Universitat de Lleida

Plant-soil feedbacks in boreal and Mediterranean forest ecosystems

Dora Štraus
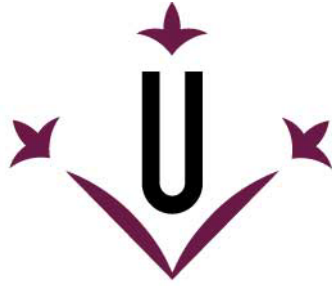
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PHD THESIS

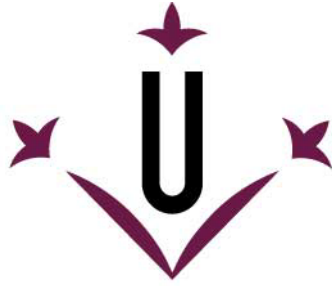# Plant-soil feedbacks in boreal and Mediterranean forest ecosystems

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To obtain the degree of Doctor at the University of Lleida
Doctorate Programme in Forestry and Natural Environment Management

Supervised by
Jonàs Oliva Palau

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TESI DOCTORAL

Plant-soil feedbacks in boreal and Mediterranean forest ecosystems

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Study **II**   Štraus, D., Caballol, M.; Juhanson, J.; Redondo, M.Á.; Bonet, J.A.; Pemán, J.; Clemmensen, K.E.; Hallin, S.; Oliva, J. Ectomycorrhizal fungi drive negative plant-soil feedbacks in Mediterranean pine-oak mixtures. *Under revision in New Phytologist.*

Study **III**   Štraus, D., Caballol, M.; Oliva, J. Biocide application relieves plant-soil feedbacks among Mediterranean forest species. *Manuscript.*

Study **IV**   Štraus, D.; Caballol, M.; Serradó, F.; Oliveras, J.; Ramis, X.; Oliva, J. Distribution of *Phytophthora* species within recreational chestnut, beech and cork oak forests. *For. Ecol. Manage.* **2023**, 529, 120674, doi: 10.1016/j.foreco.2022.120674.

Contributions in other manuscripts:

i.   Caballol, M.; Štraus, D.; Macia, H.; Ramis, X.; Redondo, M.Á.; Oliva, J. *Halophytophthora fluviatilis* pathogenicity and distribution along a Mediterranean-subalpine gradient. *J. Fungi* **2021**, *7*, 112. https://doi.org/10.3390/jof7020112

ii.   Lade, S.B.; Štraus, D.; Oliva, J. Variation in fungal community in grapevine (*Vitis vinifera*) nursery stock depends on nursery, variety and rootstock. *J. Fungi* **2022**, *8*, 47. https://doi.org/10.3390/jof8010047

iii.   Lade, S.B.; Štraus, D.; Buñol, A.; Oliva, J. Hot water treatment causes lasting alteration to the grapevine (*Vitis vinifera* L.) mycobiome and reduces pathogenic species causing grapevine trunk diseases. *J. Fungi* **2022**, *8*, 485. https://doi.org/10.3390/jof8050485

ABSTRACT

Trees form relationships with soil microbiota that can affect their growth. These associations can be beneficial or detrimental and do not affect the fitness of all tree species growing on a given site equally. Therefore, feedbacks between plants and soil microbiota (plant-soil feedbacks, PSFs) are considered to regulate diversity in many ecosystems, from tropical forests to grasslands. However, the contribution of PSFs to tree diversity in many forest biomes is still poorly understood, for instance in boreal or Mediterranean conditions, in which climatic constraints such as cold or drought may also limit species diversity. In the face of global climate change, it is important to characterise the contribution of soil microbiota to forest stand dynamics in order to better predict the outcome of a warming climate on vulnerable ecosystems. Therefore, the main objectives of this thesis were (i) to describe the PSFs in forest ecosystems where climate change is expected to pose an important impact, such as boreal or Mediterranean forests; (ii) to examine the effect of PSFs on tree species diversity and stand composition; (iii) to determine which microbial guilds drive the PSFs; and (iv) to explore whether invasive species can be part of those feedbacks as well.

This thesis combines the results of three experimental glasshouse studies (studies **I**, **II** and **III**) and one field survey (study **IV**). The three experimental studies were aimed at characterising the PSFs among common boreal forest tree species (*Betula pendula*, *Alnus glutinosa*, *Pinus sylvestris* and *Picea abies*) and Mediterranean forest tree species. Mediterranean species included four species from the genus *Quercus* and four from the genus *Pinus*, which were selected based on their widespread presence in Mediterranean forests, where they often form pine–oak mixtures. Seedlings of those species were planted into pots with a mixture of sterilized soil and a small amount of soil collected under mature trees. The soil microbial community in the pots was analysed by metabarcoding and biocides targeting specific microbial guilds were included in studies **I** and **III** to determine their contribution to the observed feedbacks. A field survey of forest soils across Catalonia was performed in study **IV** to examine native and invasive *Phytophthora* pathogen presence in the area and assess its impact on tree health and regeneration. The survey included forests with varying degrees of recreational pressure to determine whether human activity could lead to an increased risk of pathogen invasion. A drought treatment was included in study **II** to determine the effect of water limitation on PSFs among Mediterranean species.

The results showed that PSFs had the potential to shape tree species assemblies in boreal and Mediterranean forests. In both ecosystems, the PSFs seemed to be driven by ectomycorrhizal fungi (EMF) and, to a lesser extent, pathogens, which promoted the establishment of other species and hampered conspecific regeneration. In boreal forests, PSFs among individual tree species operated in a way that corresponded with tree species succession in nature. Negative PSFs in Mediterranean forests acted at genus level and showed a putative capacity to promote the formation of mixed pine

and oak stands. However, results showed that drought and the introduction of invasive pathogens could affect naturally occurring PSFs and potentially influence future tree species composition of Mediterranean forests.

In summary, this thesis characterises PSFs in forests and provides insight into how PSFs could change under drought or through human influence. It demonstrates the contribution of soil microbiota to forest stand diversity and sheds light on the processes driving the observed feedbacks. Finally, it shows that beneficial EMF, not just pathogens, can drive negative feedbacks. The fact that EMF beneficial for mature trees may not necessarily be beneficial for conspecific recruits is a novel and important contribution that may lead to new hypotheses and experiments in the field of plant–soil feedbacks in a range of forest environments.

RESUMEN

Los árboles establecen relaciones con la comunidad microbiana del suelo que pueden afectar a su crecimiento. Estas asociaciones pueden ser beneficiosas o perjudiciales y no afectan a todas las especies que crecen en un determinado sitio por igual. Por lo tanto, se cree que las retroalimentaciones entre las plantas y los microorganismos del suelo ('plant-soil feedbacks', PSF) regulan la diversidad en muchos ecosistemas, desde los bosques tropicales hasta las praderas. Sin embargo, la contribución de los PSF a la diversidad arbórea en muchos biomas forestales sigue siendo poco conocida, por ejemplo, en condiciones boreales o mediterráneas, en las que restricciones climáticas como el frío o la sequía pueden también limitar la diversidad de especies. De cara al cambio climático global, es importante caracterizar la contribución de la comunidad microbiana del suelo a la dinámica de las masas forestales para poder predecir mejor las consecuencias de un clima más cálido en ecosistemas vulnerables. Por lo tanto, los principales objetivos de esta tesis eran (i) describir los PSF de los ecosistemas forestales en los que se espera que el cambio climático tenga un impacto importante (como los bosques boreales o mediterráneos), (ii) examinar el efecto de los PSF en la diversidad de especies arbóreas y la composición de las masas forestales, (iii) determinar qué grupos microbianos impulsan los PSF y (iv) explorar si las especies invasoras también pueden formar parte de los PSF.

Esta tesis combina los resultados de tres estudios experimentales realizados en el invernadero (estudios **I**, **II** y **III**) y un estudio de campo (estudio **IV**). Los tres estudios experimentales tenían como objetivo caracterizar los PSF entre especies arbóreas típicas de los bosques boreales (*Betula pendula*, *Alnus glutinosa*, *Pinus sylvestris* y *Picea abies*) y especies arbóreas de los bosques mediterráneos. Las especies mediterráneas incluían cuatro especies del género *Quercus* y cuatro del género *Pinus*, seleccionadas por su presencia generalizada en los bosques mediterráneos, donde suelen formar mezclas de pino y roble. Los plantones de esas especies se plantaron en macetas con una mezcla de suelo esterilizado y una pequeña cantidad de suelo recogido bajo árboles maduros. Se analizó la comunidad microbiana del suelo en las macetas mediante secuenciación masiva y se incluyeron biocidas dirigidos a grupos microbianos específicos en los estudios **I** y **III** para determinar su contribución a los PSF observados. En el estudio **IV** se realizó un estudio de campo de los suelos forestales de Cataluña para examinar la presencia de patógenos del género *Phytophthora* nativos e invasores en la zona y evaluar su impacto en la salud y regeneración de los árboles. El estudio incluía bosques con distintos grados de uso recreativo para determinar si la actividad humana podía conllevar un aumento del riesgo de invasión de patógenos. En el estudio **II** se incluyó un tratamiento de sequía para determinar el efecto de la limitación hídrica en los PSF entre las especies mediterráneas.

Los resultados mostraron que los PSF tenían el potencial de afectar a la composición de las masas forestales en los bosques boreales y mediterráneos. En ambos ecosistemas, los PSF parecían estar impulsados por hongos ectomicorrícicos (EMF) y, en menor medida, por patógenos que promovían

el establecimiento de otras especies y dificultaban la regeneración conespecífica. En los bosques boreales, los PSF entre especies arbóreas individuales parecían funcionar del modo que se correspondía con la sucesión de esas especies en la naturaleza. Los PSF negativos en los bosques mediterráneos actuaban a nivel de género y parecían tener la capacidad de promover la formación de rodales mixtos de pino y roble. Sin embargo, los resultados mostraron que en bosques mediterráneos la sequía y la introducción de patógenos invasores podrían afectar a los PSF y afectar la composición de especies arbóreas.

En resumen, esta tesis caracteriza los PSF en los bosques y proporciona una visión de cómo los PSF podrían cambiar con la sequía o por influencia humana. Demuestra la contribución de la comunidad microbiana del suelo a la diversidad de las masas forestales y desvela los procesos que impulsan los PSF observados. Por último, muestra que los EMF beneficiosos, y no solo los patógenos, pueden impulsar los PSF negativos. El hecho de que los EMF que son beneficiosos para los árboles maduros no lo sean necesariamente para los plantones conespecíficos es una contribución novedosa e importante de esta tesis que puede conducir a nuevas hipótesis y experimentos en el campo de la retroalimentación planta suelo en el ámbito forestal.

RESUM

Els arbres interaccionen amb la comunitat microbiana del sòl. Aquestes associacions poden ser beneficioses o perjudicials i a més poden no afectar a totes les espècies d'arbres que creixen en un determinat lloc per igual. És per això que es creu que les retroalimentacions entre les plantes i els microorganismes del sòl (plant-soil feedbacks, PSF) regulen la diversitat en molts ecosistemes, des dels boscos tropicals fins als prats. Tot i això, la contribució dels PSF a la diversitat arbòria en molts biomes forestals continua sent poc coneguda, per exemple, en condicions boreals o mediterrànies, en les quals restriccions climàtiques com el fred o la sequera poden també limitar la diversitat d'espècies. En un context d'escalfament global és important caracteritzar la contribució de la comunitat microbiana del sòl a la dinàmica de les masses forestals per poder predir millor les conseqüències d'un clima més càlid. Per tant, els principals objectius d'aquesta tesi han estat (i) descriure els PSF dels ecosistemes forestals on es preveu que el canvi climàtic tingui un impacte negatiu més notori, com es el cas dels boscos boreals i els boscos mediterranis, (ii) examinar l'efecte dels PSF en la diversitat d'espècies arbòries i la composició de les masses forestals, (iii) determinar quins grups microbians impulsen els PSF i (iv) explorar si les espècies invasores també poden formar part dels PSF.

Aquesta tesi combina els resultats de tres estudis experimentals realizats a l'hivernacle (estudis **I**, **II** i **III**) i un estudi de camp (estudi **IV**). Els tres estudis experimentals tenien com a objectiu caracteritzar els PSF entre espècies arbòries típiques dels boscos boreals (*Betula pendula*, *Alnus glutinosa*, *Pinus sylvestris* i *Picea abies*) i espècies arbòries dels boscos mediterranis. Les espècies mediterrànies incloïen quatre espècies del gènere *Quercus* i quatre del gènere *Pinus*, seleccionades per la seva presència generalitzada als boscos mediterranis, on solen formar barreges de pi i roure. Els plançons d'aquestes espècies es van plantar en testos amb una barreja de sòl esterilitzat i una petita quantitat de sòl recollit sota arbres madurs. Es va analitzar la comunitat microbiana del sòl als tests mitjançant seqüenciació massiva i es van incloure biocides dirigits a grups microbians específics als estudis **I** i III per determinar la seva contribució als PSF observats. A l'estudi **IV** es va realitzar un estudi de camp dels sòls forestals de Catalunya per examinar la presència de patògens nadius o invasors del gènere *Phytophthora* i avaluar-ne l'impacte en la salut i regeneració dels arbres. L'estudi incloïa boscos amb diferents graus d'ús recreatiu per determinar si l'activitat humana podia comportar un augment del risc d'invasió de patògens. A l'estudi **II** es va incloure un tractament de sequera per determinar l'efecte de la limitació hídrica als PSF entre les espècies mediterrànies.

Els resultats van mostrar que els PSF tenien el potencial d'afectar la composició de les masses forestals als boscos boreals i mediterranis. En tots dos ecosistemes, els PSF semblaven estar impulsats per fongs ectomicòrrics (EMF) i patògens que promovien l'establiment d'altres espècies i dificultaven la regeneració coespecífica. Als boscos boreals, els PSF entre espècies arbòries individuals semblaven funcionar d'acord amb la successió natural que aquestes espècies mostren a la natura. Els PSF

negatius als boscos mediterranis actuaven a escala de gènere i semblaven tenir la capacitat de promoure la formació de rodals mixtos de pi i roure. Tot i això, els resultats van mostrar que en boscos meditarranis la sequera i la introducció de patògens invasors podrien afectar els PSF i afectar la composició d'espècies arbòries.

En resum, aquesta tesi caracteritza els PSF als boscos i proporciona una visió de com els PSF podrien canviar amb la sequera o per influència humana. Demostra la contribució de la comunitat microbiana del sòl a la diversitat de les masses forestals i desvetlla els processos que impulsen els PSF observats. Per acabar, mostra que els EMF beneficiosos, i no només els patògens, poden impulsar els PSF negatius. El fet que els EMF que són beneficiosos per als arbres madurs no ho siguin necessàriament per als plançons de la mateixa espècie és una contribució nova i important d'aquesta tesi que pot conduir a noves hipòtesis i experiments en el camp de la retroalimentació planta sòl en l'àmbit forestal.

1   INTRODUCTION

1.1   Boreal and Mediterranean forests

Forests cover almost a third of the world's land area, providing a range of ecosystem services that improve human well-being ("Ecosystems and Human Well-being: Synthesis," 2005). Besides offering traditional material resources such as wood, forests are important regulators of climate change, acting as major sinks of atmospheric carbon (Nave et al., 2018). Forests prevent erosion by stabilising slopes, assist with water regulation and offer many non-material benefits, such as raising the aesthetic value of a landscape or providing spaces for recreation. Finally, trees are foundation organisms of highly diverse ecosystems, including shrubs and understory herbs, which provide habitat for myriad animals and other organisms.

Forests can be formed by one or more species. Some appear to be dominated by a single tree species, as is the case with European beech (*Fagus sylvatica*) in some central European forests. However, mixed forests in which several tree species coexist at different stages of forest development seem to be more frequently found in nature. Species diversity contributes to the resistance of a forest ecosystem and increases its capacity to adapt to change. As biodiversity increases, so does the resilience of a system because diverse populations that are adapted to a variety of conditions are more likely to survive both abiotic and biotic disturbances. Tree diversity can also be the result of a dynamic process in which some species are replaced by others due to interspecific competition.

Fitness traits such as growth, survival or reproduction of an individual tree species are affected by abiotic factors, such as light or water availability, and by biotic factors, such as herbivore density or the presence of beneficial and harmful microbes. It is generally accepted that life traits (e.g., light or nutrient requirements) determine which plants will dominate a particular successional stage (Petrokas et al., 2020). For example, in the early stages of forest development, stands tend to be dominated by light-demanding species, which are gradually outcompeted by species better adapted for later-successional stages with different light and nutrient availability (Angelstam & Kuuluvainen, 2004). Coexisting species occupy different ecological niches in order to minimise competition for resources (Silvertown, 2004). However, as trees grow, they also change the environmental conditions in their surroundings. For example, the structure and density of their crown affect the amount of light that reaches the ground, or they can change soil properties via their demands for nutrients and water or through the decomposing leaf litter. All these factors affect the establishment of conspecific seedlings (i.e., seedlings belonging to the same species as the adult tree) in the area. For instance, species that are more shade-tolerant can more successfully establish under light-demanding species dominating the forest crown than seedlings of those light-demanding species. Soil physical and chemical properties are also major drivers of tree species composition, as they influence species establishment and growth (Aponte et al., 2011; Soong et al., 2020). Trees not only interact directly

with the environment but also indirectly via mutualistic associations with soil microorganisms, which can affect their growth and alter their chances for establishment. Evidence from the last decades of research (Bennett et al., 2017; Domínguez-Begines et al., 2020; Klironomos, 2002; Mangan et al., 2010; Teste et al., 2017) shows that soil microorganisms play an important role in regulating species diversity and coexistence.

Tree species diversity can vary greatly between different forest ecosystems. For example, boreal forests covering large swaths of Scandinavia and the Baltic countries are relatively poor in species, which usually coexist only during the transition between successional stages. In European boreal regions, early successional forests are dominated by light-demanding species such as silver birch (*Betula pendula*) or alder (*Alnus glutinosa*). Whereas birch is a typical early coloniser that inhabits areas that were cleared of tree cover as a result of a disturbance, alder often forms stands along rivers and lakes but can also be found away from water sources, particularly in the relatively humid boreal forests (Condé *et al.*, 2003; Sundseth *et al.*, 2009). As the forest stand matures, birch is outcompeted by the more common Scots pine (*Pinus sylvestris*) which in the final stages of succession either forms a stable mixture with Norway spruce (*Picea abies*) or is simply superseded by it. Old forests in boreal regions therefore tend to be dominated by Norway spruce and Scots pine, whereas areas recovering from disturbance (e.g., a forest fire) are most often colonised by birch (Beck et al., 2016).

In contrast to boreal forests, Mediterranean forests cover a relatively small part of the Mediterranean but are some of the most diverse ecosystems on earth and host to a large number of endemic flora (WWF, 2001). In areas such as NE Spain, the characteristic Mediterranean forest tree species are predominantly oaks and pines, which naturally appear in two-species mixed stands lying on an altitude gradient. The altitude gradient includes both a precipitation and temperature gradient. Aleppo pine (*Pinus halepensis*) is among the most common Mediterranean pines and often forms mixed stands with holm oak (*Quercus ilex*) in dry areas located at sea level along the coast and up to 1000 m above sea level in the interior. The pair is displaced by mixed stands of maritime pine (*Pinus pinaster*) and cork oak (*Quercus suber*) in areas along the coast with sandy soils and by mixtures of black pine (*Pinus nigra*) and Portuguese oak (*Quercus faginea*) at higher altitudes and in areas with higher precipitation further inland. As precipitation increases and summer temperatures become milder, black pine is outcompeted by Scots pine and pubescent oak (*Quercus pubescens*) mixtures, which are common inland at 1000–1600 m. Silver fir (*Abies alba*) and European beech (*Fagus sylvatica*) dominate the altitudes near the tree line in cool sites with abundant precipitation during the summer months (GeoPortal MAPA/MITECO, 2023).

Knowing how forest stands are formed and which factors drive species coexistence is crucial to being able to predict how forests might react to climate change. In both boreal and Mediterranean forests, climatic conditions can limit vegetation growth either due to low temperatures or water limitations, making the forests susceptible to global warming. In the last few decades, soil microbiota has begun

to be viewed as one of the key players in forest biodiversity. However, the relationships between trees and soil microorganisms are still poorly described for boreal and Mediterranean forests.

1.2 Trees and soil microorganisms

As a tree grows, it attracts a range of microorganisms that benefit from its resources in various ways. Microbial saprotrophs, often belonging to fungi, degrade fallen leaves and other litter, driving the global carbon cycle by returning the otherwise inaccessible carbon stored in plant tissues back into the trophic chain. Parasites, many of them agents of disease, can damage plant roots, hijack parts of the plant metabolism for their own purposes, or simply kill off their host to extract nutrients from the necrotic tissue. Finally, symbionts, such as mycorrhizal fungi or many bacteria from the phylum Actinomycetota, associate with plants and provide them with competitive advantages over other species that do not form these symbioses.

The microbial community that associates with an individual tree changes throughout its life. The microorganisms recruited by seedlings are different from those recruited by adult trees (Cline et al., 2005; Jones et al., 2003; Reverchon et al., 2012). Soil microorganisms also compete with each other to form interactions with trees. Such competition has led to adaptation and specialisation so pathogens and symbionts and their preferred hosts often display a level of phylogenetic signal (Gilbert & Webb, 2007). These interactions can range from narrow specialists, such as *Phytophthora quercina*, which infects only *Quercus* trees, or *Suillus grevillea*, which forms ectomycorrhizal symbioses only with larch, to cosmopolitan generalists such as *Phytophthora cinnamomi*, which is capable of infecting species from more than 260 different genera. However, such extreme cases are rare, and most pathogens and symbionts are able to associate with a moderate number of closely related plant species (Gilbert & Webb, 2007).

1.2.1 Soil-borne pathogens

A growing seedling is threatened by a multitude of pathogenic soil microorganisms. Soil-borne fungal pathogens are some of the most destructive plant diseases of modern times, causing a range of commercially significant plant diseases (e.g., *Fusarium* species or *Rhizoctonia solanii*) (Loria et al., 2021; Loria & Tuttle McGrath, 2021; Molla et al., 2020; Sharma et al., 2021; Sherf & Tuttle McGrath, 2021). However, some of the most harmful and invasive soil-borne pathogens are not fungi but rather fungal-like oomycetes from the genera *Phytophthora* and *Pythium*. The name of the former, derived from the Greek '*phyto*,' meaning plant, and '*pthora*,' meaning destroyer, illustrates the pathogenic potential of these organisms. Indeed, the genus *Phytophthora* contains pathogens responsible for some of the most devastating crop and forest epidemics to date, such as the Irish potato famine in the 19th century caused by *P. infestans* or the sudden oak death caused by *P. ramorum*. *Phytophthora* species have a great capacity for surviving harsh conditions in the soil due to specialised resistant

structures called oospores. This survival capacity in the soil has likely been central to their spread worldwide via nursery trade (Bienapfl & Balci, 2014; Liebhold et al., 2012; Sims & Garbelotto, 2021). Once introduced in a new region, their capacity to spread via soil movements due to construction, recreational activities or planting (Donaldson & Bennett, 2004; Giordana et al., 2020; Lewis & Colquhoun, 2000) has allowed them to find suitable hosts and establish permanent populations as invasive species in native forests globally. Currently, *P. cinnamomi* is one of the world's most invasive species and one of the worst pathogens that exist today ('Global Invasive Species Database' 2023). Together with other *Phytophthora* species, such as *P. cactorum* or *P. plurivora*, it has been linked to forest decline across Europe (Jung, 2009; Jung et al., 2018) and is particularly threatening oak forests in the Mediterranean region (Brasier et al., 1993; Colangelo et al., 2018; Sánchez et al., 2002).

1.2.2   *Fungal and bacterial symbioses*

Naturally, trees can also benefit from the soil microbiota by forming symbioses with certain soil fungi and bacteria. Symbiotic nitrogen-fixing bacteria (N-fixers) convert atmospheric nitrogen into forms that are accessible to plants. Bacteria, such as many Rhizobia or Actinomycetota, can provide a large fraction of nitrogen to the plant and increase nitrogen availability in forests where they are abundant (Steidinger et al., 2019). In contrast to N-fixers that assimilate atmospheric nitrogen, mycorrhizal fungi extract nutrients from organic material in the soil. Mycorrhizal symbionts, specifically those forming ectomycorrhiza or ericoid mycorrhiza, play a crucial role in forest ecosystems worldwide (Gill & Finzi, 2016). Many boreal and Mediterranean trees form symbiotic relationships with ectomycorrhizal fungi (EMF), which facilitate seedling establishment and growth by providing nutrients to the host. EMF envelop tree roots in a sheath of fungal tissue (the mantle), spreading a network of hyphae into the root between the epidermal and cortical cells (the Hartig net) and extending a system of hyphae growing outwards to form connections with the soil (extraradical mycelium) (Smith & Read, 2008). The Hartig net expands the area of contact between the two symbionts (i.e., the tree and the fungus) and improves the exchange of nutrients and water, whereas the extraradical mycelium formed by hyphae extending from the EMF mantle greatly increases the areas of the soil in contact with the colonised root.

The structure of the extraradical mycelia differs between fungal species, which affects the way each fungus proliferates through the soil, interacts with the environment and acquires nutrients (Smith & Read, 2008). These exploration types range from 'contact' exploration types, where fungi send out only a few short hyphae, to 'long-distance' types, where mostly hydrophobic rhizomorphs (thread-like structures of parallel hyphae) emanate up to several decimetres from the root. EMF with hydrophobic mycelia tend to be very good at extracting nitrogen from complex organic sources and are more common in environments where nitrogen is primarily bound in organic compounds, such as boreal forests (Lilleskov, Hobbie, and Horton 2011).

Nitrogen has two stable isotopes: ^{14}N, which makes up the majority of all naturally occurring nitrogen, and ^{15}N, which is far less abundant. After extracting nitrogen from the soil, EMF assimilate the two isotopes differently based on whether the compound is meant to be transferred to the plant or retained within the fungus (Hobbie & Hobbie, 2008). Because EMF preferentially retain ^{15}N-enriched nitrogen compounds, ectomycorrhizal plants tend to be depleted in ^{15}N relative to the source (i.e., they have negative δ^{15}N values) (Hobbie & Hobbie, 2008). Non-mycorrhizal plants generally have higher δ^{15}N signatures than mycorrhizal plants because there is little discrimination against ^{15}N during nitrogen uptake via the roots (Craine et al., 2015). Therefore, δ^{15}N values of plant tissues can provide insight into nitrogen partitioning between EMF and host plants and indicate whether the nitrogen in plant tissues was provided by the EMF.

While the extraradical mycelium is a sink for tree carbohydrates transferred to the root system, it is also an important extension of the root system, granting trees access to a larger pool of nutrients and easier access to water (Agerer, 2001). In fact, this relationship with EMF is so important to the tree that in nutrient-limited ecosystems (such as boreal forests, where most of the belowground nitrogen is bound in organic matter inaccessible to plants), more than half of the carbon produced by photosynthesis is allocated to roots and EMF fungi (Gill & Finzi, 2016). In exchange, EMF supply the plant with nitrogen and phosphorous (Smith & Read, 2008) and protect the tree from soil-borne pathogens owing to the mantle they form around vulnerable feeder roots (Bennett et al., 2017; Colinas et al., 1994).

1.3 Plant–soil feedbacks regulating biodiversity

Feedback mechanisms between plants and soil microbiota are thought to be key players in shaping species diversity in plant communities in grasslands (Bauer et al., 2015; Klironomos, 2002), Mediterranean-climate shrublands (Teste et al., 2017) and tropical and temperate forests (Anthony et al., 2022; Bennett et al., 2017; Mangan et al., 2010). Plant–soil feedbacks (PSFs) occur when plants alter abiotic and biotic soil characteristics in their immediate vicinity, affecting the performance of individuals of the same species (direct PSF) as well as the performance of surrounding plant populations (indirect PSF) (van der Putten et al., 2013).

PSFs are negative when they make the soil under an adult tree less suitable for conspecific seedlings than for heterospecific individuals. Therefore, negative PSFs favour the establishment of heterospecific seedlings and limit the spread of the more dominant species, thus acting as stabilising forces in the system. The Janzen-Connell hypothesis suggests that tree species diversity is a result of negative PSFs. It proposes that tree species biodiversity is regulated by species-specific pathogens and other natural predators that accumulate near adult host trees and decrease their regeneration capabilities. More abundant species accumulate higher concentrations of specific pathogen inoculum, which hampers their ability to regenerate in places they have colonised and promotes the formation

of mixed-species stands. The Janzen-Connel hypothesis works with certain assumptions, such as that trees can disperse further than pathogens and that pathogens are able to infect only some of the multiple coexisting species.

Negative PSFs can therefore maintain species coexistence when feedbacks between species are reciprocal (meaning that the growth of each species is better in the soil of the other species than in conspecific soil) (Mangan et al., 2010) or promote species change, for instance in a successional progression, when they are not reciprocal (van der Putten et al., 2013). In the latter case, different tree species coexist temporarily during the transition between successional stages.

Conversely, when PSFs are positive, they enhance the performance of conspecific seedlings under an adult tree and increase the chances of that species dominating the area. As a result, positive feedbacks often result in the dominance of one tree species over the others (Lance et al., 2020). Positive feedbacks are thought to prevail in temperate forests because many temperate trees associate with EMF, which offer them a competitive advantage over non-mycorrhizal species (Bennett et al., 2017; Bennett & Klironomos, 2019).

The direction of PSFs likely depends on the type and strength of interactions different tree species establish with soil microorganisms. As a result, feedbacks that promote coexistence and succession in tropical stands probably differ from PSFs that occur in boreal or Mediterranean forests. However, little information is currently available about how PSFs operate in such ecosystems.

1.4   Forests and global change

1.4.1   *PSFs under climate change*

Global climate change represents one of the major drivers of ecological change and is threatening forest ecosystems worldwide. Climate change alters abiotic conditions, such as temperature and water availability, and has the capacity to increase the severity and frequency of extreme weather events, such as heavy rainstorms or extreme droughts (Gardiner et al., 2010; Lange, 2020). If forests are unable to cope with increased environmental pressure as a result of changing environmental conditions, their ability to provide natural ecosystem services will inevitably diminish (Thom & Seidl, 2016).

The geographical ranges that tree species occupy are predominantly limited by the species' requirements for water and their tolerance of temperature minima. Trees establish in new areas more readily than they evolve a new set of climate tolerances (Davis & Shaw, 2001). Therefore, as the climate warms, many species in the northern hemisphere are predicted to shift their ranges to higher altitudes or to higher geographical latitudes, either to avoid reduced water availability caused by the warming climate or because areas previously inaccessible due to low temperatures will become available as the temperatures rise. Apart from environmental conditions driving this expansion, soil

microbiota could promote range shifts by making the new area more welcoming to shifting species. It has been proposed in the context of invasive species that range expansion is associated with a release from specific pathogens (Inderjit & van der Putten, 2010; Rout & Callaway, 2012). However, little is known about how PSFs operate between forest tree species occupying different altitudinal ranges in the same ecosystem.

Rising temperatures as a result of climate change will likely result in increased water needs, which can exacerbate the severity and length of drought stress. Many forests are already under climate-induced stress, which is causing tree mortality worldwide (Allen et al., 2010). Regions where multiple detrimental effects of climate change come together are likely to be the most vulnerable. One such region is the Mediterranean, where decreased precipitation together with rising temperatures is expected to exacerbate the effects of climate change (Giorgi, 2006; Lange, 2020). Tolerance to periods of low water availability is characteristic of most Mediterranean tree species. However, young seedlings are often more susceptible to drought than conspecific adults, which can hamper tree regeneration, as has been shown for Mediterranean oaks (Gómez-Aparicio et al., 2008; Pulido & Díaz, 2005).

Besides directly affecting plants, climate warming and changes in water availability can cause shifts in the microbial community composition associated with the plant (Castaño et al., 2018; Kaisermann et al., 2017). The interactions between plants and microorganisms are therefore susceptible to climate change, as both plants and the soil microbial communities that associate with them can be sensitive to environmental changes (Castro et al., 2010; de Vries et al., 2018; Sterkenburg et al., 2015).

Soil moisture, which is tightly linked to temperature, can have a strong effect on microbial community composition and activity (Li et al., 2017). Waterlogging of soil as a result of sudden abundant rainfall can facilitate the spread of pathogens (such as many *Phytophthora* species), which require periods of high water availability for the release of spores and the start of the infection process. Flash flooding and extreme weather could therefore indirectly affect tree survival and establishment, causing or strengthening negative PSFs by creating soil conditions that promote pathogen activity (Domínguez-Begines et al., 2020). On the other hand, drought can have long-lasting effects on the microbial community in the soil, likely as a result of changes in the plant community (de Vries et al., 2018; Kaisermann et al., 2017). EMF have been found to enhance plant tolerance to drought either by improving access to water or by facilitating nutrient uptake under limiting conditions (Karlowsky et al., 2018; Mariotte et al., 2017; Querejeta, 2017). However, plants under drought stress are limited in their ability to continue providing photosynthates to EMF and other root-associated symbionts, which can in turn severely hamper the ability of these organisms to effectively supply the plant with nutrients (Chomel et al., 2019). Even though the responses of plants and microbial communities to the effects of climate change are relatively well documented, little is known about how plant-microbial interactions change in response to limiting conditions.

1.4.2 PSFs under globalisation

Naturally, the introduction of alien species into geographically distant areas is very rare. However, with globalisation connecting almost all parts of the world through trade routes and international travel, areas that were previously geographically isolated have become much easier to reach. As a result, many plants and plant pathogens have been introduced into new regions that would have otherwise been inaccessible. There, exotic species can become introduced into natural environments and, in the case of pathogens, can become highly invasive if they encounter susceptible hosts.

Plant species can be relatively well adapted to pathogens that share their native ranges and can show little to no symptoms of infection even if the pathogen is present in the soil (Jung et al., 2016; Vettraino et al., 2011). Even in such cases, however, the age of the host can affect its susceptibility, and, particularly for trees, the effects of pathogens can change dramatically from adults to young recruits. Seedlings without woody tissues tend to be more susceptible to pathogen attack than adult trees and can succumb to pathogens that adult trees may be able to ward off (Hansen et al., 2005). Invasive pathogens can often be much more devastating than native ones precisely because plants have not had the opportunity to develop defences through generations of coexistence (Freeman et al., 2019).

However, exotic pathogens, such as many *Phytophthora* species, do not invade all areas equally and do not present the same level of threat to all areas they invade. Instead, natural areas subject to high levels of anthropogenic disturbance are often at much higher risk of *Phytophthora* invasion than undisturbed regions. Soil movement due to construction, planting of nursery plants and the movement of soil on the boots and tyres of hikers and mountain bikers all raise the likelihood of introducing a potentially devastating exotic *Phytophthora* species into the environment (Davidson et al., 2005; Elliot et al., 2015; Garbelotto et al., 2018; Sims & Garbelotto, 2021; Tjosvold et al., 2002).

When introduced, the impact of an invasive pathogen can be exacerbated if it invades a region where plants are under stress from limiting environmental conditions, as environmental stress can make plants more susceptible to pathogen attack (Oliva et al., 2014). In the Mediterranean, where water availability is expected to decrease with climate change, highly visited forests may therefore be particularly at risk from invasive pathogens.

However, despite the threat *Phytophthora* presents to many forest trees, with several Mediterranean oak species among them, little is known about the distribution of the pathogen in the Mediterranean forests of NE Spain. Moreover, the impact of *Phytophthora* pathogens on tree regeneration and forest composition in boreal or Mediterranean forests is not well characterised.

1.5   Thesis objectives

In order to correctly predict the response of forest ecosystems to climate change and to appropriately address them in a way that maintains the ecosystem services they provide, we need to understand which factors drive forest stand formation and diversity and how climate change might affect them. In this thesis, I focused on the contribution of plant–microbial relationships to tree species diversity in different forest ecosystems. The thesis combines the work of four studies: one on PSFs among boreal forest species (study **I**), two that focus on determining the PSF processes among Mediterranean forest species and the effect of drought on the PSFs (study **II** and **III**) and one survey to determine invasive pathogen presence in the forests of Catalonia (study **IV**).

To increase our understanding of plant–soil feedbacks among forest tree species, the **first objective** of this thesis was:

> **1)** to describe the effects of PSFs on forest trees in boreal and Mediterranean forests.
>
> Glasshouse experiments were performed using seedlings of different tree species common to boreal forests (study **I**) and Mediterranean forests (studies **II** and **III**) to determine the role of PSFs in those ecosystems.
>
> Many PSF experiments follow a similar design where a small amount of field soil is added to a pot filled with sterilised bulk soil (detailed description in section **2.6**). However, the differences in nutrient content of the different field soils might affect initial seedling growth. An experiment with an alternative design was set up in study **III** to determine whether a small amount of inoculum mixed into the bulk soil confers any observable nutritional benefit to the seedling and whether such homogenisation allows for the recruitment of specific microorganisms from the field soil. In the same experiment, pots with sterilised inoculum mixed into the bulk soil served as controls to account for soil fertility. This design was not used in experiments **I** and **II** due to time and space constraints, as such a design effectively doubles the size of an experiment.

To gain knowledge on the type and strength of interactions that different boreal and Mediterranean tree species establish with soil microorganisms and to increase our understanding of the mechanisms behind the PSFs that structure plant communities, the **second objective** of this thesis was:

> **2)** to identify the microbial community driving the PSFs in boreal and Mediterranean forests.
>
> The microbial community in the rhizosphere of the seedlings (i.e., the thin region of soil close to the plant root that is directly influenced by root exudates) was analysed by metabarcoding to identify the microbial guilds underlying the observed PSFs (studies **I** and **II**). An oomycete-targeting biocide treatment was included in the experiment of study **I** and a general fungicide

treatment was included in the experiment of study **III** to determine whether the observed effects were due to a specific group of soil microbiota.

To more accurately predict the effects of climate change and design effective management strategies in Mediterranean forests, it is important that both biotic and abiotic facets of stand formation are characterised. In order to describe how PSFs might change in response to severe drought and to explore how PSFs operate between forest tree species occupying different altitudinal ranges in the same ecosystem and whether they might contribute to species' range expansions to higher altitudes, the **third objective** of this thesis was:

3) to determine the effect of drought on PSFs among Mediterranean forest tree species and to determine the possible role of PSFs in the upward shift of Mediterranean forest trees.

To examine the effect of severe drought on biotic PSFs among Mediterranean tree species, half of the seedlings in the experiment of study **II** were subjected to a drought treatment. To determine whether PSFs could play a role in species' altitudinal shifts, the seedlings in study **III** were inoculated with soil from trees growing at a different altitude.

Many forests are under threat from invasive pathogens, which are expected to widen their ranges as the climate warms and they are able to establish in more areas where they are introduced. Invasive pathogens can affect tree regeneration and modify existing PSFs. In order to identify which forests are at a higher risk of invasion and better assess the impact of invasive pathogens, the **fourth objective** of this thesis was:

4) to identify invasive pathogen prevalence in Mediterranean forests, describe the effects of invasive pathogens on tree regeneration and identify whether an increased risk of invasion is associated with human activity.

To address the lack of information regarding the distribution of *Phytophthora* pathogens in the forests of NE Spain, forests with previously unknown *Phytophthora* incidence rates were surveyed to examine the distribution of native and invasive *Phytophthora* in this region. Pathogenicity trials were combined with field observations to determine how *Phytophthora* pathogens might affect tree health and regeneration and thus alter PSFs in those forests. Finally, recreational and non-recreational areas were included in the survey to determine whether recreational activities increase the risk of invasion.

2 METHODOLOGY

2.1 Studied tree species

To study the PSFs in boreal forests, soil from four common boreal forest species was collected for the experiment: silver birch (*Betula pendula*), Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and alder (*Alnus glutinosa*). The species from which soils were collected in the studies on PSFs among Mediterranean trees were predominantly oaks and pines naturally appearing in two-species mixed stands on an altitude gradient (Figure **1**; Supplementary Information, Table **S1**). The species pairs were Scots pine and pubescent oak (*Quercus pubescens*), black pine (*Pinus nigra*) and Portuguese oak (*Q. faginea*), Aleppo pine (*Pinus halepensis*) and holm oak (*Q. ilex*), and maritime pine (*Pinus pinaster*) and cork oak (*Q. suber*). Study **III** also included European beech (*Fagus sylvatica*) and silver fir (*Abies alba*). Species were selected on an altitude gradient to allow us to study potential upward shifts (Figure **1**). Briefly, seedlings of each of these species were grown in pots with sterilised bulk soil and a small amount of inoculum soil collected under adults of both species from a higher altitude (high 'away' soil) or from a lower altitude in the case of *A. alba* and *F. sylvatica* seedlings (low 'away'; details in section **2.6**).

2.2 Site selection and soil collection

2.2.1 Plant-soil feedback experiments

In the three experiments focusing on PSFs, the seedlings were planted into pots containing a mixture of sterilised bulk soil and forest soil collected under mature trees. The bulk soil for the experiment focusing on boreal species was collected from old agricultural land near Uppsala, Sweden (study **I**) and for the studies involving Mediterranean species, it was collected near Lleida, Spain (studies **II** and **III**). In both cases, the soil was poor in organic matter and had not been recently colonised by vegetation. Bulk soil and part of the inoculum soil for study **III** were sterilised by gamma radiation (25–90 kGy) and stored in sealed bags at ambient temperature until planting. Gamma irradiation was preferred over other techniques such as steam sterilisation or autoclaving to avoid changing the nutrient profile (Berns et al., 2008). Sterilised nutrient-poor soil was used to prevent naturally-present microbiota from interfering with the interpretation of the experimental results and to stimulate root associations with microbiota from the forest soils. The soils used as inoculum were collected from natural mixed forests in central Sweden (study **I**) and NE Spain (studies **II** and **III**). Inoculum soil was taken under mature trees of the investigated species. In study **I**, the soil was taken from six trees of each species from a single forest in which all four species coexisted, whereas the sampling in NE Spain (studies **II** and **III**) was done in stands with the four previously described pine-oak pairs or fir-beech mixtures, sampling three stands per species pair for a total of 15 plots (Figure **1**, Table **S1**).
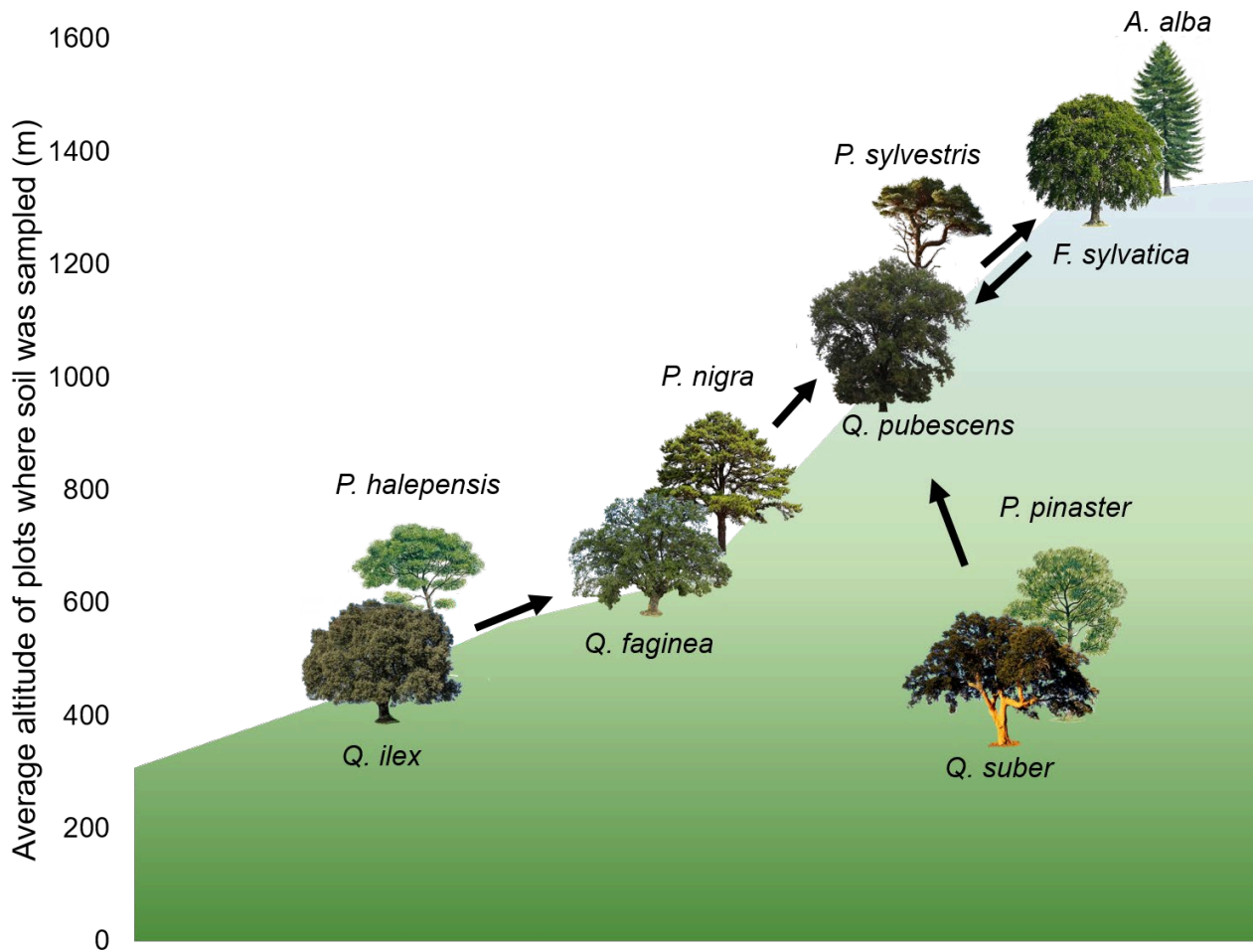
Figure 1: The altitudinal distribution of the species pairs in studies **II** and **III** (see also Table **S1**). *Abies alba* and *Fagus sylvatica* were not included in study **II**. The arrows indicate which species' soil was used as 'high away' inoculum in the experiment of study **III** (e.g., 'high away' inoculum soil for *Q. ilex* seedlings was from *Q. faginea* and *P. nigra*); because *A. alba* and *F. sylvatica* occupy the highest sampled altitudes, their seedings were inoculated with soil collected under *P. sylvestris* and *Q. pubescens*, which was labelled as 'low away' soil (details in section **2.6**).

In each mixed stand, soil was taken under three trees of each species and pooled to obtain one sample per tree species from each location. In all soil samplings, the litter layer was first removed, and soil in contact with the root system of a single adult tree was carefully collected. The soil was sieved through a 2–5 mm mesh and then stored at room temperature for 12–24 h (study **I**) or at 4 °C (studies **II** and **III**) until the seedlings were planted. The soil from individual trees of the same species in study **I** and soil from the same species in different locations in studies **II** and **III** were considered independent soil samples (ISS) to avoid mixing soil communities between biological entities as much as possible (Gundale et al., 2019; Reinhart & Rinella, 2016).

Two field surveys were conducted to assess the impact of invasive pathogens on adult trees and regeneration of forests in NE Spain and to assess the invasion risk associated with human activities (study **IV**). The first survey involved a targeted sampling of 44 plots in forests near the city of Barcelona, distributed between recreational areas (20 plots) and areas with low recreational use (24 plots), whereas the second survey included a systematic sampling of 277 plots across Catalonia (Figure **2**; Tables **S2** and **S3**). The plots of the targeted sampling were selected based on local knowledge and included only forests where the dominant tree species was chestnut (*Castanea sativa*), European beech or cork oak. Recreational forests included tourist attractions and areas frequently visited at weekends. The targeted sampling comprised 13 plots in chestnut forests, 19 plots in European beech forests, and 12 plots in cork oak forests, distributed across areas of high and low recreational pressure. Although plots with low recreational use were usually located in the same vicinity as plots with high recreational use, they were at least 100 m away from the recreational plot. Two soil samples were taken from each plot of the targeted sampling: one under a seemingly healthy tree and one under a tree showing symptoms of a possible *Phytophthora* infection. The trees were located in undisturbed areas of the plot. The targeted sampling was carried out in two rounds. In the first round, 24 plots were sampled in the late spring of 2020. An additional set of 20 plots was sampled in the spring of 2022, together with eight plots where *Phytophthora* was absent in 2020, to determine whether *Phytophthora* absence was year-dependent. The sampling in 2022 also included the assessment of *Phytophthora* incidence on seedling regeneration. Roots from three young conspecific seedlings were collected from each plot sampled in 2022, along with the two soil samples.

In 2021, a systematic sampling of 277 plots across Catalonia was conducted in addition to the targeted sampling (Figure **2**). The systematic sampling covered ca. 200 × 200 km of land in forests of more than 20 tree species (Table **S3**). The majority of the tree species growing in the systematic sampling plots were common Mediterranean species such as Aleppo pine (35% of plots) or holm oak (15% of plots), with less than 5% composed of the less abundant chestnut, beech or cork oak. Soil samples were collected from under three dominant trees in each of the 277 plots and pooled to create one sample for each plot. In 32 plots that were located in mixed forests, two pooled soil samples were collected: one from the dominant tree species and one from the other present species. In total, 309 pooled soil samples were collected from 277 plots (Table **S2**).

2.3 **Field measurements**

In study **IV**, the degree of human influence in each plot of the targeted sampling was assessed through quantitative variables such as the presence of anthropogenic litter (e.g., pieces of plastic or paper, bottles), the distance from a plot to the closest walking path, and the number of documented trails in the area based on Wikiloc (© 2021 Wikiloc Outdoor; www.wikiloc.com). The number of documented

trails was used as a proxy for visitor frequency. Wikiloc is an app commonly used to store and share outdoor trails, indicating the density of routes in a selected location on the map. Areas with tourist attractions tend to have more trails and receive more visitors than areas without such attractions.
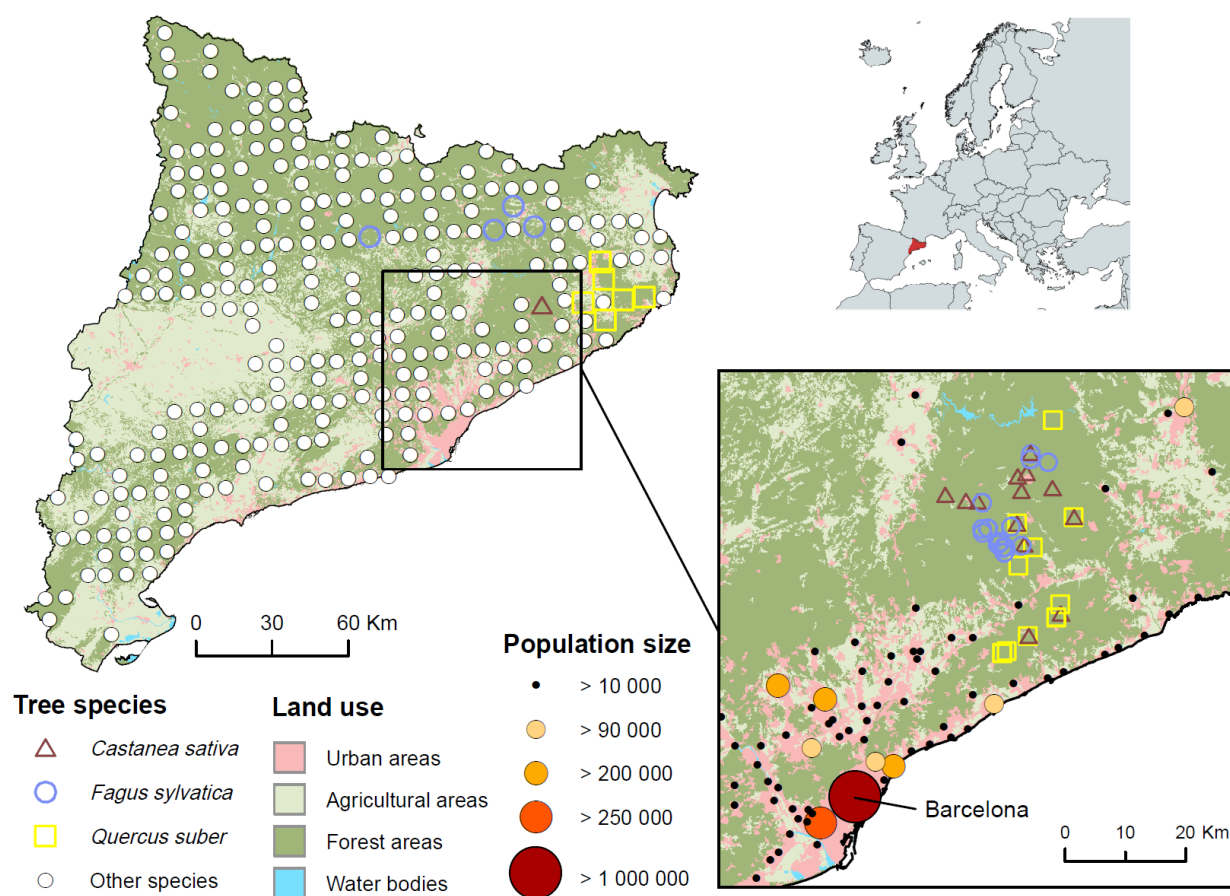
Figure 2: Systematic and targeted sampling plots in Catalonia (NE Spain) for study **IV**. Locations of the 277 systematic sampling plots in non-recreational forests across Catalonia (left-hand map), and locations of the 44 targeted sampling plots in forests with high and low recreational use near Barcelona (inset).

Additionally, the degree of defoliation of the 10 dominant trees closest to the centre of the plot was measured and the number and species of seedlings taller than 50 cm were recorded along a 10 × 4 m transect. Tree density, basal area, altitude, orientation and signs of recent management were also documented. No stand measures were taken in the systematic sampling, and the sampling did not differentiate between healthy and symptomatic trees.

2.4 Soil and root baiting

To recover *Phytophthora* from the soil and seedling samples collected in study **IV**, *Phytophthora* baiting was carried out following a procedure described by Jung (2009) using healthy young leaves of

Quercus robur L., *Q. ilex* and *Q. suber*. During baiting, soil or root samples are submerged in water to encourage the potential *Phytophthora* colonies present in the sample to develop sporangia and release zoospores. Within one week, the zoospores infect the young leaves used as bait, which are floating on the surface. Isolation is then carried out from the localised lesions that appear on the leaves. To bait *Phytophthora* from the seedling roots, the roots were thoroughly rinsed, cut into small pieces and placed in baiting trays with water, which were incubated in a growth chamber at 20 °C under a 16/8 h light/dark photoperiod and 90% humidity. The floating leaves were removed upon showing symptoms of *Phytophthora* infection. They were rinsed with distilled water and briefly surface-sterilised with 70% ethanol. Small fragments of necrotic tissue were then excised and placed on selective PARBPH-CMA medium plates (Jeffers & Martin, 1986). The plates were kept in the dark at 20°C and checked periodically for three weeks. Colonies displaying *Phytophthora*-like growth were subcultured onto plates of 20% V8A medium to confirm their identity.

2.5 Seedling production

The species used in PSF experiments in studies **I**, **II** and **III**, as well as the seedlings used in pathogenicity tests in study **IV**, are detailed in Table **1**.

Table 1: Seedling species used in glasshouse experiments in studies **I**–**III** and in pathogenicity tests in study **IV**.

Seedling species (common name)	Seedling species (Latin name)	Study I	Study II	Study III	Study IV
Alder	*Alnus glutinosa* (L.) Gaertn	X			
Silver birch	*Betula pendula* Roth	X			
Norway spruce	*Picea abies* (L.) H. Karst	X			
Scots pine	*Pinus sylvestris* L.	X	X	X	
Black pine	*Pinus nigra* J.F. Arnold		X	X	
Aleppo pine	*Pinus halepensis* Mill.		X	X	
Maritime pine	*Pinus pinaster* Aiton		X	X	
Pubescent oak	*Quercus pubescens* Willd.		X	X	X
Portuguese oak	*Quercus faginea* Lam.		X	X	X
Holm oak	*Quercus ilex* L.		X	X	X
Cork oak	*Quercus suber* L.		X	X	X
Silver fir	*Abies alba* Mill.			X	
European beech	*Fagus sylvatica* L.			X [a]	X
Chestnut	*Castanea sativa* Mill.				X

[a] seedlings were excluded from the analyses due to widespread mortality.

All seeds were surface sterilised before sowing. For use in study **I**, Scots pine and Norway spruce seeds were immersed in a solution containing 4.7% NaOCl and one drop of TWEEN® 20 for 15 minutes, followed by thorough rinsing with sterile water and a 24-h soak in sterile water. Floating seeds were discarded and the remaining seeds were sown in vermiculite. Alder and birch seeds were sterilised in

a mixture of 2.5% NaOCl and one drop of TWEEN® 20 for 10 minutes, rinsed and then immediately sown in vermiculite. In study **I**, seeds were germinated in a clean growing facility at 20°C with a 16 h/8 h light/dark photoperiod for one month, during which they were watered weekly with distilled water. Two weeks after sowing, they were watered with a nutrient solution instead. After that, the seedlings were not fertilised again. Seedlings of Portuguese oak and maritime pine used in study **II** and cork oak seedlings used in study **IV** were produced in the glasshouse. The seeds were first soaked in 5% NaOCl for 15 minutes, rinsed and soaked in sterile water for 24 hours. Any floating seeds were discarded and the remaining seeds were sown into trays with vermiculite. The trays were kept in a glasshouse at ca. 20°C with a 16 h/8 h light/dark photoperiod for three months and watered twice per week. Seedlings of the remaining species in studies **II** and **IV** and all seedlings for study **III** were obtained from a commercial nursery in Girona, Spain. Before planting, all the substrate coming from the nursery was thoroughly removed from the roots of each seedling.

2.6   Design of the plant–soil feedback experiments

The experiments focusing on PSFs followed a so-called 'home-away' experimental design based on the experiment by Mangan et al. (2010) (Figure **3**). In a 'home-away' experiment, seedlings are planted into pots containing ca. 95% sterilised bulk soil mixture and ca. 5% forest soil inoculum. Depending on the source of the inoculum, the seedlings can be classified as growing in either 'home' soil (when the inoculum is from the same species as the seedling, i.e., conspecific) or 'away' soil (when the inoculum is from a different species, i.e., heterospecific). The PSF is positive when seedlings show better growth in 'home' soil than in 'away' soil (Figure **3c, d**) and negative when they grow better in 'away' soil than in 'home' (Figure **3f, g**).

Glasshouse 'home-away' experiments were established to investigate PSFs among boreal tree species (study **I**, 336 pots) and Mediterranean tree species (study **II**, 624 pots; study **III**, 780 pots), with one seedling per pot (Figures **4** and **5**). The experiments in studies **I** and **II** were fully reciprocal. Each pot contained gamma-sterilised bulk soil (in study **II,** the soil was mixed with vermiculite and perlite in a 2:2:1 ratio) with the addition of either no additional soil (to simulate uncolonised soil) or 5–6% V/V inoculum soil. Inoculum soil was added around the roots of the seedling at planting (Figure **4a**, **b**)**.**

Only a small portion of inoculum soil, together with the same bulk soil, was used across all pots to control for abiotic differences between inoculum soils (Mangan et al., 2010). Hereafter, I will use the term 'inoculated soil' to refer to this mixture of sterile and inoculum soil and 'uninoculated soil' to refer to pots containing only sterile bulk soil. Table **2** displays the number of seedlings in studies **I** and **II** that were planted into pots inoculated with each type of soil, namely 'home' and 'away', and those without inoculum.
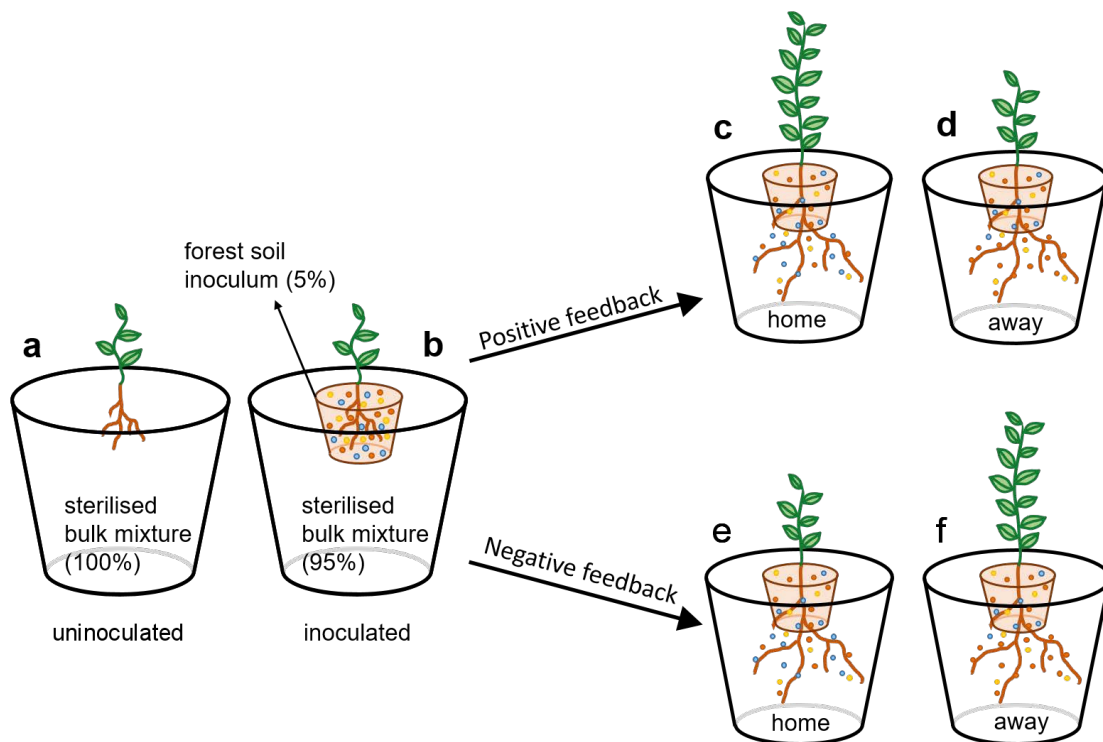
Figure 3: The design of the 'home-away' experiments used in studies **I** and **II**. Seedlings are planted into pots with sterilised bulk mixture and either no additional inoculum (**a**) or a small amount (ca. 5% V/V) of field soil inoculum (**b**), from which they recruit microorganisms as they grow. 'Home' refers to inoculum soil collected under the same species as the seedling; 'away' refers to inoculum soil collected under a species other than the seedling. Seedlings exhibiting positive plant–soil feedback will grow better in 'home' soil than in 'away' soil (**c, d**), whereas the opposite is expected for seedlings showing negative plant–soil feedback (**e**, **f**).
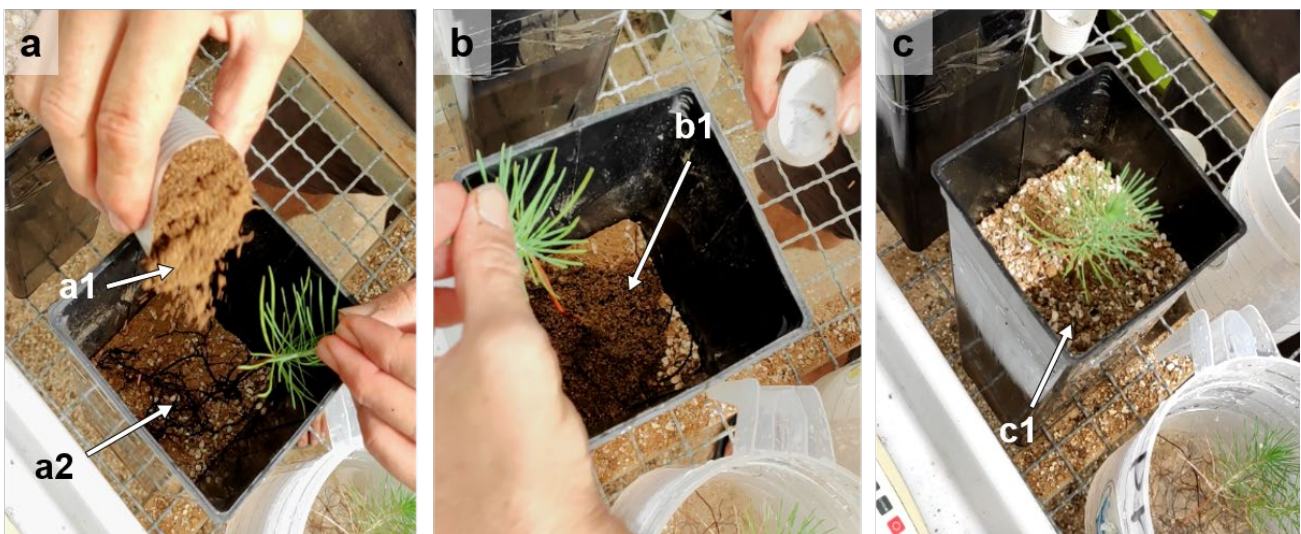
Figure 4: Seedling planting in study **II**. First, the pot was filled with most of the bulk soil mixture. Then, a seedling was added and its roots (**a2**) were covered with 5% V/V inoculum soil (**a1**, **b1**). Finally, the remaining bulk soil was used to cover the seedling roots (**c1**).

Table 2: The number of seedlings of each species used in studies **I** and **II** inoculated with 'home' or 'away' soil or without inoculum. The number in parenthesis indicates the number of seedlings inoculated with soil from each 'away' species.

	study **I** (4 species)	study **II** (8 species)
'home' soil	24	18
'away' soil	36 (12)	42 (6)
no inoculum	24	18
total (per species)	**84**	**78**

Figure 5: A section of the glasshouse experiments in studies **I** and **II**; (**a**) seedlings in the glasshouse experiment of study **I** in mid-September 2018, two months after planting; (**b**) seedlings in the glasshouse experiment of study **II** in late April 2021, ten months after planting.

The glasshouse experiment in study **III** followed a 'home-away' design, which differed from the previous two. First of all, seedlings were planted into 2-l pots with 95% V/V of a potting mixture containing gamma-sterilised bulk soil, vermiculite and perlite in a 2:2:1 ratio and 5% V/V of either gamma-sterilised or unsterilised inoculum soil, so any fertilisation effects were further controlled. Second, instead of covering seedling roots with inoculum soil, the inoculum was thoroughly mixed with the potting mixture before planting. Table **3** displays the number of seedlings of each species that were planted into pots with different types of inoculum. Briefly, seedlings of each species were planted into 36 pots with conspecific inoculum (18 sterilised, 18 unsterilised; Table **3**) and 42 pots with heterospecific inoculum. Each seedling species received heterospecific inoculum from its stand neighbour (18 pots, 'local "away" soil'; Table **3**) and from both species from a higher altitude (or lower

for the species growing at the highest sampled altitudes; 12 pots each; 'low/high "away" soil'; Table **3** and Table **S1**, Figure **1**).

In all experiments, the acorn was removed from oak seedlings before planting and the pots were elevated from the glasshouse table surface to prevent contamination of roots through contact with run-off water (Figure **5**). None of the seedlings in any of the three studies were fertilised at any point after planting. Growth conditions during the experiments in studies **I**, **II** and **III** are presented in Table **S4**.

Table 3: The number of seedlings of each species used in study **III** inoculated with sterilised or unsterilised 'home' or 'away' soil. Local 'away' soil refers to heterospecific inoculum from the seedling's stand neighbour; low/high 'away' soil refers to soil from one conifer and one broadlead species from a higher altitude (or lower for the species growing at the highest sampled altitudes).

study **III** (10 species)		**control**		**biocide**		
		sterile	unsterilised	sterile	unsterilised	**total**
'home' soil		9	9	9	9	**36**
local 'away' soil		3	6	3	6	**18**
low/high 'away' soil	conifer	3	3	3	3	**12**
	broadleaf	3	3	3	3	**12**
total		**18**	**21**	**18**	**21**	**78**

All pots were watered regularly to keep the soil moist. In study **I**, half of the pots were treated with 150 ml of Subdue® MAXX® (metalaxyl) at a concentration of 0.05 ml/l every two weeks to prevent oomycete growth, while the remaining plants were watered normally. In studies **II** and **III**, the seedlings were watered with an iron chelate solution (Ultraferro® WG by ASCENZA, Spain) with a concentration of 0.5 g/l one month after planting to adjust the pH of the soil. Eleven months after planting, half of the plants in study **II** stopped being watered to simulate drought conditions. In study **III**, half of the plants were treated with a fungicide solution applied to the soil (Table **3**, 'biocide') five weeks after planting, while the other half were watered normally (Table **3**, 'control'). The fungicide treatment was repeated every two weeks until the end of the experiment. The experiments in studies **I** and **II** were harvested five and twelve months after planting, respectively.

2.7 Measurements and harvest of the PSF experiments

Table 4: An overview of the measurements and sampling performed during each of the three 'home-away' experiments.

Measurement	Time of measurement	Study I	Study II	Study III
Seedling height	at planting	X	X	X
	after 2 months	X		
	after 3 months		X [a]	
	after 10 months		X	X
	at harvest	X [a]	X	
Seedling weight	at planting	X	X	X
	at harvest		X	
Shoot biomass (dry weight)	at harvest	X	X	
Root biomass (dry weight)	at harvest	X	X	
Number of *Frankia* nodules [b]	at harvest	X		
Number of leaves [c]	at harvest	X	X	
Number of side shoots	at harvest		X	
Root collar diameter	at harvest		X	
Leaf ^{15}N	*see comment*	X	X	
Transpiration rate	after 2 weeks of drought			
Pot soil fertility	after 11 months		X	
	at harvest	X		
Inoculum soil fertility			X	X
Rhizosphere community	at harvest	X	X	

[a] at this point, leaves for ^{15}N isotope analysis were also sampled; [b] only for alder seedlings; [c] only for broadleaf seedlings

2.7.1 Seedling measurements

In all studies, seedling height was measured at various stages of the experiment and seedling weight was measured at planting and at harvest. (Table **4**). Seedling growth in study **III** was calculated as shoot height increase between July 2022 and February 2023 relative to shoot height in July 2022. When harvesting the experiments of studies **I** and **II**, the aboveground part of each plant was separated from the root system. Samples of rhizosphere soil were taken from each seedling for soil community profiling (see section **2.7.5** for details). The rest of the soil was discarded and the root system was washed with water to remove all the remaining soil. In study **I**, and because this was the only study that included alder seedlings, clusters of *Frankia* nodules on the roots of alder seedlings were counted. Shoot height and root collar diameter of the harvested seedlings were measured and the number of side shoots was recorded for all seedlings in study **II**, as was the number of leaves on broadleaf seedlings. Shoots and roots were then dried separately at 60°C for a week (study **I**) or at 65°C for 72 hours (study **II**) and dry matter was determined.

2.7.2 Leaf ^{15}N isotope

Leaf ^{15}N isotope signatures were analysed in studies **I** and **II** (Table **4**) to estimate the fraction of nitrogen in the plants that may have been provided by ectomycorrhizal fungi (EMF). EMF discriminate against the ^{15}N isotope when creating compounds to transfer to the plant, resulting in plants that are depleted in ^{15}N (compared to ^{14}N) when nitrogen is provided by the fungi (Hobbie & Hobbie, 2008). The leaves for the analysis were collected three months after planting (study **II**) or at harvest (study **I**). In study **II**, one to three mature leaves were collected from each seedling (depending on leaf size) near the tip of the main shoot, whereas in study **I**, two to three random leaves were selected after harvest from all the leaves of each seedling. In study **II**, leaf samples from seedlings of the same species with inoculum from the same species and location were pooled, giving a total of 312 samples. The leaves were dried and ground to a fine powder in a ball mill. Subsamples were encapsulated in tin capsules and sent to the UC Davis Stable Isotope Facility (Davis, CA, USA) for analysis.

2.7.3 Soil fertility

Samples of pot soil were analysed at the end of study **I** and eleven months after planting in study **II** (Table **4**) to determine whether there were abiotic differences between the inoculated soils. In study **II**, three soil cores were extracted from each pot using an auger (1 cm diameter), whereas in study **I**, a sample of pot soil was taken from each pot at random at harvest. The samples were taken from parts of the pot that did not contain roots or inoculum soil. The nutrient content of the original inoculum soil was analysed in studies **II** and **III** (Table **4**). The chemical properties of the soil, such as phosphorous and potassium content (studies **I**, **II** and **III**), total nitrogen content (studies **I**, **II** and **III**), total carbon content (study **I**) and pH (study **I**), were analysed at the Department of Soil and Environment at the Swedish University of Agricultural Sciences in Uppsala (study **I**) and at the Department of Environment and Soil Sciences and Chemistry at the University of Lleida (studies **II** and **III**).

2.7.4 Physiological measurements

Three weeks after the start of the drought treatment in study **II**, stomatal conductance to water vapour (Table **4**) was measured in each plant using a LI-600 Porometer/Fluorometer (LI-COR®, Inc.) to assess the transpiration rate of the seedlings.

2.7.5 Microbial community

To collect the rhizosphere soil for microbial community analyses, three (study **I**) or five (study **II**) random fine roots were selected from each seedling at harvest and placed in 50-ml centrifuge tubes with 30 ml of phosphate-buffered saline (PBS). The tubes were vortexed to detach the soil from the roots, which were then removed before centrifuging. The tubes were centrifuged the same day at

10,000 $\times$ g for 10 minutes (study **I**) or at 5000 $\times$ g for 20 minutes (study **II**). The PBS was discarded and the soil pellet was stored at −20 °C until DNA extraction.

2.8 DNA extraction, library preparation and isolate identification

DNA was extracted from pellets of rhizosphere soil produced in studies **I** and **II** and from the original inoculum soils used in study **I** using a NucleoSpin® Soil kit (Macherey-Nagel). To extract DNA from isolates produced in study **IV**, a fast hot water protocol described by Grünwald et al. (2011) was used. A NucleoSpin® Plant II kit (Macherey-Nagel) was used to extract the DNA from isolates that were contaminated with bacteria and from isolates that had little mycelium. All kit extractions were carried out following the manufacturer's instructions.

2.8.1 Fungal library preparation

In studies **I** and **II**, the fungal ITS2 region was amplified using tagged primers (Table **S5**) to enable de-multiplexing, as described in Ihrmark et al. (2012). PCR reactions were conducted using the cycling conditions specified in Table **S5**. In study **II**, PCR reactions were done in duplicate and then pooled. PCR products were purified using the AMPure PCR purification kit (Beckman Coulter) (study **I**) or NucleoMag® NGS Clean-up and Size Select (Macherey-Nagel) (study **II**) following the manufacturer's instructions. DNA concentration was assessed with the Qubit™ DNA quantification kit (Thermo Fisher Scientific) and PCR products were pooled in equimolar mixtures. Each pool was purified with the EZNA Cycle Pure kit (Omega Bio-Tek, Norcross) (study **I**) or with the MicroElute® Cycle-Pure kit (Omega Bio-Tek) (study **II**). Four pools from study **I** were sequenced at SciLifeLab (Uppsala, Sweden) using two Pacific Biosciences Sequel SMRT cells per pool after the addition of sequencing adapters by ligation. 17 pools from study **II** were sequenced using 2 $\times$ 300 base-pair (bp) paired-end sequencing chemistry on three lanes of Illumina® MiSeq at the Genomics Unit of the Centre for Genomic Regulation (Barcelona, Spain).

2.8.2 Bacterial library preparation

To study the bacterial and archaeal communities in study **I**, the V3–V4 region of the 16S rRNA gene was amplified following a two-step PCR protocol (Berry et al., 2011). In the first reaction, amplification was performed using primers with Nextera adaptor sequences in duplicate reactions. The second amplification step was performed in duplicate reactions with 10% of the final concentration of purified product from the first PCR and primers with Nextera barcoding regions. Table **S5** specifies the reaction conditions of the first and second amplification steps ('reaction 1' and 'reaction 2', respectively). After each reaction, the products were pooled and purified using the AMPure kit (Beckman Coulter). After the second amplification step, the DNA concentration of pooled products was assessed with Qubit™ (Thermo Fisher Scientific) and the products were pooled in equimolar

ratios. Three pools were sequenced on one Illumina® MiSeq lane per library at SciLifeLab using 2×250 bp paired-end sequencing chemistry.

2.8.3   Oomycete library preparation

In study **I**, the ITS1 region of oomycete DNA was amplified using the oomycete-specific primers with one primer tag for each soil sample and PCR cycling conditions as specified in Table **S5**. The PCR products were purified with NucleoMag® NGS Clean-up and Size Select (Macherey-Nagel) following the manufacturer's protocol. The concentration of the purified PCR product was measured using the Qubit™ DNA quantification kit (Thermo Fisher Scientific) and the products were pooled in equimolar ratios. The four pools were sequenced on one lane of Ilumina® MiSeq at SciLifeLab.

2.8.4   Isolate identification

The *Phytophthora*-specific ITS A2-I2 primers were used to amplify the ITS region of isolate DNA from study **IV**, with reaction conditions as specified in Table **S5**. Where this primer pair failed to produce a good sequence, ITS6 and ITS4 primers were used following the protocol by Grünwald et al. (2011) (Table **S5**). Purification and sequencing were performed by Macrogen. Sequences were identified using the BLAST search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For isolates that could not be identified at species level with only ITS, a multi-locus analysis was performed using five marker genes as described by Aghighi et al. (2012). The genes included were the cytochrome *c* oxidase subunit 1 gene (*cox*1), heat shock protein 90 gene (HSP90), β-tubulin gene (β-*tub*), NADH dehydrogenase subunit 1 gene (*nadh*1) and ITS. The primers used to amplify the genes of interest were the same as in Jung et al. (2017). Reaction conditions are presented in Table **S5**. The DNA sequences of the isolates were compared with *Phytophthora* sequences obtained from GenBank. Each locus was aligned with MEGAx (Kumar et al., 2018) using the ClustalW algorithm with the default parameters. The five loci dataset consisted of 3588 characters (cox1 = 758, HSP90 = 710, ITS = 712, *nadh*1 = 541, β-*tub* = 867) and contained sequences from 20 reference isolates from GenBank and 17 unidentified isolates obtained in study **IV**. To identify the isolates, a Maximum Likelihood phylogenetic tree was used. The tree was constructed in MEGAx with the default parameters and 300 bootstrap replicates to test branch support (Figure **S1**). Accession numbers for the sequences used in the multi-locus analysis are provided in Table **S6**.

2.9   Pathogenicity tests

To determine the pathogenicity of the *Phytophthora* species isolated from the soils and seedling roots collected in study **IV**, pathogenicity trials were performed on seedlings of six Fagaceae species that are common in NE Spain (*C. sativa*, *F. sylvatica*, *Q. suber*, *Q. faginea*, *Q. pubescens* and *Q. ilex*). The average stem diameter of the seedlings ranged from 3.2 mm for *Q. suber* to 6.3 mm for *C. sativa*. The pathogenicity of six *Phytophthora* isolates representing four species was assessed: *P. plurivora*,

P. x cambivora, *P. castanetorum*, and three putative *P. cactorum* isolates with differing growth rates, which were later analysed together as *P. cactorum* since they did not display any significant differences in pathogenicity or host specificity. *Phytophthora quercina* was excluded from the inoculation experiment because none of the isolates had grown sufficiently by the beginning of the trial.

To inoculate the seedlings with *Phytophthora*, a small section of the stem bark of the seedling was removed and a 5-mm agar plug (from a 3-week-old *Phytophthora* culture growing on 20% V8A medium) was placed onto the exposed plant tissue with the mycelium facing downwards. The inoculation point was then wrapped with Parafilm® (Figure **6**). Seedlings inoculated with sterile V8A plugs served as controls. Each isolate and control were replicated six times per tree species, totalling 252 seedlings (42 per tree species). The plants were kept in a growth chamber for seven weeks at 25 °C with a 16/8 h light/dark photoperiod and 80% relative humidity. After seven weeks, the bark was carefully scraped to reveal the lesion and the vertical length of necrosis below and above the inoculation spot was measured. To confirm *Phytophthora* presence in the lesion, pieces of necrotic tissue were transferred onto plates of PARBPH-CMA medium, which were incubated at 20 °C and inspected for *Phytophthora* after two to three days.
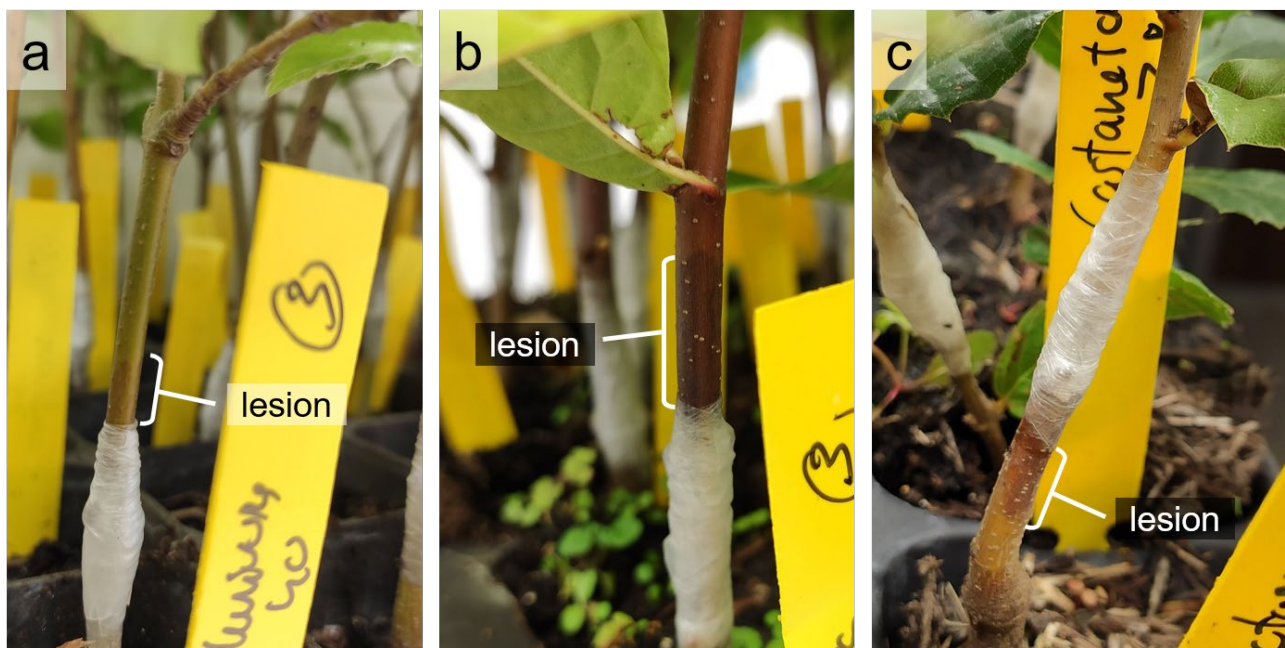

Figure 6: Examples of necrotic stem lesions formed after the inoculation of seedlings with *Phytophthora*. Parafilm® on the stem covers the point of inoculation. (**a**) *F. sylvatica* and (**b**) *C. sativa* inoculated with *P. plurivora;* (**c**) *Q. ilex* inoculated with *P. cactorum*.

2.10 Processing of sequencing data

Quality control, screening and clustering of fungal and oomycete sequences from studies **I** and **II** into operational taxonomic units (OTUs) were performed using the bioinformatics SCATA pipeline (scata.mykopat.slu.se).

2.10.1 Fungal sequences

Sequences with less than 200 bases, an average base quality score of < 20 or an individual base with a quality score of < 10 were discarded from the dataset. Only sequences containing both primers (≥ 90% primer match) and the same tag (100% match) in the forward and reverse primers were retained. Homopolymers were collapsed to three bases and the dataset of study **I** was further processed as in Castaño et al. (2020). Sequences from both studies were clustered into OTUs using a similarity threshold of 98.5%. In total, 594,134 reads in study **I** and 10,596,063 reads in study **II** passed quality control. Post-clustering curation of amplicon data was performed with the LULU package (Frøslev et al., 2017) in R version 4.1.2 (R Core Team, 2021), with a minimum match of 98.5% in study **I** and a minimum co-occurrence of 90% in study **II**.

The dataset was further cleaned in study **II** to exclude any OTU with less than 10 reads or present in less than two samples. Plant OTUs were removed and the remaining fungal OTUs (456 in study **I** and 1422 in study **II**) were taxonomically and functionally classified. The PROTAX-fungi tool (Somervuo et al., 2016) in PlutoF was used to obtain the first taxonomical profile of each OTU using a 50% classification probability. Finally, taxonomic identities and functional classifications were assigned to OTUs using the FungalTraits database (Põlme et al., 2020). In study **I**, the classifications were further curated using the UNITE database, DEEMY (Agerer & Rambold, 2017) and published literature (Clemmensen et al., 2015; Sterkenburg et al., 2015).

2.10.2 Bacterial sequences

To trim the 16S rRNA gene sequences produced in study **I**, the FASTX-Toolkit (available at http://hannonlab.cshl.edu/fastx_toolkit) was used and the paired-end sequences were merged using PEAR (Zhang et al., 2014). The assembled sequences were quality-filtered (maximum expected error value of 1) and clustered into OTUs using VSEARCH (Rognes et al., 2016). The SINA alignment tool (Pruesse et al., 2012) was used to align the OTUs and their taxonomy was assigned by using the SILVA database as a reference (release 138.1). The OTUs classified as 'Chloroplast' or 'Mitochondria' were removed from the dataset to obtain 7300 OTUs. This dataset was then divided into core and satellite communities following Jeanbille et al. (2016), keeping only the core community (4041 OTUs representing 99% of all reads), which is predominantly shaped by environmental factors. The satellite communities, which are mostly dispersed randomly, were not used in further analyses.

Oomycete sequences produced in study **I** with a length < 150 bp and sequences with a < 90% primer match, mismatched sample tags (requiring 100% match), average base quality < 10 or individual base quality < 2 were discarded. Homopolymers were collapsed into three bases and the remaining sequences were clustered into OTUs using a similarity threshold of 99%. The OTUs were matched to reference sets from *Phytophthora*-ID and a list of sequences obtained from NCBI. Tag jumps and OTUs with less than two reads or with an incidence of less than two were removed from the resulting dataset. The remaining OTUs were identified using BLAST and the resulting file was analysed in MEGAN (Huson et al., 2007) using a Least Common Ancestor (LCA) analysis with a minimum score of 50 and a minimum identity of 70%. Sequences that were assigned to the SAR supergroup (a group of phyla that includes Stramenopiles, Alveolates and Rhizarians) were then manually identified with BLAST. Only sequences with more than 80% coverage and a similarity of > 95% were retained. Sequences with ≥ 99% similarity were identified at species level, while those with 97–98.9% similarity were identified at genus level. The remaining OTUs were identified only as oomycetes. The final dataset was composed of 61 OTUs.

2.11 Statistical analyses

All statistical analyses were performed with R version 4.1.2 (R Core Team, 2021).

2.11.1 *Samples excluded from analyses*

Seven seedlings that died during study **I** were excluded, as were the 21 seedlings from study **II** that died before the start of the drought treatment. In study **III**, most beech seedlings died during the experiment, so all beech seedlings were excluded from the analyses (78 samples). In study **III**, seven seedlings from other tree species also died and were removed from the analysis. Three seedlings that died before the necrosis assessment in the pathogenicity trial in study **IV** were excluded from the analysis.

2.11.2 *Effects on seedling growth (PSF experiments)*

To determine the effect of soil inoculum, soil type (uninoculated or inoculated), and metalaxyl treatment on the growth of seedlings in study **I**, a one-way ANOVA was used, analysing each seedling species separately. Post-hoc comparisons were made using Fisher's LSD test (the 'LSD.test' function from the package agricolae) (de Mendiburu, 2021) with a false discovery rate adjustment of $\alpha = 0.05$.

In studies **II** and **III**, all species were analysed together. However, the analyses were done separately for watered and drought-exposed seedlings in study **II** and for seedlings with and without biocide application in study **III**.

A linear mixed-effects model ('lmer' function, lme4 package) (Bates et al., 2015) was used to determine whether there were differences in seedling shoot biomass (study **II**), growth (study **III**) and foliar $\delta^{15}N$ (both studies) between seedlings growing in different soils. Seedling height at planting and artificial light intensity above the seedling were included as covariates in the model used in study **II**, whereas the model in study **III** included seedling weight at planting and total nitrogen content in the inoculum soil as covariates.

To analyse differences in the survival of drought-exposed seedlings growing in different soils (study **II**), a generalised linear mixed-effects model ('glmer' function) was used. A logistic model was used to model survival (1) or death (0), with logit as the link function. Leaf water content of seedlings at harvest was used to classify the drought-exposed seedlings as 'dead' or 'alive' (Figure 7). This classification was further confirmed with visual observations of wilting or crispiness. Seedling height before the start of the drought treatment and artificial light intensity above the seedling were included as covariates. All mixed-effects models included seedling species as a random effect. Post-hoc comparisons in studies **II** and **III** were made using the 'emmeans' function from the emmeans package (Lenth, 2021).
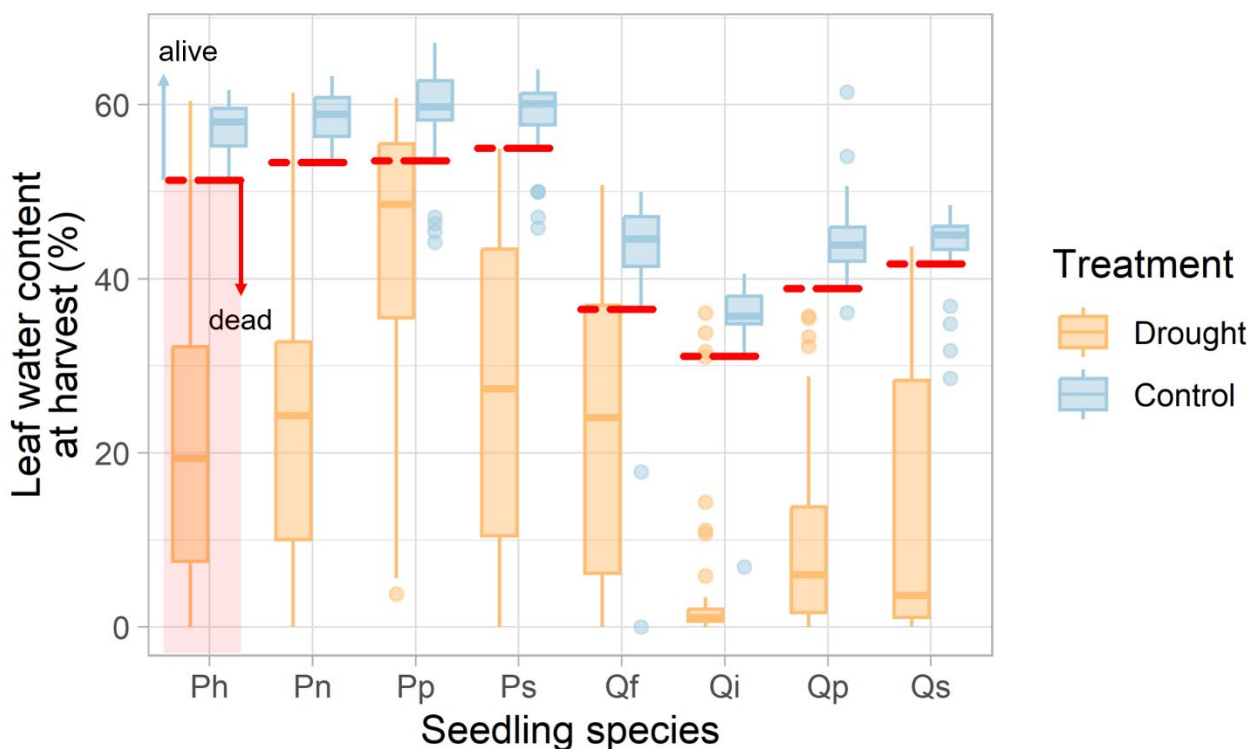
Figure 7: Leaf water content (LWC) at harvest in seedlings from study **II** that were watered regularly ('Control') or exposed to drought ('Drought'). The red line below the 'Control' boxplot for each seedling represents the line chosen as the survival limit for drought-exposed seedlings based on leaf water content ranges of control seedlings. This limit was used to classify the seedlings as 'dead' (LWC below the limit) or 'alive' (LWC above the limit) as a result of drought which was confirmed with visual observations.

2.11.3 Microbial community analyses

In study **I**, a permutational multivariate analysis of variance (PERMANOVA) was used to assess the effect of seedling species, soil inoculum and metalaxyl treatment on microbial community composition. The analysis was performed separately for fungal, bacterial and oomycete communities using the 'adonis2' function from the vegan package (Oksanen et al., 2020), set to test the marginal effects of the variables. Before the analysis, OTU abundances were standardised using the Hellinger transformation.

A principal coordinate analysis based on the Bray-Curtis dissimilarity index was used to visualise differences in community composition. The association between root and shoot biomass of seedlings in study **I** and the composition of the microbial communities was analysed using the 'envfit' function of the vegan package.

To analyse frequency shifts of microbial taxa between different seedling-inoculum combinations in study **II**, the 'multipatt' function from the indicspecies package (De Caceres & Legendre, 2009) was used to perform an indicator species analysis. The same analysis was performed in study **II** to annotate rhizosphere OTUs as specific or non-specific. The analysis was done separately for oaks and for pines. OTUs were annotated as specific if they were identified as indicator species for the particular seedling growing in either 'away', 'home' or uninoculated soil.

Seedling biomass and the relative abundance of each fungal guild or bacterial phylum in study **I** were correlated and the effects of inoculum and seedling species on microbial community composition were analysed. Seedling biomass was square-root transformed to fit normality assumptions. In study **II**, OTUs were annotated in terms of their effect on growth. For that, a linear model ('lm' function) was used to test whether the relative abundance of an individual OTU correlated with shoot biomass of oaks and pines separately, including seedling height at planting and artificial light intensity above the seedling as covariates. If the correlation was not significant, the OTUs were annotated as 'neutral'. For significant interactions at $p \leq 0.05$, the OTUs were annotated as 'positive' (i.e., having a positive effect) if the correlation was positive and as 'negative' if the correlation was negative. A quasibinomial linear model was used ('glm' function, family = 'quasibinomial') to determine whether there were differences in the relative abundance of specific and non-specific OTUs in study **II**, as well as OTUs with positive, negative or neutral effects. Post-hoc comparisons were made using the 'emmeans' function in the emmeans package.

2.11.4 Analyses of Phytophthora incidence in Catalan forests

The *Phytophthora* species isolated from forest soils and seedling roots in study **IV** were divided into invasive and non-invasive species following their respective species descriptions and applying the definition by Blackburn et al. (2011). They proposed that an invasive pathogen is a non-native

pathogen that has been introduced into an environment where it has been able to establish and spread successfully from the point of introduction. Accordingly, *P. plurivora*, *P. cactorum* and *P. xcambivora* were classified as invasive pathogens, whereas *P. castanetorum* and *P. quercina* were classified as non-invasive species. This division was supported by existing literature, which suggests that *P. plurivora*, *P. cactorum* and *P. xcambivora* originated outside Europe, where they are now widespread (CABI, 2022; Huai et al., 2013; Jung, 2009; Pánek et al., 2016; Vettraino et al., 2011), whereas *P. castanetorum* and *P. quercina* are believed to be native, as they are widespread in European forests and have lower incidence rates on other continents (Jung et al., 2017).

The incidence of native and invasive *Phytophthora* between plots of the systematic and targeted sampling in study **IV** was compared using a binomial model. Before fitting the model, the 321 plots were divided into four groups: targeted sampling plots with high recreational use (n = 19) or low recreational use (n = 25) and systematic sampling plots with chestnut, beech or cork oak species (n = 11) or with other species (n = 266). To test for differences between groups, a Fisher's LSD test was performed using the function 'LSD.test' from the package agricolae (de Mendiburu, 2021). To compare the incidence of invasive *Phytophthora* between forests of high or low recreational use in the targeted sampling, only chestnut and beech plots were included in the analysis, excluding cork oak plots due to the absence of invasive *Phytophthora* in those plots. A one-way ANOVA was used to test the association between *Phytophthora* incidence and plot variables, log-transforming the variables Wikiloc, distance from a path, regeneration dominance, density and altitude to meet the normality assumptions. A one-way ANOVA was also used to determine how the plot variables were associated with high or low recreational use and to test the association between defoliation and *Phytophthora* presence. The 'emmeans' function was used for pairwise comparisons.

To assess the putative impact of *Phytophthora* on regeneration, a log response ratio (lnRR) (Goldberg et al., 1999) was calculated in each plot using the equation $\ln(same + 1)/(\ln(other + 1))$, where *same* refers to the number of conspecific seedlings in the plot and *other* refers to the number of heterospecific seedlings in the plot. The lnRR value indicates whether there is a higher abundance of conspecific or heterospecific seedlings. A value above zero indicates a greater abundance of conspecific seedlings, whereas a value below zero indicates the opposite. The lnRR values in plots with and without *Phytophthora* in areas with varying levels of recreational use were compared.

2.11.5 Pathogenicity analyses

The length of the lesion above and below the inoculation point was averaged to obtain a single value, which was then log-transformed to meet the normality assumptions. An ANOVA was performed to compare lesion lengths caused by different *Phytophthora* species.

3 RESULTS

3.1 Effect of soil inoculum on seedling growth

Soil inoculum had an effect on seedling growth in all 'home-away' experiments. Most seedlings grew better in inoculated soil than in uninoculated soil. However, the overall pattern of PSFs among boreal tree species was different than the PSF patterns observed among Mediterranean species.

3.1.1 PSFs among boreal tree species (study I)

Each of the four boreal tree species showed a unique response when growing in soil without inoculum or with inoculum collected under each of the other species in the study (Figure **8**, Table **5**). Norway spruce, Scots pine and alder grew significantly better in inoculated soil than in uninoculated soil. In contrast, no significant differences in birch growth were observed between uninoculated and inoculated soil.

Alder was the only species growing better in 'home' soil than in 'away' soil, and thus the only species exhibiting a positive PSF (Table **5**). Moreover, alder growth was associated with a depletion of foliar $\delta^{15}N$ and a lower foliar carbon to nitrogen (C : N) ratio, suggesting that part of the nitrogen was provided by microbial symbionts. Birch grew comparably well in almost all soils, including uninoculated soil, and had the highest foliar C : N ratios of the four species in study **I**. Birch grew the least in alder soils, where it also exhibited the lowest foliar $\delta^{15}N$ values (Figure **8**). Scots pine seedlings grew best in birch soil, and spruce grew best when inoculated with Scots pine soil. Scots pine and spruce seedlings showed higher foliar $\delta^{15}N$ values when grown in inoculated soils than in uninoculated soils. In contrast, foliar $\delta^{15}N$ values in alder seedlings were higher for seedlings grown in uninoculated soil than in inoculated soil.

Table 5: Effect of soil on seedling biomass in study **I**, shown as R^2 values from one-way ANOVA. Uninoculated soil is only included in the analysis of uninoculated versus inoculated soil. Inoculated soil is a factor with four levels (i.e., alder, *Alnus glutinosa*; birch, *Betula pendula*; pine, *Pinus sylvestris*; and spruce, *Picea abies*). **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

Seedling	Uninoculated vs inoculated	Conspecific vs heterospecific	Inoculated soil
Alder	0.39 ***	0.15 **	0.29 ***
Birch	0.01 ns	0.03 ns	0.26 ***
Pine	0.45 ***	0.02 ns	0.36 ***
Spruce	0.27 ***	0.04 ns	0.08 ns

Birch inoculum soil seemed to contain more nitrogen and potassium than some of the other soils. However, there were no significant differences in nitrogen or potassium in the pots where Scots pine seedlings had been planted (Figure **S2**), discarding the possibility that pine growth in birch soil was enhanced because of soil nutrient content. Soil phosphorous content did not differ significantly between inoculated soils. Metalaxyl treatment did not affect the growth of any tree species.
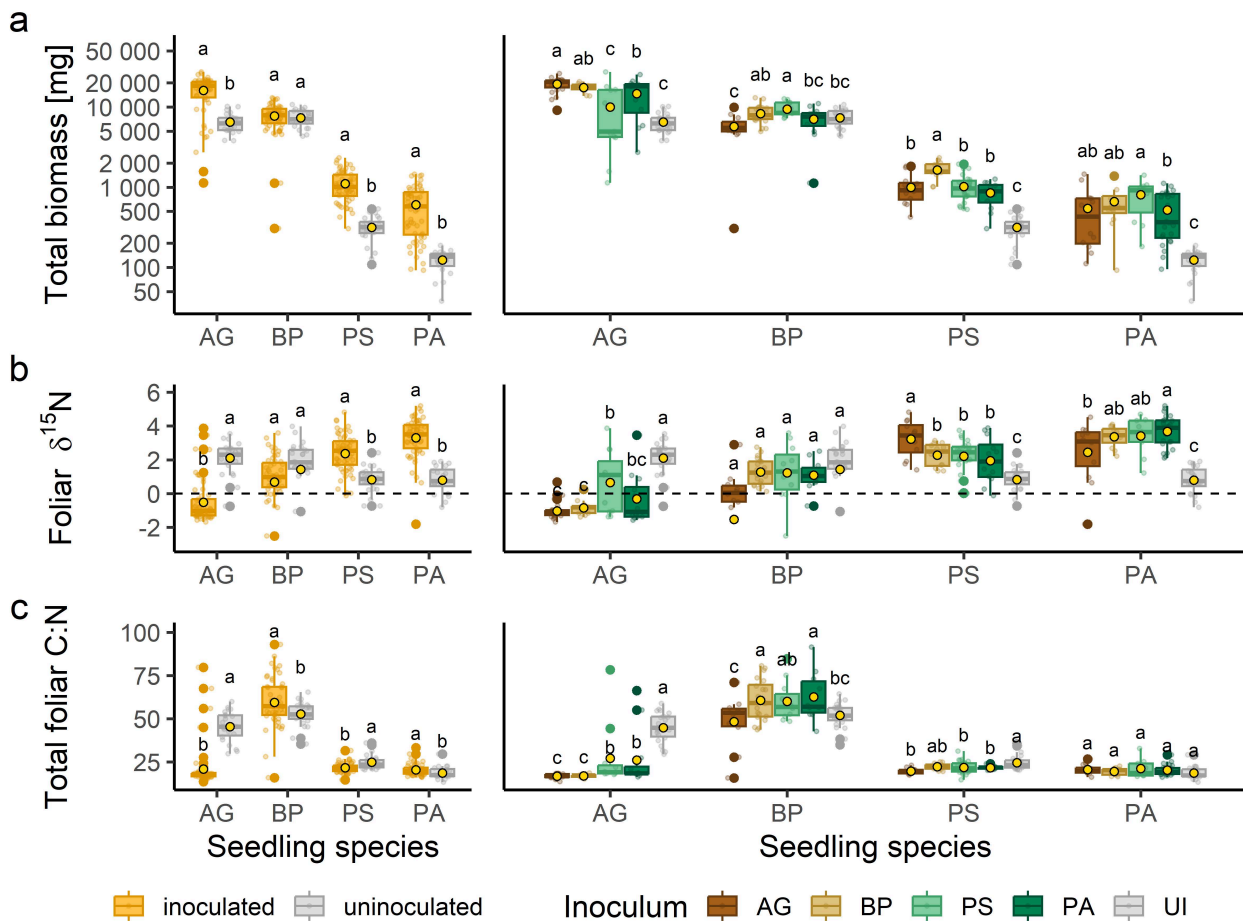
Figure 8: Performance of study **I** seedlings of *Alnus glutinosa* (AG), *Betula pendula* (BP), *Pinus sylvestris* (PS) or *Picea abies* (PA) in sterile bulk soil inoculated with soil from adult trees of the same four species and in uninoculated soil (UI). Total biomass (mg at harvest; **a**), foliar δ^{15}N (**b**) and total foliar C : N ratio (**c**) of each seedling species (x axis) in different soil types (fill colour). Yellow dots show mean values, while the dots below and above the boxplots show outliers. The letters above the plots were obtained through a Fisher's LSD test with a false discovery rate adjustment performed separately for each seedling species; different letters indicate significant differences ($p \leq 0.05$).

Seedling biomass at harvest (standardised by tree species) was used to predict biomass accumulations for different successional scenarios, transitioning from uninoculated soil (Figure **9**). In that way, two successional pathways that resulted in the greatest accumulation of biomass were identified. Both pathways shared the initial transition from uncolonised land (uninoculated soil was considered to approximate uncolonised land in the experiment) to birch forest. From there, the pathways diverged and resulted either in a succession to a pine forest and finally to a spruce forest (Figure **9a**) or in a transition to an alder forest due to a positive feedback promoting monospecific alder stands (Figure **9b**). Alternative succession pathways resulted in lower cumulative biomass values.
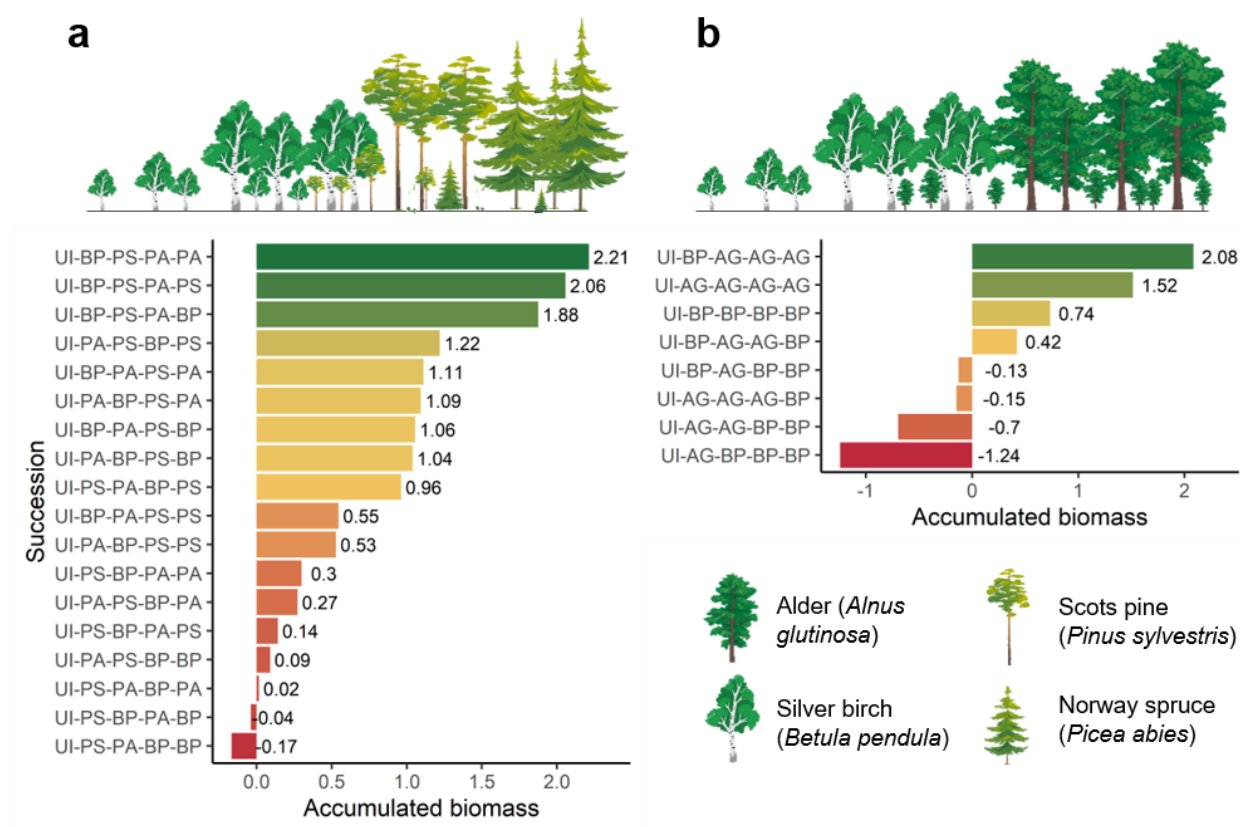
Figure 9: Biomass accumulation along different successional pathways of boreal species (study **I**) starting from uninoculated soil (UI) that resembles the soil of a post-disturbance site, which is colonised by different tree species that replace each other in four steps; (**a**) succession with birch (BP) and the conifers pine (PS) and spruce (PA); (**b**) succession with broadleaved birch and alder (AG).

3.1.2   PSFs among Mediterranean tree species (studies **II** and **III**)

Seedling growth in study **II** (measured as shoot biomass at harvest) was better in inoculated soil than in uninoculated soil (Figure **10a**). Seedlings growing in inoculated soil showed lower foliar $\delta^{15}N$ values than seedlings growing in sterile soils (Figure **10d**), indicating a higher nitrogen supply by EMF in inoculated soil. Also, seedlings in inoculated soils displayed higher rates of stomatal water

conductance (Figure **S3**), suggesting they had a higher rate of photosynthesis than the seedlings growing in uninoculated soil.

Most interestingly, seedlings grew better in 'away' soil than in 'home' soil (Figure **10b**). No significant differences in foliar δ15N values (Figure **10e**, **f**) or rates of stomatal water conductance (Figure **S3**) were observed in seedlings growing in pots with either 'home' or 'away' inoculum. Soil nitrogen, potassium or phosphorous concentrations in the original inoculum soils and in the pot soil at harvest did not differ significantly between soil types in study **II** ($p > 0.05$), so confounding effects of soil fertility explaining the observed negative feedbacks were discarded.

The inoculated soils in study **II** were further analysed at genus level by grouping all 'away' oak soils into one group and all 'away' pine soils into another, which revealed a phylogenetic signal in the negative feedback. Seedlings inoculated with 'away' soil from phylogenetically distant species (e.g., an oak seedling inoculated with pine soil) grew better than seedlings with 'home' inoculum (e.g., an oak seedling inoculated with soil from the same oak species, Figure **10c**), indicating that PSFs may promote the establishment of pine-oak mixtures in Mediterranean forests. No significant differences were observed between the growth of seedlings inoculated with 'away' soil from trees of the same genus (e.g., an oak seedling inoculated with soil from a different oak species) and seedling growth in 'home' soil (Figure **10c**).
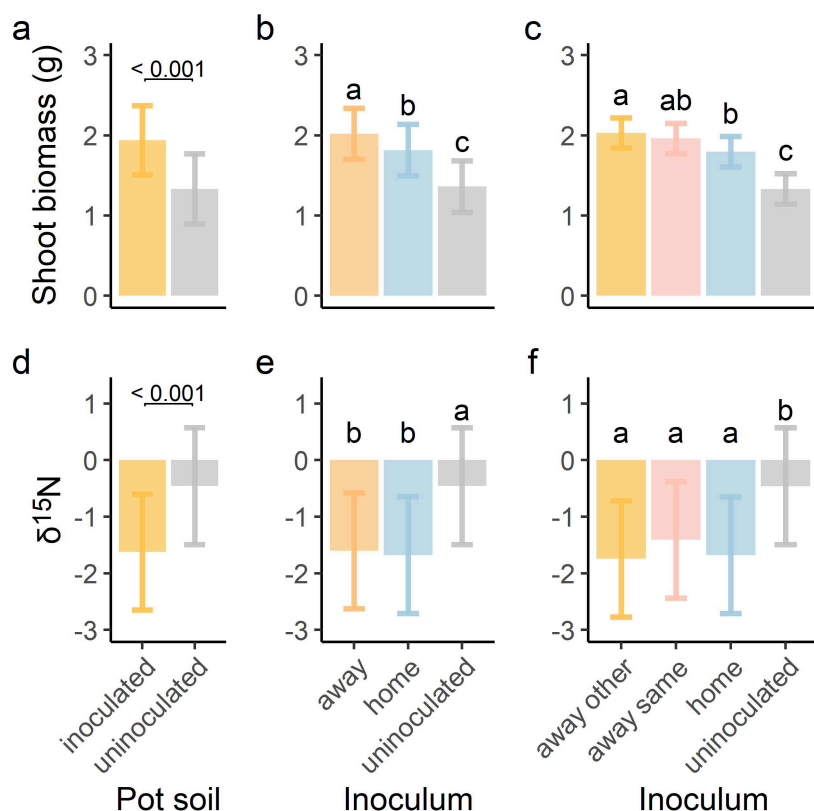
Figure 10: Shoot biomass at harvest (**a–c**) and foliar δ15N (**d–f**) of study **II** seedlings growing in

different soil types: (**a, d**) inoculated soil or uninoculated soil; (**b, e**) soil inoculated with heterospecific inoculum ('away') or with conspecific inoculum ('home'); (**c, f**) soil inoculated with heterospecific inoculum from trees of the same genus ('away same') or with heterospecific inoculum from trees of a different genus ('away other'). Different letters above the bars indicate significant differences between treatments at $p \leq 0.05$, which were determined by an emmeans analysis of a linear mixed-effects model. Error bars represent standard error.

The growth of seedlings in study **III** (measured as shoot height increase 10 months after planting) was better in soil inoculated with unsterilised inoculum than in soil with sterilised inoculum (Figure **11a**, **b**), suggesting the involvement of growth-promoting soil microbiota. No differences between the growth in 'home' and 'away' soils were observed ($p = 0.299$). The 'away' soils were split into three groups based on the altitudinal range where they were collected. 'High away' and 'low away' soils were collected from trees dominating in forests at higher or lower altitude strata than the inoculated seedling species, respectively, whereas 'local away' soils were collected from trees at a similar altitude as the seedling species (Figure **1**).

Interestingly, seedlings inoculated with unsterilised 'high away' soils grew better than seedlings inoculated with either unsterilised 'local away' soil or unsterilised 'home' soil (Figure **11b**), indicating that PSFs could promote the appearance of heterospecific species from vegetation strata situated at lower altitudes, i.e., promoting the movement of species upwards. Seedling growth in sterilised soils was lower than in unsterilised soils, suggesting that soil microbiota promoted growth. The addition of a biocide changed the growth patterns of seedlings growing with different types of soil inoculum. In biocide-treated seedlings, no differences in growth were observed between the seedlings growing in 'home' soil and in any of the three types of 'away' soil (Figure **11e**), further suggesting that the differences observed in control seedlings were due to soil microorganisms. The biocide treatment also nullified growth differences between seedlings growing with different types of sterilised inoculum (Figure **11f**).
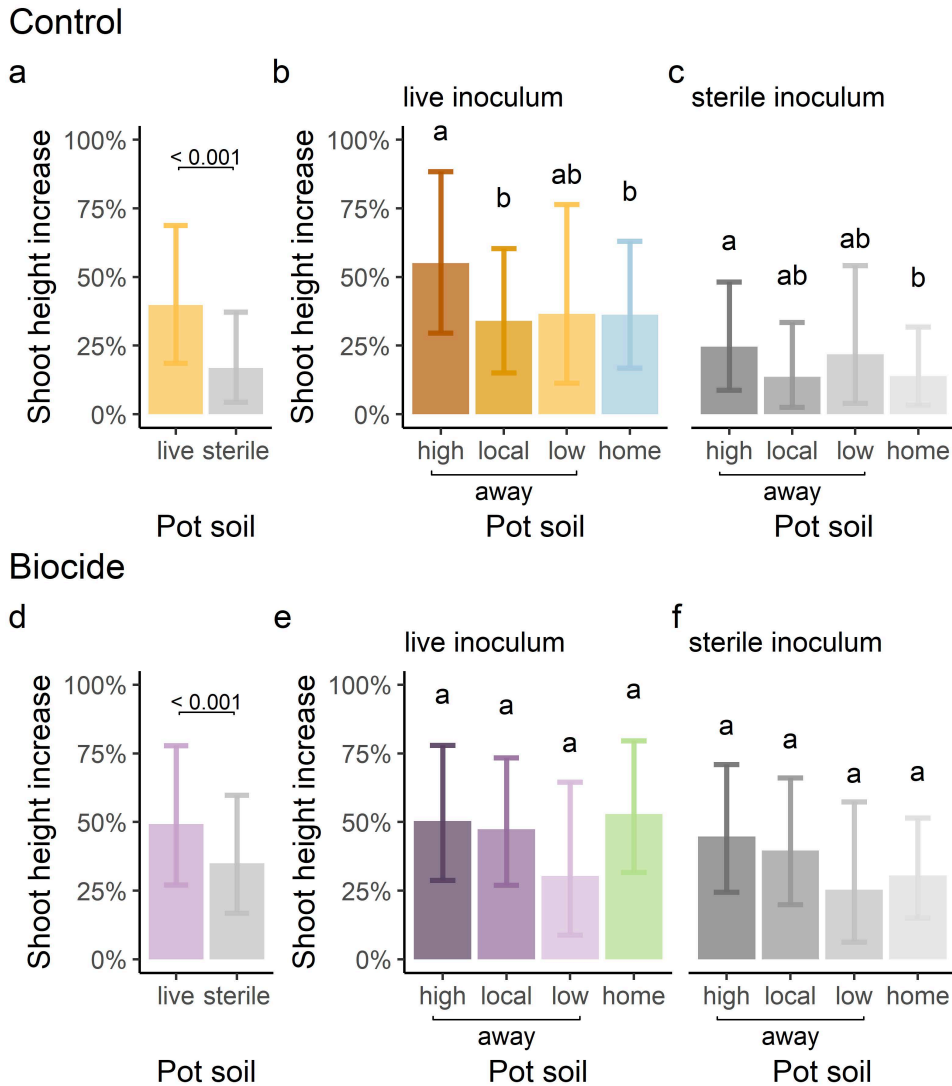
Figure 11: Seedling growth (shown as % shoot height increase between March 2022 and January 2023; study **III**) with and without biocide treatment ('Biocide', 'Control', respectively) in pots inoculated with different types of soil: (**a, d**) with unsterilised ('live') or sterilised ('sterile') inoculum; (**b, c, e, f**) unsterilised ('live') or sterilised ('sterile') inoculum from conspecific trees ('home') and from heterospecific trees ('away') growing at the same altitudinal range as the seedling species ('local') or at a higher or lower altitudinal range than the seedling species ('high' and 'low', respectively).

3.2   Effect of drought on seedling growth and survival

Subjecting seedlings to severe drought (study **II**) resulted in altered growth and extensive mortality. Before death and when subjected to drought conditions, seedlings grew worse in uninoculated soil than in inoculated soil (Figure **12a**), but no significant differences in growth were observed between seedlings inoculated with 'home' soil or with 'away' soil (Figure **12b, c**). Survival of drought-exposed seedlings growing in inoculated soil correlated negatively with seedling shoot biomass at harvest, indicating that seedlings that grew more prior to the onset of drought had a higher risk of dying once

drought struck. This pattern was not observed in drought-exposed seedlings growing in uninoculated soils. Under drought conditions, seedlings growing in inoculated soil had significantly lower rates of survival than seedlings in uninoculated soil (4.2% and 11.3%, respectively) (Figure **12d**). Furthermore, seedlings growing in 'away' soil had significantly higher mortality rates than seedlings growing in uninoculated soil. In contrast, seedling survival rate in 'home' soil was not significantly different from the survival rate in 'away' soil or in uninoculated soil (Figure **12e**). No differences in stomatal water conductance were observed in drought-exposed seedlings growing in different soils.
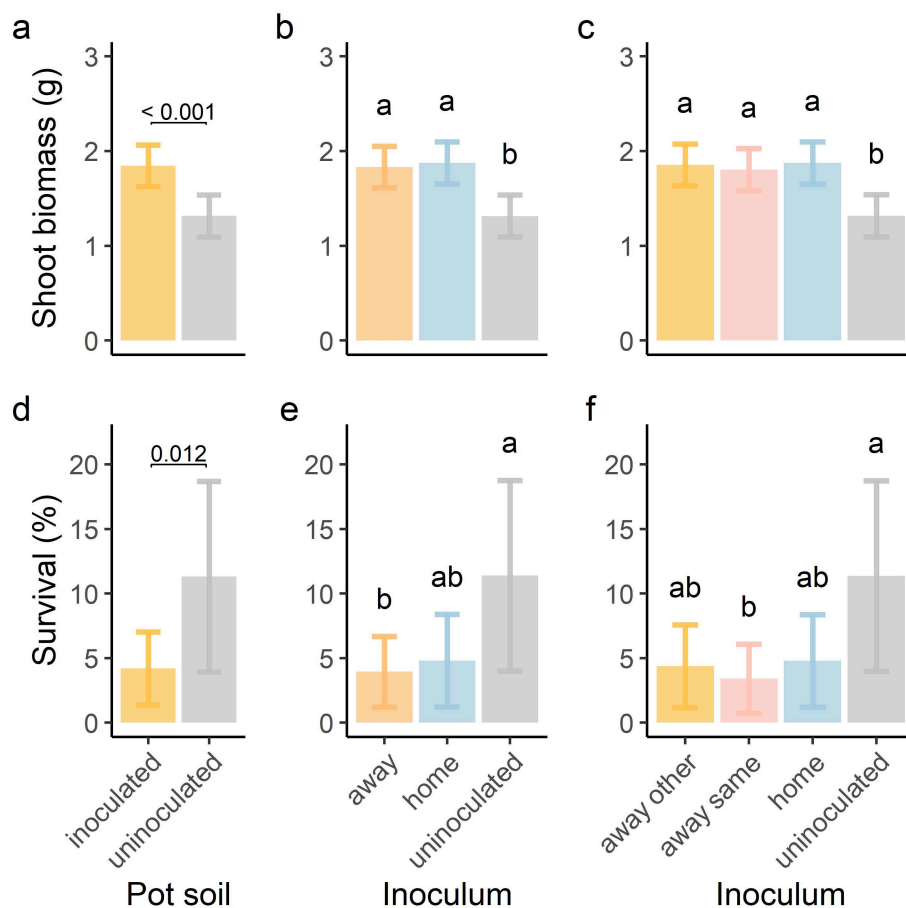
Figure 12: Shoot biomass (g at harvest, **a–c**) and survival (**d–f**) of drought-exposed seedlings from study **II** growing in different soil types: (**a, d**) inoculated soil or uninoculated soil; (**b, e**) soil inoculated with heterospecific inoculum ('away') or with conspecific inoculum ('home'); (**c, f**) soil inoculated with heterospecific inoculum from trees of the same genus ('away same') or with heterospecific inoculum from trees of a different genus ('away other'). Different letters above the bars indicate significant differences between treatments at $p \leq 0.05$, which were determined by an emmeans analysis of a linear mixed-effects model. Error bars represent standard error.

3.3 Soil microbial communities

The microbial community in studies **I** and **II** differed between uninoculated and inoculated soils. Seedling growth was related to microbial community composition in both studies, although the PSFs operated at different phylogenetic scales (i.e., at species- or genus-level). EMF were the main drivers of the observed feedbacks in both studies.

The soil microbial community in the pots in study **I** was shaped mainly by seedling species (Table **6**) which explained 18% of fungal and bacterial variation, whereas the inoculum species (the tree species under which inoculum soil was taken) explained 4% and 8%, respectively (Table **6**). The oomycete community in study **I** did not seem to be affected by inoculum species (Table **6**). In study **II**, the fungal community in the rhizosphere of Mediterranean pines and oaks was shaped primarily by seedling genus. Little differentiation of communities was observed between species of the same genus (Figure **S4**) and so the OTU composition was analysed at genus level (i.e., pines in any pine soil were considered to be growing in conspecific soil and pines in oak soil were considered to be growing in heterospecific soil).

Table 6: Effect of experimental factors on fungal, bacterial and oomycete community composition in the rhizosphere of seedlings in study **I** shown as R^2 values from PERMANOVA. Uninoculated soil is only included in the analysis of uninoculated versus inoculated soil. **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

	Fungi	**Bacteria**	**Oomycetes**
Uninoculated vs inoculated soil	0.13 ***	0.09 ***	0.04 ***
Seedling species	0.18 ***	0.18 ***	0.05 ***
Conspecific vs heterospecific soil	0.01 **	0.01 ns	0.00 ns
Inoculum species	0.04 ***	0.08 ***	0.02 ns
Metalaxyl	0.00 ns	0.00 ns	0.01 ns

3.3.1 Microbial community associated with boreal tree species (study I)

In all seedlings of study **I**, the largest differences in terms of fungal community composition were observed between inoculated and uninoculated soils (Figures **13** and **14**). Uninoculated soil contained primarily saprotrophs in broadleaf seedlings or saprotrophs and pathogens in conifers. EMF were the dominant fungal guild in the rhizosphere of all seedlings. The relative abundance of EMF correlated positively with the growth of alder, Scots pine and spruce seedlings (alder, $p = 0.003$, $R = 0.38$; Scots pine, $p = 0.027$, $R = 0.29$; spruce, $p = 0.003$, $R = 0.39$) but not with the growth of birch seedlings ($p = 0.418$, $R = 0.11$), which suggested that birch might rely less on EMF to provide nutrients than the

other three species. Scots pine growth correlated negatively with the relative abundance of fungal pathogens ($p = 0.048$, $R = -0.26$).

An indicator species analysis was performed to determine whether particular soil fungi were preferentially associated with any of the four tree species. Results showed that while all seedlings were able to recruit at least some EMF indicator species, they did not recruit them equally from all soils (Table **S7**). Broadleaf seedlings recruited EMF from most inoculated soils, but only one EMF species associated with alder seedlings and one EMF species associated with beech seedlings correlated positively with seedling biomass (Table **S7**). Scots pine seedlings recruited some EMF and root-associated fungi from beech soil but only moulds and saprotrophs in 'home' soil (Table **S7**). One EMF species (*Trichophaea* spp.) and one root-associated fungus (*Oidiodendron* spp.), which were indicator species for Scots pine growing in birch soil, correlated positively with seedling growth, indicating that pine was able to recruit growth-promoting fungi from soil conditioned by birch. Many EMF and root-associated fungi were identified as indicator species for spruce growing in Scots pine soil, but none of them correlated positively with seedling growth (Table **S7**). Only five out of 14 indicator taxa for spruce growing in 'home soil were EMF or root-associated fungi, which indicates that spruce was able to recruit more specific EMF and root-associated fungi from pine soils than from conspecific soil.

The main bacterial phyla recruited by the seedlings were conserved across all soils (Figure **14**). However, the community differed between inoculum types, and several phyla showed a strong correlation with seedling biomass. For alder and birch, growth correlated positively with the relative abundance of bacteria from the phylum Actynomycetota (alder, $p < 0.001$, $R = 0.59$; birch, $p = 0.037$, $R = 0.27$). Root nodule formation in alder was correlated with Actinomycetota relative abundance ($p < 0.001$, $R = 0.58$), which was correlated with a higher soil nitrogen content ($p = 0.04$, $R = 0.26$). Nodule formation was highest in alder seedlings growing with alder inoculum and correlated with seedling biomass ($p < 0.001$, $R = 0.69$). Concerning alder, these results suggest that nitrogen-fixing *Frankia* endophytes recruited from conspecific soil promoted seedling growth by increasing their access to nitrogen.

The oomycete community composition was similar in all seedlings and across all soils. Only one oomycete genus (*Isoachlya*) associated with alder soils showed a significant negative correlation with spruce growth ($p = 0.002$, $R = -0.42$).
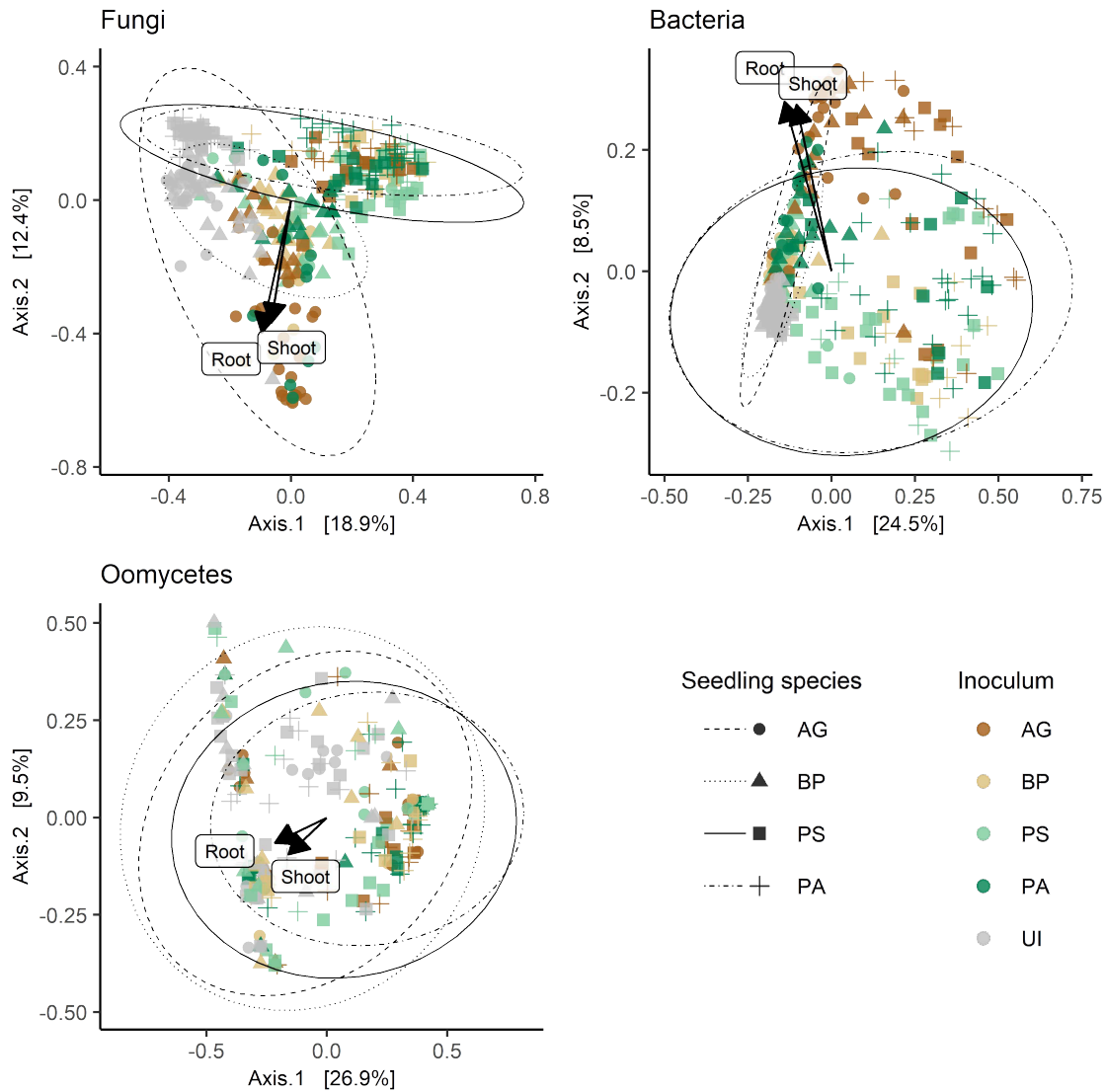
Figure 13: Principal coordinate analysis plots of fungal, bacterial and oomycete communities from study **I** based on Bray–Curtis distances. Differences in the length and direction of vectors indicate the relative strength of the association between ordination axes and the root or shoot biomass (i.e., 'Root' and 'Shoot', respectively). For fungi and bacteria, vector axes were multiplied by 0.5 to fit better within the plot. Ellipses correspond to the seedling species indicated by the symbol shape (AG, *Alnus glutinosa*; BP, *Betula pendula*; PS, *Pinus sylvestris*; PA, *Picea abies*). Inoculum soil is indicated by the symbol colour corresponding to the soil from adult trees of the same four species as the seedlings and uninoculated soil (UI) is sterile bulk without inoculum. The percentage of the total variance explained by each PC is indicated in parentheses.
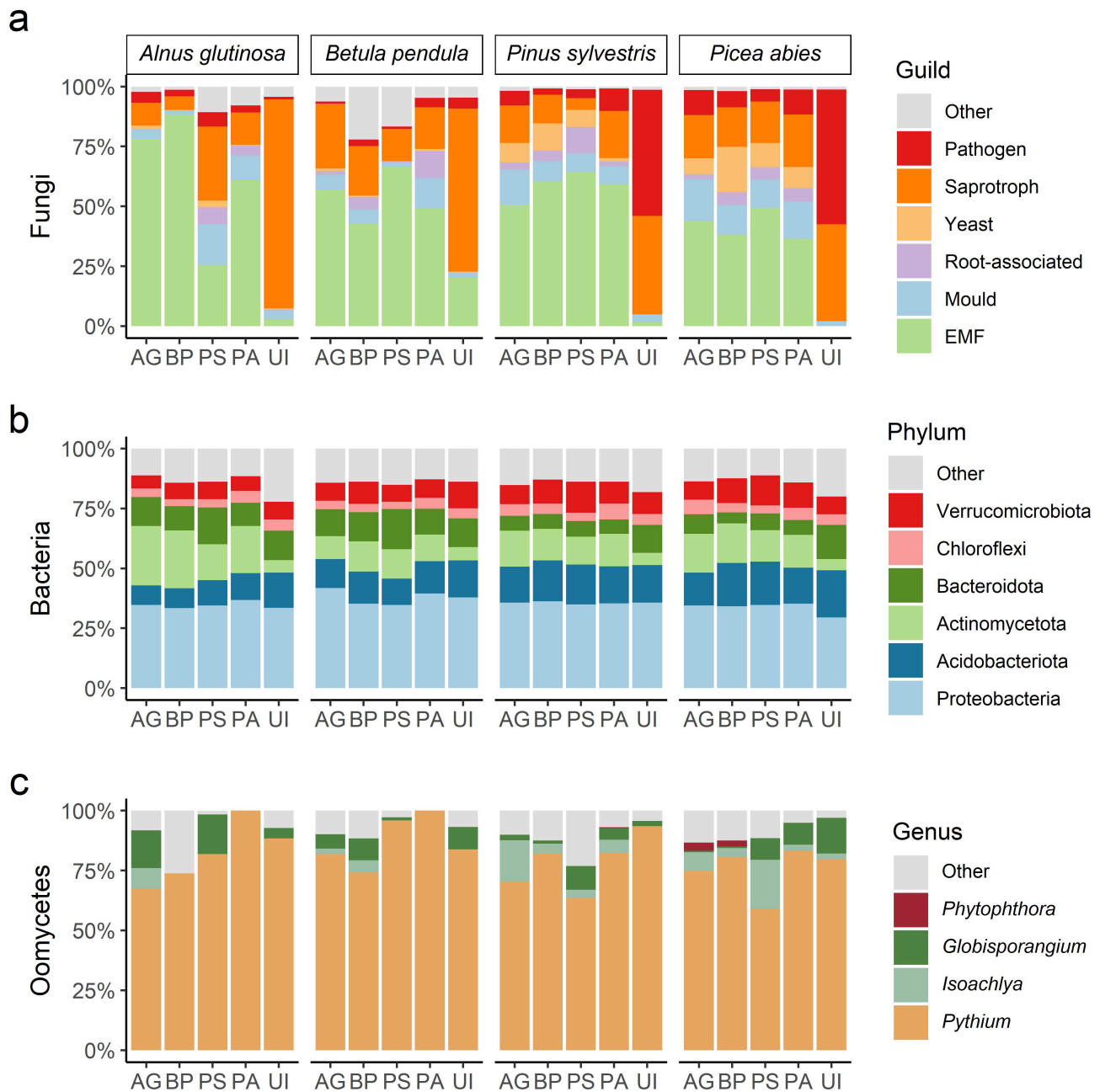
Figure 14: Relative abundance of each fungal guild (**a**), bacterial phylum (**b**) and oomycete genus (**c**) in the rhizosphere of *Alnus glutinosa*, *Betula pendula*, *Pinus sylvestris* and *Picea abies* seedlings in study **I** growing in uninoculated sterile soil (UI) or in sterile soil inoculated with soil collected under mature trees of *Alnus glutinosa* (AG), *Betula pendula* (BP), *Pinus sylvestris* (PS) or *Picea abies* (PA).

3.3.2 *Fungal community associated with Mediterranean oaks and pines (study II)*

The microbial community of study **II** was analysed in regards to how it was associated with seedlings and their growth. Fungal OTUs were annotated as specific if they were identified as indicator species for a particular seedling and 'non-specific' if they were not identified as such. Separately, the same

OTUs were annotated as 'positive' or 'negative' if they showed a positive or negative correlation with seedling growth, respectively.

OTU relative abundance patterns differed between seedlings that survived the drought treatment and those that either died as a result of drought exposure or were not exposed to it at all (i.e., seedlings grown in control conditions without a drought treatment). In control seedlings, there was a higher relative abundance of non-specific OTUs in the rhizosphere of seedlings growing in 'away' soil than in uninoculated soil. No differences in the relative abundance of specific or non-specific OTUs were observed in the rhizosphere of drought-exposed seedlings (Figure **15**).

In all seedlings, negative OTUs were more abundant in uninoculated soil than in inoculated soil ($p < 0.001$), but no differences were observed within inoculated soils (Figure **15**). Negative OTUs were predominantly attributed to pathogens (Figure **16**). In control seedlings and in seedlings that died from drought exposure, the relative abundance of negative pathogens was higher in uninoculated than inoculated soils ($p < 0.001$) but did not differ within inoculated soils ($p = 0.448$ in control, $p = 0.095$ under drought). However, in control seedlings growing in inoculated soils, pathogen relative abundance correlated negatively with seedling shoot biomass ($p = 0.032$, R = -0.15), indicating that under conditions of optimal soil moisture, fungal pathogens might hamper seedling growth.

One relevant association between PSFs and soil fungi was that the relative abundance of positive OTUs was higher in 'away' soils than in 'home' or uninoculated soils of control seedlings and seedlings that died due to drought (Figure **15**). Most positive OTUs were identified as EMF. However, EMF abundance in inoculated soils did not correlate with the shoot biomass of control seedlings or of those subjected to drought, signifying that not all EMF were able to contribute equally to seedling growth. Nevertheless, positive EMF were proportionally more abundant in 'away' soils than in 'home' soil (Figure **16**), indicating that seedlings may be able to recruit more beneficial EMF from heterospecific soils than from conspecific soil. Following the same pattern, seedlings growing in 'away' soil from a different genus had a higher relative abundance of positive EMF than seedlings in soils from the same genus.

In seedlings that survived drought exposure, the relative abundance of positive OTUs did not vary significantly between the different soil types (Figure **15**). In contrast with well-watered conditions, in seedlings that survived drought, no differences in the relative abundance of positive EMF were found. Also, neither EMF relative abundance nor pathogen relative abundance correlated with seedling survival under drought conditions.
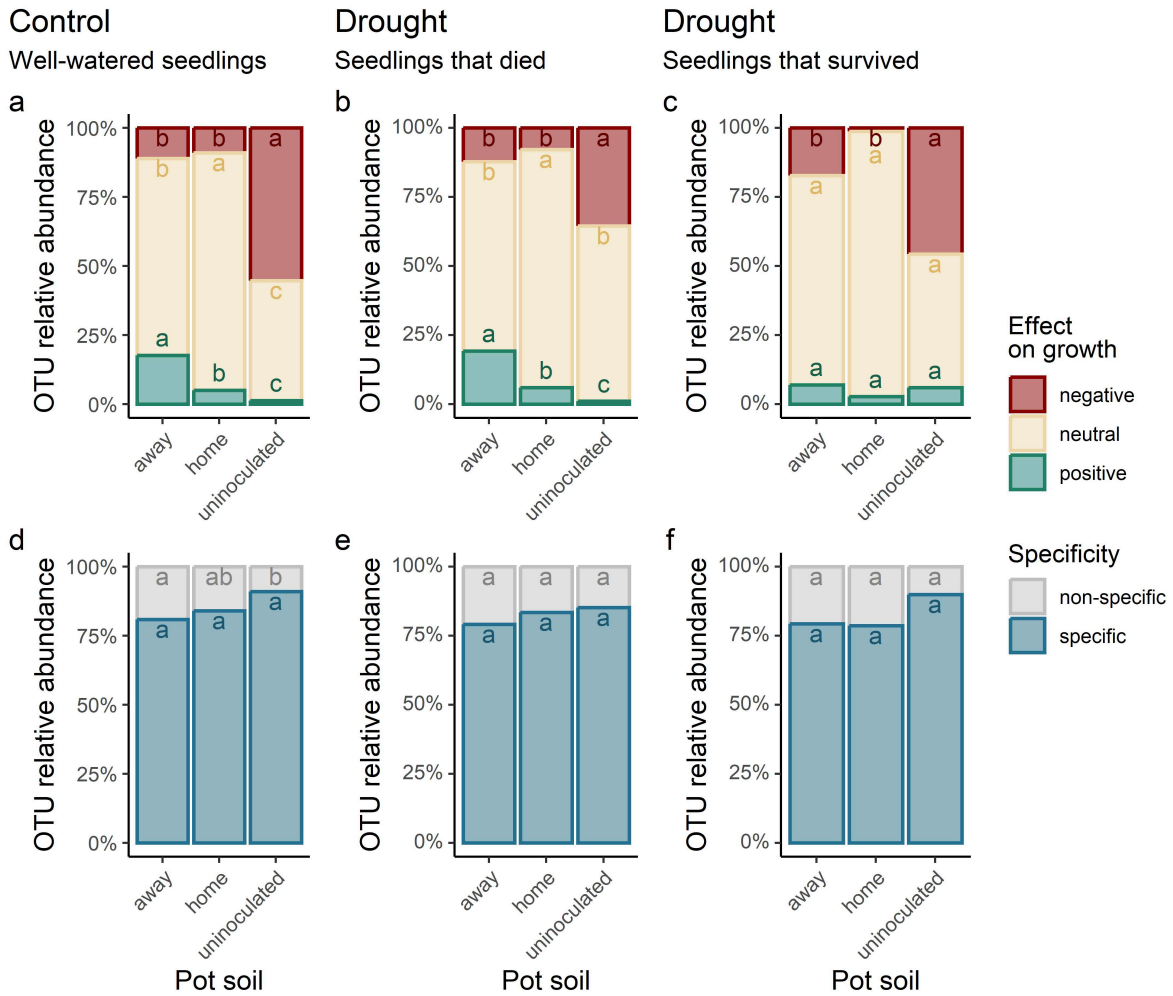
Figure 15: Relative abundance of fungal operational taxonomic units (OTUs) in the rhizosphere of seedlings in study **II** growing in different inoculated soils ('home', 'away') or in uninoculated soil under different experimental conditions (control or drought). The OTUs are divided by the type of correlation they display with seedling shoot biomass ('Effect on growth'; **a–c**) and by whether they were treatment specific ('Specificity'; **d–f**). Different letters above the bars indicate significant differences between soils at $p \leq 0.05$, which were determined by an emmeans analysis of a linear mixed-effects model.
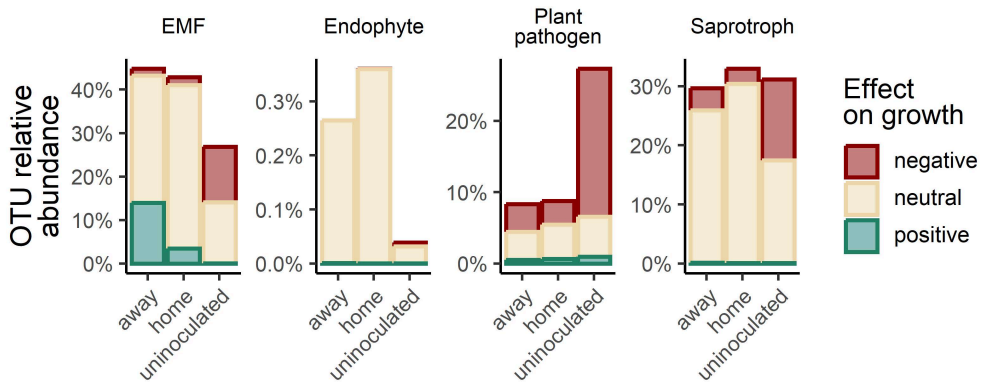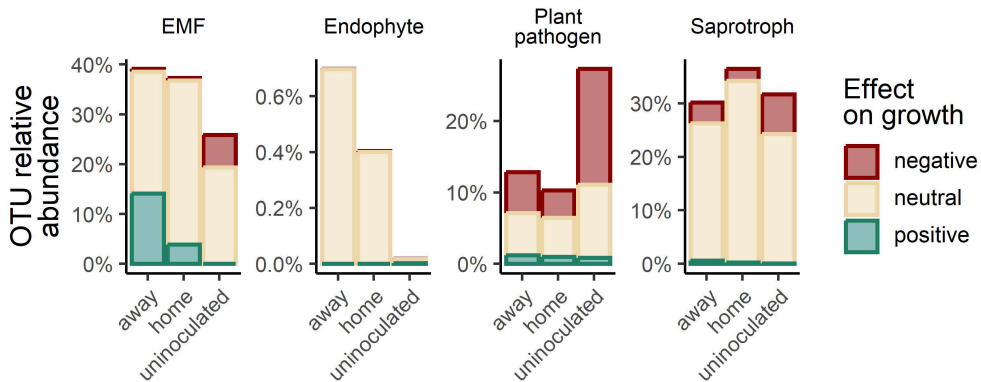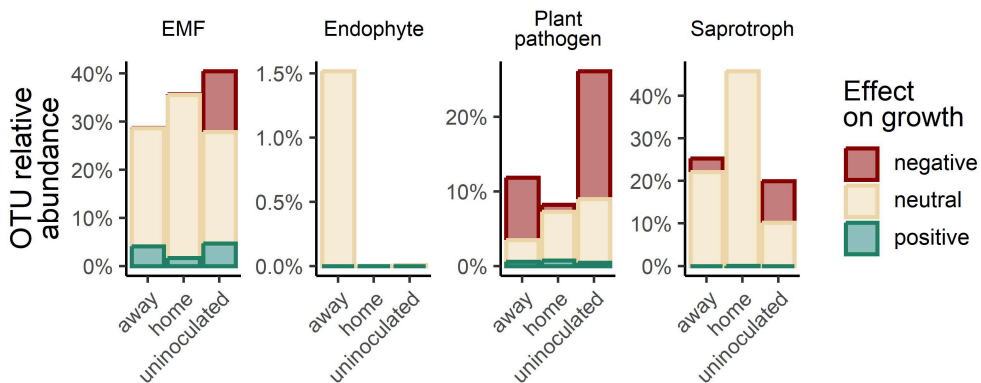
Figure 16: Relative abundance of fungal operational taxonomic units (OTUs) belonging to the four most abundant fungal guilds in the rhizosphere of seedlings in study **II** growing in different inoculated soils ('home', 'away') or uninoculated soil under different experimental conditions (control or drought). The OTUs are divided by the type of correlation they display with seedling shoot biomass ('Effect on growth'). EMF: ectomycorrhizal fungi.

3.4 Presence of *Phytophthora* root pathogens in Catalan forests

The overall *Phytophthora* incidence in Catalan forests was relatively low; only four out of 277 plots in the systematic sampling were found to host *Phytophthora*, whereas in the targeted sampling near the metropolitan area of Barcelona, *Phytophthora* was present in roughly a quarter of all non-recreational plots (Figure **17**). Most of the forests included in the systematic sampling were dominated by coniferous species (Table **S3**). In contrast, forests in the targeted sampling only included broadleaf trees, namely beech, chestnut and cork oak. When comparing *Phytophthora* incidence in the non-recreational plots of the targeted sampling with the incidence in forests of chestnut, beech or cork oak in the systematic sampling, no significant differences were observed (24% vs 9%, p = 0.32) (Figure **17**). In the targeted sampling, no significant differences in terms of *Phytophthora* incidence were found between asymptomatic and symptomatic trees (39% and 22%, respectively, p = 0.15).

Seven *Phytophthora* species were isolated from the sampled plots. The *Phytophthora* species that were isolated differed depending on the main tree species in the stand. *Phytophthora cactorum* and *P. plurivora* were isolated from beech and chestnut forests, whereas *P. x cambivora* was isolated only from chestnut plots. *Phytophthora castanetorum* and *P. quercina*, which are likely native to Europe, were isolated from chestnut and cork oak forests, respectively. *Phytophthora syringae* and *P. pseudocryptogea* were isolated from plots of other species in the systematic sampling.
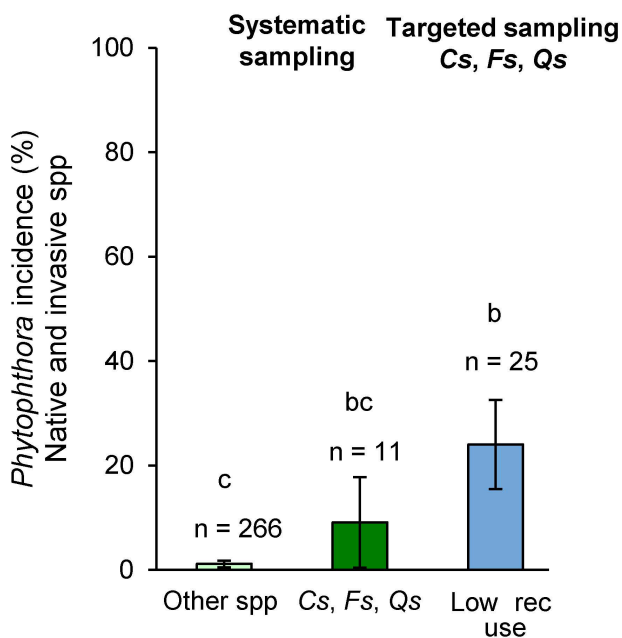
Figure 17: Incidence of native and invasive *Phytophthora* species in the systematic and targeted sampling of plots with low recreational use in study **IV**. Error bars represent the standard error. The letters above the bars were obtained using a Fisher LSD test; the levels not connected by the same letter are significantly different at p < 0.05. 'n' indicates the number of plots. Abbreviations: *Cs*, *C. sativa*; *Fs*, *F. sylvatica*; *Qs*, *Q. suber*; rec. use, recreational use.

3.4.1 Impact of Phytophthora *presence on forest regeneration*

Phytophthora presence affected tree health and regeneration of both conspecific seedlings (i.e., seedlings belonging to the dominant tree species in the stand) and heterospecific seedlings (i.e., seedlings belonging to species other than the dominant one). The ratio of conspecific to heterospecific regeneration in chestnut and cork oak plots was lower in areas with *Phytophthora* than in those without *Phytophthora* (Figure **18a**), signifying that *Phytophthora* could potentially strengthen negative PSFs by hampering conspecific regeneration. In beech forests, *Phytophthora* presence did not affect the ratio of conspecific to heterospecific regeneration (Figure **18a**). When considering individual *Phytophthora* species separately, beech stands with *P. plurivora* had a lower ratio of conspecific to heterospecific regeneration than stands without *Phytophthora* (-0.1 and 1.7, respectively; $p = 0.03$), whereas no differences were observed between *P. cactorum*-invaded and non-invaded beech forests (1.5 and 1.7, respectively; $p = 0.82$). *Phytophthora cactorum* was the only *Phytophthora* species isolated from the roots of naturally regenerating seedlings growing in one chestnut plot and two beech plots. Invasive *Phytophthora*, which were only found in beech and chestnut forests, were associated with a higher severity of crown defoliation in beech trees but not in chestnut trees (Figure **18b**), which implies that invasive *Phytophthora* species can affect adult trees as well as seedlings.
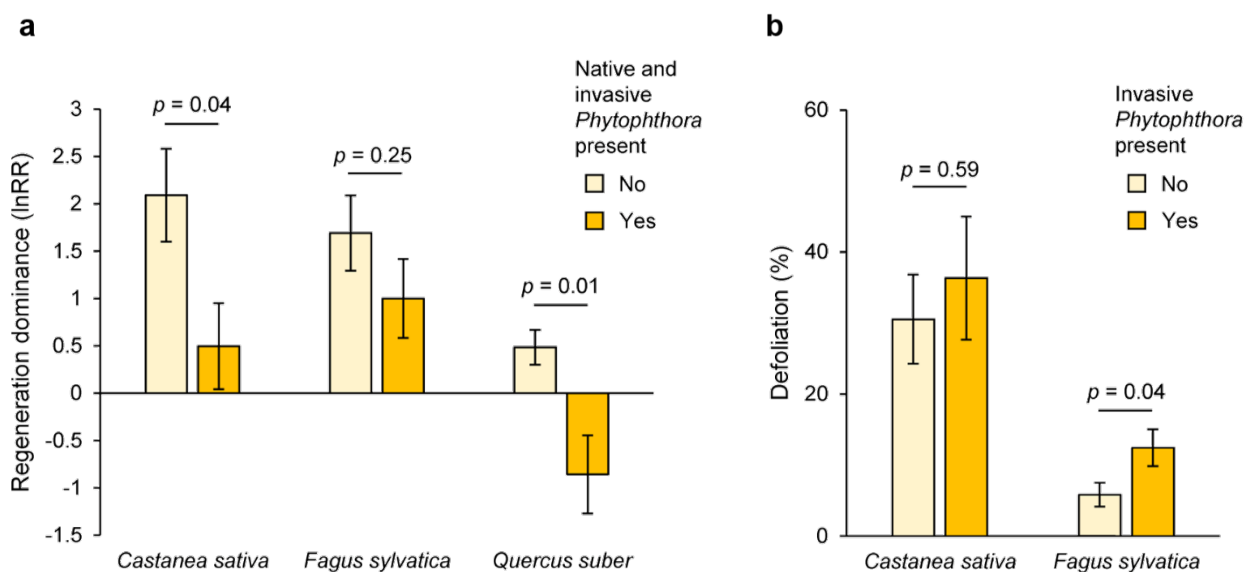
Figure 18: Impacts of *Phytophthora* presence in study **IV**: (**a**) Regeneration dominance (lnRR) values for regeneration under chestnut, beech and cork oak in plots with and without native and invasive *Phytophthora*. Values higher than zero indicate a higher abundance of conspecific seedlings (i.e., the same species as the adult tree) rather than heterospecific (i.e., species other than the adult tree), whereas values below zero indicate the opposite. (**b**) Defoliation of chestnut and beech in plots with and without invasive *Phytophthora*. Error bars represent the standard error.

3.4.2 Pathogenicity of isolated pathogens to Mediterranean tree species

The results of the inoculation trials revealed differences in terms of pathogenicity and specificity of the isolated *Phytophthora* species to seedlings of different tree species. *Phytophthora xcambivora* and *P. plurivora* were the most pathogenic species, consistently causing the longest lesions across all tree species (Figure **19**). *Phytophthora cactorum* displayed a higher degree of specificity for beech than the other *Phytophthora* species. In beech seedlings, it caused lesions comparable to those caused by *P. xcambivora* and *P. plurivora*. In all the other tree species, lesions caused by *P. cactorum* were significantly smaller than those caused by *P. xcambivora* and *P. plurivora*. The putatively native *P. castanetorum* showed specificity for cork oak seedlings. Cork oak and pubescent oak were the only species in which the lesions caused by *P. castanetorum* differed from those caused by mock inoculations used as controls.
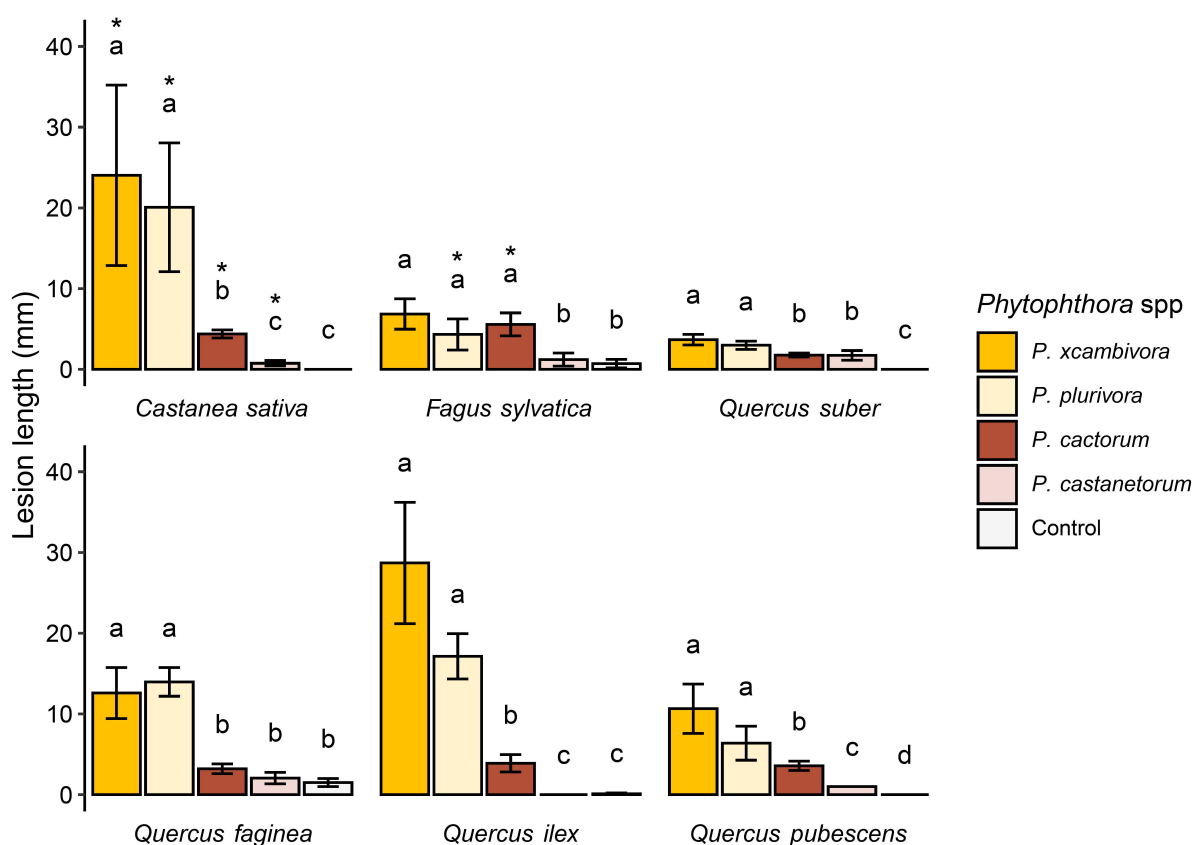
Figure 19: Pathogenicity of the isolated *Phytophthora* species (study **IV**) to different tree species, measured as lesion length seven weeks after inoculation. The letters above the bars were obtained using a Fisher's LSD test; the levels not connected by the same letter are significantly different at $p < 0.05$. Asterisks indicate the *Phytophthora*–host combinations found in nature. Error bars represent the standard error.

3.4.3 Phytophthora *incidence and recreational activities*

The targeted sampling revealed that *Phytophthora* incidence was associated with recreational use of forests. Areas used for recreational activities had a higher incidence of *Phytophthora* than non-recreational areas (63% and 24%, respectively) (Figure **20a**). Plots in recreational areas were characterised by a lesser distance to the nearest path and a higher number of recreational trails (according to Wikiloc) compared to non-recreational plots. Recreational plots were also more likely to contain anthropogenic litter than non-recreational plots. In turn, plots with litter were more likely to host *Phytophthora* than plots where no litter was found. (p = 0.04).

Invasive *Phytophthora* species were more commonly found in recreational forests of beech and chestnut than in non-recreational ones (76% and 21%, respectively) (Figure **20b**). Moreover, invasive *Phytophthora* were detected more often in plots that were close to a walking path and that contained anthropogenic litter than in plots further from a path and those without litter (p = 0.03 and p = 0.01, respectively).
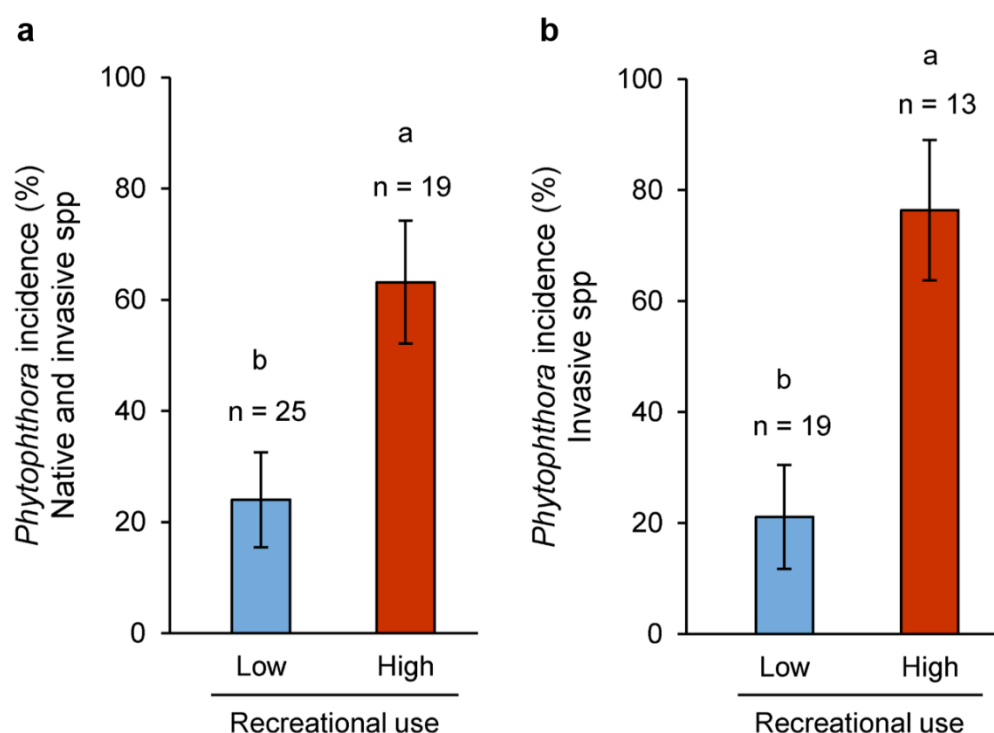
Figure 20: Incidence of *Phytophthora* species in forests with low or high recreational use in the targeted sampling in study **IV**; (**a**) incidence of native and invasive *Phytophthora* species in chestnut, beech and cork oak forests; (**b**) incidence of invasive *Phytophthora* species in chestnut and beech forests. Cork oak plots are not included in (**b**) since no invasive *Phytophthora* were detected in those areas. Error bars represent the standard error. The letters above the bars were obtained using a Fisher LSD test; the levels not connected by the same letter are significantly different at $p < 0.05$. "n" indicates the number of plots.

4 DISCUSSION

The results of the three experimental studies as well as the results of the forest soil surveys revealed that soil microbiota may be one of the mechanisms shaping forest stand dynamics in boreal and Mediterranean forests. Negative PSFs were the norm among the investigated tree species, with alder being the only species exhibiting a positive PSF. Boreal tree species showed species-specific PSFs. Each species recruited a unique microbial community from the different inoculated soils, and the responses of different seedling species varied depending on the origin of the soil inoculum. PSFs among Mediterranean tree species were negative and operated at genus level. Seedlings were able to recruit a higher proportion of EMF from heterogeneric soils (i.e., soils from trees of a different genus) than from congeneric soils. Fungal and oomycete pathogens seemed to play a relatively minor role in regulating the PSFs in studies **I** and **II**. In study **I**, only Scots pine seedling growth was hampered by pathogen presence, and although pathogen relative abundance correlated with seedling biomass in study **II**, it did not account for differences in growth between different soils. Instead, results suggest that EMF were the primary drivers of the observed feedbacks in both studies. Seedling biomass correlated with EMF abundance in study **I**, whereas in study **II**, the soils where seedlings grew more contained a higher proportion of EMF. The ability of trees to recruit beneficial root-associated microbial communities from heterospecific soils may be an important factor underlying biotic PSFs and one of the mechanisms maintaining tree species diversity.

4.1 Soil microbial community underlies tree species succession in boreal forests

Plant–soil feedbacks among boreal trees operated at the level of individual species and followed succession patterns seen in nature. The growth of silver birch, an early coloniser species (Condé et al., 2003; Špulák & Kacálek, 2020), was comparable between inoculated and uninoculated soil. This suggests that birch establishment in a disturbed area may depend more heavily on abiotic conditions, such as mineral nutrients, light or temperature, than on growth-enhancing microorganisms recruited from the soil. Moreover, the weak reliance on soil microbiota may give birch seedlings an advantage over other species when colonising areas that have been cleared by a disturbance, such as a forest fire. Scots pine often colonises birch communities that establish in a disturbed area, forming stands that represent the next stage of boreal forest succession.

Scots pine is a relatively shade-intolerant species (Gaudio et al., 2011; Houston Durrant et al., 2016b) and so the light shade cast by the birch canopy allows it to successfully establish in birch-colonised areas. However, the results of study **I** suggest that soil microbiota may be aiding pine establishment in birch-conditioned areas. Scots pine seedlings grew best in soil with birch inoculum and were able to recruit specific EMF and root-associated fungi from those soils, some of which showed a positive correlation with growth. Across all soils, pine seedling growth was positively correlated with the

proportion of EMF in the soil. Fungal pathogens, which were least abundant in birch soil, potentially inhibited pine growth in other soils, such as spruce soil. These observations indicate that the microbial community in the soil may support Scots pine's intermediate position in the boreal succession.

Norway spruce follows Scots pine in succession. It is a late successional species that can establish in more densely colonised areas due to its shade tolerance (Angelstam & Kuuluvainen, 2004). Just as Scots pine grew best in birch soil, Norway spruce in the experiment also grew best in the soil of its successional predecessor, Scots pine. The microbial community associated with spruce growing in pine soils indicated that the tendency of stands to transition from pine to spruce may, in part, be driven by soil microbiota. Norway spruce growing in pine soil associated with several fungal species known to form extensive mycelia with a high capacity to capture nitrogen from the soil and retain it (Agerer, 2001). Some of the recruited species have high δ^{15}N signatures and are associated with late stages of forest development (Hobbie & Högberg, 2012; Varenius et al., 2017), where the majority of nitrogen tends to be bound in organic compounds inaccessible to plants (Lilleskov, Hobbie, and Horton 2011). Pine and spruce seedlings growing in inoculated soils had high foliar δ^{15}N signatures, which suggest a shift in the nitrogen source potentially towards ^{15}N-enriched organic matter (Hobbie & Högberg, 2012). Although no individual EMF species associated with spruce growth were found, spruce seedlings were able to recruit more specific EMF and root-associated fungi from pine soil than from any other soil, suggesting soil microbiota may support spruce establishment in mature pine stands and thus drive successional transition towards a forest dominated by Norway spruce. This is additionally supported by the results of the simulated biomass accumulation in different successional pathways, where the pathway that resulted in the highest biomass accumulation was the one observed in nature (i.e., a succession from birch to pine and finally to spruce). Traditionally, successional patterns have been attributed to the varying light or nutrient demands of different tree species (Angelstam & Kuuluvainen, 2004; Petrokas et al., 2020). However, the experiment of study **I** revealed a possible effect of microbiota on seedling growth, suggesting that successional patterns could be partly driven by the interaction of trees with specific microorganisms, particularly EMF.

Alder was the fourth species included in the 'home-away' experiment of study **I**. It did not fit into a successional model with the other three species, which is in line with how it exists in nature. Alder is not usually part of the successional transition from birch to spruce (Angelstam & Kuuluvainen, 2004), but tends to form highly shaded single-species stands near water bodies or in soils with a high water table (Houston Durrant et al., 2016a). In the experiment, alder was the only species that showed a positive PSF, growing best when inoculated with its own soil. Additionally, the other species showed the poorest growth in alder soils, indicating that the microbial community recruited by alder may hamper the establishment of other species, allowing alder to exist in pure stands. Alder is known to establish symbioses with nitrogen-fixing bacteria from the genus *Frankia* (phylum Actinomycetota) (Benson & Silvester, 1993) which form root nodules and facilitate the plant's access to nitrogen. This

may aid the establishment of alder seedlings in alder-conditioned soils, and indeed, in the experiment, alder growth correlated strongly with the relative abundance of Actinomycetota and the presence of root nodules. Although the relative abundance of *Frankia* in alder soil was very low and did not correlate with biomass, this is likely due to *Frankia* being an endophyte (Benson & Silvester, 1993) which was not present in the rhizosphere but instead within the root nodules. Finally, alder associated with EMF species of the short-exploration type such as *Tuber* and *Tomentella* (the former was linked to higher seedling biomass), which tend to dominate in conditions of high nitrogen availability (Lilleskov et al., 2002; Sterkenburg et al., 2015). Foliar δ^{15}N depletion of alder mirrored growth in different soils, indicating that some of the nitrogen may have been provided to the plant by the microbial symbionts (Craine et al., 2015; Hobbie & Hobbie, 2008). Combining these results provided an indication that the monospecific nature of alder stands is at least partially driven by the soil microbiota alder associates with.

4.2   Microbial community shapes forest stands in the Mediterranean

Plant–soil feedbacks among Mediterranean species operated at genus level. The composition of the fungal community in the rhizosphere of seedlings was conserved across seedling genera, which has previously been observed in Mediterranean pine forests (Adamo et al. 2021).

The observed PSFs were negative and seemed to be driven primarily by EMF and pathogens. The capacity of pathogens to affect PSFs was further confirmed with field surveys, which showed that invasive pathogens were able to decrease conspecific regeneration.

4.2.1   Phytophthora *pathogens affect tree health and regeneration*

In study **IV**, *Phytophthora* presence was associated with tree defoliation and a lower ratio of conspecific to heterospecific regeneration. Beech defoliation was higher in areas with invasive *Phytophthora* than in forests without it, but no association between invasive *Phytophthora* presence and the defoliation of chestnut was found. It should be noted that it can sometimes be challenging to link defoliation with *Phytophthora* because the pathogen presence in an area can be very patchy or it may not be present under very damaged trees (Colangelo et al., 2018; Davison & Tay, 2005; Jönsson et al., 2005; Khaliq et al., 2021; McDougall et al., 2002; Pryce et al., 2002; Weste & Marks, 1987). However, in this study, chestnut defoliation was high in both invaded and uninvaded plots. In the surveyed area, chestnut blight (*Cryphonectria parasitica*) is a widespread invasive pathogen that also causes dieback and defoliation of chestnut trees (Oliva et al. 2016). The presence of chestnut blight might therefore help explain the lack of correlation between *Phytophthora* invasion and chestnut defoliation.

Phytophthora presence also seemed to strengthen negative PSFs by affecting tree regeneration. In chestnut, beech and cork oak, the proportion of conspecific regeneration was lower in forests with

Phytophthora than in areas without *Phytophthora*. The fact that *Phytophthora* seemed to affect regeneration in all trees but not defoliation suggests that the pathogen may affect seedlings more strongly than adult trees, likely because seedlings can be more susceptible to root pathogen attack compared to adult trees (Crandall, 1948; Hansen et al., 2005). Observing the putative effects of *Phytophthora* on chestnut regeneration but not on adult trees was theoretically possible because soil suppresses the growth of *C. parasitica* (Weidlich, 1978).

The assumption that the isolated *Phytophthora* have the capacity to cause disease in seedlings was supported by the results of the pathogenicity trial. All isolated *Phytophthora* species used in the pathogenicity trial were able to cause lesions longer than the control but varied in pathogenicity and specificity towards different seedling species. The differences were in line with the classification of these *Phytophthora* species as generalist or specific pathogens. *Phytophthora cambivora* and *P. plurivora* were the most pathogenic to all seedlings, which fits the current classification of these two species as broad generalists (Jung & Burgess, 2009; Vannini & Vettraino, 2011). *Phytophthora cactorum*, which is also considered a generalist (Hudler, 2013), was less pathogenic than *Phytophthora cambivora* and *P. plurivora* to all seedlings except *Q. faginea* but still caused significantly larger lesions than controls.

These three exotic *Phytophthora* species, considered to be invasive in Europe, were more pathogenic than the putatively native *P. castanetorum*. The three species have also been consistently associated with forest decline problems across Europe (Jung et al., 2018; Redondo et al., 2018). In study **IV**, their presence was associated with increased defoliation and a decrease in conspecific regeneration, indicating that invasive *Phytophthora* introduction could affect the recreational and environmental value of chestnut and beech forests in the region. Altogether, the results of study **IV** suggest that *Phytophthora* pathogens have the capacity to hamper seedling performance and may play a role in regulating the biotic PSFs among tree species in Mediterranean forests.

4.2.2 *Role of fungal pathogens and ectomycorrhizal fungi in pine-oak mixtures*

In study **II**, the relative abundance of fungal pathogens in the rhizosphere correlated negatively with seedling shoot biomass. Pathogens are generally considered to be the drivers of negative PSFs because their accumulation around adult trees makes 'home' soil less hospitable to conspecific seedlings, thus hampering their establishment and allowing other non-host species to establish in the area (Klironomos, 2002; Mangan et al., 2010; van der Putten, 2017). In an experiment with grassland species, soil pathogens caused negative feedbacks and seemed to affect community assembly (Klironomos, 2002). That study and a separate study involving tropical trees by Mangan et al. (2010) found that plants with stronger negative feedbacks were less common in nature than plants with a weak negative feedback or a positive one. In Mediterranean forests, oomycete pathogens have been linked to decreased conspecific regeneration of Mediterranean oaks (Domínguez-Begines et al.,

2020). The results of study **IV** also show that *Phytophthora* presence has the capacity to strengthen negative PSFs among susceptible species.

However, in the experiment of study **II**, pathogen abundance alone could not explain the differences in seedling growth between 'home' and 'away' soils. Instead, EMF, which form symbiotic relationships with many oak and pine species (Smith & Read, 2008), seemed to underlie the observed negative feedbacks. Seedlings in inoculated soils had lower foliar δ^{15}N signatures than those growing in uninoculated soil, which indicated that part of the nitrogen was supplied by the EMF (Hobbie & Colpaert, 2003; Hobbie & Hobbie, 2008), possibly explaining the differences in seedling growth between inoculated and uninoculated soils.

In the context of PSFs, mycorrhizal fungi have generally been considered to facilitate the establishment of conspecific seedlings and, in contrast to pathogens, drive positive feedbacks (Bennett et al., 2017; Segnitz et al., 2020; Teste et al., 2017). In addition to promoting seedling growth, EMF may have the capacity to directly mitigate the negative impacts of pathogen invasions by protecting seedling roots from pathogen attack (Bennett et al., 2017). However, the results of study **II** suggest that EMF that are beneficial for the adult tree may not necessarily benefit conspecific seedlings. The proportion of EMF was higher in 'away' soils than in 'home' soils, which suggests that EMF may have been able to promote the growth of heterospecific seedlings, potentially reducing the successful establishment of conspecific seedlings. Indeed, a higher proportion of beneficial EMF was found in the rhizosphere of seedlings growing in heterogeneric soil than in congeneric or uninoculated soil, which further points to the involvement of certain EMF in seedling growth.

These results show that the EMF community recruited by adult trees may not necessarily benefit conspecific seedlings but could instead underlie the negative PSFs, allowing other species to establish in the area. Seedlings could therefore benefit from dispersal in areas previously conditioned by trees of a different genus, favouring the formation of pine-oak mixed stands common in Mediterranean forests (GeoPortal MAPA/MITECO, 2023).

4.3   Global change and PSFs

One of the goals of each of the three studies focusing on Mediterranean forests was to determine the influence of climate change and anthropogenic disturbance on natural pathogen presence and on the feedbacks observed in the experiments. In study **II**, the impact of severe drought on PSFs among Mediterranean tree species was examined. Study **III** was designed in part to examine whether soil microbiota could contribute to predicted altitudinal changes thought to be driven primarily by rising temperatures as a result of climate change. Finally, in study **IV,** the impact of human recreational activity in forests on the likelihood of pathogen invasion was assessed.

4.3.1 Climate-driven range shifts facilitated by soil microbiota

Climate warming as a result of anthropogenic activity is predicted to affect the distribution of many species worldwide (Marx et al., 2016). Many plant species in the northern hemisphere are predicted to shift their ranges either further north or to higher altitudes to stay within their optimal rainfall and temperature ranges (Davis & Shaw, 2001). Though environmental conditions are largely considered to drive this expansion, biotic PSFs could also play a role.

In study **III**, compelling evidence was found suggesting that the migration of tree species may be aided by soil microbiota. Seedlings grew best when inoculated with unsterilised soil from trees dominating at a higher altitude. This signal disappeared in fungicide-treated seedlings, which all grew equally well, pointing to the involvement of soil fungi in the feedbacks. Previous research on invasive species has suggested that species introduced into a new area can become invasive partially because they are released from their specific pathogens while continuing to associate with beneficial microbiota (Inderjit & van der Putten, 2010; Rout & Callaway, 2012). In study **III**, biocide addition eliminated growth differences by seemingly enabling seedlings in local 'away' and 'home' soils to grow as well as the seedlings in 'high away' soil. These results could imply that better growth in range-expanding plants is not associated with beneficial microbiota promoting establishment at target sites but instead with a release from area-specific pathogens. This is in agreement with a study by Ramirez et al. (2019) which found that range-expanding species were exposed to fewer pathogens than native species. It is therefore possible that the range expansion of Mediterranean tree species may be facilitated by a putatively lower abundance of antagonistic microbiota supporting establishment at target sites.

4.3.2 Recreational activities aiding spread of invasive pathogens

The migration of novel pathogens to new areas would likely be very slow without external influences. For example, *Phytophthora* pathogens can spread with runoff water, but this spread is slow and relatively limited (Cahill et al., 2008). However, most known *Phytophthora* species have been able to spread far beyond their native environment, owing primarily to human activities.

In the survey of study **IV**, recreational forests had a much higher risk of harbouring exotic *Phytophthora* species than non-recreational forests. However, the underlying reasons for the higher prevalence of *Phytophthora* in recreational forests remain elusive. Globally, plant trade is one of the major drivers of invasive plant pathogen spread, and *Phytophthora* pathogens are often introduced into new environments via out-planting of infected plants (Bienapfl & Balci, 2014; Hulbert et al., 2019; Jung et al., 2016; Liebhold et al., 2012; Agnes V. Simamora et al., 2018; Sims & Garbelotto, 2021). In the surveyed area, *Phytophthora* inoculum may have been introduced in similar ways, for example, via the planting of amenity trees or through soil movements due to the construction of paths or car parks. Also, visitors in recreational forests may have aided the local dispersal of *Phytophthora* by carrying the inoculum in the soil on their hiking boots or bike tyres (Balm, 2017; Tjosvold et al., 2002;

Vélez et al., 2020), while soil compaction or root damage as a result of human activity in the area could have contributed to the establishment of introduced *Phytophthora* (Landa et al., 2021; Rhoades et al., 2003). In study **IV**, the distance to walking paths and the presence of anthropogenic litter were the best predictors of *Phytophthora* presence, further pointing to the role of humans in the invasion process. However, all these factors tend to occur together, so it is not possible to determine which activity increases invasion risk the most.

Particularly in the case of high-value recreational forests, such as those in nature reserves, pathogens spread through recreational activities can present a serious threat to the diversity of local flora (Cahill et al., 2008; Elliot et al., 2015; Lewis & Colquhoun, 2000). If invasive pathogens are introduced into a new environment, their interactions with the local flora could shift the PSFs and change the composition of the plant community. Apart from directly affecting plants and other soil microorganisms, the presence of invasive pathogens could cause shifts in the soil microbial community indirectly by affecting tree health and performance. For example, in a mountain birch forest defoliated by an outbreak of a birch-feeding moth, ectomycorrhizal abundance declined dramatically with increasing defoliation intensity (Saravesi et al., 2015). A simultaneous decrease in the abundance of EMF and an increase in pathogen abundance could substantially alter plant–soil relationships in an area and possibly lead to a restructuring of the plant community. Moreover, if tree species shifts to higher altitudes could be supported by a release from specific soil pathogens, as suggested by the results of study **III**, invasive pathogens introduced to those sites could interfere with this process and potentially prevent trees from migrating to a more suitable climactic range.

4.3.3   *Effect of beneficial microbiota negated by severe drought*

Mediterranean trees have been known to have trouble regenerating (Gómez-Aparicio et al., 2008; Pulido & Díaz, 2005), an issue exacerbated by drought, which can strongly affect young seedlings (Engelbrecht et al., 2005; Khurana & Singh, 2001). Many Mediterranean trees associate with EMF and rely on them to increase their nutrient acquisition capabilities. Though the microbial community in an area can be relatively stable, soil communities can be sensitive to environmental changes (Bardgett & Caruso, 2020; de Vries et al., 2018).

EMF can not only support seedling growth by providing them with nutrients but can also help plants survive periods of limited water availability and improve their drought tolerance (Castaño et al., 2023; Osonubi et al., 1991; Wang et al., 2021). However, the results of study **II** suggest that under severe drought conditions, plant–EMF symbioses could increase seedling susceptibility to drought. In the study, shoot biomass was the main predictor of seedling mortality under drought, and although no direct effect of soil microbiota on seedling survival was observed, it seemed that the beneficial EMF in the soil hampered seedling survival indirectly by promoting growth.

Seedlings that grow more as a result of their association with beneficial EMF likely have higher demands for water, which EMF may not be able to satisfy when water availability is severely limited. Indeed, a study by Villar-Salvador et al. (2013) showed that pines that grew more following nitrogen addition showed increased susceptibility to drought. Under conditions of severe drought, association with EMF, which supply nitrogen to the plant and promote growth, may prove disadvantageous and could hamper seedling establishment in 'away' soils. The warming climate and predicted increase in severity of droughts in the Mediterranean (Lange, 2020) could therefore seriously affect the ability of Mediterranean tree species to coexist.

4.4   Study limitations and future perspectives

All experiments in this thesis were done exclusively with young seedlings of the studied species. It is important to note that seedlings may not reflect adult tree responses. For example, seedlings may be more susceptible to pathogens or harsh environmental conditions than adult trees because of their small size and the presence of young succulent tissues (Hansen et al., 2005). It has been shown that tree mortality rates tend to decline as the tree ages (Simamora et al. 2017) and indeed, we saw indications of higher pathogen resistance in adults in study **IV**. Moreover, study **II** suggests that seedlings may benefit from a different soil microbiota than conspecific adults. Examining the PSFs at the seedling level is informative because seedling growth and survival determine the number of trees that become established and that will potentially develop into adults. Knowing seedling growth patterns in different soils therefore allows us to predict how a forest stand may evolve. However, trees associate with different microorganisms throughout their lives and exploring how the soil community evolves as a tree matures and how that affects the PSFs could be an interesting avenue for further research.

In study **II**, the effect of severe drought on PSF among seedlings of Mediterranean oaks and pines was examined and correlated with the abundance of different fungal guilds. Future studies could attempt to assess the long-term impacts of drought on PSFs by establishing experiments with multiple drought treatments across several growing seasons. Also, because previous research has shown that EMF have the capacity to protect seedlings from drought stress, it would be interesting to explore how strong this protection is and at which point in the drought process EMF associations begin to negatively affect the seedlings, as was observed here in study **II**. Another interesting possibility lies in the exploration of the metabolically active fraction of the microbiome (via RNA metabarcoding), which could lend insight into which microbial guilds operate at certain stages of the drought process and how the active community changes with drought or other stressors. Finally, establishing 'home-away' field trials would shed light on the importance of the experimentally observed feedbacks in nature.

In study **IV**, pathogenicity was assessed by inoculating seedling stems, which is generally accepted as a proxy for assessing resistance to pathogens. It is possible, however, that the seemingly low

pathogenicity of *P. castanetorum* was a consequence of the method of inoculation, which did not exactly reflect how these trees and pathogens interact in nature, namely *via* zoospores infecting fine roots. For example, in a previous study by Jung et al. (2017), *P. castanetorum* did not cause lesions when inoculated under bark. Nevertheless, in the inoculation trial of study **IV**, *Q. suber* and *Q. pubescens* developed significantly larger lesions than the controls after inoculation with *P. castanetorum*, which could indicate that apart from infecting its main host, *C. sativa* (Jung et al., 2017), *P. castanetorum* might also be capable of infecting other species. Soil inoculations approximating natural infection pathways would be needed to further explore the infectivity of *P. castanetorum* towards other tree species.

The surveys in study **IV** determined *Phytophthora* presence in forest soils by baiting. This method can miss *Phytophthora* present in the soil, especially if the species are difficult to culture (Vannini et al. 2013; Català et al. 2017). In study **IV**, we did not find *Phytophthora cinnamomi* in any of the plots, which is in contrast with other areas of Spain, for example, the south, where *P. cinnamomi* is considered to be one of the most destructive pathogens affecting oak forests (Brasier et al., 1993; Domínguez-Begines et al., 2020). Considering that we did not find *P. cinnamomi* in any of the plots in the targeted or systematic sampling, it is unlikely that this is due to the fact that baiting was used to assess *Phytophthora* presence. Instead, alkaline, calcium-rich soils and steep mountainous terrain where host species grow might hamper *P. cinnamomi* establishment in the area (Burgess et al., 2017; Falcon et al., 1984; Serrano et al., 2012; Shearer & Crane, 2014). Metabarcoding analyses of the soils would provide valuable information on the presence of any potentially undetected *Phytophthora* in the sampled region.

4.5   Conclusions

This thesis finds that PSFs are one of the drivers of species compositions in boreal and Mediterranean forests. In both ecosystems, negative PSFs dominated and seemed to be driven by beneficial EMF, which encouraged the establishment of heterospecific seedlings, and pathogens, which hampered conspecific regeneration. The thesis further finds that climate change and human activity have the potential to affect these relationships and consequently affect tree species diversity in Mediterranean forests. Considering the potential impact of soil microbiota on forest stand composition, it is imperative that PSFs in vulnerable ecosystems be characterised and their microbial drivers determined to enable informed management decisions.

5 Supplementary information

5.1 Tables

Table S1: Location, dominant tree species and altitude of plots where soil inoculum was sampled in studies **II** and **III**.

Study	Tree	Latitude	Longitude	Altitude
Study **III**	*Fagus sylvatica*	42.6174	0.76747	1572
Study **III**	*Abies alba*	42.6174	0.76747	1572
Study **III**	*F. sylvatica*	42.8433	-0.7105	1190
Study **III**	*A. alba*	42.8433	-0.7105	1190
Study **III**	*F. sylvatica*	42.844	-0.8278	1105
Study **III**	*A. alba*	42.844	-0.8278	1105
Studies **II** and **III**	*Quercus pubescens*	42.4637	0.77963	1115
Studies **II** and **III**	*Pinus sylvestris*	42.4637	0.77963	1115
Studies **II** and **III**	*Q. pubescens*	42.3251	0.98178	1123
Studies **II** and **III**	*P. sylvestris*	42.3251	0.98178	1123
Studies **II** and **III**	*Q. pubescens*	41.8838	2.11127	782
Studies **II** and **III**	*P. sylvestris*	41.8838	2.11127	782
Studies **II** and **III**	*Q. faginea*	42.1467	1.02061	605
Studies **II** and **III**	*P. nigra*	42.1467	1.02062	605
Studies **II** and **III**	*Q. faginea*	42.0449	1.03754	883
Studies **II** and **III**	*P. nigra*	42.0449	1.03754	883
Studies **II** and **III**	*Q. faginea*	41.9834	1.29255	447
Studies **II** and **III**	*P. nigra*	41.9834	1.29255	447
Studies **II** and **III**	*Q. suber*	41.8414	2.51078	440
Studies **II** and **III**	*P. pinaster*	41.8414	2.51078	440
Studies **II** and **III**	*Q. suber*	41.8603	2.56373	457
Studies **II** and **III**	*P. pinaster*	41.8603	2.56373	457
Studies **II** and **III**	*Q. suber*	41.8293	2.51295	371
Studies **II** and **III**	*P. pinaster*	41.8293	2.51295	371
Studies **II** and **III**	*Q. ilex*	41.3685	1.06148	563
Studies **II** and **III**	*P. halepensis*	41.3685	1.06148	563
Studies **II** and **III**	*Q. ilex*	41.2832	1.13733	645
Studies **II** and **III**	*P. halepensis*	41.2832	1.13733	645
Studies **II** and **III**	*Q. ilex*	41.0282	0.79284	488
Studies **II** and **III**	*P. halepensis*	41.0282	0.79284	488

Table S2: The design of the targeted and systematic sampling in study **IV**.

Sampling	Year	Sample type	Number of plots	Sampling strategy	Number of samples	Missing samples
Targeted	2020	Soil	24 [a]	Soil taken under one healthy and one symptomatic tree	63	Soil from under one tree
	2022	Soil	20	Soil taken under one healthy and one symptomatic tree	40	
		Seedlings' roots		Pool of roots of three conspecific seedlings	26 [a]	Seedlings' roots from two plots
Systematic	2021	Soil	277 [b]	Pool of soil taken under three trees	309	
Total			**321**		**438**	

[a] Eight plots sampled in 2020 were re-sampled again in 2022. In the re-sampled plots, along with the soil, the roots of conspecific seedlings were also collected.

[b] 32 plots were located in mixed stands. In those stands, two pools of soil were taken, i.e. one from the dominant tree species, and one from the other tree species.

Table S3: Summary of the 277 plots of the systematic sampling from study **IV** categorized by dominant tree species and the number of plots in which *Phytophthora* was present. The number of mixed plots is specified in parentheses.

Dominant tree species	Number of plots	Number of plots with *Phytophthora*
Abies alba	4 (1)	0
Acer campestre	1 (1)	0
Castanea sativa	1	0
Celtis australis	1	1
Fagus sylvatica	4	1
Fraxinus angustifolia	1 (1)	0
Fraxinus excelsior	2	0
Picea abies	1	0
Pinus halepensis	96 (13)	0
Pinus nigra	25 (3)	0
Pinus pinaster	3 (1)	0
Pinus pinea	8 (2)	0
Pinus sylvestris	38 (3)	1
Pinus uncinata	19 (1)	0
Populus sp.	1	0
Quercus faginea	9	1
Quercus ilex	41 (2)	0
Quercus petraea	2	0
Quercus pubescens	11 (1)	0
Quercus robur	2 (1)	0
Quercus suber	6 (2)	0
Ulmus spp.	1	0
Total	**277 (32)**	**4**

Table S4: Growing conditions in the glasshouse during the experiments of studies **I**, **II** and **III**. 'Light/dark hours' refers to the hours with and without artificial illumination, respectively.

	Month (year)	Light/dark hours	Temperature (°C)
Study **I**	July – August (2018)	no artificial light	no temperature control
	September – November (2020)	16/8	20
Study **II**	June – September (2020)	16/8	25 – 30
	October (2020)	12/12	20 – 25
	November (2020)	8/16	15 – 20
	December (2020)	6/18	10 – 15
	January (2021)	8/16	15
	February (2021)	12/12	20
	March – July (2021)[a]	16/8	20 – 30
Study **III**	March – May (2022)	16/8	20 – 25
	June – October (2022)	16/8	25 – 30
	November – December (2022)	12/12	15 – 20
	January – February (2023)	16/8	20
	March – April (2023)	16/8	25 – 30

[a] Half of the seedlings stopped being watered in May 2021 (to simulate drought).

Table S5: Primers and PCR reaction conditions for the amplification of different DNA regions for metabarcoding (studies **I** and **II**) and *Phytophthora* isolate identification (study **IV**).

Study	Organism	Region amplified	Primers	Initial denaturation	Denaturation	Annealing	Elongation	Final elongation	Cycles
study **I**									
	fungi	ITS2	gITS7[a] – ITS4[b]/ITS4arch[c]	5 min at 95°C	30 s at 95°C	30 s at 58°C	30 s at 72°C	10 min at 72°C	24–31
	bacteria – reaction 1	V3-V4 region of 16S rRNA gene	pro341F – pro805R[d]	3 min at 98°C	30 s at 98°C	30 s at 55°C	30 s at 72°C	10 min at 72°C	25
	bacteria – reaction 2	PCR product tagging		3 min at 98°C	30 s at 98°C	30 s at 55°C	45 s at 72°C	10 min at 72°C	8
	oomycetes	ITS1	ITS6 – ITS7[e]	30 s at 95°C	1 min at 94°C	30 s at 55°C	1 min at 72°C	6 min at 72°C	32–35
study **II**	fungi	ITS2	ITS7f[a] – ITS4[b]	5 min at 94°C	30 s at 94°C	30 s at 57°C	30 s at 72°C	7 min at 72°C	29–35
study **IV**	*Phytophthora*	ITS1 and 2	ITS A2 – I2[f]	3 min at 94°C	30 s at 94°C	45 s at 60°C	2 min at 72°C	10 min at 72°C	35
study **IV**	*Phytophthora*	ITS1 and 2	ITS4 – ITS6[g]	3 min at 94°C	30 s at 94°C	30 s at 55°C	1 min at 72°C	10 min at 72°C	32
	Phytophthora	*cox*1	COXF4N – COXR4N[h]	2 min at 94°C	15 s at 94°C	30 s at 52°C	1 min at 72°C	10 min at 72°C	35
	Phytophthora	HSP90	HSP90_F1int – HSP90_R1[i]	2 min at 94°C	30 s at 94°C	30 s at 62°C	2 min at 72°C	5 min at 72°C	35
	Phytophthora	β-*tub*	TUBUF2 – TUBUR1[h]	2 min at 94°C	15 s at 94°C	30 s at 60°C	1 min at 72°C	10 min at 72°C	35
	Phytophthora	*nadh*1	NADHF1 – NADHR1[h]	2 min at 94°C	15 s at 94°C	30 s at 53°C	1 min at 72°C	10 min at 72°C	35

[a] Ihrmark et al. (2012); [b] White et al. (1990); [c] Kyaschenko et al. (2017); [d] Takahashi et al. (2014); [e] Vannini et al. (2013); [f] Drenth et al. (2006); [g] Grünwald et al. (2011); [h] Blair et al. (2008); [i] Kroon et al. (2004).

Table S6 GenBank accessions of the isolates used in the multi-locus analysis in study **IV**; 'this study' indicates the isolates that were obtained during study **IV**.

Species	Isolate	GenBank Accession				
		Btub	NADH	Cox1	HSP90	ITS
P. cactorum	isolate P6183	AY564052.1	AY563994.1	AY564167.1	n. a.	n. a.
P. cactorum	isolate P0715	EU080285.1	n. a.	n. a.	EU080288.1	n. a.
P. cactorum	isolate P11184	EU080292.1	n. a.	n. a.	EU080295.1	n. a.
P. cactorum	isolate Cs106 (this study)	MW924715	MW924749	MW924765	MW924732	MW927118
P. cactorum	isolate Cs107 (this study)	MW924716	MW924750	MW924766	MW924733	MW927119
P. cactorum	isolate Cs108 (this study)	MW924717	MW924751	MW924767	MW924734	MW927120
P. castanetorum	isolate BD292	MF036214.1	MF036292.1	MF036266.1	MF036240.1	MF036182.1
P. castanetorum	isolate BD293	MF036215.1	MF036293.1	MF036267.1	MF036241.1	MF036183.1
P. castanetorum	isolate BD476	MF036216.1	MF036294.1	MF036268.1	MF036242.1	MF036185.1
P. castanetorum	isolate BD484	MF036217.1	MF036295.1	MF036269.1	MF036243.1	MF036186.1
P. castanetorum	isolate P14	MF036218.1	MF036296.1	MF036270.1	MF036244.1	MF036189.1
P. castanetorum	isolate P17	MF036219.1	MF036297.1	MF036271.1	MF036245.1	MF036190.1
P. castanetorum	isolate P18	MF036220.1	MF036298.1	MF036272.1	MF036246.1	MF036191.1
P. castanetorum	isolate Cs76 (this study)	MW924708	MW924741	MW924758	MW924724	MW927094
P. castanetorum	isolate Cs77 (this study)	MW924709	MW924742	MW924759	MW924725	MW927095
P. castanetorum	isolate Cs78 (this study)	MW924710	MW924743	MW924760	MW924726	MW927096
P. castanetorum	isolate Cs109 (this study)	MW924718	MW924752	MW924768	MW924735	MW927121
P. castanetorum	isolate Cs111 (this study)	MW924720	MW924754	MW924770	MW924737	MW927123
P. castanetorum	isolate Cs112 (this study)	MW924721	MW924755	MW924771	MW924738	MW927124
P. castanetorum	isolate Cs113 (this study)	MW924722	MW924756	MW924772	MW924739	MW927125

P. castanetorum	isolate Cs114 (this study)	MW924723	MW924757	MW924773	MW924740	MW927126
P. hedraiandra	isolate 33F3	KX250384.1	n. a.	n. a.	KX250387.1	n. a.
P. hedraiandra	isolate 38C2	KX250391.1	n. a.	n. a.	KX250394.1	n. a.
P. hedraiandra	isolate 62A5 (ex-type)	KX250398.1	n. a.	n. a.	KX250401.1	n. a.
P. hedraiandra	isolate P11056	EU080073.1	n. a.	n. a.	EU080076.1	n. a.
P. quercina	isolate P10334	EU080490.1	n. a.	n. a.	EU080493.1	HQ261659.1
P. quercina	isolate P10441	EU080592.1	n. a.	n. a.	EU080595.1	HQ261658.1
P. quercina	isolate BD10	MF036221.1	MF036299.1	MF036273.1	MF036247.1	MF036192.1
P. quercina	isolate BD549	MF036222.1	MF036300.1	MF036274.1	MF036248.1	MF036193.1
P. quercina	isolate PL7	MF036223.1	MF036301.1	MF036275.1	MF036249.1	MF036194.1
P. quercina	isolate PL9	n. a.	MF036302.1	MF036276.1	MF036250.1	MF036195.1
P. quercina	isolate Qs98 (this study)	n. a.	MW924744	MW924761	MW924727	MW927112
P. quercina	isolate Qs100 (this study)	MW924711	MW924745	n. a.	MW924728	MW927114
P. quercina	isolate Qs102 (this study)	MW924712	MW924746	MW924762	MW924729	MW927115
P. quercina	isolate Qs104 (this study)	MW924713	MW924747	MW924763	MW924730	MW927116
P. quercina	isolate Qs105 (this study)	MW924714	MW924748	MW924764	MW924731	MW927117
P. quercina	isolate Qs110 (this study)	MW924719	MW924753	MW924769	MW924736	MW927122

Table S7: Fungal indicator species in study **I** associated with each seedling in each soil and the correlation of their relative abundance with seedling growth; statistically significant correlations ($p \leq 0.05$) are in bold.

Seedling	Soil	Indicator species		Correlation with growth	
		Functional guild	Species	R	p
Birch	AG	EMF	*Tomentella*	−0.12	0.290
Birch	AG	EMF	*Tuber*	−0.01	0.926
Birch	AG	EMF	*Serendipita*	−0.16	0.139
Birch	AG	Saprotroph	*Clonostachys rosea*	−0.05	0.675
Birch	AG	Saprotroph	*Chaetomium*	−0.04	0.718
Birch	BP	EMF	*Hebeloma leucosarx*	0.14	0.195
Birch	BP	Root-associated	*Serendipita*	−0.18	0.101
Birch	**PA**	**Animal parasite**	***Arthrobotrys***	**0.23**	**0.033**
Birch	PA	Animal parasite	*Arthrobotrys*	0.04	0.718
Birch	PA	EMF	*Wilcoxina*	0.17	0.131
Birch	PA	EMF	*Tuber*	0.16	0.137
Birch	PS	Animal parasite	*Arthrobotrys*	−0.04	0.718
Birch	**PS**	**EMF**	***Hyaloscypha finlandica***	**0.22**	**0.042**
Birch	PS	Saprotroph	*Tetracladium*	0.16	0.162
Birch	UI	Saprotroph	*Stachybotrys chartarum*	−0.02	0.890
Alder	**AG**	**EMF**	***Tomentella testaceogilva***	**0.55**	**0.000**
Alder	AG	Mould	*Trichoderma viride*	0.16	0.150
Alder	AG	Saprotroph	*Clonostachys rosea*	0.17	0.121
Alder	PA	Arbuscular mycorrhizal	*Funneliformis*	0.14	0.214
Alder	PA	Arbuscular mycorrhizal	*Funneliformis*	0.13	0.257
Alder	PA	Arbuscular mycorrhizal	*Funneliformis*	0.13	0.248
Alder	PA	EMF	*Wilcoxina*	−0.15	0.173
Alder	PA	EMF	*Tuber*	−0.20	0.080
Alder	PA	Mould	*Mortierella*	0.08	0.486
Alder	**PA**	**Mould**	***Mortierella alpina***	**−0.25**	**0.022**
Alder	**PA**	**Mould**	***Umbelopsis***	**−0.32**	**0.003**
Alder	**PA**	**Mould**	***Umbelopsis***	**−0.32**	**0.003**
Alder	**PA**	**Root-associated**	***Archaeorhizomyces***	**−0.32**	**0.004**
Alder	**PA**	**Root-associated**	***Archaeorhizomyces***	**−0.26**	**0.019**
Alder	**PA**	**Saprotroph**	***Gymnostellatospora***	**−0.35**	**0.001**
Alder	**PA**	**Saprotroph**	***Pseudeurotium***	**−0.24**	**0.028**
Alder	**PA**	**Yeast**	***Solicoccozyma aeria***	**−0.30**	**0.006**
Alder	PS	Saprotroph	*Apiotrichum*	0.01	0.931
Alder	**UI**	**Animal parasite**	***Arthrographis***	**−0.30**	**0.007**
Alder	UI	EMF	*Trichophaea*	−0.20	0.067
Alder	**UI**	**Saprotroph**	***Aspergillus***	**−0.58**	**0.000**
Alder	UI	Saprotroph	*Schwanniomyces vanrijiae*	−0.10	0.360
Alder	**UI**	**Saprotroph**	***Stachybotrys chartarum***	**−0.42**	**0.000**
Pine	AG	EMF	*Cortinarius*	0.17	0.135

Pine	AG	EMF	*Inocybe maculata*	0.07	0.538
Pine	AG	EMF	*Tomentella*	0.10	0.382
Pine	AG	EMF	*Rhizopogon luteolus*	0.13	0.260
Pine	AG	EMF	*Thelephora alnii*	0.09	0.414
Pine	AG	EMF	*Cortinarius*	0.19	0.088
Pine	AG	EMF	*Inocybe*	0.19	0.090
Pine	AG	EMF	*Cortinarius fulvoconicus*	0.04	0.736
Pine	AG	Ericoid mycorrhizal	*Phialocephala fortinii*	0.12	0.297
Pine	AG	Mould	*Mortierella*	0.16	0.149
Pine	AG	Mould	*Mortierella*	0.01	0.897
Pine	AG	Mould	*Penicillium bialowiezense*	0.10	0.352
Pine	AG	Mould	*Mortierella*	0.08	0.471
Pine	**AG**	**Mould**	***Umbelopsis***	**0.22**	**0.048**
Pine	AG	Mould	*Mortierella antarctica*	0.11	0.309
Pine	AG	Mould	*Penicillium*	0.17	0.120
Pine	AG	Mould	*Umbelopsis*	−0.01	0.919
Pine	AG	Mould	*Mortierellales*	0.08	0.494
Pine	AG	Pathogen	*Ganoderma applanatum*	0.21	0.055
Pine	AG	Pathogen	*Verticillium*	0.07	0.528
Pine	**AG**	**Root-associated**	***Oidiodendron flavum***	**0.33**	**0.002**
Pine	AG	Saprotroph	*Neonectria*	0.07	0.540
Pine	AG	Saprotroph	*Hypocreales*	0.04	0.713
Pine	AG	Saprotroph	*Clonostachys rosea*	0.02	0.872
Pine	AG	Saprotroph	*Apiotrichum*	0.04	0.744
Pine	AG	Saprotroph	*Cylindrocarpon*	0.18	0.108
Pine	AG	Saprotroph	*Pholiota alnicola*	0.12	0.275
Pine	AG	Saprotroph	*Neobulgaria*	−0.03	0.807
Pine	AG	Saprotroph	*Acremonium*	−0.06	0.619
Pine	AG	Saprotroph	*Pseudeurotium*	0.01	0.902
Pine	AG	Saprotroph	*Tetracladium*	0.04	0.720
Pine	AG	Saprotroph	*Tetracladium*	0.02	0.876
Pine	AG	Saprotroph	*Atractospora*	0.01	0.895
Pine	AG	Saprotroph	*Pseudeurotium*	0.08	0.461
Pine	**BP**	**EMF**	***Trichophaea***	**0.35**	**0.001**
Pine	BP	EMF	*Clavulina*	0.09	0.430
Pine	BP	EMF	*Cenococcum geophilum*	0.11	0.309
Pine	**BP**	**Mould**	***Umbelopsis***	**0.42**	**0.000**
Pine	**BP**	**Mould**	***Penicillium***	**0.45**	**0.000**
Pine	**BP**	**Root-associated**	***Oidiodendron***	**0.33**	**0.002**
Pine	**BP**	**Yeast**	***Saitozyma podzolica***	**0.43**	**0.000**
Pine	PA	EMF	*Amphinema*	−0.01	0.946
Pine	**PA**	**EMF**	***Wilcoxina***	**0.25**	**0.025**
Pine	PA	EMF	*Hyaloscypha finlandica*	−0.01	0.907
Pine	PA	EMF	*Rhizopogon mohelnensis*	0.17	0.121
Pine	PA	Mould	*Trichoderma viride*	0.00	0.989
Pine	PA	Mould	*Umbelopsis*	0.04	0.741

Pine	PA	Mould	*Umbelopsis*	0.11	0.339
Pine	PA	Root-associated	*Archaeorhizomyces*	0.00	0.973
Pine	PA	Root-associated	*Archaeorhizomyces*	−0.04	0.720
Pine	**PA**	**Yeast**	***Solicoccozyma terricola***	**0.31**	**0.004**
Pine	PA	Yeast	*Solicoccozyma aeria*	0.19	0.091
Pine	PS	Mould	*Penicillium*	−0.09	0.421
Pine	PS	Saprotroph	*Pseudogymnoascus pannorum*	0.03	0.773
Pine	PS	Saprotroph	*Fibulochlamys chilensis*	−0.14	0.199
Pine	PS	Saprotroph	*Entoloma*	0.00	0.983
Pine	**UI**	**Animal parasite**	***Arthrographis***	**−0.32**	**0.003**
Pine	UI	Animal parasite	*Arthrobotrys*	−0.15	0.190
Pine	**UI**	**Mould**	***Mortierella***	**−0.29**	**0.007**
Pine	**UI**	**Mould**	***Mortierella zychae***	**−0.34**	**0.001**
Pine	**UI**	**Pathogen**	***Fusarium***	**−0.70**	**0.000**
Pine	**UI**	**Saprotroph**	***Aspergillus***	**−0.48**	**0.000**
Pine	**UI**	**Saprotroph**	***Fusarium***	**−0.29**	**0.008**
Pine	**UI**	**Saprotroph**	***Blastobotrys***	**−0.30**	**0.007**
Pine	**UI**	**Saprotroph**	***Candida***	**−0.31**	**0.004**
Spruce	AG	EMF	*Tuber*	−0.16	0.158
Spruce	AG	EMF	*Inocybe maculata*	−0.18	0.113
Spruce	AG	EMF	*Clavulina cinerea*	−0.05	0.644
Spruce	AG	EMF	*Thelephora alnii*	0.03	0.787
Spruce	AG	EMF	*Cortinarius*	−0.09	0.448
Spruce	AG	EMF	*Hymenogaster griseus*	−0.07	0.536
Spruce	AG	Mould	*Penicillium bialowiezense*	−0.14	0.247
Spruce	AG	Mould	*Mortierella*	−0.12	0.321
Spruce	AG	Mould	*Mortierellales*	−0.18	0.128
Spruce	AG	Pathogen	*Ganoderma applanatum*	−0.15	0.214
Spruce	AG	Saprotroph	*Neonectria*	−0.19	0.106
Spruce	AG	Saprotroph	*Hypocreales*	−0.08	0.500
Spruce	AG	Saprotroph	*Clonostachys rosea*	−0.16	0.165
Spruce	AG	Saprotroph	*Clitopilus*	−0.14	0.219
Spruce	AG	Saprotroph	*Pholiota alnicola*	−0.06	0.615
Spruce	AG	Saprotroph	*Acremonium*	−0.19	0.094
Spruce	AG	Saprotroph	*Cheilymenia*	−0.13	0.270
Spruce	AG	Saprotroph	*Pseudeurotium*	−0.13	0.268
Spruce	AG	Saprotroph	*Atractospora*	−0.15	0.187
Spruce	BP	EMF	*Clavulina*	0.06	0.585
Spruce	BP	EMF	*Cenococcum geophilum*	0.03	0.802
Spruce	BP	EMF	*Inocybe*	−0.14	0.233
Spruce	BP	EMF	*Amphinema byssoides*	0.08	0.471
Spruce	BP	Ericoid mycorrhizal	*Pezoloma*	−0.02	0.855
Spruce	BP	Ericoid mycorrhizal	*Pezoloma*	0.00	0.994
Spruce	BP	Ericoid mycorrhizal	*Meliniomyces vraolstadiae*	−0.07	0.547
Spruce	BP	Mould	*Umbelopsis*	−0.16	0.178
Spruce	BP	Mould	*Penicillium*	−0.13	0.264

Spruce	BP	Mould	*Mortierella*	0.03	0.799
Spruce	BP	Mould	*Mortierella*	0.08	0.508
Spruce	BP	Pathogen	*Venturia*	0.05	0.676
Spruce	BP	Root-associated	*Oidiodendron*	−0.14	0.215
Spruce	BP	Root-associated	*Chaetothyriales*	−0.15	0.214
Spruce	BP	Root-associated	*Archaeorhizomyces*	0.06	0.604
Spruce	BP	Saprotroph	*Acephala*	0.09	0.441
Spruce	BP	Yeast	*Saitozyma podzolica*	−0.04	0.748
Spruce	BP	Yeast	*Solicoccozyma terricola*	−0.02	0.898
Spruce	**PA**	**Animal parasite**	***Arthrobotrys***	**0.31**	**0.006**
Spruce	PA	EMF	*Rhizopogon mohelnensis*	−0.01	0.927
Spruce	PA	EMF	*Russula integra*	0.07	0.535
Spruce	PA	Mould	*Mortierella alpina*	−0.18	0.115
Spruce	**PA**	**Mould**	***Trichoderma viride***	**0.27**	**0.017**
Spruce	PA	Mould	*Umbelopsis*	0.11	0.328
Spruce	PA	Mould	*Umbelopsis*	0.06	0.628
Spruce	PA	Mould	*Penicillium*	0.01	0.963
Spruce	PA	Mould	*Umbelopsis*	−0.09	0.451
Spruce	PA	Root-associated	*Archaeorhizomyces*	0.09	0.448
Spruce	PA	Root-associated	*Oidiodendron*	−0.06	0.631
Spruce	PA	Root-associated	*Archaeorhizomyces*	−0.06	0.592
Spruce	PA	Saprotroph	*Gymnostellatospora*	−0.13	0.285
Spruce	PA	Yeast	*Solicoccozyma aeria*	0.13	0.261
Spruce	PS	EMF	*Piloderma byssinum*	0.13	0.273
Spruce	PS	EMF	*Hydnellum peckii*	−0.06	0.584
Spruce	PS	EMF	*Tretomyces lutescens*	0.06	0.587
Spruce	PS	EMF	*Russula*	0.03	0.768
Spruce	PS	EMF	*Pseudotomentella mucidula*	0.01	0.944
Spruce	PS	EMF	*Amphinema*	−0.07	0.538
Spruce	PS	Mould	*Mortierella*	−0.05	0.698
Spruce	PS	Mould	*Penicillium*	−0.09	0.439
Spruce	PS	Mould	*Penicillium*	−0.02	0.876
Spruce	PS	Mould	*Umbelopsis*	−0.07	0.544
Spruce	PS	Pathogen	*Volutella*	−0.04	0.739
Spruce	PS	Root-associated	*Oidiodendron chlamydosporicum*	0.03	0.818
Spruce	PS	Root-associated	*Oidiodendron*	−0.06	0.626
Spruce	PS	Root-associated	*Archaeorhizomyces*	0.16	0.180
Spruce	PS	Saprotroph	*Pseudogymnoascus pannorum*	−0.07	0.553
Spruce	PS	Saprotroph	*Apiotrichum*	−0.01	0.955
Spruce	PS	Saprotroph	*Cylindrocarpon*	0.03	0.795
Spruce	PS	Saprotroph	*Ramariopsis crocea*	−0.08	0.511
Spruce	PS	Saprotroph	*Pseudogymnoascus*	0.11	0.348
Spruce	UI	Animal parasite	*Arthrographis*	−0.22	0.059
Spruce	**UI**	**Pathogen**	***Fusarium***	**−0.45**	**0.000**

AG, *Alnus glutinosa*; BO, *Betula pendula*; PS, *Pinus sylvestris*; PA, *Picea abies*; UI, uninoculated soil; EMF, ectomycorrhizal fungi
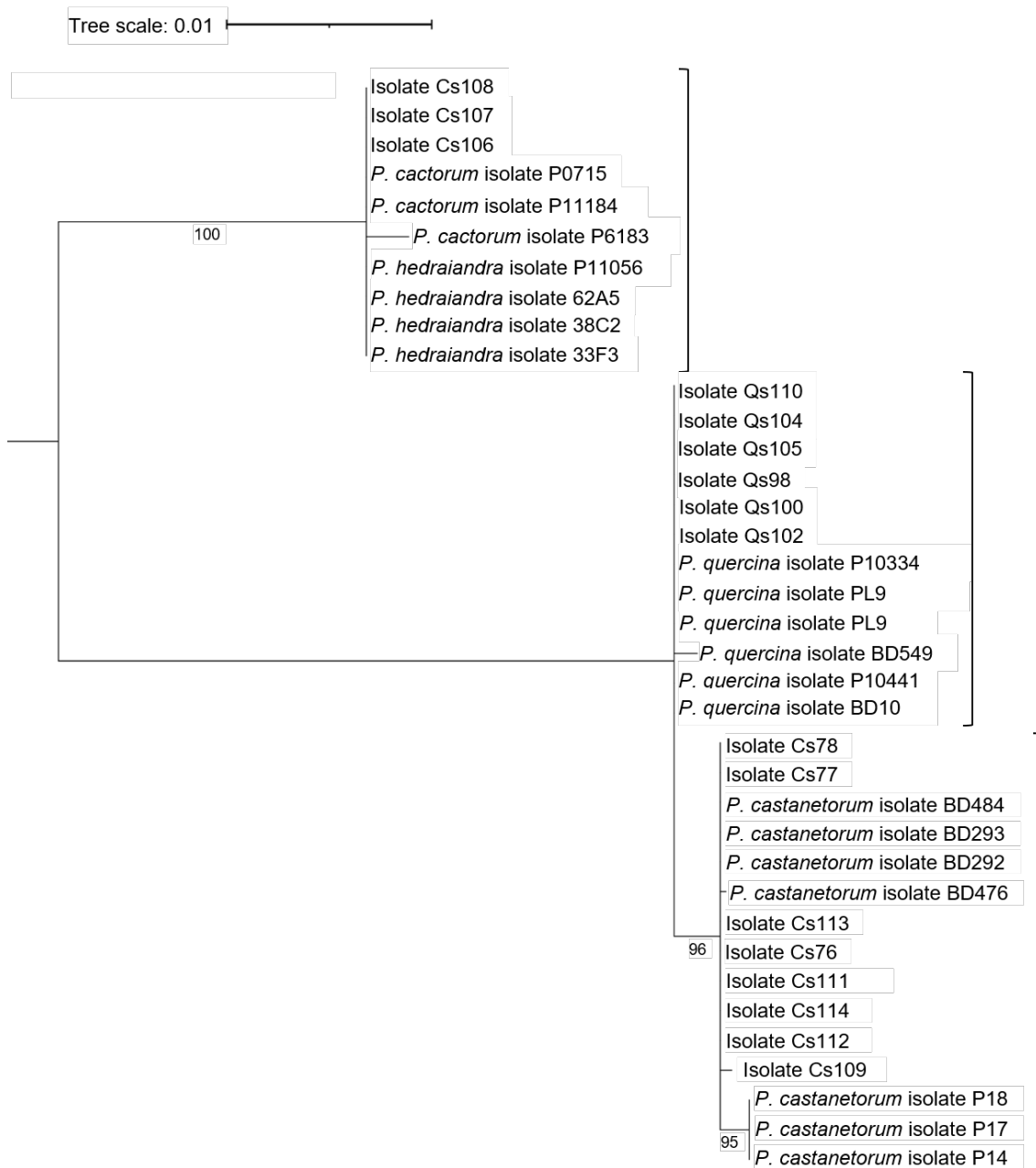
5.2 Figures

Figure S1: Maximum Likelihood phylogenetic tree determined by performing a multi-locus analysis (ITS-Btub-NADH1-cox1-HSP90) with isolates from study **IV**. Numbers indicate the bootstrap support values for each branch after re-sampling 300 times. Isolates with species names are type material obtained from GenBank and isolates named 'Isolate' were obtained in study **IV**.
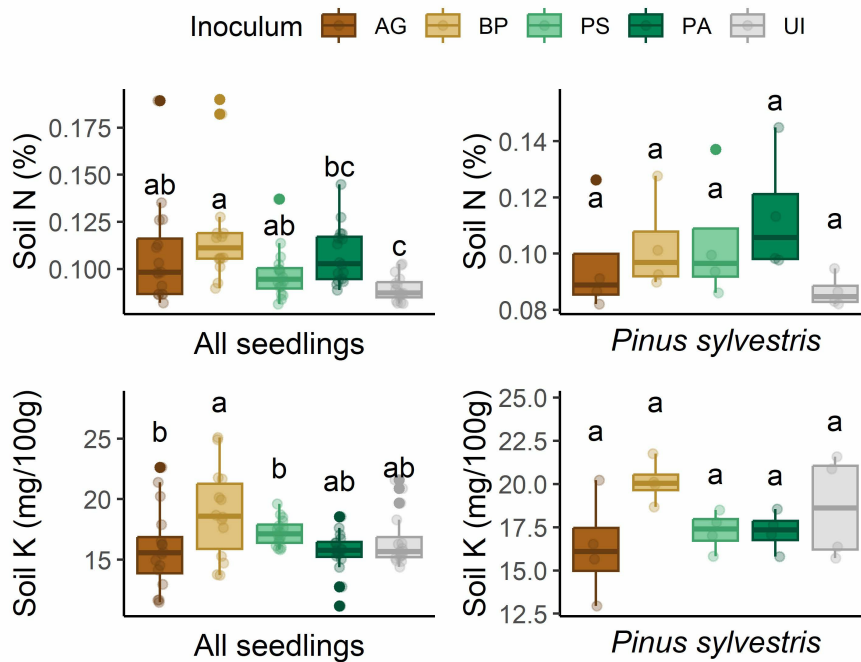
Figure S2: Nitrogen (N) and potassium (K) content in inoculated and uninoculated soils from study **I** across all seedlings (left) and in pots with Scots pine seedlings (*Pinus sylvestris*, right). Letters above the bars were obtained through a Fisher's LSD test with a false discovery rate adjustment; different letters indicate significant differences ($p \leq 0.05$)
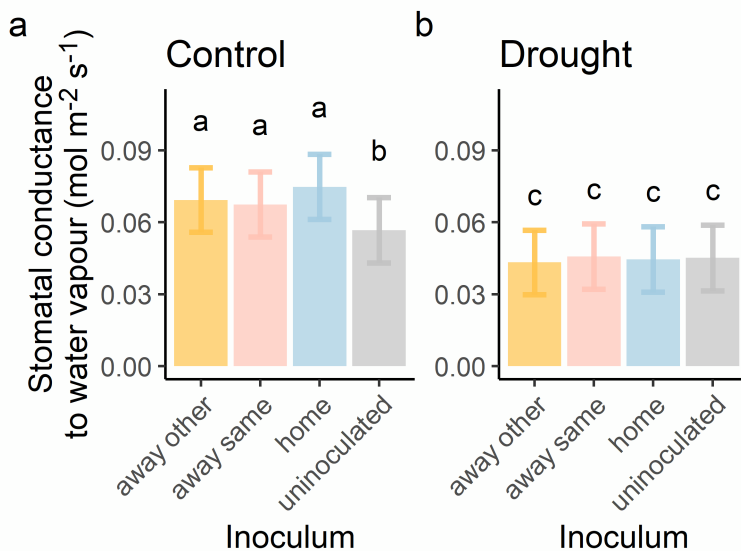

Figure S3: Leaf stomatal conductance to water vapour in well-watered seedlings (Control) and in seedlings exposed to drought during study **II**. The measurement was taken two weeks after the start of the drought treatment. Different letters above the bars indicate significant differences between treatments at $p \leq 0.05$, which were determined by an emmeans analysis of a linear mixed-effects model. Error bars represent standard error.
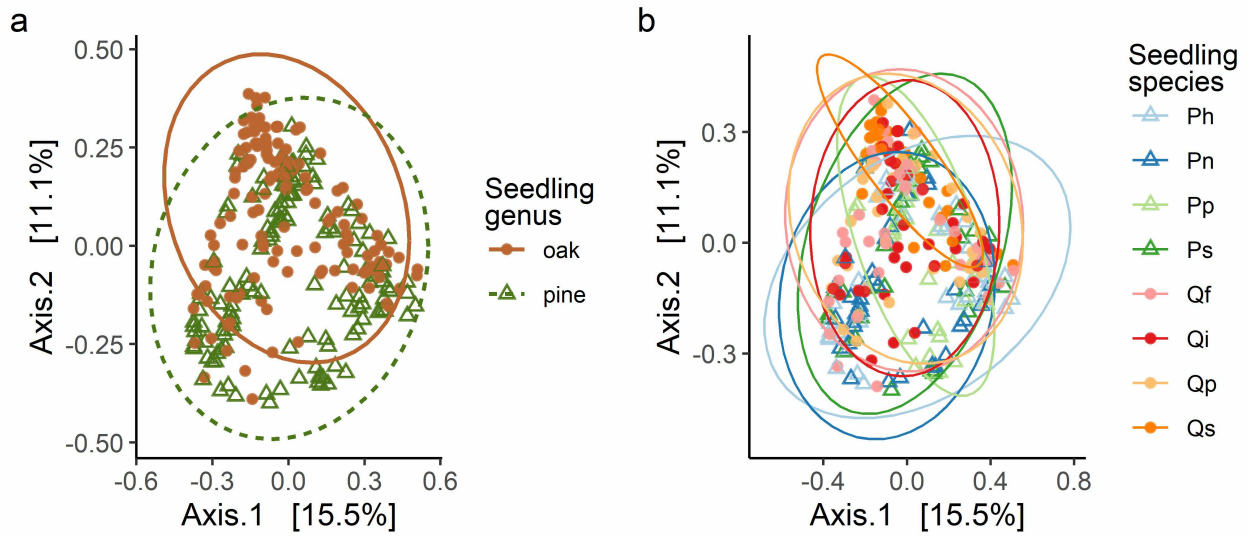
Figure S4: Principal coordinate (PC) analysis plots of fungal communities from study **II** based on Bray–Curtis distances. **(a)** Seedling genus is indicated by the symbol shape. Ellipses correspond to the seedling genus (oak, *Quercus*; pine, *Pinus*). **(b)** Ellipses correspond to the seedling species indicated by the symbol colour (Ph, *Pinus halepensis*; Pn, *Pinus nigra*; Pp, *Pinus pinaster*; Ps, *Pinus sylvestris*; Qf, *Quercus faginea*; Qi, *Quercus ilex*; Qp, *Quercus pubescens*; Qs, *Quercus suber*). The percentage of the total variance explained by each PC is indicated in parentheses.

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