil aggregates were broken (n = 72 samples in total). Nematodes were extracted following well-established protocols from a subsample of 100 ml by the sugar centrifugation method (Jenkins, 1964). After heat killed and fixed in 4% formalin, all nematodes in the samples were counted at 100×

magnification. In addition, 100 individuals per sample were identified in temporary slides to the family and genus level (Andrássy, 2005, 2007, 2009; Bongers, 1994) at 400× and 1000× magnification. Estimated nematode abundance was expressed as number of individuals per g of soil dry weight (ind/g). Richness and the Shannon index (H', Shannon & Weaver, 1949) of genera were calculated as measures of nematode diversity. The same sampling protocol was used at the Spanish site in spring 2016 to control for pre-treatment differences in the nematode community. Pre-treatment values of nematode communities were not available for the French site.

Each nematode was assigned to a trophic group (bacterivore, fungivore, herbivore, omnivore or predator, according to Yeates et al., 1993) and a c-p group (colonizer-persister scale with five levels) indicative of their life-history strategy (Bongers & Ferris, 1999). Trophic and c-p groups were assigned using the NINJA automated calculation system (Sieriebriennikov et al., 2014). Six indices indicative of food web characteristics were also calculated using NINJA (Sieriebriennikov et al., 2014): the maturity index for free-living taxa (MI), the plant parasitic index (PPI), the structure index (SI), the enrichment index (EI), the fungivores:bacterivores ratio (F:B) and the prey:predator ratio. The maturity index is an indicator of the ecological successional status of a soil community, considering the relative abundance of nematodes with different life-history strategy: those in the higher c-p scale (longer life cycles and K-strategists) vs. those in the lower c-p scale (opportunistic, fast-growing, and r-strategists) (Bongers, 1990; Bongers & Ferris, 1999). The plant parasitic index is the equivalent of the maturity index for plant-feeding nematodes. The interpretation of the plant parasitic index is the inverse to the maturity index, with undisturbed systems showing a high maturity index but a low plant parasitic index (Bongers et al., 1997). The structure index measures the complexity of the soil food web, with high scores indicating a structured and complex food web (Ferris et al., 2001). The enrichment index is a measure of nutrient enrichment, generally associated with nutrient addition or with the mortality of other organisms reflecting a nutrient flush, describing therefore the resource availability in the soil (Ferris & Bongers, 2006). The fungivores:bacterivores ratio (F:B) was calculated as indicator of energy use efficiency in the soil decomposition process, with low values suggesting a dominance of the fast bacterial decomposition channel and high values of the slower fungal channel. Finally, the prey:predator ratio was calculated as a proxy for top-down pressure within the nematode community, considering the sum of the abundances of fungivore, bacterivore and herbivore nematodes as prey and the sum of omnivore and predator nematodes as predators. We considered omnivores as predators due to the predatory feeding habit of *Dorilaimida* (Yeates et al., 1993), which was the dominant order of omnivore nematodes in our study (see Results).

Characterization of soil abiotic properties, microbial biomass and root chemistry

At the short-term site, we measured soil organic matter, microbial biomass and tree root chemistry to explore possible mechanisms behind the effect of the rainfall exclusion treatment on the nematode community. Data were obtained from soil and root samples collected in spring 2018 in the same trees where nematodes were quantified. Soils (0-10 cm depth) were sampled following the same protocol than for nematodes. Tree roots were sampled in the first 0-20 cm of the soil by carefully tracing them from the tree trunk in at least two different cardinal directions. Soil and root samples were kept moist in sealed plastic bags and transported inside an ice box to the laboratory, where they were stored at 4º C until processing. Soil samples were sieved through a 2 mm mesh sieve and used for quantification of organic matter content using the calcination method (Howard & Howard 1990), and of soil microbial biomass in terms of C and N following the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Roots were gently washed to remove soil particles, dried at 45 ºC during 48 hours and finely grounded with liquid nitrogen. Secondary compounds (total phenols and condensed tannins) and non-structural carbohydrates (soluble sugars and starch) were extracted from 300 and 20 mg (respectively) of dry tissue of each root sample. Total phenols were quantified colorimetrically by the Folin-Ciocalteu assay (Moreira et al., 2009), condensed tannins following the procyanidine method (Sampedro et al., 2011), and non-structural carbohydrates using the anthrone method (Hansen & Møller, 1975).

Data analysis

We first used Generalized Linear Models (GLMs) to test the effect of the rainfall exclusion infrastructure on the richness, diversity (H'), total abundance, abundance of different trophic groups, abundance of the dominant families (> 0.1 indv/g), and indices (maturity index, plant parasitic index, structure index, enrichment index, F:B and prey:predator) of the soil nematode community. Richness was modelled using a Poisson error distribution, whereas the remaining variables were modelled using a normal distribution. Site, rainfall exclusion and their interaction were included as fixed factors. To control for the nested structure of the experimental design, block was included as a fourth fixed factor nested in site. Block was included as a fixed factor because it did not have enough levels (i.e. > 4) to be considered as a random factor (Ieno & Zuur, 2015). For the Spanish experimental site, pre-treatment differences in the nematode community between control and rainfall exclusion plots were explored also using GLM. Since some pre-treatment differences appeared (Table S5, Fig. S5), models testing the effect of the rainfall exclusion treatment were also run for this site including pre-treatment values as covariables (Table S6).

To test for statistical differences on the composition of the nematode community across sites and rainfall exclusion treatments, we performed a PERMANOVA analysis with 999 randomizations. Prior to the analysis, the matrix containing abundances for the different taxonomic groups identified across all sites was squared-root transformed to reduce the weight of the most common taxa in the analysis (Santonja et al., 2017). Distances between groups were calculated using the Bray-Curtis distance index (Bray & Curtis, 1957).

Finally, we used structural equation models (SEMs) to explore the mechanisms underlying the effects of short-term rainfall exclusion on the nematode community at the Spanish site. SEMs were not conducted for the French site due to unavailability of enough data about the abiotic and biotic determinants of nematodes abundance. Before the analysis, pairwise correlations were conducted to check for the lack of strong correlations (Pearson r > 0.8) among the environmental predictors. We also transformed (log, square root) several variables to fulfil normality and homoscedasticity assumptions. We hypothesised that rainfall exclusion would cause alterations of soil abiotic properties, microbial biomass and tree root chemistry. Changes in soil abiotic properties would affect abundance of all nematode trophic groups, whereas changes in microbial biomass would affect mostly bacterivores and fungivores, and changes in root chemistry would affect only herbivores. Regarding relationships between the different nematode trophic groups, we tested the different possible directionalities to determine if the trophic food web had a top-down or bottom-up regulation. Hypothesized mechanisms are summarized in Table S1. According to this causal structure, the initial path diagram was tested (Fig. S1), selecting or discarding the different relationships until we reached consensus of a model providing biological realism a nd a good statistical fit following several indices (root mean square error of approximation (RMSEA), comparative fit index (CFI) and standardized root mean square residual (SRMR)) (Kline, 2016). The rainfall exclusion treatment was introduced in the model as a dummy variable (0, 1). Soil abiotic properties, microbial biomass and root chemistry were introduced as three composite variables. Soil abiotic properties was composed by soil water content and organic matter, microbial biomass by microbial biomass measured in terms of carbon and nitrogen, and root chemistry by secondary compounds (total phenols and condensed tannins) and non-structural carbohydrates. As an integrative measure of soil water content, we used the cumulative value during the fall and winter months previous to the sampling date (October-March) in the first 40 cm of the soil profile.

All statistical analyses were conducted using the R software (R version 3.3.2, 2017-10-31). Linear Models were performed using package “nlme” (Pinheiro et al., 2019), PERMANOVA analyses were performed using package “vegan” (Oksanen et al., 2013), and structural equation models (SEMs) were conducted using package “lavaan” (Rosseel, 2011).

Results

Impact of rainfall exclusion on nematode diversity, abundance and composition

The rainfall exclusion treatment did not affect the richness of the nematode community at any of the two study sites, and had only marginal positive effects on diversity (H’) (Table 1). However, rainfall exclusion had an effect on the total abundance of nematodes, but only at the short-term site (significant Site × Rainfall exclusion interaction, Table 1). At this site, nematode abundance was 39% lower in rainfall exclusion plots than in control plots (Fig. 1). Results for nematode richness, diversity, abundance and indices at the short-term site were not significantly affected by the introduction of pre-treatment values as covariables (Table S6), suggesting that initial differences did not influence the effect of the rainfall exclusion treatment.

Bacterivores and fungivores were the most abundant groups at both sites and the most affected by the rainfall exclusion treatment (Fig. 1). The effect of rainfall exclusion on bacterivore abundance differed among sites (significant Site × Rainfall exclusion interaction, Table 1), being largely negative (43% reduction) at the short-term site but slightly positive (14% increase) at the long-term site (Fig. 1). Regarding fungivores, the rainfall exclusion treatment had a consistent negative effect on its abundance at both sites (Table 1), but of larger magnitude at the short-term site (38% vs. 7% reduction, Fig. 1). The abundance of herbivore and predator nematodes was not affected by rainfall exclusion at any site, whereas omnivores abundance showed a marginal, positive response to the experimental treatment at both sites (Table 1, Fig. 1). When explored at the family level, rainfall exclusion had consistent negative effects across sites on some dominant families of bacterivores (*Cephalobidae*) and fungivores (*Aphelenchidae*). However, other dominant families were only affected at one of the sites (e.g. the bacterivorous *Rhabditidae* at the long-term site, the fungivore *Filenchus* at the short-term site) or showed contrasting responses among sites (bacterivores *Plectidae* and *Monhysteridae*) (Table S7, Fig. 2). Among herbivores, treatment effects were detected on the abundance of just one family (*Criconematidae*), which decreased under rainfall exclusion but only significantly at the short-term site (Table S7, Fig. 2). The effect of rainfall exclusion on omnivores was restricted the dominant family *Qudsianematidae*, which increased under rainfall exclusion at both sites, and the family *Nordiidae* which also increased under rainfall exclusion but only at the long-term site (Table S7, Fig. 2). These differential impacts across trophic groups and families translated into nematode communities of contrasting composition at control versus rainfall exclusion plots at both sites, as supported by the results of the PERMANOVA analysis (Table S8, Fig. S6).

Impact of rainfall exclusion on nematode indices

The rainfall exclusion treatment had a significant effect on the maturity index, the structure index, the prey:predator ratio, and (only marginally) the F:B ratio (Table 1). Moreover, these effects were always consistent across sites (i.e. non-significant Site **×** Rainfall exclusion interactions, Table 1), except for the F:B ratio (marginally significant Site × Rainfall exclusion interaction). Rainfall exclusion caused an increase in both the maturity index and the structure index, as well as a decrease in the prey:predator ratio (Fig. 3). The effect on the F:B ratio was only detected at the long-term site, where rainfall exclusion caused a 26% decrease in the ratio (Fig. 3). Rainfall exclusion had no effects on the plant parasitic index or the enrichment index at any site (Table 1).

Drivers of the short-term impact of rainfall exclusion on nematode communities

The final SEM model adequately fit the data on the soil food web at the short-term site (RMSEA = 0.032, SRMR = 0.113, p-value = 0.412). Rainfall exclusion caused a direct significant negative effect on soil abiotic properties (soil water content and organic matter), which in turn had a positive effect on the abundance of fungivore, and bacterivore nematodes (Fig. 4). Rainfall exclusion had also a negative effect on the concentration of root compounds, which in turn had a positive effect on herbivore nematodes abundance. Rainfall exclusion had no direct effects on soil microbial biomass, although microbial biomass had direct positive effects on the abundance of bacterivore and fungivore nematodes. Among the several path diagrams tested, the final SEM did not include directional relationships among nematode trophic groups, but positive correlations. Particularly strong were correlations among fungivores and bacterivores, as well as among predators (omnivores and predators) and lower trophic levels (fungivores and bacterivores) (Fig. 4).

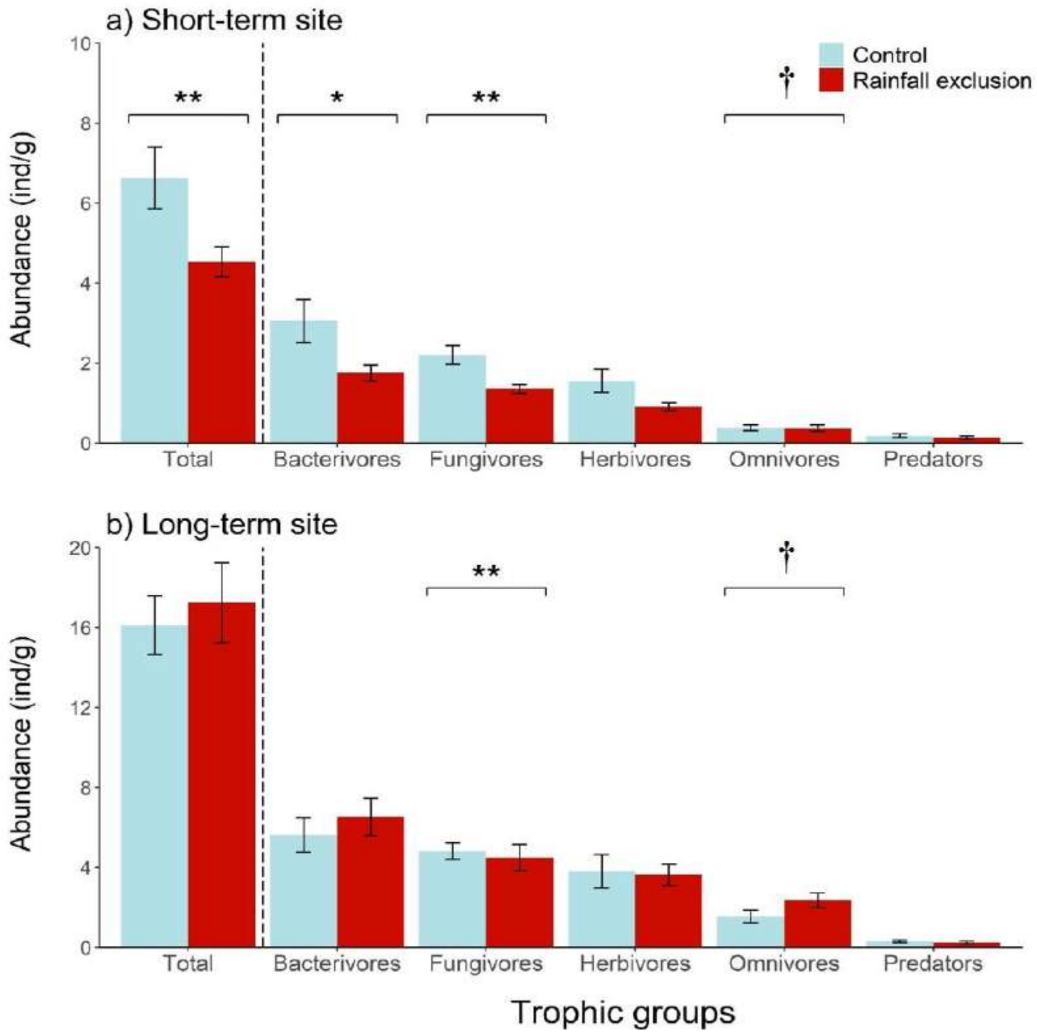


Figure 1. Effects of rainfall exclusion on the abundance (mean ± SE) of soil nematodes, both total and separated by trophic groups, at the short-term (a) and long-term (b) experimental sites. Abundances are expressed as the number of individuals per gram of soil. Asterisks denote significant effects: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.01$**.** (see Table 1). When significant differences for Site x Rainfall exclusion interaction were found, we performed separate analysis for each site and the differences were represented in the graph with the same symbols.

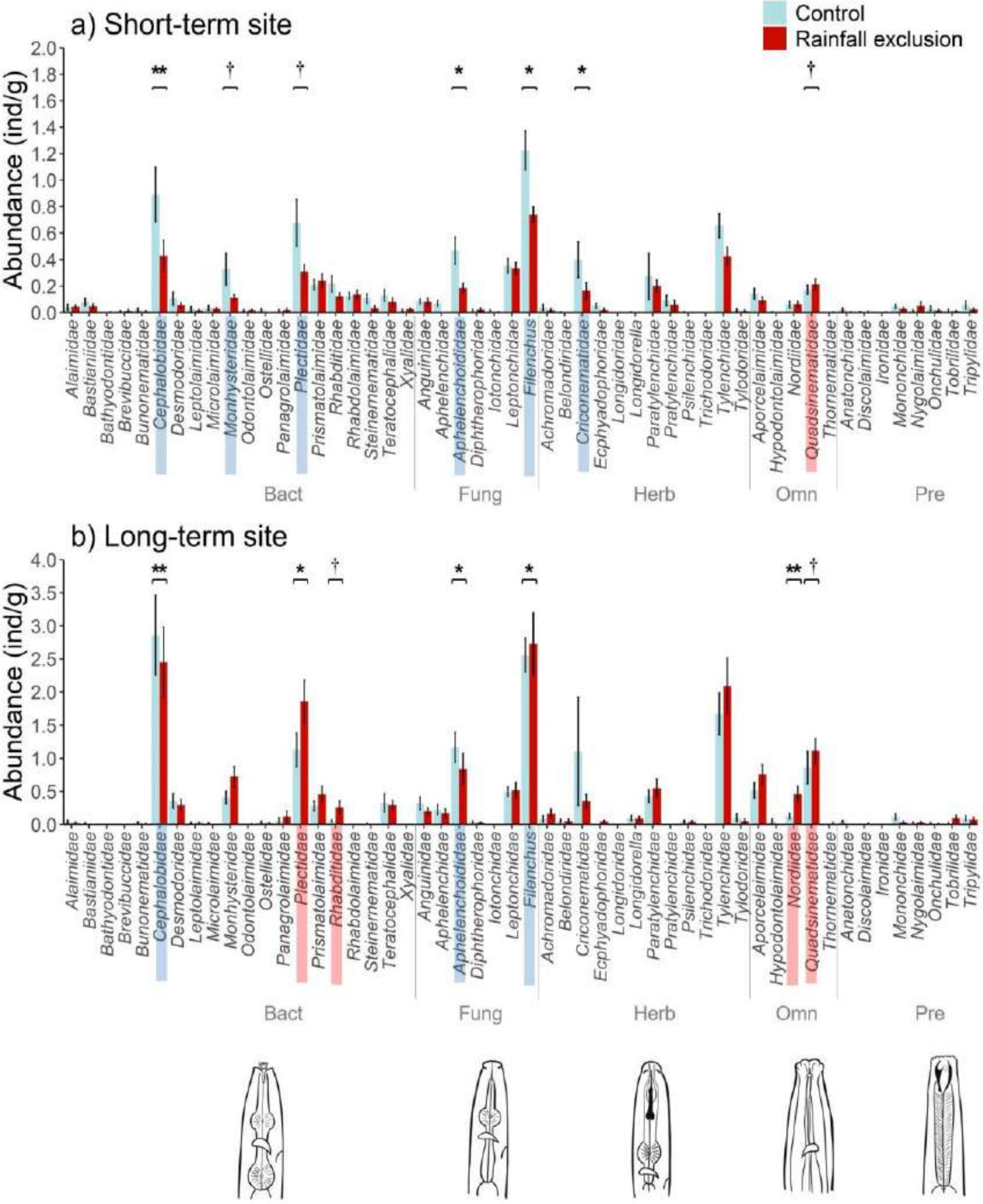


Figure 2. Effects of rainfall exclusion on the total abundance (mean ± SE) of nematode families, grouped by functional groups (Bact: bacterivores; Fung: fungivores; Herb: herbivores; Omn: omnivores; Pre: predators) present at the short-term (a) and at the long-term (b) experiment sites. The *Filenchus* genus is represented as fungivore, separately from the *Tylenchidae* family due to their different trophic habits. Families for which a significant effect of the rainfall exclusion was found are indicated in the colour of the treatment where they reached the largest abundance. Asterisks denote significant effects: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.01$**.** (see Table S7).

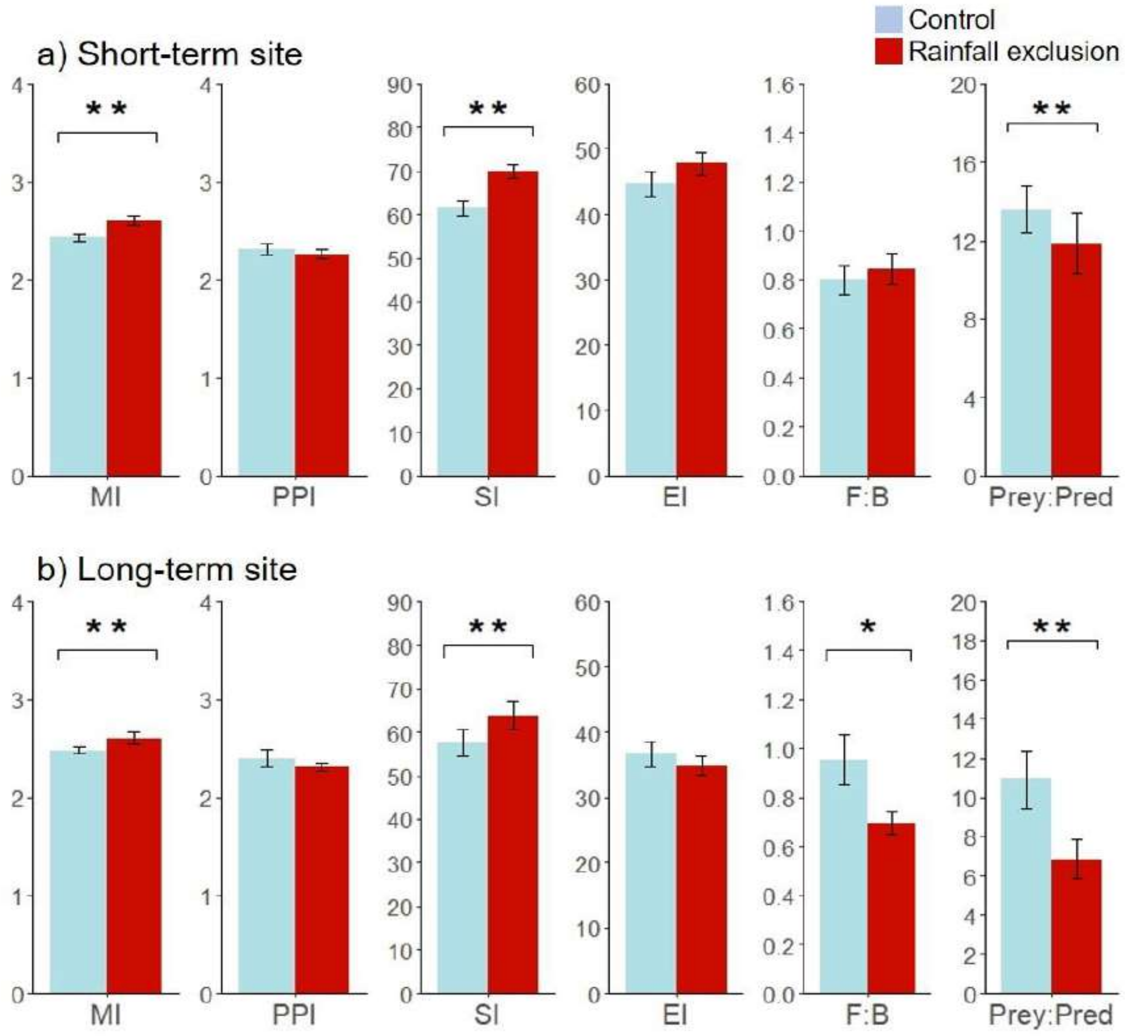


Figure 3. Effects of rainfall exclusion on the nematode-based indices (mean ± SE) at the short-term (a) and long-term (b) experimental sites. MI = maturity index, PPI = plant parasitic index, SI = structure index, EI = enrichment index, F:B = fungivores:bacterivores ratio; Prey:Pred = prey:predator ratio. Asterisks denote significant effects: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.01$**.** (see Table 1). When significant differences for Site x Rainfall exclusion interaction were found, we performed separate analysis for each site and the differences were represented in the graph with the same symbols.

Table 1. Results of the Generalized Linear Models analysing the effect of rainfall exclusion on nematode diversity, abundance, and indices at the short- and long-term sites. Values represent Z-values for Richness analysis and F-values for the rest of the variables. Block was introduced as a fixed factor to control for the nested structure of the experimental design. Asterisks denote significant effects: **** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05, † p < 0.01.

Variable	**Site**	**Block [Site]**	**Rainfall exclusion**	**Site × Rainfall exclusion**	**R^2**
Diversity					
Richness	0.08	3.82	0.99	0.74	0.07
H’	0.33	1.27	2.97†	1.33	0.12
Abundance					
Total	106.47****	2.67*	3.46†	4.02*	0.64
Bacterivore	62.69****	3.47*	1.47	5.55*	0.54
Fungivore	94.49****	7.51***	10.34**	1.76	0.66
Herbivore	47.79***	1.18	1.78	1.21	0.44
Predator	2.19	1.76	1.31	0.14	0.13
Omnivore	84.06***	3.22*	3.21†	2.62	0.59
Indices					
Maturity index	0.32	1.99	10.37**	0.38	0.21
Plant Parasitic index	1.17	0.78	1.03	0.02	0.07
Structure index	4.75**	1.66	9.73**	0.42	0.23
Enrichment index	45.71****	4.14**	0.01	0.79	0.47
Fungivores/Bacterivores	0.09	2.98*	3.39†	3.31†	0.21
Prey/predator	15.81**	5.28**	10.59**	0.51	0.40

Discussion

This study provides novel experimental evidence showing that moderate rainfall reductions as those predicted by climate change models for the Mediterranean region (i.e. 30% reduction in annual precipitation) can induce fast and long-lasting impacts on soil food webs of forest ecosystems. Our results therefore demonstrate the sensibility of water-limited forests to further reductions in soil water availability, which might substantially alter their soil communities and likely affect the many ecosystem processes that they control.

Impact of rainfall exclusion on the diversity, abundance and composition of nematode communities

Experimental evidence regarding the impacts of rainfall reduction on the abundance and diversity of soil nematodes is very scarce, with available studies showing a variety of complex responses including negative, neutral and positive impacts (Stevnbak et al., 2012; Sylvain et al., 2014; Cesarz et al., 2015; Torode et al., 2016; Franco et al., 2019; Ankrom et al., 2020; Siebert et al., 2020). Because nematodes are aquatic organisms physiologically active only when water is available, we expected rainfall reduction to have a negative effect on their abundance and diversity in Mediterranean forests, where soil water content is extremely low during a large part of the year (June-Octuber) (Fig. S4 and S5). Our results supported this prediction for nematode abundance, with a moderate rainfall reduction (30%) translating into a 39% reduction in the abundance of the nematode community, but only at the short-term site. These results are consistent with those found in, to our knowledge, the only previous study that have explored the impacts of rainfall reducion on soil nematodes in forest ecosystems (Landesman et al., 2011), although in such study the magnitude of the rainfall exclusion was particularly extreme and short-term (100% rainfall exclusion during 1 year). Our results therefore show for the first time that nematode abundance in water-limited Mediterranean forests show low resistance to moderate rainfall reductions predicted by climate change models.

We also hypothesized that rainfall exclusion effects would vary among trophic groups, following previous literature showing higher trophic levels to be particularly sensitive to environmental changes (Preisser & Strong, 2004; Franco et al., 2019). Our results supported the prediction of differential responses among trophic groups consistently across sites, but in the opposite direction than expected. Thus, rainfall reduction had in general larger negative effects on lower trophic groups (bacterivores and fungivores) than on higher trophic groups (omnivores and predators), which even showed a marginal positive response to rainfall exclusion. A plausible explanation for the absence of negative effects on higher trophic groups might rely on their composition, since they were largely dominated by the family *Qudsianematidae*, a group with high drought tolerance (Landesman et al., 2011; Bakonyi et al., 2007) and plasticity to support extreme climates (Hoschitz, 2003), probably due to a remarkable anhydrobiosis ability (i.e. ability to survive the loss of most water and enter a state of suspended metabolism; Treonis et al., 2000). Moreover, it is likely that long life cycles together with a broad feeding strategy might not represent a disadvantage to deal with increasing drought as it has been reported for other stress sources, since they might allow the exploitation of a larger variety of resources during favourable seasons, entering at diapause status when conditions are adverse (Landesman et al., 2011).

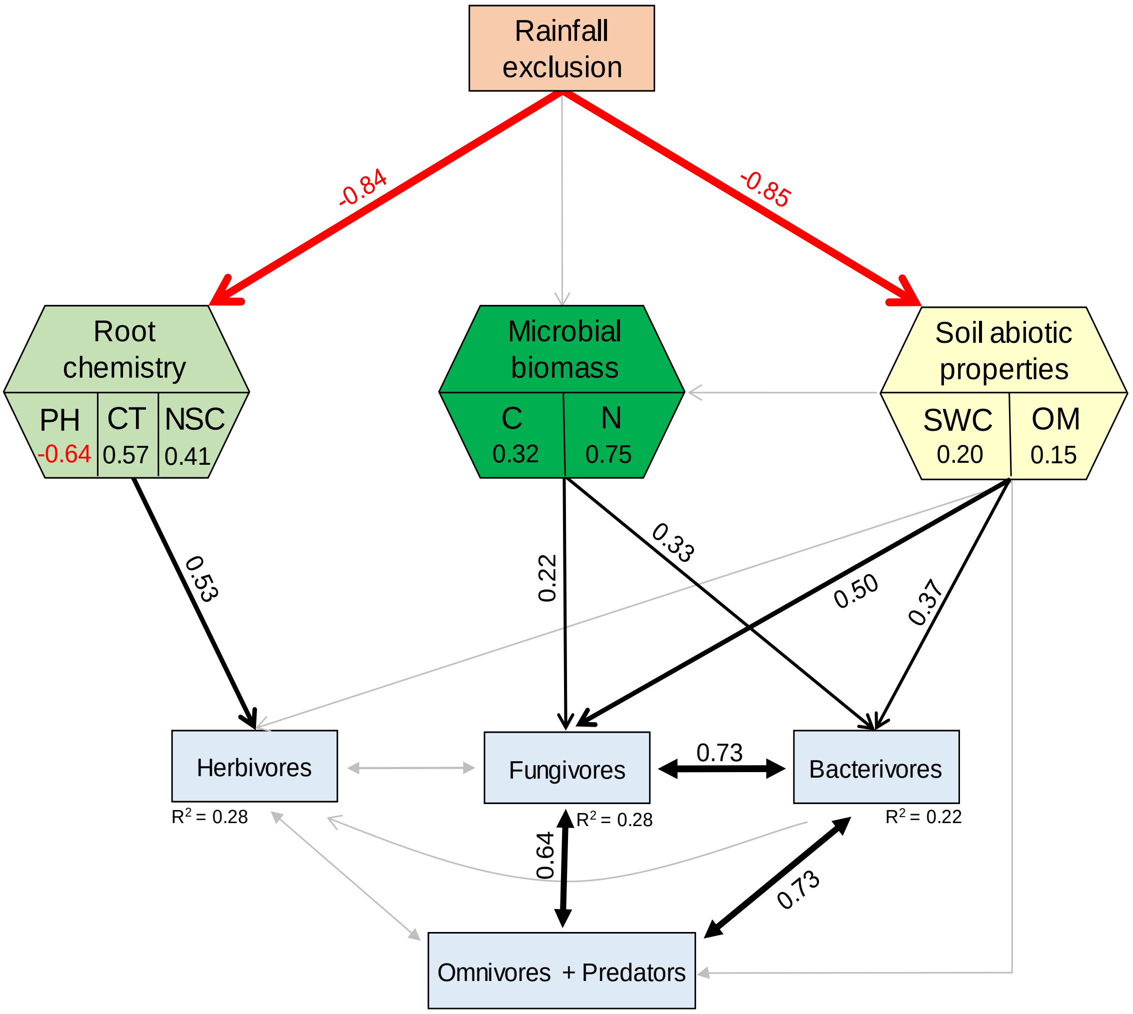


Figure 4. Results of the structural equation model (SEM) linking rainfall exclusion with abiotic, microbial and plant drivers of the abundance of different nematode trophic groups. Hexagons represent composite variables and rectangles observed variables. Numbers adjacent to arrows are standardized path coefficients. Width of arrows is proportional to the strength of path coefficients. Red arrows indicate negative relationships, black arrows positive relationships, and grey arrows relationships not included in the final model. Numbers inside hexagons are the coefficients of the different indicator variables used to construct the composite variables (PH, total phenols; CT, condensed tannins; NSC, non-structural carbohydrates; C, microbial biomass carbon; N, microbial biomass nitrogen; SWC, soil water content; OM, organic matter). R^2 represent the variance explained by the model for each endogenous observed variable.

A closer look at the family level revealed taxa specific responses of nematodes to predicted rainfall reduction, likely reflecting a large variation within the nematode community in their drought tolerance. In fact, a recent study along an aridity gradient found that, although most genera were more abundant in the wet part of the gradient, some reached their higher abundance in the drier part (Xiong et al., 2020). In our study, the negative effects of rainfall reduction on fungivores across sites were largely driven by the response of the *Aphelenchoididae* family, which seems to have a great susceptibility to drought (Landesman et al., 2011), whereas the positive effect on omnivores were driven by the drought-tolerant *Qudsianematidae*. The response of bacterivores was, however, much more complex, since the two most abundant families, *Cephalobidae* and *Plectidae*, showed contrasting responses to rainfall exclusion both within and across sites, even though they belong to the same functional guild (i.e. c-p 2, Cesarz et al., 2015). Previous studies have also reached contrasting conclusions about the drought tolerance of these bacterivore families (Bakonyi et al., 2007; Landesman et al., 2011; Xiong et al., 2020). Clearly, further studies are needed to improve our understanding of the large variability present within nematode trophic and functional groups in their response to a drier climate. Such variability might be a key determinant of contrasting responses of nematode communities with different composition to a given climatic stressor.

Impact of rainfall exclusion on soil food webs based on nematode indices

A main advantage of using nematode communities as target group to understand climate change impacts on soils is that they allow the calculation of multiple indices that reflect the overall state of soil food webs and the ecosystem. Based on previous studies, we hypothesized that rainfall reduction would translate into a simplified and less structured soil food web (i.e. lower values of the maturity and structure index) where top-down regulation of lower trophic levels (opportunistic and plant feeders) might be impaired (i.e. higher prey:predator ratio). However, we found just the opposite and in a consistent manner across both experimental sites. Thus, the lower vulnerability to rainfall exclusion of omnivores and predators compared to lower trophic levels translated into an increase in the maturity and structure index, as well as in a reduction of the prey:predator ratio. These changes implied a shift towards mature and complex soil food webs with many trophic links and a large top-down control within the nematode community at both short and long temporal scales (Bongers, 1990; Ferris et al., 2001; Dominguez-Begines et al., 2019). Interestingly, a similar increase in the top-down control of soil food webs under rainfall reduction was found in a parallel study conducted at the short-term site with litter mesofauna (Homet et al., 2021). These findings suggest that the predicted rainfall reduction for Mediterranean areas cause persistent changes in the soil food web that might improve its resistance to further disturbances.

We also predicted that rainfall exclusion would cause a change towards the slow fungal decomposition channel, as indicated by higher fungivore:bacterivore ratios (F:B ratio). This prediction was based on accumulated evidence showing that bacteria are more sensitive to a drier climate than fungi due to a higher dependence on moisture availability to access resources for growth (Ren et al., 2018). However, our results did not support this prediction at any site. At the short-term site, both bacterivores and fungivores showed large decreases (30-40%) induced by drought causing no alterations of the decomposition channel, whereas in the long-term site only fungivores were negatively affected by drought whereas bacterivores were stimulated, causing a decrease of the F:B ratio. Thus, the decomposition channel was affected only after long-term exclusion towards a dominance of the fast bacterial pathway. A plausible explanation that needs to be tested is that this impact was driven by changes in the structure and diversity of the plant community after more than a decade of rainfall exclusion. In fact, the long-term site had a diverse understorey that might have benefited from the negative effects of long-term drought on tree performance (Limousin et al., 2009; Gavinet et al., 2019). Previous studies have shown that soil bacterial communities in Mediterranean forests can benefit from a decline in tree health and the subsequent increase in the diversity and cover of understorey species with higher litter quality (Curiel-Yuste et al., 2012; Lloret et al., 2015). Our results therefore support limited direct effects of rainfall reduction on decomposition pathways in Mediterranean forests, but point towards possible indirect long-term impacts mediated by changes in the plant community that would imply increase flows through the bacterial-decomposition channel and therefore less efficient nutrient and carbon cycling (de Vries et al., 2006, 2011; Sylvain et al., 2014).

Impact of rainfall reduction on soil nematodes at short vs. long temporal scales

We expected impacts of rainfall reduction to be restricted mainly to the short-term. However, we found consistent changes at both short (2 years) and long temporal scales (15 years) in the composition of the nematode community (decrease of fungivores, marginal increase of omnivores) and the structure of the soil food web (higher maturity and structure index, lower prey:predator ratio). These results therefore show the existence of fast responses in the soil food web of Mediterranean forests to a drier climate and their persistence through time, indicative of a low resilience. Unfortunately, the scarcity of experimental studies focused on soil nematodes precludes the comparison of our results with those from other systems. In fact, the literature of rainfall exclusion effects on nematode communities is dominated by short-term studies (< 5 years) and virtually absent of long-term studies. To our knowledge, only the study by Holmstrup et al., (2017) reported data after 2 and 8 years of rainfall exclusion in a heathland ecosystem of Northern Europe, finding a decrease of drought effects on nematode diversity through time that might be indicative of a high resilience to climate change. In view of these contrasting results, further long-term studies are urgently needed to allow reaching sound conclusions on the resilience of soil nematodes to a drier climate.

Drivers of the short-term impact of rainfall exclusion on nematode communities

Our SEM provided relevant insights into the pathways through which rainfall exclusion affected the different nematode trophic groups at the short-term site. Our experimental rainfall exclusion had a strong direct negative effect on the abundance of bacterivore and fungivore nematodes mediated by a reduction in soil abiotic properties (soil water content and organic matter), supporting the importance of abiotic conditions for these organisms (Ferris and Bongers, 2006; Wang et al., 2018; Xiong et al., 2020). Contrary to our expectations, rainfall exclusion did not indirectly affect the abundance of bacterivore and fungivore nematodes through changes in microbial biomass. This is because, although microbial biomass had a positive effect on the abundance of fungivores and bacterivores, it was not affected by the rainfall exclusion treatment. These results suggest that negative rainfall exclusion effects on bacterivore and fungivore nematodes were most likely due to decrease survival influenced by restrictions in movement and access to prey, and not indirectly mediated by changes in prey abundance (Landesman et al., 2011).

Contrary to the results observed for bacterivores and fungivores, negative effects of rainfall reduction on herbivore nematodes were not directly affected by changes in soil abiotic properties, but indirectly by changes in the chemistry of tree roots. Rainfall exclusion translated into a higher content of total phenols, which confer resistance against herbivores in *Quercus* species (Moreira et al., 2018 a,b), as well as into a lower concentration of non-structural carbohydrates that might affect the nutritional value of root tissues (Hoffman & Grundler, 2007). The mechanistic explanation behind the positive relationship between condensed tannins and herbivore nematodes deserves further study, and might be related with their induction after herbivore attack (Barbenhenn & Constabel, 2011; Li et al., 2016).

Previous studies have shown that, in nematode food webs, higher trophic levels (omnivores and predators) are usually limited by resource availability and lower trophic levels (fungivores, bacterivores, herbivores) by predation pressure (Yeates et al., 1997). However, our final model did not include directional relationships between lower and higher trophic groups, but strong correlations. Because these correlations were all positive, they could reflect shared environmental requirements that promoted overall higher nematode abundance at some microsites in our study forests. Therefore, our results suggest that the soil food web in the studied Mediterranean forest was more intensively influenced by environmental conditions than by trophic relationships.

Concluding remarks

Our study took advantage of standardized rainfall exclusion protocols to explore the short- (2 years) and long-term (15 years) effects of a drier climate (30% rainfall reduction) in Mediterranean forests of southern Europe. We recognize that the two study forests showed differences in soil characteristics of relevance for the nematode community (e.g. texture, soil organic matter; van den Hoogen et al., 2020) that limited our ability to directly compare the results from both sites. For example, the detection of negative rainfall exclusion effects on nematode abundance only at the short-term site might be indicative of some adaptation process of the population abundance to a drier climate over time, or just the result of a larger protection against drought offered by soil aggregates in the fine texture soils of the long-term site (Görres et al., 1999; Neher et al., 1999; Yeates et al., 2002).

Despite these limitations, most results were consistent among sites, suggesting that the nematode community of Mediterranean forests show a low resilience to climate change, with fast and sustained responses in composition and structure to predicted scenarios of rainfall reduction. Yet, the responses detected were totally different to those previously reported for temperate grasslands, where a drier climate translated into degraded and poorly structured soil food webs (Franco et al., 2019; Siebert et al., 2020). In the studied forests, the changes measured might improve the resistance of the soil food web to further disturbances thanks to a higher complexity and top-down control. Whether these patterns can be generalizable to other forests or water-limited systems needs to be tested. We firmly believe that the increasing number of manipulative climate change experiments throughout the world, often in coordinated networks (i.e. DroughtNet, Knapp et al., 2017), offer a unique opportunity to improve this knowledge gap. Given the pivotal role of soil nematodes in the regulation of carbon and nutrient dynamics (Ingham et al., 1985; Ferris, 2010; Wagg et al., 2014) and their value as bioindicators of soil health (Neher et al., 2001), a better understanding of their resistance and resilience to climatic alterations will offer very valuable insights into the short- and long-term implications of climate change for the functioning of terrestrial ecosystems.

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Appendix

Table S1. Hypothesized causal mechanisms linking rainfall exclusion and the abundance of the different nematode trophic groups.

#	Path	Justification	Ref
1	Rainfall exclusion → Soil abiotic properties	Rainfall exclusion translates into lower soil moisture. Lower soil moisture levels are known to be negatively associated with soil organic matter.	2, 7, 9
2	Rainfall exclusion → Microbial biomass	Soil microbes are dependent on soil water content. Reductions in soil moisture can have negative effects soil microbial biomass.	3,7, 16
3	Rainfall exclusion → Root compounds	Drought can change the allocation and composition of root compounds. Plants subjected to drought have shown an increase in root sugars and carbohydrates and changes in root tannins composition.	6, 15
4	Soil abiotic properties → Microbial biomass	Soil microbes are highly dependent on soil moisture. Organic matter helps to retain soil moisture favouring soil microbes, and is an important resource for many microbes which feed on it.	3, 7, 16
5	Soil abiotic properties → Nematode trophic groups	Nematodes strongly depend on soil water content for movement and access to resources. Sensitivity to drought varies among trophic groups. Organic matter is known to affect soil biodiversity due to its control of resources availability.	1, 10, 17, 19
6	Microbial biomass → Fungivores and Bacterivores	Fungivore and bacterivore nematodes show strong dependence on microbial biomass as food resource.	12, 20
7	Root compounds → Herbivores	Tannins and phenolic compounds are defensive metabolites against herbivores. Herbivore nematodes could get energetic food through root sugars and carbohydrates.	4,5, 11, 14
8	Relationships between nematode trophic groups	Directional relationships among nematodes in higher (omnivores, predators) and lower (fungivores, bacterivores, herbivores) trophic levels suggest top-down or bottom-up trophic controls of nematode abundance. Bi-directional relations between different trophic groups could reflect similar preferences for particular abiotic and biotic microsite conditions.	8, 13, 18, 20

Table S2. Summary of main characteristics of the two study forests. For stand structure and soil variables, values represent mean ± SE (n = 36, except for tree density and basal area where n = 3). There were no significant differences among control and drought plots in any of the variables analysed (p > 0.05) at any of the two sites.

	Short-term rainfall exclusion Los Alcornocales (Spain)		**Long-term rainfall exclusion Puéchabon (France)**	
Latitude	36º 34’ 07’’ N		43º 44’ 29’’ N	
Longitude	5º32’21’’ O		03º 35’ 46’’ E	
Altitude (m a.s.l)	270		209	
Mean annual temperature (ºT)	16.4		13.5	
Mean annual precipitation (mm)	910		883	
Stand structure	Control	Drought	Control	Drought
Tree density (stems/ha)	233.33 ± 0.02	244.44 ± 11.11	5933 ± 874	6433 ± 546
Basal área (m^2/ha)	28.89 ± 8.46	27.58 ± 1.73	24.9 ± 0.92	29.8 ± 1.71
Tree height (m)	11.59 ± 0.62	11.63 ± 0.61	3.84 ± 1.29	4.05 ± 1.25
Diameter at breast height (cm)	37.54 ± 3.13	35.07± 3.06	6.91 ± 0.61	7.21 ± 0.22
Soil variables				
Organic matter (%)	11.36 ± 0.73	10.42 ± 0.71	23.07 ± 0.79	23.86 ± 1.03
Soil Water Content (%)	33.93 ± 2.24	31.21 ± 3.01	34.52 ± 1.16	38.78 ± 1.55
Texture (% sand)	72.33 ± 1.69	72.84 ± 1.66	20.71 ± 1.23	21.88 ± 0.95
pH	5.53 ± 0.36	5.33 ± 0.21	7.23 ± 0.11	7.42 ± 0.17

Table S3. Results of the Linear Mixed Models that analyses the effect of the rainfall exclusion treatment on soil moisture at different depths in the Spanish short-term site. Block and year were included as fixed factors to control for spatial and temporal autocorrelation. Texture was included as a covariate to control for the effect of the differences in geological composition between plots. Month was included as a random factor. Df shows the degrees of freedom of the numerator and denominator.

Depth	**Factor**	**df**	**F**	**p-value**
10 cm	Block	2,1026	7.60	**<0.001**
	Year	2,1027	12.41	**<0.001**
	Texture	1,1026	10.57	**0.001**
	Rainfall exclusion	1,1026	13.09	**<0.001**
20 cm	Block	2,993	35.28	**<0.001**
	Year	2,994	17.67	**<0.001**
	Texture	1,993	4.05	**0.045**
	Rainfall exclusion	1,993	61.75	**<0.001**
30 cm	Block	2,1027	38.21	**<0.001**
	Year	2,1028	33.01	**<0.001**
	Texture	1,1027	4.46	**0.035**
	Rainfall exclusion	1,1027	146.73	**<0.001**
40 cm	Block	2,1025	41.93	**<0.001**
	Year	2,1026	32.01	**<0.001**
	Texture	1,1025	18.53	**<0.001**
	Rainfall exclusion	1,10250	155.93	**<0.001**

Table S4. Results of the Linear Mixed Models performed to test the effect on soil moisture of the rainfall exclusion treatment at the French long-term site. Year and month were included as random factors. Df shows the degrees of freedom of the numerator and denominator.

Depth	Factor	df	F-value	p-value
15 cm	Rainfall exclusion	1,10658	6851.1	**<0.001**
30 cm	Rainfall exclusion	1,10057	714.17	**<0.001**

Table S5. Results of the Generalized Linear Models that analyze the initial differences between treatments in nematode richness, diversity (H'), abundances and indices at the short-term site. Values represent Z-values for Richness analysis and F-values for the rest of the variables. Asterisks denote significant effects: **** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05, † p < 0.01.

Variable	Block	Rainfall exclusion	R^2
Diversity			
Richness	0.17	8.81**	0.20
H'	0.15	9.68**	0.22
Abundance			
Total	2.74†	3.45†	0.20
Bacterivore	1.58	0.42	0.09
Fungivore	2.36	6.04*	0.24
Herbivore	3.24†	3.85*	0.22
Predator	0.26	0.56	0.03
Omnivore	1.76	0.01	0.09
Indices			
Maturity index	1.29	0.93	0.09
Plant Parasitic index	0.35	1.65	0.06
Structure index	2.47	0.06	0.12
Enrichment index	0.56	1.85	0.08
F:B	0.05	3.20†	0.09
Prey:predator	3.14†	4.76*	0.24

Table S6. Results of the Generalized Linear Mixed Models that analyze the effect of rainfall exclusion on nematode diversity, abundance and indices at the short-term site, controlling for pre-treatment differences between control and rainfall exclusion plots. Only variables that showed marginal or significant differences between treatments before the start of the experiment were analyzed (see Table S5). Asterisks denote significant effects: **** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05, † p < 0.01. R^2_m represents marginal R^2 values associated only with the fixed effects of the model.

Variable	**Block**	**Pre-treatment values**	**Rainfall exclusion**	**R^2**
Diversity				
Richness	2.39	0.13	0.04	0.12
H’	2.73†	0.65	0.18	0.15
Abundance				
Total	2.33	0.07	7.41*	0.26
Fungivore	4.87*	1.59	11.89**	0.40
Herbivore	0.21	1.49	3.35†	0.13
Indices				
F/B	0.16	3.71†	0.01	0.10
Prey/predator	2.62†	2.64	3.33†	0.24

Table S7. Results of the Generalized Linear Models that analyze the effect of the rainfall exclusion treatment on the abundance of the dominant families of nematodes (i.e. > individual/g soil). The *Filenchus* genus was analyzed separately from the *Tylenchidae* family due to their different trophic habits. Values represent F-values. Asterisks denote significant effects: **** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05, † p < 0.01.

Variable	Site	Block [Site]	Rainfall exclusion	Site × Rainfall exclusion	R^2
Bacterivores					
Cephalobidae	87.21****	1.71	7.15**	3.21	0.61
Plectidae	29.74****	2.22†	0.64	9.04***	0.41
Rhabitidae	5.01*	1.97	0.85	5.14*	0.21
Monhysteridae	19.14****	0.69	0.02	4.15*	0.27
Prismatolaimidae	1.04	0.39	0.97	0.65	0.06
Teratocephalidae	6.63*	1.56	0.11	0.46	0.16
Fungivores					
Filenchus	50.49****	5.63****	5.04*	1.74	0.53
Aphelenchoididae	14.28***	0.67	5.01*	0.01	0.24
Aphelenchidae	12.33***	1.71	4.01*	1.08	0.25
Anguinidae	9.59**	1.06	1.18	1.03	0.18
Hervibores					
Tylenchidae	34.71****	0.63	0.26	1.44	0.35
Criconematidae	0.01	1.58	0.83	2.81†	0.12
Paratylenchidae	9.33**	4.13**	0.51	0.86	0.27
Omnivores					
Aporcelaimidae	44.43****	0.58	0.29	2.76	0.41
Noordidae	14.63***	3.17*	7.73**	7.52**	0.37
Qudsianematidae	44.52****	2.74*	3.03†	1.33	0.46

Table S8. Results of the PERMANOVA test that analyses the effect of rainfall exclusion on the ordination and composition of nematodes communities at the short- and long-term sites.

Factors	Df	F	p-value	R^2
Site	1	18.37	0.001	0.20
Block [Site]	4	1.90	0.003	0.08
Rainfall Exclusion	1	1.89	0.038	0.02
Site × Rainfall exclusion	1	1.74	0.071	0.02
Residuals	67			0.68
Total	71			

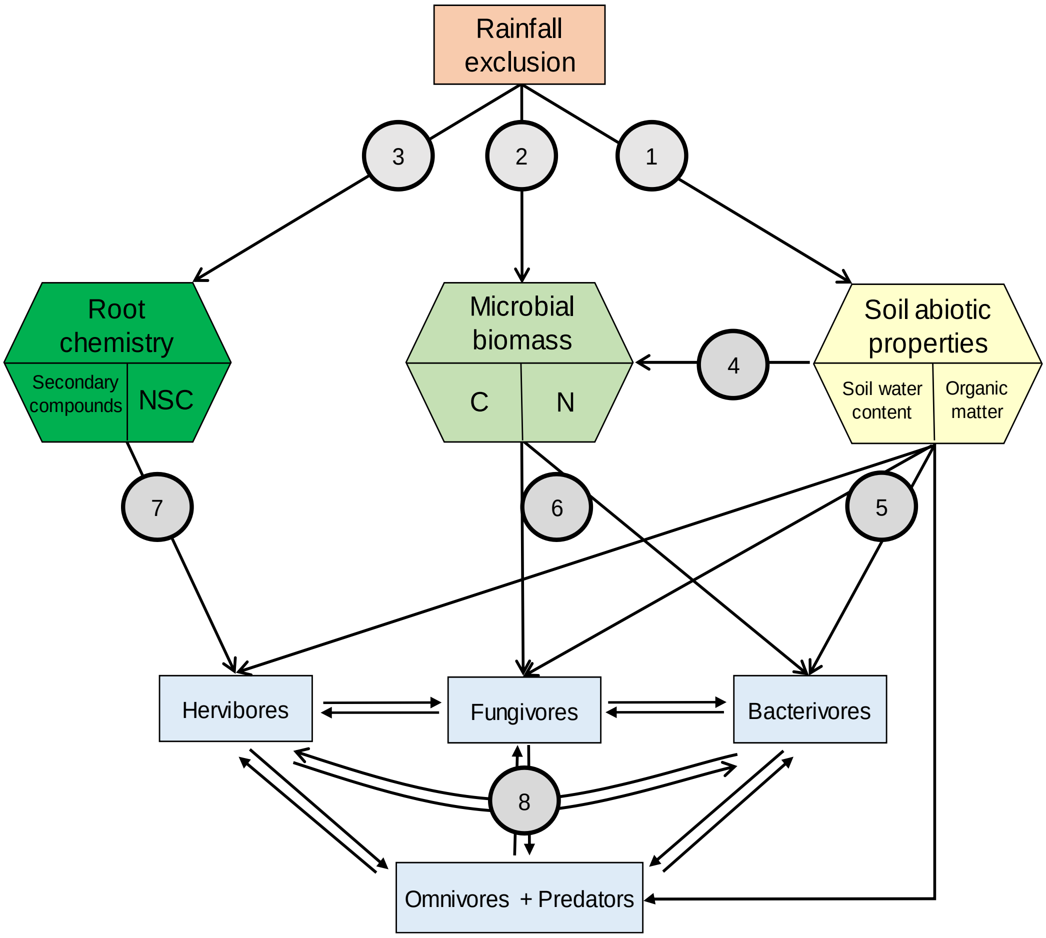

Figure S1. Full a-priori structural equation modelling (SEM) evaluating the effects of rainfall exclusion on the abundance of different nematode trophic groups mediated by changes in soil abiotic, microbial and plant drivers. Numbers in circles represent the different paths and hypothesised mechanisms explained in Table S1.

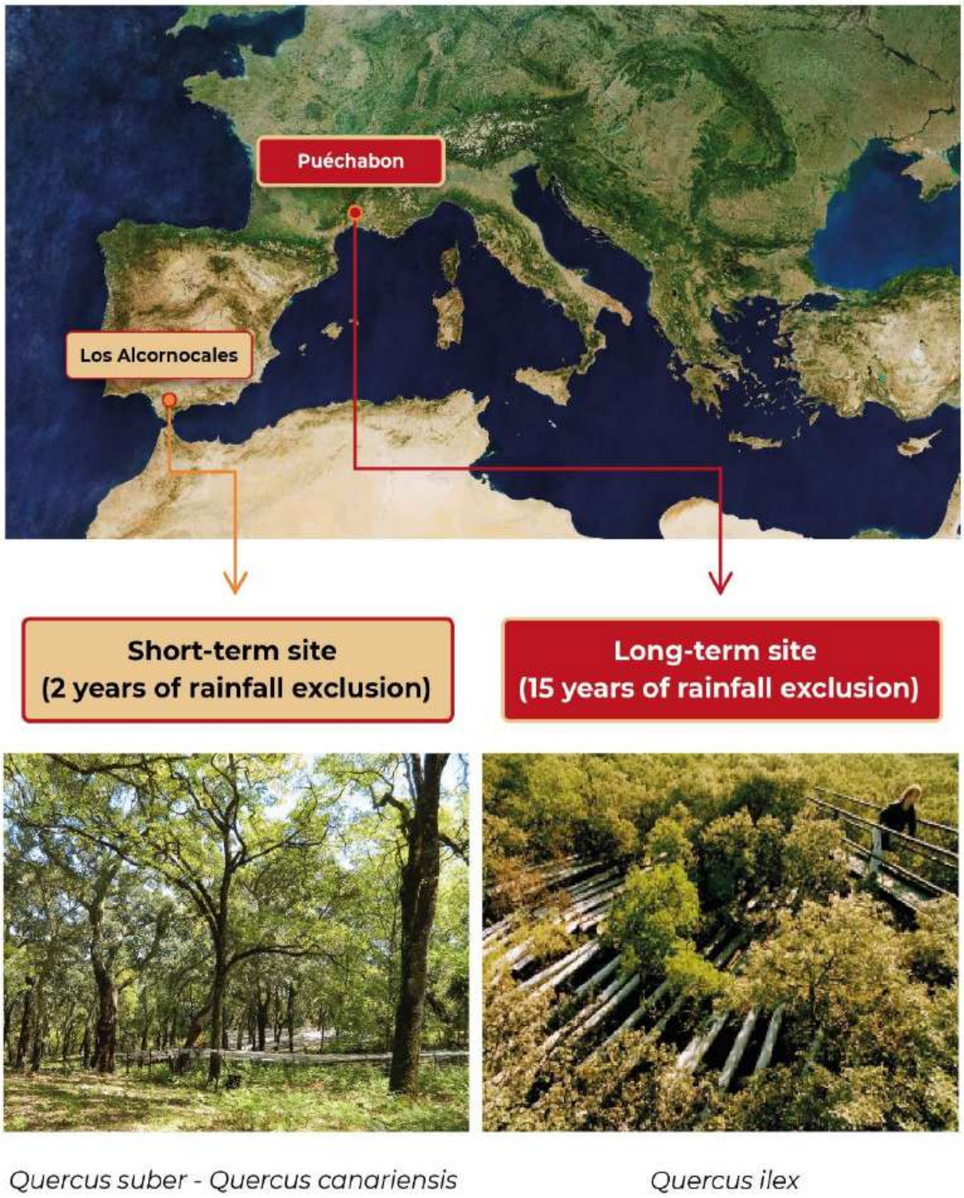

Figure S2. Location of the two experimental sites in southern Europe.

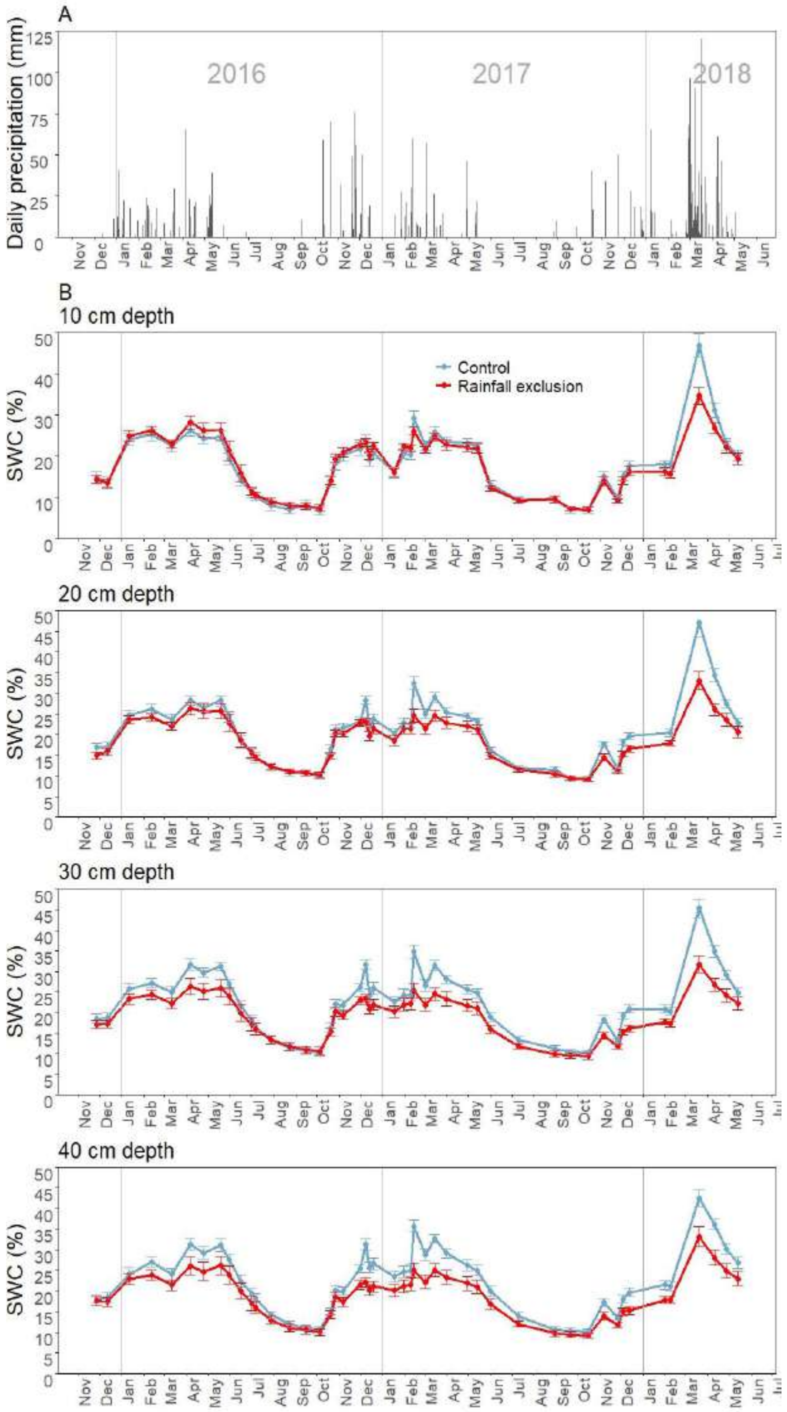

Figure S3. a) Daily precipitation at the short-term experimental site in southern Spain; b) Average soil water content (SWC) at different soil depths (10, 20, 30 and 40 cm) in rainfall exclusion and control plots (mean ± SE). Vertical grey lines separate different years. Rainfall exclusion structure was built and start working on March 2016.

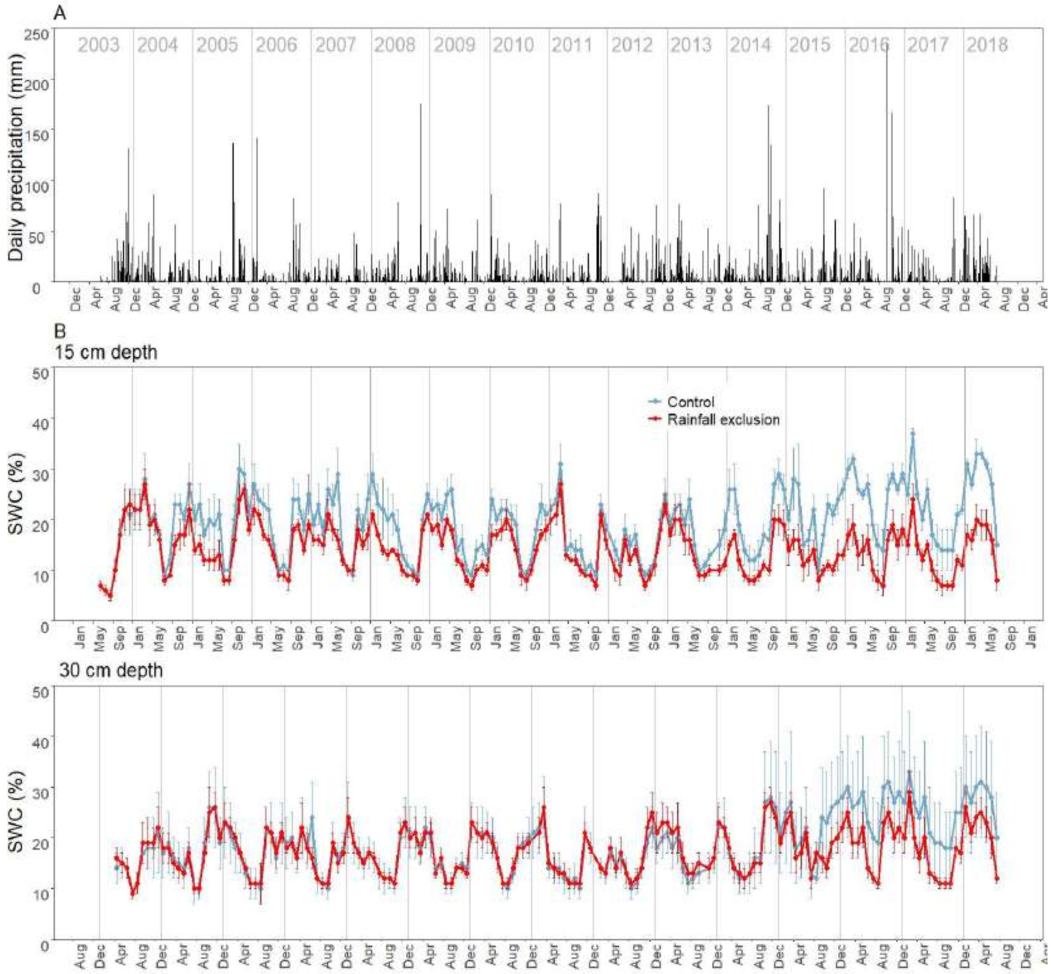

Figure S4. A) Daily precipitation at the long-term experimental site in France; B) Average soil water content (SWC) at different soil depths (0-15 cm, and 0-30 cm) in rainfall exclusion and control plots (mean ± SE) since rainfall exclusion structure installation. Vertical grey lines separate different years.

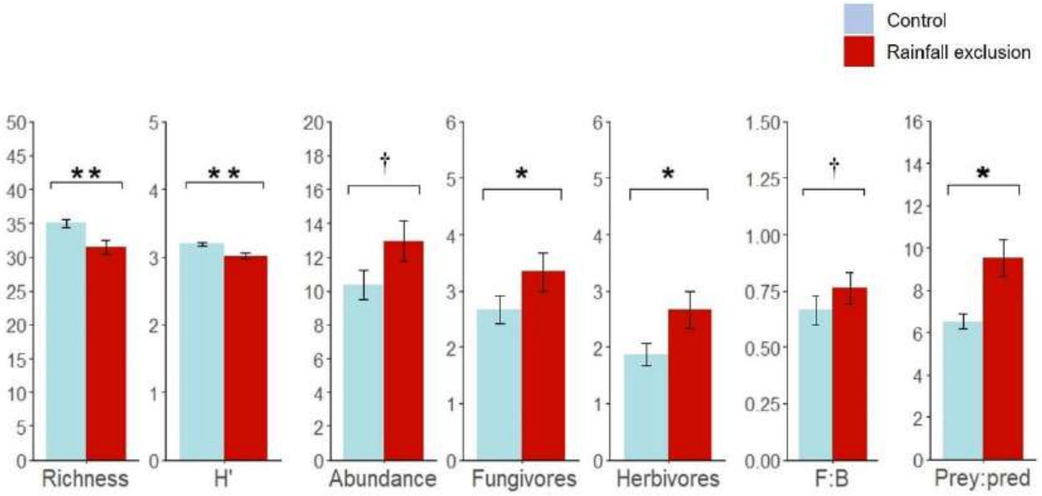

Figure S5. Pre-treatment differences at the short-term site in the richness, diversity (H') and total abundance of soil nematodes, as well as on the abundance of different trophic groups (fungivores and herbivores), the F:B ratio (fungivores:bacterivores ratio) and the prey:predator ratio. Abundances are represented as number of individuals per gram of soil. Error bars represent ±SE. Asterisks denote significant effects: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, † $p < 0.01$. (see Table S5).

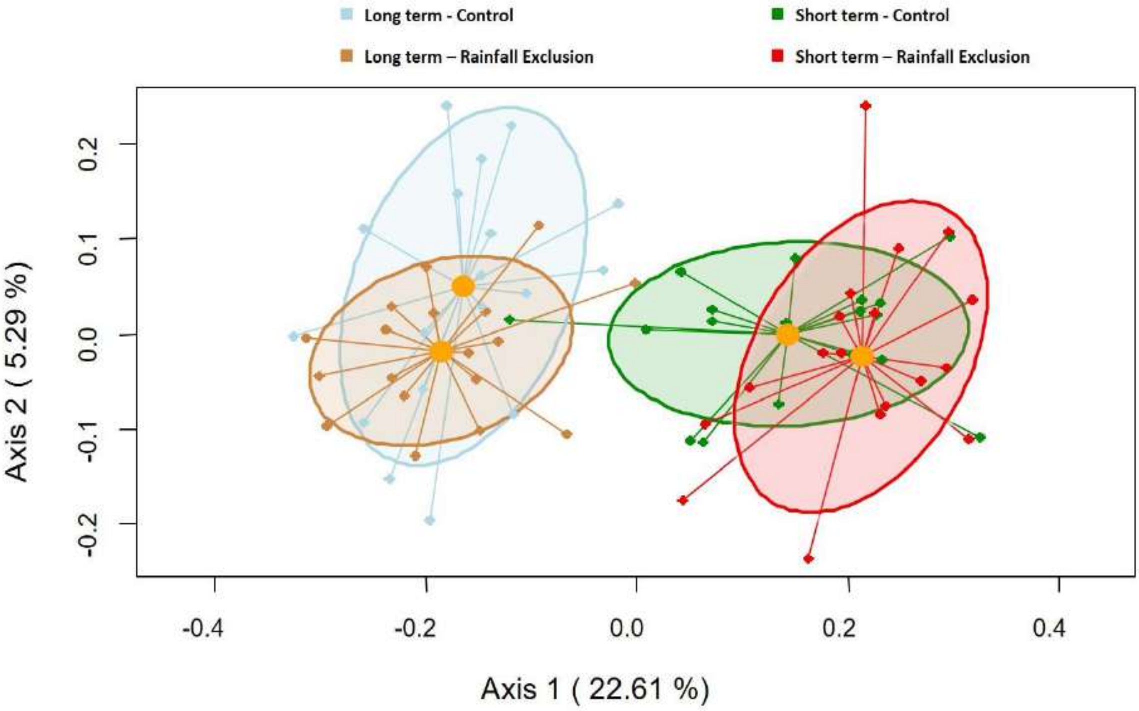

Figure S6. Ordination plot of the nematode community structure for the four combinations of site and treatment. Yellow points represent the centroids of the ellipses. Ellipses are painted with 95% margin of confidence.

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Discusión general

Discusión general

En el actual contexto de cambio global, es fundamental estudiar el efecto simultáneo de los distintos motores de cambio ya que estos pueden interactuar de diversas maneras (aditiva, sinérgica, antagónica), de modo que su efecto conjunto puede diferir de la suma de los efectos de cada motor de manera independiente. En la región mediterránea, el cambio climático y los patógenos exóticos son dos de las principales amenazas de sus bosques. En esta tesis, estudiamos la interacción de estos dos motores de cambio global y su efecto sobre procesos demográficos y ecosistémicos de los sistemas forestales mediterráneos. Nuestros resultados muestran el importante papel de los efectos indirectos de la reducción de las precipitaciones mediados tanto por el patógeno *P. cinnamomi* como por otros organismos del suelo, modulando tanto la regeneración del bosque como los procesos de descomposición de la hojarasca. Se trata de uno de los pocos estudios realizados hasta el momento en el que se ponen de manifiesto efectos antagonistas entre dos motores de cambio global, proporcionando unos resultados consistentes que nos dan una importante y novedosa información sobre el futuro del bosque mediterráneo y su funcionamiento.

Efectos de la acción combinada del cambio climático y *Phytophthora cinnamomi* sobre la salud de plántulas y la regeneración del bosque

Los experimentos realizados durante el desarrollo de la presente tesis han puesto de manifiesto que la reducción de las precipitaciones, esperada con motivo del cambio climático, y la invasión del patógeno exótico *P. cinnamomi* interactúan afectando a los procesos demográficos que influyen en la regeneración del bosque de manera distinta al efecto independiente de cada una de las fuentes de estrés (**capítulos 1 y 2**). Nuestra hipótesis de partida postulaba que, pese a que ambos motores de cambio global puedan perjudicar a las plántulas de las especies arbóreas dominantes, la dependencia de *P. cinnamomi* de la humedad (Erwin & Ribeiro, 1996) hace muy posible la existencia de efectos interactivos antagonistas entre ambos. De esta manera la reducción de las precipitaciones relacionada con el cambio climático limitará la capacidad de dispersión e infección del patógeno, influyendo indirectamente de manera positiva en la emergencia y supervivencia de las plántulas. Los resultados de los capítulos 1 y 2 muestran cómo el patógeno *P. cinnamomi* limita la regeneración de las especies estudiadas, principalmente a *Q. suber*. Sin embargo, observamos cómo una reducción moderada de la humedad del suelo puede reducir la capacidad infectiva del patógeno, disminuyendo de esta manera sus efectos negativos sobre la regeneración de las especies arbóreas. Estos efectos indirectos redujeron los daños producidos sobre las raíces de las plántulas en condiciones de sequía, traduciéndose además en una mayor biomasa radicular e influyendo positivamente de este modo sobre su emergencia y supervivencia (Fig.1). La interacción antagonista entre los dos motores de cambio llegó en algunos casos a cambiar el signo del efecto de la sequía sobre las plántulas en presencia del patógeno. Es decir, la sequía pasó de tener un efecto negativo a un efecto positivo. Esto supone que, en bosques infectados por el patógeno, las especies susceptibles a él van a verse fuertemente beneficiadas por la reducción de las precipitaciones, ya que ésta afectará más negativamente al patógeno que a las especies vegetales. Se trata por tanto de una de las primeras evidencias constatadas de efectos antagónicos de dos motores de cambio global sobre procesos demográficos de bosques mediterráneos, que normalmente son aditivos o en menor medida sinérgicos (Yue et al., 2017a, 2017b; Song et al., 2019; Ma et al., 2020; Ogle et al., 2021; Peng et al., 2021). La consistencia de resultados tanto bajo condiciones controladas de invernadero como en condiciones naturales de campo dan una gran robustez a este hallazgo, poniendo de manifiesto la importancia del estudio conjunto de los factores que alteran los ecosistemas desde distintas aproximaciones. Un aumento de este tipo de estudios podría suponer un incremento en el número de interacciones antagonistas detectadas entre distintos componentes de cambio global, que pueden estar siendo subestimadas hasta el momento.

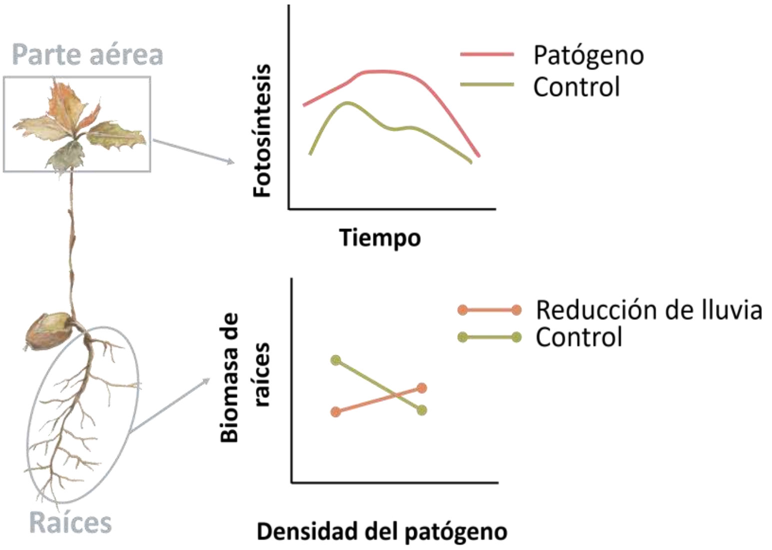

Figura 1. Resumen de los efectos interactivos detectados entre los dos motores de cambio global sobre las pántulas de *Quercus suber* y sus respuestas adaptativas.

El estudio de las posibles respuestas adaptativas de las plántulas a los motores de cambio global es fundamental ya que pueden determinar la capacidad de las plantas para compensar los efectos negativos de estas fuentes de estrés. En este trabajo hemos observado como las plántulas de *Q. suber* parecen responder a la sequía y al patógeno de manera adaptativa, morfológica y sobre todo fisiológicamente, fijando más carbono a través de la fotosíntesis para tratar de compensar los daños sufridos en las raíces que perjudican su capacidad de absorción de nutrientes (Fig. 1). Estos resultados muestran una cierta capacidad adaptativa de las plántulas de *Q. suber* a la infección por *P. cinnamomi* que no se había detectado en otros estudios previos (Luque et al., 1999; Robin et al., 2001; Maurel et al., 2001; Sghaier-Hammami et al., 2013). La capacidad adaptativa de las plántulas a una fuente de estrés va a depender de su susceptibilidad al mismo, pero también de la intensidad con la que se manifiesta dicho estrés (Halpern,1988; Allison & Martiny, 2008). En nuestro estudio hemos observado que cuando la infección es severa (alta cantidad del patógeno), la planta no es capaz de desarrollar esta respuesta fisiológica adaptativa. De esta manera, se confirma que las plántulas pueden tolerar la infección hasta cierto umbral, por encima del cual no hay respuesta compensatoria posible. Por tanto, es importante remarcar que los efectos negativos de la reducción de las precipitaciones sobre el patógeno no solo disminuirán los daños provocados por el mismo, si no que pueden mantener la abundancia de este por debajo de un umbral que permita la respuesta compensatoria de las plantas a su infección.

En los bosques mixtos, el impacto de los factores de cambio global sobre la regeneración de las distintas especies arbóreas puede variar en función del tipo de bosque y la identidad de la especie de dosel. Este es un hecho relevante para nuestra comprensión de los mecanismos que hacen que los bosques mixtos sean más resistentes y resilientes a las sequías y plagas que los bosques monoespecíficos (Bravo-Oviedo et al., 2018, Pardos et al., 2021). También nos permite conocer cómo las distintas especies arbóreas coexistentes podrían amortiguar en mayor o menor grado el impacto de los motores del cambio global sobre la regeneración en este tipo de bosques. En nuestro estudio, hemos constatado la importancia de la influencia de la especie del dosel sobre la regeneración y cómo su efecto varió entre los distintos tipos de bosque estudiados, mostrando la importancia del estudio de los impactos de los motores de cambio global en distintos contextos. Este hallazgo implica no sólo que el patrón espacial de establecimiento de las plántulas en el sotobosque podría verse alterado bajo el cambio global, sino también que la heterogeneidad de los bosques mixtos mediterráneos podría ofrecer lugares más favorables para el reclutamiento de las especies más susceptibles a las distintas fuentes de estrés que otros ecosistemas menos diversos. La variación en los efectos de los dos motores de cambio global sobre las distintas especies de plántulas estudiadas posiblemente se traducirá en cambios en la estructura y dominancia de la vegetación en un futuro.

Los dos capítulos de esta tesis que tratan de conocer los efectos interactivos de la humedad del suelo y *P. cinnamomi* sobre las plántulas de especies arbóreas sugieren que el efecto del patógeno está modulado incluso por pequeñas variaciones de la humedad. Así, un clima más seco podría implicar condiciones sub-óptimas para el patógeno que le perjudicarían. Sin embargo, debemos de reconocer que los efectos del cambio climático no solo van a depender de la progresiva reducción de las precipitaciones, sino también del aumento de la frecuencia de eventos extremos (sequías e inundaciones), las cuales podrían tener un papel muy importante en el futuro de estos bosques. El incremento de lluvias torrenciales puede provocar momentos de saturación de agua del suelo que provoquen picos de infección y propagación del patógeno, contrarrestando al menos parcialmente la pérdida de sus capacidades infectivas y dispersivas bajo condiciones generales más secas.

Efectos de la reducción de la precipitación en las comunidades bióticas del suelo y en los ciclos de nutrientes

Está sobradamente demostrado que la biodiversidad del suelo tiene una importancia capital en el funcionamiento de los ecosistemas terrestres, participando en numerosos procesos fundamentales para el mantenimiento de dichos ecosistemas y que repercutirán incluso en la salud humana (Wall et al., 2015). Dada su importancia, en el contexto de esta tesis doctoral nos planteamos la cuestión de cómo afectará el cambio climático, y más concretamente la reducción de las precipitaciones, a la fauna edáfica, así como a su papel en procesos ecosistémicos tan importantes como la descomposición de la hojarasca en bosques invadidos por *P. cinnamomi*. Debido a la influencia que tienen la vegetación (Baldrian, 2016; Prescott & Grayston, 2013; Urbanova et al., 2015), y las condiciones ambientales sobre los organismos del suelo (Preisser & Strong, 2004; Franco et al., 2019), es de esperar que los dos motores de cambio global estudiados en esta tesis (patógenos exóticos y cambio climático) afecten a la biodiversidad del suelo y su funcionamiento, directa o indirectamente a través de cambios sobre dicha vegetación. Trabajos realizados en estos mismos bosques muestran cómo ambos motores de cambio global pueden modificar los factores determinantes de la presencia y abundancia de la fauna del suelo (Jimenez-Chacón et al., 2018). En el desarrollo de esta tesis no hemos podido observar apenas efectos indirectos de ambos factores sobre la biodiversidad del suelo mediados tanto por cambios en la vegetación, referidos tanto a la composición de especies como a su abundancia, como por los compuestos de sus hojas y raíces, ya que posiblemente se expresen a más largo plazo. Por el contrario, sí hemos detectado importantes efectos directos de la sequía sobre la fauna del suelo, con notables consecuencias sobre el funcionamiento de los procesos ecológicos del suelo (Fig.2).

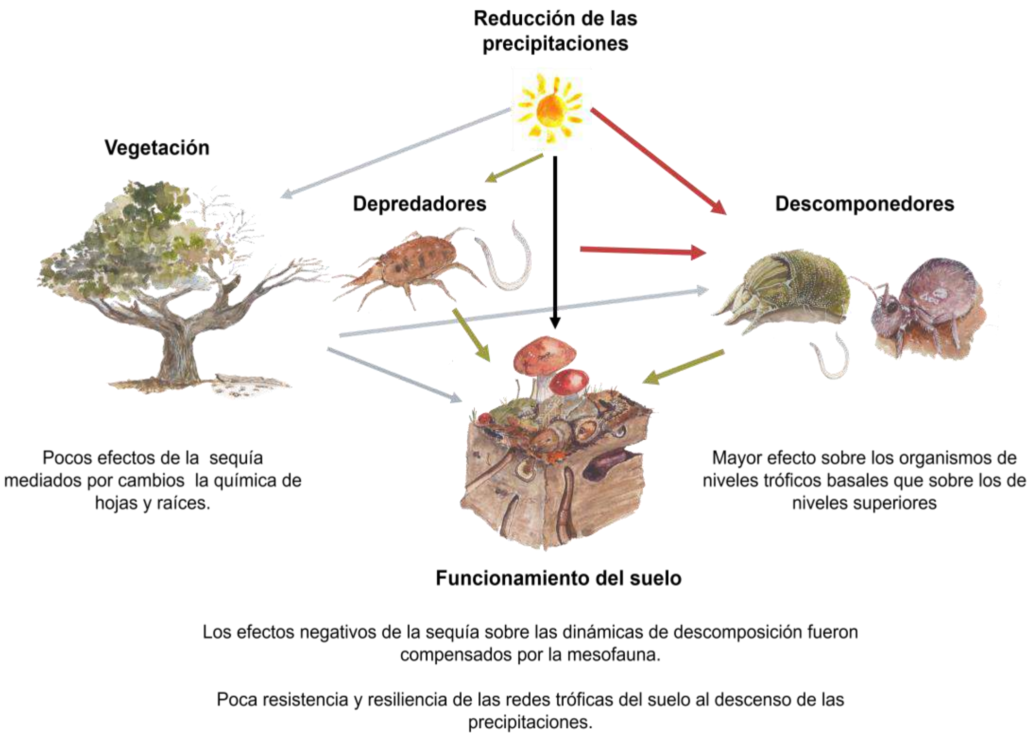

Figura 2. Resumen de los efectos directos e indirectos de la sequía sobre las comunidades bióticas del suelo y sus efectos en la funcionalidad del ecosistema.

En los capítulos 3 y 4 estudiamos la capacidad que tienen los motores de cambio global de afectar a los organismos edáficos (mesofauna y nematodos) y su funcionalidad dentro del ecosistema. En ambos capítulos hemos observado cambios significativos de las comunidades bióticas del suelo, que muestran una gran sensibilidad a la reducción de las precipitaciones. Estos efectos se produjeron principalmente sobre los organismos pertenecientes a los grupos tróficos más bajos, mientras que los grupos tróficos superiores apenas se vieron afectados. De esta forma, la reducción de lluvia produjo un aumento de la presión de depredación en la fauna del suelo (tanto para la mesofauna como para los nematodos). Esto provocó un aumento en los índices basados en los nematodos de estructura y madurez del ecosistema que muestran un incremento de la complejidad de las redes tróficas bajo el tratamiento de sequía. Los cambios producidos por la sequía sobre la fauna del suelo se observaron tanto sobre la mesofauna como sobre los nematodos, y se produjeron tanto a corto como a largo plazo, demostrando una baja resistencia (impacto a corto plazo) y resiliencia (impacto también a largo plazo) de estos sistemas. Sin embargo, los resultados de los índices de los nematodos parecen sugerir que los cambios sufridos en la estructura de la red trófica podrían cambiar hacia estados que les confieran una mayor resistencia a futuras perturbaciones.

El efecto de la reducción de las precipitaciones sobre la biodiversidad edáfica provoca cambios en los procesos de descomposición tanto a corto como a largo plazo. Esto nos muestra, como ya se ha visto en algún estudio previo, (Santonja et al., 2017; Ashton et al., 2019) que los organismos del suelo van a ser capaces de modular los efectos del cambio climático sobre los ecosistemas y su funcionamiento. A corto plazo estos cambios están mediados por los cambios sufridos por la mesofauna (capítulo 3). El incremento en la presión de depredación sobre la fauna edáfica supone paradójicamente un aumento en las tasas de descomposición de la hojarasca. Esto se puede deber a que la fauna depredadora ejerce su presión sobre ciertos grupos con una menor importancia en las dinámicas de descomposición de la hojarasca (Lawrence & Wise, 2004; Zhou et al., 2018), favoreciendo por el contrario a otros grupos que aceleran las dinámicas de descomposición (Frouz et al., 2008; Hättenschwiler et al., 2005). A largo plazo (capítulo 4), la reducción de las precipitaciones parece producir alteraciones en las vías microbianas de descomposición favoreciendo las vías bacterianas, más rápidas, pero menos eficientes que las fúngicas (de Vries et al., 2006, 2011; Sylvain et al., 2014), posiblemente asociados a cambios en la vegetación (Curiel-Yuste et al., 2012; Lloret et al., 2015). Estos resultados que requieren de investigaciones futuras más profundas podrían indicar preocupantes procesos de retroalimentación con el cambio climático. Nuestros resultados ponen de manifiesto la importancia del estudio de los efectos indirectos de la reducción de las precipitaciones sobre el funcionamiento de los ecosistemas, y del primordial papel que juegan los organismos del suelo regulando estos procesos ecológicos.

Los resultados obtenidos en esta tesis demuestran también la importancia de la identificación taxonómica a distintos niveles en el estudio de las redes tróficas del suelo y sus implicaciones funcionales. Por un lado, la identificación a nivel de género de los nemátodos nos permite explorar el grado de afectación de los distintos géneros y familias por el cambio climático. De esta manera, hemos podido observar cómo los géneros y familias predominantes disminuyen su abundancia siendo sustituidas en muchos casos por familias menos abundantes del mismo grupo funcional, mostrando cierta resiliencia funcional de las redes tróficas del suelo. Esto también nos ha permitido el cálculo de los índices indicativos del estado del ecosistema a partir del conocimiento de las redes tróficas del suelo. Por otro lado, la clasificación en grupos tróficos/funcionales utilizada tanto en el capítulo 3 como el 4, nos ha ayudado a comprender los cambios producidos en el funcionamiento del ecosistema debidos principalmente a las diferentes respuestas mostradas por los distintos grupos tróficos. Por tanto, la identificación y clasificación a los dos niveles (género y grupo funcional), combinando la explicación de los resultados obtenidos por ambas aproximaciones, nos ofrece una información muy completa que nos permite identificar qué grupos taxonómicos y funcionales son los más importantes para determinar el funcionamiento de los suelos de ecosistemas forestales y el efecto que va a tener el cambio climático sobre los organismos que viven en él.

El hecho de estudiar el efecto de la reducción de las precipitaciones sobre las dinámicas del suelo en un sistema donde está presente *P. cinnamomi* nos permite investigar las relaciones directas o indirectas de este patógeno en los procesos del suelo. Si bien es difícil demostrar una relación directa (por ejemplo, trófica) entre el patógeno y la fauna del suelo, existen estudios previos que han puesto de manifiesto la capacidad del patógeno para modificar los ecosistemas, produciendo efectos indirectos sobre la biodiversidad del suelo y sus funciones (Postle et al., 1986; Domínguez-Begines et al., 2019; Jimenez-Chacón et al., 2018). En este estudio no hemos encontrado efectos relevantes del patógeno en el paisaje (poco tiempo de evolución de la infección), ni efectos sobre los árboles adultos que se transfieran a los organismos del suelo (calidad de la hojarasca, compuestos de reserva de las plantas, etc.). Sin embargo, hemos encontrado interesantes correlaciones entre la abundancia del patógeno y los distintos grupos de fauna del suelo. Si bien no es fácil explicar biológicamente estas correlaciones, consideramos que pueden reflejar cierta preferencia de ambos organismos por condiciones ambientales concretas; o tal vez que estemos ante algún tipo de interacción biológica que está aún por explicar. Por tanto, creemos que esta relación entre fauna del suelo y patógenos exóticos es una línea a explorar en el futuro que puede conducir a resultados muy interesantes sobre el papel de la biodiversidad del suelo en la invasión y daños provocados por patógenos del suelo. Dicha información podría ser potencialmente usada para entender la diversidad de ciertos compartimentos del ecosistema como elementos claves para modular los efectos del patógeno sobre las especies de árboles.

Conclusiones generales

- La presente tesis pone de manifiesto la existencia de efectos interactivos antagonistas entre dos motores de cambio global - la reducción de la precipitación debido al cambio climático y el patógeno exótico *Phytophthora cinnamomi* - sobre la regeneración del bosque.

- En especies arbóreas susceptibles a *P. cinnamomi*, como el alcornoque, la respuesta fotosintética y en menor medida las adaptaciones morfológicas pueden compensar hasta cierto punto los daños producidos por el patógeno. La ralentización de la infección del patógeno por la sequía puede facilitar estas respuestas compensatorias y también proporcionarnos un valioso tiempo para trata de investigar posibles soluciones (estrategias de biocontrol).

- La diferente susceptibilidad de las distintas especies arbóreas coexistentes tanto a la reducción de las precipitaciones como a *P. cinnamomi* determinan el efecto combinado de ambos motores de cambio global sobre ellas, y por tanto van a modular la regeneración de los bosques mixtos mediterráneos produciendo posibles cambios en su estructura y composición.

- El proceso de descomposición de la hojarasca se vio afectado directa e indirectamente por la reducción de las precipitaciones. Aunque el incremento de la sequía tuvo un efecto negativo sobre las dinámicas de descomposición, los cambios producidos en la comunidad de la mesofauna modularon indirectamente estos efectos acelerando el proceso de descomposición y poniendo de manifiesto la importancia de estos organismos en los ciclos de nutrientes y su respuesta frente a los distintos motores de cambio global.

- La reducción de las precipitaciones provocó cambios rápidos en las redes tróficas del suelo que también se observaron a largo plazo, indicando una baja resistencia y resiliencia de estos organismos a los efectos del cambio climático. Dichos cambios se centraron principalmente en los organismos de niveles tróficos inferiores y paradójicamente podrían conferir una mayor resistencia a estas comunidades frente a futuras perturbaciones.

- Tanto los efectos antagonistas de la reducción de las precipitaciones sobre *P. cinnamomi* como los efectos de la sequía sobre la fauna edáfica se han mostrado consistentes entre diferentes metodologías (invernadero y estructura de exclusión de lluvia) y a lo largo del tiempo (3 años y 15 años), demostrando pues un buen grado de robustez de los resultados obtenidos en esta tesis.

- Los efectos indirectos de la reducción de lluvia mediados por los organismos que participan en los distintos procesos ecológicos, pueden ser de tanta o mayor importancia que los efectos directos, demostrando la importancia de su estudio para el entendimiento del funcionamiento de los ecosistemas en condiciones futuras.

- A pesar de los efectos encontrados de la reducción de la precipitación sobre la vegetación y los organismos y procesos edáficos, es importante tener en cuenta que las consecuencias de un clima promedio más seco en la dinámica de las enfermedades emergentes estarán fuertemente moduladas por el aumento predicho de los eventos climáticos extremos (sequías e inundaciones). Nuestros resultados sugieren que una mayor frecuencia de eventos de lluvia extrema que saturan el suelo y causan encharcamientos temporales serán particularmente beneficiosos para las infecciones por *P. cinnamomi*, aumentando su densidad más allá de cualquier capacidad de respuesta posible de los huéspedes susceptibles.

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