MASTERARBEIT | MASTER'S THESIS

Titel | Title

Fungi and tree growth facilitation in European forests under drought conditions.

verfasst von | submitted by

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angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 830
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Molecular Microbiology, Microbial Ecology and Immunobiology
Betreut von | Supervisor:	Ass.-Prof. Mark Anthony PhD

Acknowledgements

Upon the completion of this research, I want to thank my mentor and supervisor Assist. Professor Mark Anthony for his guidance and teaching, the members of the lab for their suggestions and all the people close to me for their mental and physical support.

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Abstract (Deutsch)

Dürreperioden in Waldökosystemen stellen eine zentrale Herausforderung für den gegenwärtigen und zukünftigen Erhalt der Biodiversität, der Kohlenstoffspeicherung und der Funktionsfähigkeit von Ökosystemen dar. Um ihre Nährstoffaufnahme zu verbessern und die Stresstoleranz zu erhöhen, sind Bäume auf symbiotische Beziehungen mit Pilzen angewiesen. Dennoch sind die Auswirkungen von Dürre auf Pflanzen-Pilz-Interaktionen bislang unzureichend erforscht und variieren je nach Zusammensetzung der Baumarten. In dieser Studie wurde untersucht, wie sich inter- und intraspezifische Wechselwirkungen zwischen drei dominanten Baumarten in europäischen Wäldern – *Fagus sylvatica* (Rotbuche), *Quercus petraea* (Traubeneiche) und *Tilia cordata* (Winterlinde) – unter simulierten Dürrebedingungen in Bezug auf ihre Rhizosphärenpilzgemeinschaften verändern. Wir stellten die Hypothese auf, dass Dürre die Diversität und funktionelle Kapazität der Pilzgemeinschaften beeinflusst, wobei diese Effekte von der Baumart und dem Konkurrenzkontext abhängen. Zur Überprüfung dieser Hypothese wurden Hochbeetexperimente mit Sämlingen der drei Arten als Mono- oder Polykulturen angelegt, die entweder natürlichen Niederschlagsbedingungen oder über zwei Jahre reduzierten Niederschlägen unter Kunststoffüberdachungen ausgesetzt waren. Das Wachstum und die Entwicklung der Baumsämlinge wurden erfasst, und am Ende des Experiments wurden Rhizosphärenproben einzelner Bäume entnommen, um die Pilzdiversität mittels ITS-DNA-Metabarcoding auf der Oxford Nanopore Technology PromethION-Plattform zu charakterisieren. Unter Dürrebedingungen bot intraspezifische Konkurrenz günstigere Voraussetzungen für das Baumwachstum als interspezifische Konkurrenz. Unsere Ergebnisse zeigen, dass die Zusammensetzung der Pilzgemeinschaften empfindlich auf Unterschiede in Pflanzenart, Konkurrenzkontext und Dürre reagierte und dass die Pilzbiodiversität spezifische Muster des Pflanzenwachstums unter Dürre- und Konkurrenzstress erklärte – insbesondere im Hinblick auf symbiotische Ektomykorrhizapilze. Diese Studie verdeutlicht die vielschichtigen Auswirkungen von Dürre auf Pilzgemeinschaften und unterstreicht die Bedeutung artspezifischer Interaktionen für die Reaktionsfähigkeit von Waldökosystemen auf klimatischen Stress. Die Ergebnisse tragen zu einem besseren Verständnis der ökologischen Rolle von Pilzen für die Resilienz von Baumarten gegenüber dem Klimawandel bei und können zukünftige Strategien im Waldmanagement zur Minderung von Dürreeffekten in gemäßigten Regionen unterstützen.

Abstract (English)

Droughts in forest ecosystems are a central concern for current and future biodiversity loss, carbon sequestration, and ecosystem functioning. Trees rely on symbiotic relationships with fungi to enhance nutrient uptake and improve stress tolerance, but the impacts of drought on plant-fungal relationships remain unclear and vary across different tree species compositions. This study investigated how inter- and intraspecific interactions among three prominent tree species in European forests—*Fagus sylvatica* (European beech), *Quercus petraea* (Sessile oak), and *Tilia cordata* (small-leaved lime)—shift under simulated drought conditions in relation to their rhizosphere fungal communities. We hypothesized that drought would shift the diversity and functional capacity of fungal communities, with these effects being dependent on the tree species and competitive context. To test this, we set up raised-bed experiments with seedlings of the three species as mono- or polycultures, exposing them to ambient rainfall conditions or two years of reduced precipitation using plastic roofing. We assessed tree seedling growth and development, and at the end of the experiment, we sampled rhizosphere soils from individual trees to characterize fungal diversity using full-length ITS DNA metabarcoding on an Oxford Nanopore Technology PromethION platform. Unique levels of interspecific competition provided more favorable conditions for tree growth. Notably, *Tilia* promoted the growth of *Fagus* and *Quercus*, *Quercus* inhibited growth of both *Tilia* and *Fagus*, and *Fagus* inhibited growth of *Tilia* but stimulated *Quercus*. Fungal community composition was resistant to drought but responsive to variation in competitive context, whereby competition with *Tilia* shifted fungal communities to be distinct from *Fagus* and *Quercus*. The magnitude to which Tilia stimulated Fagus and Quercus growth was quantified using relative interaction intensities. Growth promotion by *Tilia*, modeled as a relative interaction intensity term tolerance, was tightly coupled to variation in fungal community composition. It was highest when *Fagus* and *Quercus* fungal communities shifted to closely resemble the community composition of *Tilia* monocultures, with a number of distinct plant-symbiotic ectomycorrhizal fungi significant indicator species of these competition levels. This study highlights the importance of species-specific interactions in forest ecosystem processes and potential responses to variation in the rhizosphere soil fungal community. These findings contribute to our understanding of the ecological roles of fungi in shaping forest species interactions and may inform future forest management strategies aimed at facilitating positive species interactions to boost biodiversity and productivity.

Background Knowledge

1. *Climate change impact*

The global climate is rapidly changing due to anthropogenic greenhouse gas emissions, and this will lead to increased frequency, duration, and severity of droughts (Craig D. Allen et al., 2010). Alterations in climate conditions impact global forest composition and structure, with potential changes in forests creating severe impacts on species diversity and local populations reliant on forests for their livelihood. While the effects of climate change are global, there will be important and diverse local changes to forests. For example, in Southern Europe the impact of drought due to climate change is shown to favor the proliferation of scrubs, although local Mediterranean forest species exhibit resilience and recovery to drought events (Peñuelas et al., 2001). Temperate and boreal forests of western North America also show specific signs of dieback among young trees in response to climate change (Westerling et al., 2006). Regional warming contributes to hydrological alterations such as declining precipitation falling as snow and earlier spring snowmelt, leading to lengthened summer droughts (Westerling et al., 2006). These examples present two main common variables: elevated temperatures and water stress caused by climate change. These climatic parameters are also occurring alongside a rise in the frequency of disease outbreaks (Craig D., Allen et al., 2010). Forests are therefore imperiled by climate change through both direct and indirect drivers.

2. *Forest resistance*

Forest resistance is the capacity of a forest to withstand abiotic and/or biotic disturbances without undergoing significant changes in species composition, structure, or ecological function. In contrast, resilience refers to the capacity of a forest to recover or regenerate after a disturbance (Thomson et al., 2009). Resistance arises in forest environments with increasing species diversity, where different species have complementary traits and are differentially adapted to the local environment (Schuler et al., 2017). Diversity can also contribute to increased productivity and resilience to herbivory events that are being increasingly observed in the last years due to the monoculture approach of forestry commonly implemented in the previous decades throughout much of the globe (Haase et al., 2015).

3. *Forests, fungal carbon sinks, and climate change impact*

Forests are environmentally important both above and below ground, and it has been recognized for decades that soil organisms play important ecological roles (Perry D. et al., 1982). Soil fungi are key regulators of forest carbon and nitrogen cycling, meaning global climate changes that affect these communities can have effects which reverberate throughout ecosystems. Additionally, fungal communities are the main decomposers of plant litter (Schneider et al. 2012), with effects that can regulate total forest soil C storage (Anthony et al., 2024). This is important globally because forests are a large and persistent carbon sink, and their ability to continue to remove carbon from the atmosphere and store it belowground depends on forest management and feedback to climate change. Prolonged droughts in boreal forests alongside warmer winters suppresses primary production and reduces forest C sinks (Kurz et al., 2008), and while young forests can sequester C despite increasing temperatures, wildfires and land use may limit this ability (Pan et al., 2011). At the moment, it is not clear whether fungal communities may be able to provide forest trees with resistance to climate change to sustain productivity under more stressful conditions.

Fungi through their extensive hyphal networks and symbiotic relationships with trees facilitate the transfer and stabilization of C in soils and aboveground via plant biomass production. Mycorrhizal fungi, including ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi, allocate a substantial portion of plant-derived C belowground (Hawkins et al 2023), where it is stored in fungal biomass, exudates, and

as necromass (Clemmensen et al., 2013). This process not only enhances soil organic matter (SOM) formation but also contributes to long-term carbon sequestration. Fungal necromass may be enriched with difficult to decompose compounds like chitin and melanin which can persist in soils for decades, further enhancing C storage (Fernandez et al., 2016). Saprotrophic fungi, while primarily involved in decomposing organic matter, also contribute to C sequestration by producing melanized hyphae and other complex compounds that resist decomposition (Kögel-Knabner et al., 2008). However, beyond the intrinsic chemical recalcitrance of these compounds, the stabilization of fungal-derived carbon is largely governed by its interaction with the soil matrix. Microbial products such as exudates and necromass can become physically protected through adsorption onto mineral surfaces or entrapment within soil aggregates, processes that represent a dominant pathway for long-term carbon stabilization (Cotrufo et al., 2013).While these examples demonstrate a capacity for fungi to promote C storage via production of recalcitrant compounds, an even greater role of fungi in promoting C storage occurs via physical stabilization mechanisms. Carbon can be stabilized in soil through physical protection mechanisms like organo-mineral interactions. Microbial-derived compounds such as exudates and necromass become stabilized when they adsorb onto mineral surfaces, become enmeshed within soil aggregates, or form strong bonds metal oxides, making them largely inaccessible to decomposers. This physical protection boosts long-term carbon storage for decades to millennia (Cortufo et al., 2013), and the necromass of fungi is major constituent of this SOM pool (Liang et al. 2019). Together, these mechanisms highlight the central role of fungi in stabilizing carbon belowground; however, their influence on carbon cycling extends beyond the soil.

In addition to their role in belowground carbon stabilization, fungi significantly influence aboveground carbon dynamics by enhancing plant growth and productivity. Through mycorrhizal associations, fungi improve plant nutrient acquisition. Particularly important is their part in nitrogen and phosphorus uptake as well as increasing water availability. These lead to greater photosynthetic capacity and biomass production (Averill et al., 2014). Moreover, these symbiotic interactions can enhance plant tolerance to abiotic stress and pathogens, indirectly sustaining higher levels of primary productivity and long-term carbon fixation in vegetation (van der Heijden et al., 2015).

4. *Fungi and tree biodiversity*

Fungi are key architects of tree biodiversity, influencing the composition, distribution, and functioning of forest ecosystems through a variety of mechanisms. By mediating resource allocation above and belowground (Read and Perez-Moreno, 2003), facilitating species coexistence (Read et al., 2003), and regulating plant-soil interactions (Bennette et al., 2003), fungi play a central role in shaping and maintaining tree diversity. Mycorrhizal fungi, such as ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi, form symbiotic relationships with >90% of plant species. Mycorrhizae can enhance nutrient and water uptake in host plants, and fungi can preferentially allocate resources to different tree species, and in turn dictate competitive exclusion and species coexistence (Bennett et al., 2017). For example, common mycorrhizal networks (CMNs) connect multiple tree species via the same mycorrhizal fungal network, enabling the transfer of nutrients and carbon between individual plants. This resource sharing has even been hypothesized to support the growth of less competitive species, fostering plant regeneration and diversity (Simard et al., 2012). Fungi also influence tree biodiversity through plant-soil feedback, which can be either positive or negative. Positive feedback occurs when mycorrhizal fungi enhance the growth of specific tree species by improving nutrient availability. In contrast, negative feedback arises when pathogenic fungi accumulate around tree species, suppressing their growth and creating opportunities for other species to establish (Mangan et al., 2010). This feedback helps maintain a balance between species, preventing any single species from dominating the ecosystem.

Variation in ectomycorrhizal fungal (EMF) communities is closely linked to tree productivity, as differences in fungal identity and functional traits can directly affect nutrient acquisition, growth, and

resilience of host trees. EMF species differ in their enzymatic capacities, nutrient foraging strategies, and associations with specific tree hosts (Kohler et al., 2015; Koide et al., 2014). For instance, EMF taxa with long-distance exploration types form extensive rhizomorph networks capable of mobilizing nitrogen and phosphorus from nutrient-poor soil patches, thereby increasing nutrient availability to trees that would otherwise experience limitation (Agerer et al., 2001). In contrast, short-distance or contact-type fungi may specialize in localized nutrient uptake, leading to different patterns of resource distribution within the forest. High EMF diversity can also promote complementarity in nutrient acquisition, as multiple fungal species exploit distinct ecological niches and collectively enhance the efficiency of resource use (van der Heijden et al., 2015). These dynamics mean that shifts in EMF community composition, which can be an outcome of natural succession, environmental stress, or competitive interactions among tree species, may also lead to cascading effects on tree growth, carbon allocation, and overall forest productivity. Importantly, under changing climate conditions or altered competitive regimes, the identity and diversity of EMF communities may mediate tree responses to stress, influencing not only individual tree performance but also broader ecosystem processes such as nutrient cycling, soil carbon storage, and forest resilience (Anthony et al., 2024). Accounting for these patterns is therefore crucial for understanding and predicting forest productivity under future environmental scenarios.

5. *Competition & ecology*

Competition occurs when organisms or species "fight" for the same limited resources, such as nutrients, space, light, or water, which can constrain their growth, survival, or reproduction (Callaway & Walker, 1997). There are two main forms: intraspecific competition (within the same species) and interspecific competition (between different species), and both play critical roles in shaping community structure. In the context of soil and plant–microbe systems, competition extends not only among plants but also among microbial taxa (like fungi) and between plants and microbes for soil resources (Peay et al., 2016). Competition matters for community assembly because it determines which species can coexist, drives resource partitioning, and influences biodiversity patterns and ecosystem functioning (Fukami et al., 2015). Ignoring competition, especially the capacity for microbial communities to influence plant-plant competition, overlooks a key force that shapes how plant, fungal, and microbial communities assemble, interact, and respond to environmental change.

Microbes influence plant competition by regulating nutrient availability, altering plant physiology, and shaping species-specific feedback in the rhizosphere. Soil microbial communities, including mycorrhizal fungi and pathogens, can either amplify or suppress competitive hierarchies among coexisting plants depending on the nature of their interactions (Bever et al., 2010). While beneficial microorganisms such as mycorrhizal fungi can enhance nutrient and water uptake, particularly in nutrient-poor environments, giving symbiotic hosts a competitive advantage (van der Heijden et al., 2008). There are also antagonistic microorganisms, including host-specific pathogens, which can impose negative plant–soil feedback that reduce dominance of abundant species and facilitate coexistence (Mangan et al., 2010).

This shows that microbial community composition acts as an ecological filter which determines what plant species thrive under specific soil conditions. Differences in microbial associations can lead to spatial resource partitioning of nutrients and variable competition–facilitation balances among species (Klironomos, 2002). Additionally, microbial-driven nutrient cycling processes such as mineralization and organic matter decomposition can indirectly modulate plant competition by regulating the timing and form of nutrient release (Philippot et al., 2013). These observations support that plant competition is not determined solely by abiotic resource availability or intrinsic plant traits, but also by the dynamic interactions that plants maintain with their associated microbiota. Understanding these microbial influences in our case fungal communities is essential for predicting how plant communities and particularly forest assemblages, respond to environmental change, disturbance, and species introductions.

“Fungi and tree growth facilitation in European forests under drought conditions “

Introduction

What facilitates species coexistence is one of the greatest questions in ecology. Fungi play key roles by promoting plant growth as mutualists and inhibiting it as pathogens. The Janzen–Connell effect suggests that high conspecific plant density increases vulnerability to natural enemies, reducing growth and survival (Janzen, 1970; Connell, 1971). In tropical forests, Bagchi et al. (2010) showed that fungal pathogens caused nearly complete mortality of *Pleradenophora longicuspis* seedlings at high densities. Such pathogen-driven negative density dependence prevents local dominance and promotes species coexistence, but this must be considered alongside fungal mutualists which can have the opposite effects.

Mycorrhizal associations can increase the survival rates of seedlings, particularly in nutrient-poor or stressful environments, by improving nutrient and water uptake, protecting against pathogens, and enhancing stress tolerance (van der Heijden et al., 2008). This support for early life stages is essential for maintaining tree diversity, as it allows a wider range of species to establish and persist in the ecosystem (Van der Heijden, 2015). These fungi also enhance tree resilience to environmental stressors such as drought, heavy metals, and pathogens, indirectly supporting biodiversity (Allsup et al., 2023). For example, inoculating *Pinus sylvestris* seedlings with ECM fungi improved their drought resistance (Li et al. 2023). The inoculated seedlings exhibited higher non-structural carbohydrate content and greater nutrient availability, which are crucial for maintaining growth and physiological functions under water-limited conditions (Li et al. 2023).

Ectomycorrhizal fungi (EMF) can shape plant competition and coexistence. By forming a sheath around roots and extensive biomass in the soil, EMF can reduce the prevalence and impact of soil-borne pathogens (Buscot et al. 1992). This can create conditions for positive density dependence for ECM-associated seedlings which boosts tree species co-existence (Bennet et al. 2017). While such patterns are observable when comparing EcM to arbuscular mycorrhizal fungal associated vegetation, it is critical to consider that EMF taxa also differ in foraging strategy (Agerer 2001), extracellular enzymatic production capacity (Kohler et al. 2015), and in other important ways that influence symbiotic outcomes with host plants (Averil et al., 2014). For example, EMF community composition is tightly coupled to variation in forest tree growth (Anthony et al. 2022), and different EMF species have distinct impacts on host tree seedling growth and defense (Anthony, 2025). These functional distinctions influence patterns of both above and belowground niche overlap and coexistence.

EMF fungi are not the only fungal functional guilds that can shape forest tree communities. Saprotrophic fungi are key mediators of forest biomass decomposition and nutrient turnover (Schneider et al. 2012). Saprotrophic fungi can regulate nutrient availability to tree seedlings and in turn, patterns of growth and co-existence (Deniau et al. 2017). There is therefore a balance among EMF functioning, saprotrophic decomposition, and pathogen pressure, which together determine the net effect on seedling recruitment, competitive hierarchies, and overall patterns of species co-existence (Perry et al., 1989). Fungal-mediated effects on nutrient cycling and pathogen resistance may therefore ripple to shape the success and distribution of temperate forest trees.

In European temperate forests, variation among EMF host trees plays a key role in shaping ecosystem function, influencing nutrient cycling, microbial communities, and resilience to stress. *Fagus sylvatica* (European beech) is one of the most widespread and ecologically influential broadleaf trees, ranging from southern Italy to southern Norway and from Spain to eastern Romania, where it thrives under mild winters and sufficient summer moisture (Fuchs et al., 2015). Despite this wide distribution, *F. sylvatica* is notably drought-sensitive and exhibits strong declines in growth and regeneration under heat and water stress, making it a key indicator species for studying climate vulnerability in European forests (Leuschner, 2020).

In contrast, *Quercus petraea* (sessile oak) is more drought-tolerant and often co-dominates with beech in mixed stands throughout central and western Europe (Ligot et al., 2013). It hosts a diverse EMF community, contributing substantially to forest biodiversity and enhancing ecosystem resilience under fluctuating water availability (Milović et al., 2021). *Tilia spp.* (limes), though less abundant in secondary forests, are ecologically and functionally important due to their frequent planting in urban and peri-urban environments and their comparatively high drought tolerance (Tedersoo et al., 2020). *Tilia* represents an atypical EMF host, producing nutrient-rich litter with high base cation concentrations and low carbon-to-nitrogen ratios, which promote faster nutrient cycling and shape distinct soil microbial communities compared to beech or oak (Heděnec et al., 2023). Together, these examples illustrate how variation among EMF hosts drives key feedback to forest nutrient dynamics, microbial composition, and ecosystem resilience.

Together, *Fagus*, *Quercus*, and *Tilia* represent contrasting functional and ecological strategies within ECM-dominated forests. These species range from drought-sensitive to drought-tolerant, and from slow to fast nutrient cycling. Even though they can co-occur across parts of Europe, the extent to which their interactions are mediated by the soil mycobiome remains unclear. In particular, the soil fungal community may alter facilitative or competitive interactions among these ECM hosts, with potential consequences for forest composition and resilience under climate change.

In this study, we investigated how fungal communities mediate tree seedling interactions and responses to drought in *Fagus sylvatica* (beech), *Quercus petraea* (oak), and *Tilia cordata* (lime) experimental mesocosms. Tree seedlings were grown in all individual pair-wise combinations to test their inter- and intra-specific feedback on growth. Specifically, we asked (1) how intra- and interspecific competition influences seedling growth and drought tolerance, (2) how tree species identity, competition, and drought affect soil fungal community composition and diversity, (3) whether soil fungi are linked to seedling variation in tree seedling growth and performance. By uncovering these dynamics, we provide new insights into the potential contributions of fungal communities in mediating tree growth and competition and how future drought conditions may affect fungal communities.

Methods and materials

1.*Experimental Design and Site Conditions*

The study was conducted in a raised bed experiment designed to investigate how varying tree species combinations shape growth and responses to drought. Eighteen wooden containers (3 m² each) were arranged in a randomized block design, with each container divided into four blocks (72 plots in total). Twelve seedlings were planted per block, comprising three tree species: *F. sylvatica* (beech), *Q. petraea* (oak), and *T. cordata* (lime). Two treatment factors were applied: (1) drought, with two levels (ambient rainfall vs. 50% exclusion using transparent roofs for two years), and (2) species composition, with monocultures of each species and their pairwise mixtures. Drought efficacy was evaluated based on soil moisture sensors, and reduced soil moisture by 20-50% across the year (Supplementary Figure 6). Each treatment combination was replicated three times per block, with two replicate trees per species. We randomly sampled individual trees from each treatment, and total of 164 samples were retained for analysis after removing dead and mislabeled trees.

2.*Soil growing conditions and sampling*

A standardized substrate was formulated to simulate nutrient-poor forest soils, consisting of 20% peat, 20% expanded shale, 16% pumice, 40% sand, 4% clay, and 1 kg sulfur beads per m³. Initial soil properties included pH 7.2, 4 mg/L N, <2 mg/L P, 51 mg/L K, and 75 mg/L Mg. To enhance microbial activity, 10 L of compost and 100 g of lime were added per compartment in winter 2022/2023.

Rhizosphere soils were collected by carefully removing each tree from the soil to maintain its root system, removing all loosely held soil by shaking and then placing the root system inside a plastic bag and shaking to remove rhizosphere soil adhered to the root system. Soil was then immediately placed on ice packs in a cooler and stored at −20°C with 12 hours of sampling.

3.*Plant Growth and Physiological Measurements*

Tree growth was monitored annually from 2020 to 2023, including height (cm) and stem diameter (mm) measurements. Derived metrics included annual height and diameter increments, predicted biomass volume (cm^3, assuming conical growth), and root-to-shoot ratios from destructive harvesting in 2023. Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and elemental composition (%C, %N, C/N) were analyzed on foliar samples to assess physiological responses. Note that the foliar chemical analyses were performed on mixed samples at the plot level versus on individual trees.

4.*Molecular Analysis of Fungal Communities*

For molecular analysis, genomic DNA was extracted from 0.25 g of soil using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek) following the manufacturer's protocol with an additional, initial 40 second bead beating step. The full ITS region was amplified using the fungal-specific primers ITS9MUN (5′-GTACACACCGCCCGTCG-3′) and ITS4ngUni (5′-CGCCTSCGCTTANTDATATGC-3′). The thermocycler conditions used as follows: 95°c 15 minutes, 35 cycles of 95°c for 30 seconds, 57°c for 30 seconds, 72°c for 10 minutes and 4°c hold. PCR products were verified by 1.5% agarose gel electrophoresis. Barcoded libraries were prepared and sequenced on an Oxford Nanopore PromethION platform (R9.4.1 flow cells).

5.*Bioinformatics*

Raw sequence data (FASTA) generated from the ONT PromethION platform were processed using a custom bioinformatics pipeline. All analyses were performed on a Linux-based high-performance computing cluster (LiSC) due to the computational intensity of the steps involved. Initial sequence files were concatenated into a single merged .fastq file. Sequence quality was assessed using FastQC, however, no further quality trimming was performed as the quality of the consensus reads was high (average quality per read = 43). Reads < 200 bp were removed.

Sample-specific sequences were demultiplexed using a custom script allowing for one mismatch in the 12 bp barcode sequences in both the forward and reverse barcode. Full-length Internal Transcribed Spacer (ITS) regions were extracted using ITSx (Bengtsson-Palme et al. 2013).

Downstream analysis was then conducted on full-length ITS extracted sequences using the vsearch plugin within QIIME2 (version 2024.10). Sequences were first dereplicated, singletons (sequences appearing only once across all samples) were filtered out to reduce the impact of sequencing errors, and de novo operational taxonomic units (OTUs) were clustered at a 98% sequence similarity threshold using the cluster-features-de-novo algorithm. Taxonomy was assigned to the representative sequences of each OTU using a trained model on the UNITE database (vers 19.02.2025) using the classify-sklearn algorithm. The classifier was trained on the UNITE reference sequences and taxonomy prior to the analysis using the fit-classifier-naive-bayes algorithm. Samples with fewer than 500 sequences were discarded from all downstream fungal and plant-fungal analyses. In total, 78 samples were retained for further analyses, including at least three samples from each experimental level. Samples were rarified to 722 sequences per sample using the rrarefy function in the vegan package.

6.*Statistical analysis*

All statistical analyses were conducted in R (2024.12.0+467; R Development Core Team, 2019), and significance was set to a p-value ≤ 0.05. We considered effects with p-values ≤ 0.1 as marginally significant. The effects of experimental factors (tree species, competition, and drought) on univariate response variables, including fungal alpha diversity indices (species richness and Shannon diversity, calculated using functions in vegan), were tested using linear-mixed effects models and the lme function from the nlme package. Block was used as a random variable. We used the Anova function in the car package to calculate type III sums of squares, and multiple comparisons were evaluated by computing estimated marginal means using the emmeans function. All models were inspected to meet the assumptions of ANOVA based on the distribution of residuals and QQnorm plots.

Relative interaction intensity indices were calculated to assess how intra- and interspecific tree species competition influenced plant growth using total plant biomass at the end of the experiment as the growth metric. Tolerance was calculated to assess how well a plant could sustain its own biomass when competing with a different tree species while the suppression index was calculated to assess the ability of a plant to suppress the growth of a competitor species *sensu* Fletcher et al. (2016). We calculated the mean conspecific value of each tree species (Con.Mean) and the separate mean heterospecific value of each tree species when grown with either tree species (Het.Mean). In other words, if Tilia was grown with Fagus, then we calculated the mean Tilia growth when grown with Fagus, and we repeated this to calculate a second value when Tilia was grown with Quercus. We also analyzed the individual growth of each replicate (Rep.Growth).

Tolerance = (Rep.growth – Con.Mean) / (Rep.growth + Con.Mean)
Suppression = -(Het.Mean – Rep.growth) / (Het.Mean + Rep.growth)

Differences in relative interaction intensity were analyzed only between treatments that made ecological sense, including across the different levels of competition and drought. These were analyzed using t-tests. ANOVA was not used because it would include experimental levels for which there is no logical ecological reason to make comparisons.

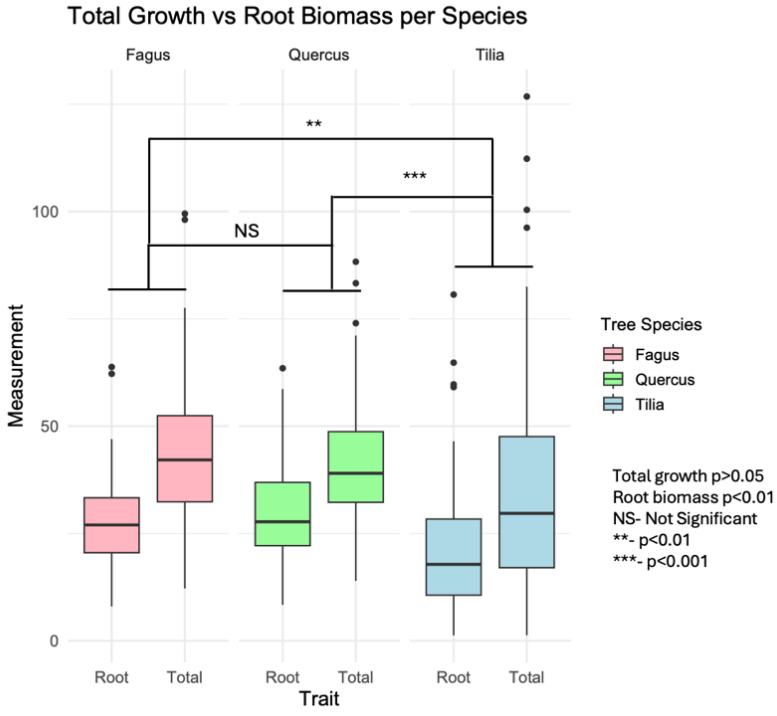
We analyzed differences in fungal community composition in response to the treatments using PERMANOVA based on Bray-Curtis dissimilarities with the adonis function in vegan and visualized these differences using principal coordinates analysis with the pcoa function in the ape package. We used indicator species analysis to identify fungal OTUs significantly associated with unique levels of significant experimental treatments using the multipatt function in the indicspecies package.

We tested the correlation between fungal community characteristics and tree physiology using pearon correlations. Because we cannot disentangle directionality in our study, we used pearon correlations versus linear regression. We specifically correlated fungal predictor variables, which included community composition (represented by PCoA axes 1 and 2), fungal OTU richness, and Shannon diversity. Separate correlations were made for every combination of tree competitor and physiology measurement.

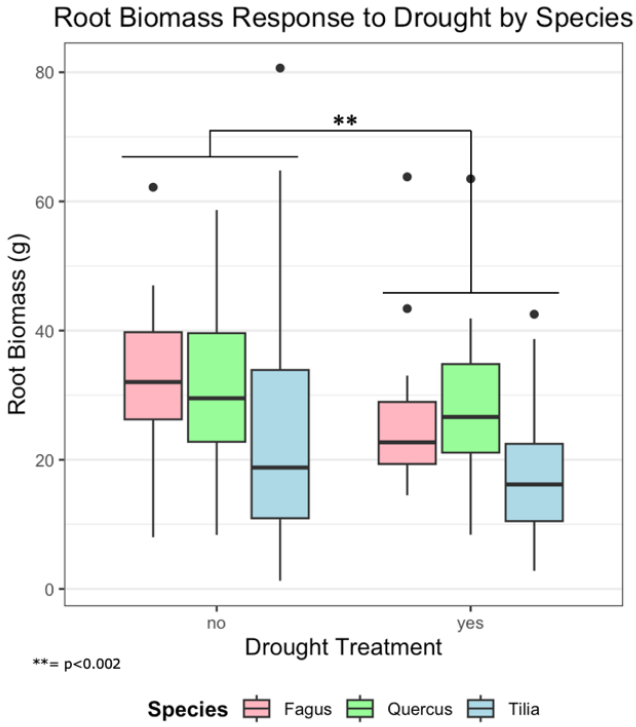
Results

1.*Plant growth across species, drought and competition.*

Tilia exhibited the highest variation in total growth, and it was on average lower than the other two species. While total growth did not vary across tree species ($p > 0.1$), *Fagus* and *Quercus* had similar root biomass ($p > 0.1$) while that of *Tilia* was lower ($p < 0.05$); Figure 1). Drought significantly reduced root biomass ($P < 0.002$), and this effect was observable for all species (Figure 2). Variation in root biomass was also significantly reduced under drought, but only for *Tilia* ($p = 0.005$). There were no differences between *Fagus* and *Quercus* when grown in monoculture or competition. On the other hand, *Fagus* and *Tilia* as competitors had a marginally significant ($0.05<p<0.1$) effect on *Fagus* growth, while *Quercus* and *Tilia* as competitors had a significant effect on *Quercus* growth ($p < 0.02$). When both *Fagus* and *Quercus* were grown with *Tilia* as their competitor, both species had higher growth (Supplementary figure 1).



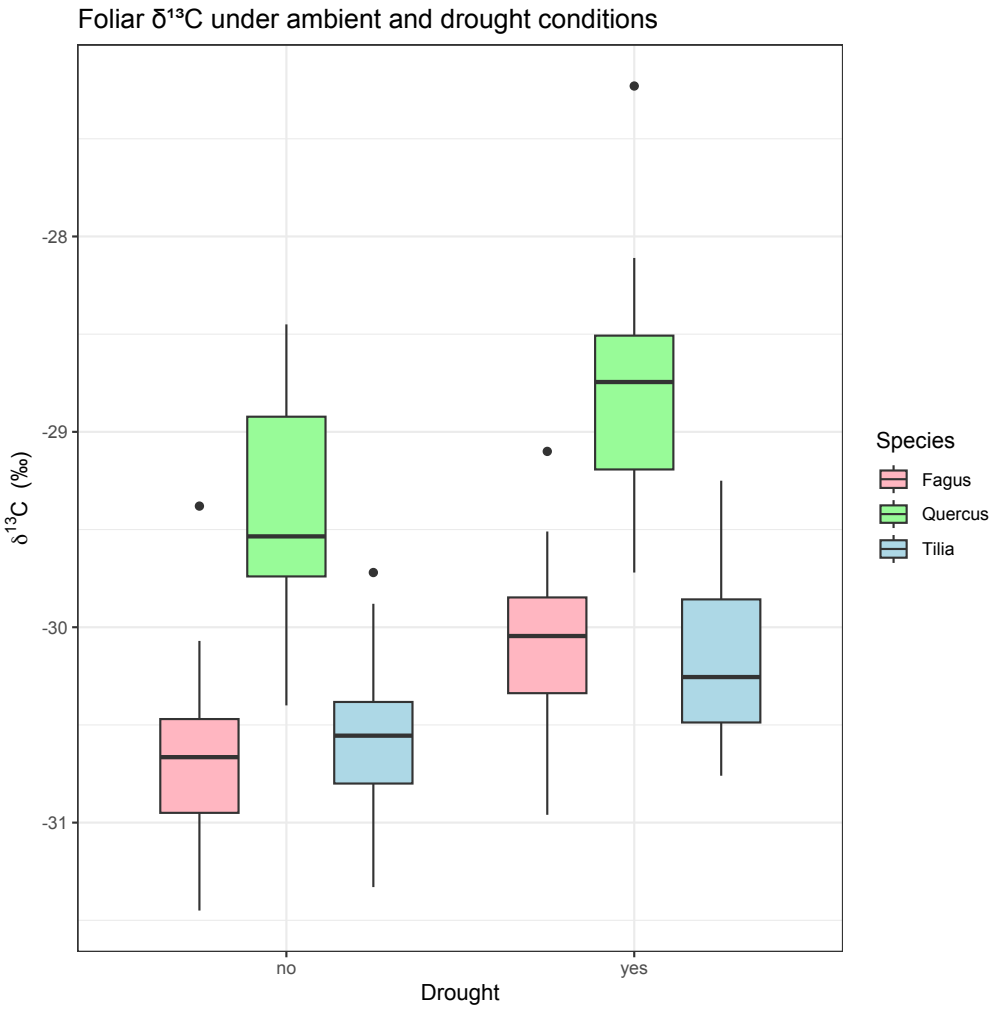
<u>Figure 1.</u> Total tree growth and root biomass (g) per species by the end of the experiment. There was no difference in total tree biomass across the tree species. Root biomass was different between Tilia-Quercus and Fagus-Tilia ($P < 0.05$).



<u>Figure 2</u>. Root biomass responses to drought by species. Root biomass was measured at the end of the experiment, and it was significantly reduced across all species.

A competitive growth advantage provided to Fagus and Quercus by Tilia is also supported by the interaction intensity indices. Suppression and tolerance interactions across species, revealed that drought altered the balance between competitive and facilitative interactions. Tilia enhanced *Quercus* and *Fagus* growth while *Quercus* reduced *Fagus* and *Tilia* growth (Figure 4). *Fagus* had little effect on Quercus, but it reduced *Tilia* growth, especially under drought. Under ambient conditions, *Fagus* grew significantly more when engaged in competition with *Tilia* compared to *Quercus* ($P = 0.02$). The same pattern was observed for *Quercus*, but this effect was marginally significant ($P = 0.06$). These positive effects of *Tilia* compared to *Fagus* and *Quercus* were reduced under drought conditions (Table 1).

Tilia seedlings exhibited the highest foliar $\delta^{15}N$ values, while both *Fagus* and *Quercus* showed less negative $\delta^{15}N$ signatures, both when grown with conspecifics and when co-occurring with *Tilia* (Supplementary Figure 2). These patterns suggest that the presence of *Tilia* did not alter N uptake among *Quercus* and *Fagus*, despite promoting their growth. Drought significantly increased $\delta^{13}C$ values across all species, indicating a consistent physiological adjustment toward reduced stomatal conductance and increased water-use efficiency under water-limited conditions. This enrichment in $\delta^{13}C$ reflects a common drought response rather than species-specific differences in carbon isotope discrimination.



<u>Figure 3</u>. Foliar δ¹³C among the tree species under ambient and drought conditions. On the y –axis the δ¹³C ratio is shown, while on the x -axis the environmental conditions. For all species under droughted conditions, foliar δ¹³C is enriched, with *Quercus* having the most enriched ratio for both conditions.

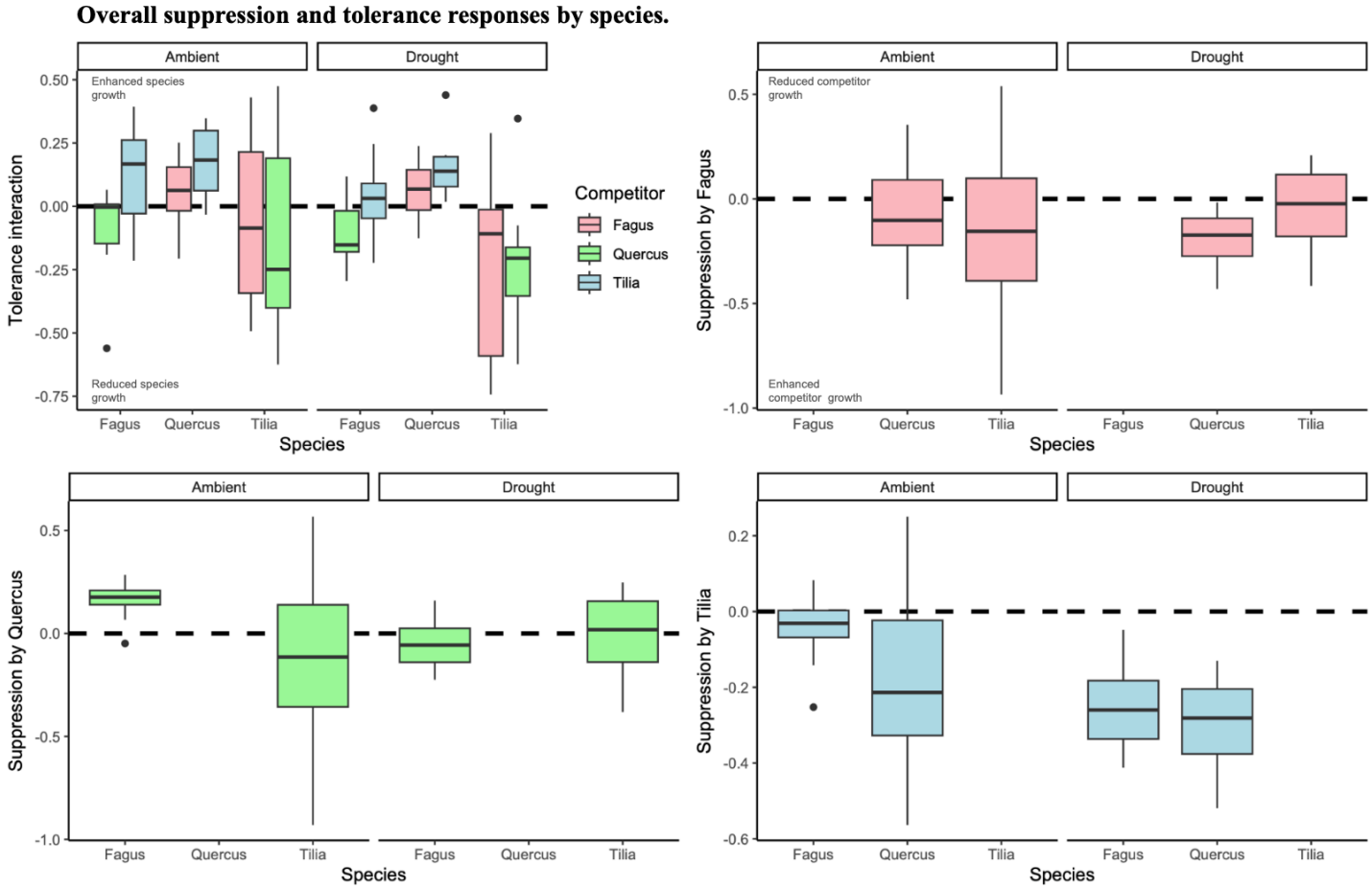


Figure 4. Overall suppression and tolerance responses by species. On the top left panel, the tolerance interaction is illustrated. On the y- axis is the tolerance values and x- axis the species. The competitors are colored accordingly in the legend. The rest of the panels show suppression per species. On the y-axis is the suppression values and on the x-axis the competitor. Horizontal dashed lines are included in each figure to denote positive and negative relative intensity interaction values. Captions to aid interpretation are also included in the panels.

Table 1. Table of multiple comparisons of tolerance among tree species combinations.
P-values show results of t-tests comparing each group and drought condition**.**

Group 1 (Species-Competition-Drought)	Group 2 (Species-Competition-Drought)	P-value
Fagus-Quercus-Ambient	Fagus-Tilia-Ambient	0.02
Quercus-Fagus-Ambient	Quercus-Tilia-Ambient	0.06
Tilia-Fagus-Ambient	Tilia-Quercus-Ambient	0.67
Fagus-Quercus-Ambient	Fagus-Quercus-Drought	0.86
Fagus-Tilia-Ambient	Fagus-Tilia-Drought	0.31
Quercus-Fagus-Ambient	Quercus-Fagus-Drought	0.8
Quercus-Tilia-Ambient	Quercus-Tilia-Drought	0.67
Tilia-Fagus-Ambient	Tilia-Fagus-Drought	0.3
Tilia-Quercus-Ambient	Tilia-Quercus-Drought	0.56

2. *Fungal community shifts upon species and competition*

Soil fungal communities shifted in response to different species competition scenarios but were resistant to drought. Overall, competition with *Quercus* and *Fagus* showed similar fungal community variation. *Tilia* significantly shifted communities as a competitor ($p<0.05$). In Supplementary Figure 5, the dominant OTUs for each tree species as a competitor are shown, along with their relative abundances in the samples. Across all competitor treatments, Thyridium (saprotroph) and Tomentella (ectomycorrhizal) were the most commonly observed OTUs. *Tilia* seedlings displayed the highest variability of interactions. Indicator species analysis revealed that competition with *Tilia* was associated with ectomycorrhizal OTUs from the genera *Inocybe* and *Tomentella*, endophytic OTUs including *Clonostachys rosea* and *Linnemannia hyalina*, and ericoid/endophytic OTUs, with *Oidiodendron periconioides* as the main representative. Additionally, putatively ectomycorrhizal connections with unidentified Ascomycota OTUs were observed.

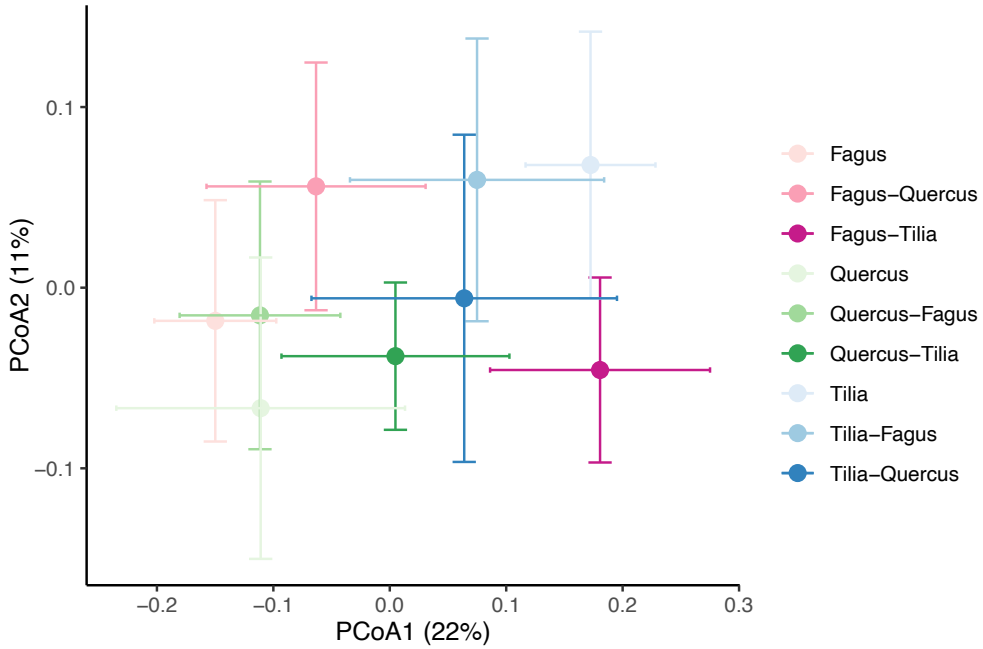


Figure 5. Principal Coordinates analysis of fungal community shifts under different tree competition scenarios. The fungal community shifts are illustrated depending on the competitor tree species. The largest fraction of variation is shown on the x-axis (PCoA1; 22%) and the additional variation at the y-axis (PCoA2; 11%). Competition with Tilia significantly shifted fungal communities while *Quercus* and *Fagus* were similar (PERMANOVA $P < 0.005$). In the legend, *Fagus*, *Quercus*, and *Tilia* alone are the monocultures, and pair-wise combinations refer to the rhizosphere mycobiome of the first species in the pairing when in the presence of the second species in the pairing (e.g., the *Fagus* mycobiome when in the presence of *Tilia* is indicated as "Fagus-Tilia").

Next, we clustered fungal OTUs into three different groups: EMF, saprotrophic fungi, and pathogenic fungi and assessed their responses to the treatments as distinct groups. Permutation based analyses of variation in community composition demonstrate that competition and species were the main effects, with drought having little impact on fungal composition. Competition was the most significant factor for ectomycorrhizal ($p<0.05$) and for saprotrophic and pathogenic fungi ($p<0.001$).

Tilia supported the highest EMF species richness of all tree species (Figure 6). There was a significant interaction between tree species and competition on EMF diversity, with Tilia tending to increase

diversity across all groups, although this effect was not statistically significant. Saprotrophic fungal richness also showed a significant interaction between species and competition ($p < 0.001$; Figure 7). *Fagus* seedlings in competition with *Tilia* exhibited an increase in saprotrophic OTUs, while *Quercus* and *Tilia* seedlings showed elevated saprotrophic richness under intraspecific competition. *Tilia* seedlings exhibited the highest saprotrophic richness across all competition scenarios. Shannon diversity of saprotrophic fungi revealed a significant interaction between species and drought, with *Fagus* and *Tilia* showing reduced diversity under drought, and *Quercus* lower diversity under ambient conditions ($p < 0.1$; Figure 7). Pathogenic fungal richness was similarly influenced by competition, with *Quercus* and *Tilia* showing increased pathogenic OTU richness under intraspecific competition, while *Fagus* maintained similar richness across all competitors ($p < 0.001$; Figure 8).

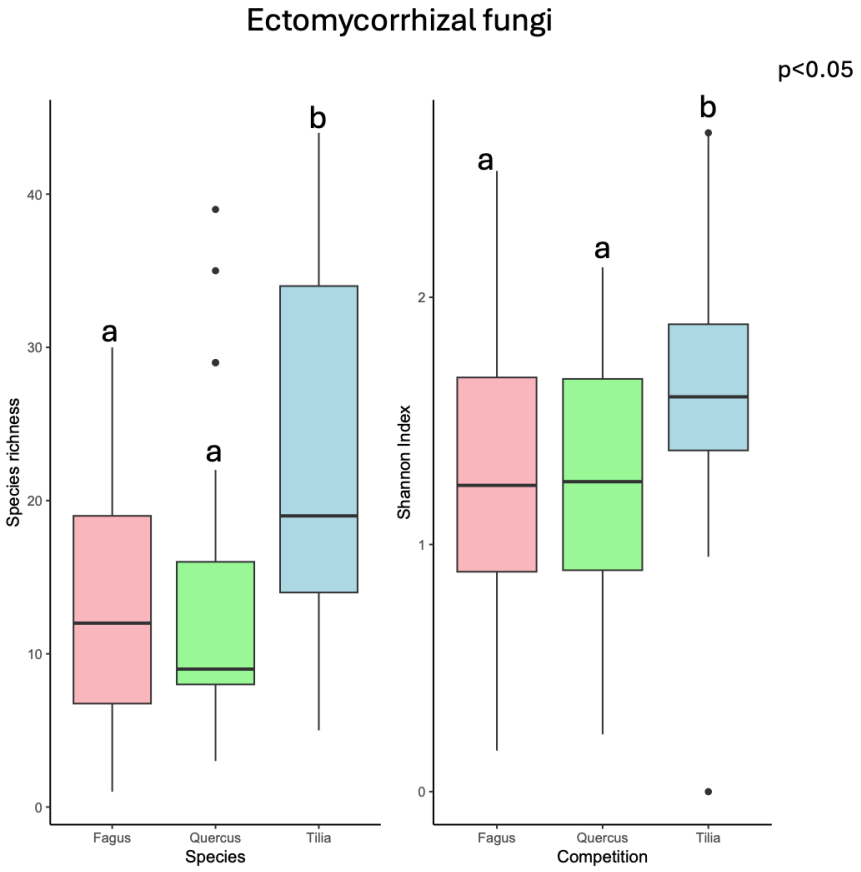
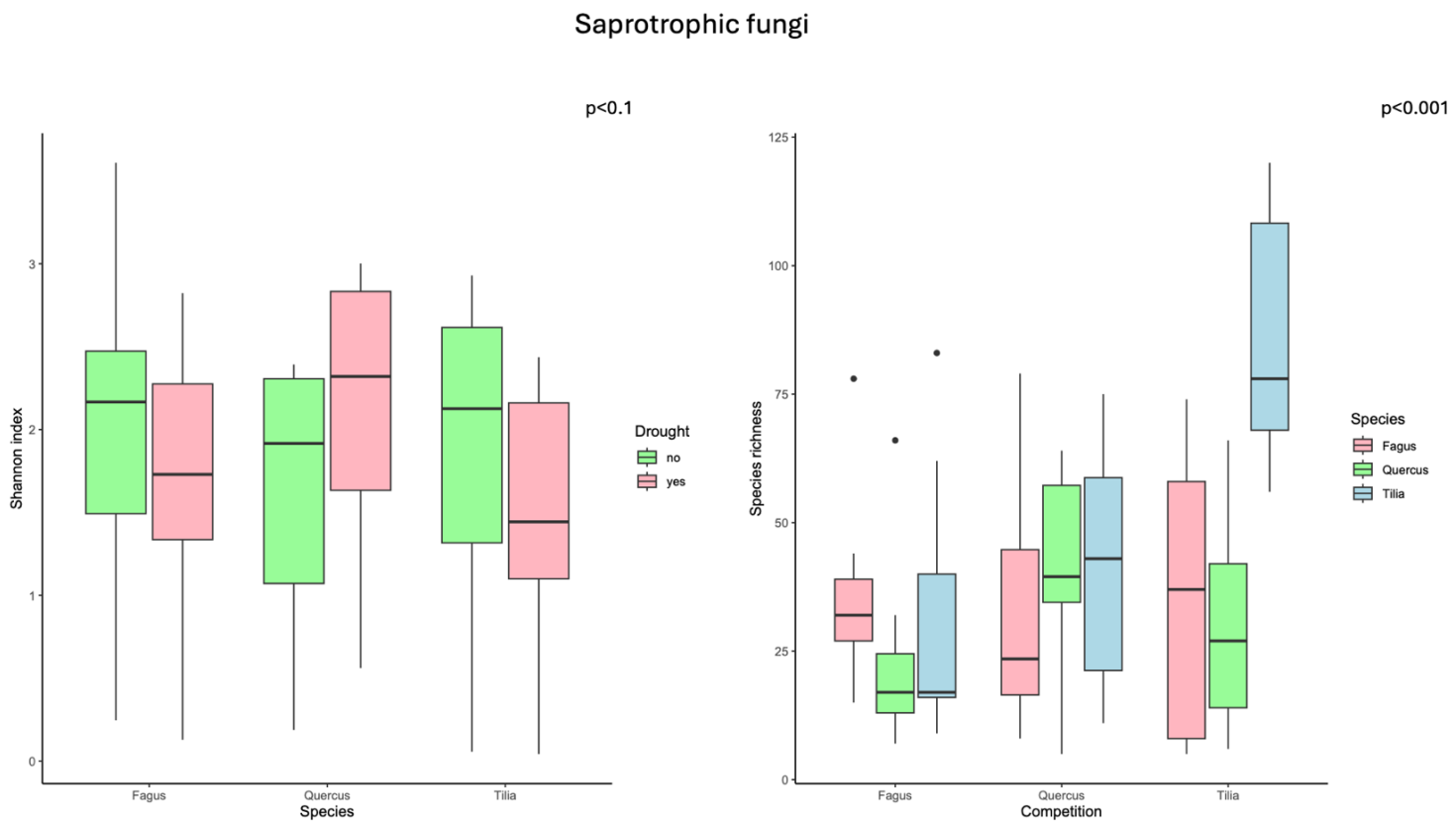


Figure 6. Species richness and Shannon diversity across species competition scenarios for ectomycorrhiza fungi. On the left side, species richness is plotted on the y -axis and the tree species on the x-axis. On the right side, Shannon diversity index is plotted across competitors. Different lowercase letters indicate significant differences ($P < 0.05$).



<u>Figure 7.</u> Shannon diversity and species richness responses to drought and competition for saprotrophic fungi. On the left side, Shannon index plotted against species under different drought conditions ($p<0.1$). On the right side, species richness plotted across competition scenario by tree species ($p<0.001$). We did not include multiple comparisons for the Shannon index because the effect was marginally significant. We also did not include them visualization purposes because there are too many levels to clearly show in one figure.

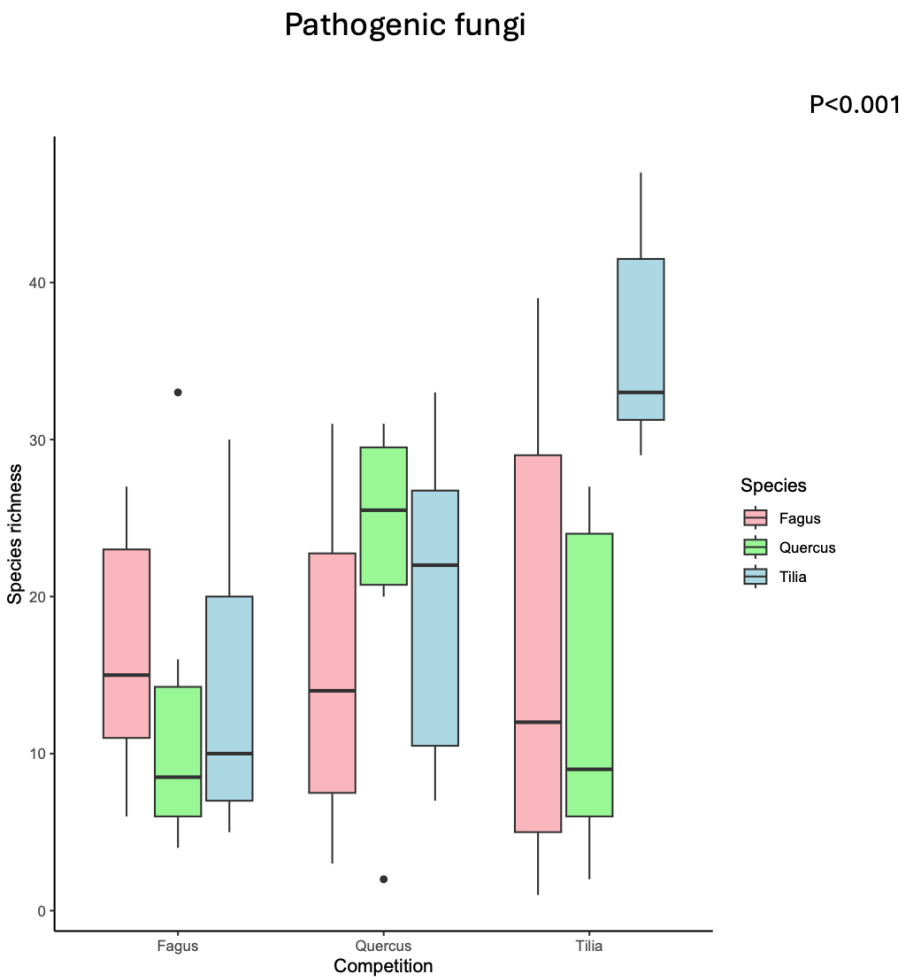


Figure 8. Species richness of pathogenic fungi across competition scenarios. Species richness of pathogenic fungal OTUs shown an increase upon intra species competition in Tilia and Quercus ($p<0.001$). We did not include multiple comparisons for visualization purposes because there are too many levels to clearly show in one figure.

3. *Fungal communities and tree growth*

Fungal richness and community composition was correlated with different tree growth metrics across the studied species. Height of *Quercus* and *Fagus* was positively correlated with fungal richness (p=0.09 and p<0.05 respectively). Tilia seedling height growth increment and root/shoot ratio were both positively correlated with fungal richness (p<0.05 and p=0.08, respectively).

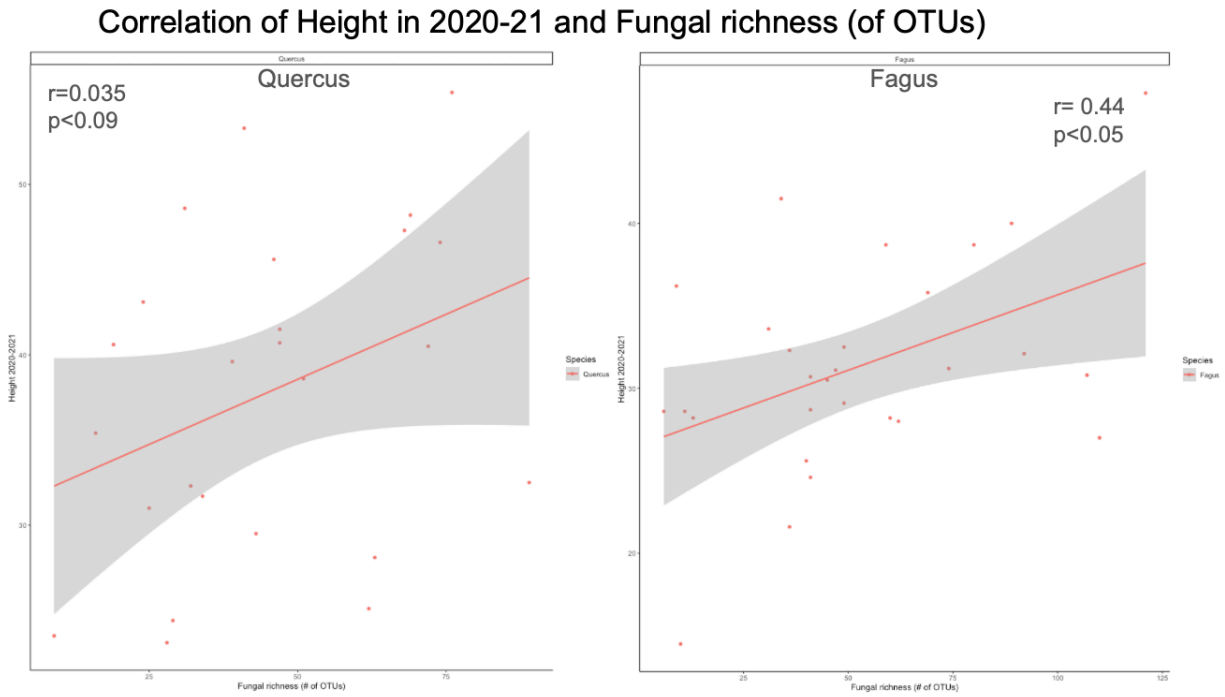
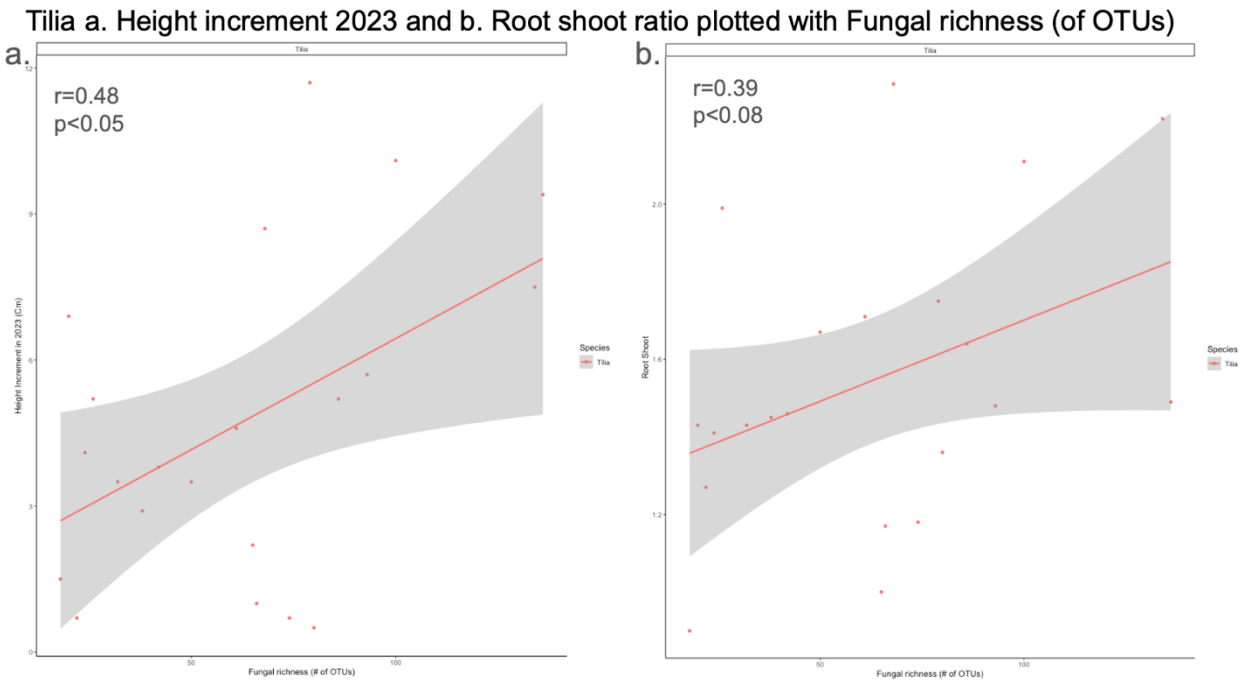
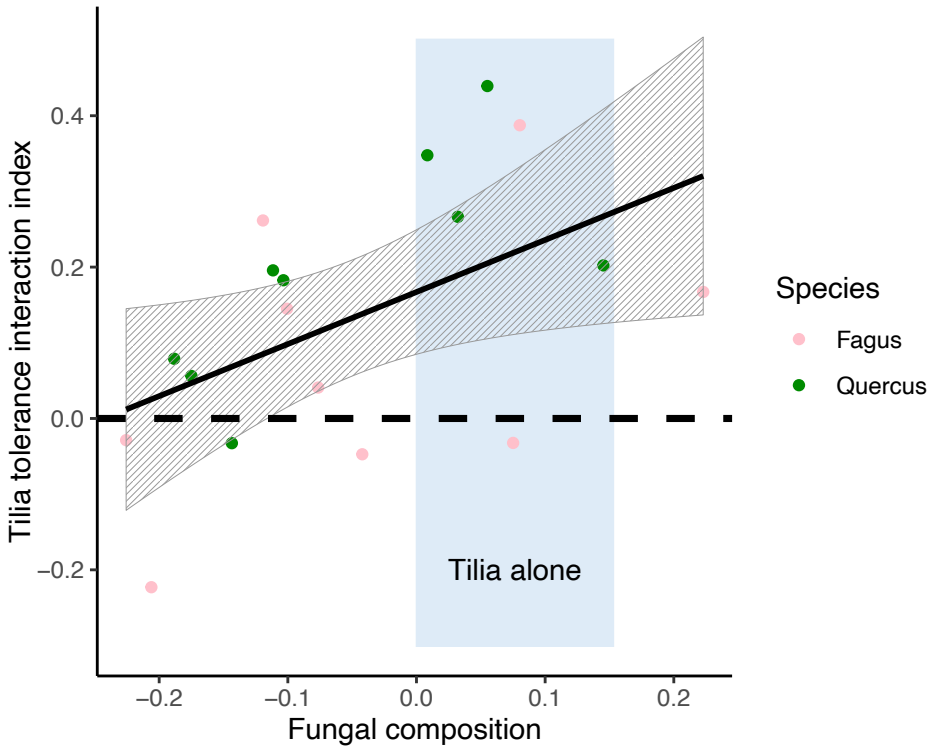


Figure 9. Correlations between plant height and fungal richness. Quercus & Fagus height in 2020-21 was correlated with total fungal richness (# of OTUs). The Pearson correlation and p-values are annotated in each plot. The shaded area around the line shows the 95% confidence interval.



<u>Figure 10.</u> Tilia growth and root and shoot ratio and fungal richness. The height increment in 2023 and root/shoot ratio were positively correlated with fungal richness. The Pearson correlation and p-values are annotated in each plot. The shaded area around the line shows the 95% confidence interval.

Fungal composition was also correlated with variation in the relative intensity index of tolerance. Tolerance, which was positive under Tilia, illustrating that it promotes *Quercus* and *Fagus* growth, was tightly linked to variation in fungal community composition (PCoA2) (Figure 11). Interestingly, there no significant correlations for tolerance when competing with *Fagus* or *Quercus*.



<u>Figure 11.</u> The correlation between tolerance competition and fungal community composition. The correlation shows fungal composition (PCoA2). The dashed line shows where tolerance is positive or negative. The solid line shows the linear correlation, and the shaded area is the 95% confidence interval. ($r = 0.56$, $P = 0.01$). The blue bar shows the mean and standard error around the composition of *Tilia* when grown alone.

Discussion

This study highlights the critical role of tree species identity, interspecific and intraspecific interactions, and to a lesser extent drought stress as key factors shaping forest tree seedling growth and soil fungal communities. Our findings reveal that *Tilia cordata* exhibits distinctive interactions with both fungal communities and neighboring tree species, distinguishing it from *Fagus sylvatica* and *Quercus petraea*. *Tilia* specifically stimulated heterospecific seedling growth, shifted soil fungal communities on co-occurring *Fagus* and *Quercus* seedlings to resemble *Tilia* communities, and these changes in fungal composition were positively coupled to plant growth responses to interacting with *Tilia*. These results underline the importance of host-specific, plant–fungal relationships for seedling performance and forest resilience under changing environmental conditions. Below, we discuss these results in more detail and identify potential mechanisms that could explain these dynamics.

Distinct tree species interspecific interactions shape seedling growth and fungal composition

The competition treatment strongly shaped seedling growth outcomes in a species-specific manner, giving rise to both facilitation and competition. *Fagus* growth was suppressed when competing with *Quercus*, even though *Fagus* had a positive effect on *Quercus*. *Quercus* and *Fagus* are two dominant mid-to-late-stage trees throughout temperate European forests, and they routinely co-occur due to forest management histories (Petritan et al. 2014, Ligot et al. 2013). While light regulates their co-existence patterns in mature forests (Ligot et al. 2013), this was unlikely in our study system given the experimental design and similar heights of the trees (Figure 1). Competition for soil resources could explain these dynamics (Pretzsch et al. 2014). Notably, *Fagus* growth is slowed more by soil nitrogen limitations than Quercus (Lévesque et al.2015), and this can give *Quercus* a competitive advantage over more nutrient demanding species like *Fagus* and possibly also *Tilia*.

In contrast to *Quercus,* which suppressed competitor tree growth, heterospecific interactions with *Tilia* promoted *Fagus* and *Quercus* growth. *Quercus* growth was improved in admixtures with *Tilia*, even though *Tilia* performed worse in the presence of *Quercus* but also *Fagus*. Collectively, these patterns suggest that *Tilia* acts as a facilitator, promoting the growth of co-occurring species but growing more slowly itself. One reason this may be is that *Tilia* produces high-quality litter with a lower C:N ratio, increasing soil N availability (Patoine et al., 2017) and in turn stimulating *Fagus* and *Quercus* growth. However, N recycling and uptake is affected by soil fungi, especially EMF communities (Read and Perez-Moreno, 2003), and their composition shifted under distinct competition scenarios. While we did not measure N cycling in this study, we were able to test how differences in fungal communities were correlated with tree growth responses to different competition scenarios.

EMF communities are compositionally distinct and more diverse under Tilia than Quercus or Fagus

Competition significantly influenced soil fungal communities, with *Tilia* acting as the strongest driver of shifts in the mycobiome. *Tilia* stimulated EMF, saprotrophic, and pathogenic fungal richness compared to the other species. *Tilia* species have been described as "generalist" hosts for EMF, and they strongly shape belowground microbial communities (Janowski et al., 2021). Increased fungal richness associated with *Tilia* may be linked to its generalist behavior in addition to specific traits such as lower leaf litter C:N ratios, which can promote fungal diversity by enhancing nitrogen availability and decomposition rates (Anthony et al., 2021; Patoine et al., 2017).

Competition with *Tilia* also restructured the rhizosphere fungal communities of *Fagus* and *Quercus* to resemble those of Tilia. *Tilia* was linked to 47 of the 50 significant indicator species (Table SX), and admixtures with *Tilia* specifically enriched relative abundances of an ectomycorrhizal taxon from the *Tomentella* (SH1282496.10FU). *Tomentella* species are known to be widespread generalists with high

extracellular enzyme activity and capacity to mobilize organic N and phosphorus (Ruess et al., 2019). When *Tilia* was competing with *Fagus* or *Quercus*, its rhizosphere fungal community appeared more like that of *Fagus* and *Quercus* (which were similar to each other). This "exchange" of fungal communities may affect plant growth responses to interspecific interactions, promoting *Quercus* and *Fagus* growth while suppressing that of Tilia.

Fungal composition and richness are linked to plant growth and relative interaction intensities

Fungal richness was positively correlated to multiple indicators of plant growth. This is consistent with established patterns across Europe demonstrating that higher forest productivity is positively linked to fungal diversity (Anthony et al. 2024). Specifically, EMF richness can affect nutrient acquisition, water relations, and seedling performance, though the magnitude and direction depend on soil nutrient context and fungal traits (Anthony, 2025). For example, under higher soil fertility, there is no link between fungal richness and tree growth (Nash et al. 2020), and these patterns are particularly influenced by host plant identity (Jonsson et al. 2001). Saprotrophic fungal richness is positively correlated with soil multifunctionality and negatively with pathogen abundance (Li et al., 2022). *Tilia* seedlings consistently hosted more diverse fungal communities, including EMF and saprotrophic fungi, and this may contribute to their facilitative effects on neighboring species.

While our study was not designed to tease apart causal mechanisms of above-belowground interactions, it establishes the idea that shifts in fungal communities are involved in tree species responses to interspecific competition. The magnitude to which *Fagus* and *Quercus* responded positively to interspecific interactions with *Tilia* was correlated with fungal community composition, and it was most stimulated when these communities shifted to resemble the community composition of Tilia associated fungi when grown in monoculture. *Tilia* monocultures were enriched in EMF *Tomentella* (SH1282496.10FU) and *Inocybe sp.* (SH1048232.10FU), in addition to other saprotrophic and potentially endophytic taxa like *Pleosporales sp.* (SH1297244.10FU) and *Debaryomycetaceae sp.* (SH1406940.10FU). These may be especially important fungi in shaping plant growth responses to interspecific interactions with *Tilia*.

Drought imposed physiological stress on all seedlings, as evidenced by reduced root biomass and foliar $\delta^{13}C$ enrichment, reflecting a shift toward conservative water-use strategies. Interestingly, *Quercus* exhibited increased saprotrophic fungal diversity under drought, suggesting a shift toward fungal taxa capable of decomposing more recalcitrant litter or exploiting limited nutrient pools under water limitation, potentially stimulating water use efficiency Previous studies have reported that drought can alter fungal abundance, enzyme activity, and taxonomic composition, indicating that fungal communities exhibit both resistance and adaptive change under stress (Canarini et al., 2024; Pena et al., 2023). These shifts likely help maintain nutrient cycling and plant access to essential elements, buffering drought impacts on seedling performance.

Conclusion

Our results have implications for forest management under future climate scenarios. Observations that *Tilia* supports diverse fungal communities and facilitates the growth of co-occurring tree species may make it a valuable component of reforestation and mixed species planting schemes. By promoting high fungal diversity and enhancing the stress tolerance of its neighbors (e.g., improved growth under drought conditions), *Tilia* could promote overall stability and development of heterospecific seedlings under both ambient and drought conditions. Importantly, *Tilia* growth decreased in admixtures with *Fagus* and *Quercus*, and its fungal communities shifted to be more resemblant of *Quercus* and *Fagus* mycobiomes. Thus, considering trade-offs between growth stimulation to *Fagus* and *Quercus* and concomitant inhibition to *Tilia* will be critical when designing future tree species admixtures for research and forestry applications. How tree species co-exist and feedback to shape forest productivity has been a major focus

of research and forestry application given its importance for ecosystem functioning, and our results demonstrate that these interactions may be further shaped by feedback to rhizosphere mycobiomes.

References

Agerer, R. (2001). Exploration types of ectomycorrhizae. *Mycorrhiza*, *11*(2), 107–114. https://doi.org/10.1007/s005720100108

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D. D., Hogg, E. H. (Ted), Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S. W., Semerci, A., & Cobb, N. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, *259*(4), 660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Allsup, C. M., George, I., & Lankau, R. A. (2023). Shifting microbial communities can enhance tree tolerance to changing climates. *Science*, *380*(6647), 835–840. https://doi.org/10.1126/science.adf2027

Anthony, M. A., Knorr, M., Moore, J. A. M., Simpson, M., & Frey, S. D. (2021). Fungal community and functional responses to soil warming are greater than for soil nitrogen enrichment. *Elementa: Science of the Anthropocene*, *9*(1). https://doi.org/10.1525/elementa.2021.000059

Anthony, M. A., Knorr, M., Moore, J. A. M., Simpson, M., & Frey, S. D. (2021). Fungal community and functional responses to soil warming are greater than for soil nitrogen enrichment. *Elementa: Science of the Anthropocene*, *9*(1). https://doi.org/10.1525/elementa.2021.000059

Anthony, M. A. (2025). Does ectomycorrhizal fungal biodiversity affect tree growth? *Fungal Ecology*, *74*, 101413. https://doi.org/10.1016/j.funeco.2025.101413

Averill, C., Turner, B. L., & Finzi, A. C. (2014). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, *505*(7484), 543–545. https://doi.org/10.1038/nature12901

Bagchi, R., Swinfield, T., Gallery, R. E., Lewis, O. T., Gripenberg, S., Narayan, L., & Freckleton, R. P. (2010). Testing the Janzen-Connell mechanism: pathogens cause overcompensating density dependence in a tropical tree. *Ecology Letters*, *13*(10), 1262–1269. https://doi.org/10.1111/j.1461-0248.2010.01520.x

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., de Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., … Nilsson, R. H. (2013). Improved software detection and extraction of ITS1 and <scp>ITS</scp> 2 from ribosomal <scp>ITS</scp> sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution*, *4*(10), 914–919. https://doi.org/10.1111/2041-210X.12073

Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., & Klironomos, J. (2017a). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, *355*(6321). https://doi.org/10.1126/science.aai8212

Bever, J. D., Platt, T. G., & Morton, E. R. (2012). Microbial Population and Community Dynamics on Plant Roots and Their Feedbacks on Plant Communities. *Annual Review of Microbiology*, *66*(1), 265–283. https://doi.org/10.1146/annurev-micro-092611-150107

Buscot, F., Weber, G., & Oberwinkler, F. (1992). Interactions between Cylindrocarpon destructans and ectomycorrhizas of Picea abies with Laccaria laccata and Paxillus involutes. *Trees*, *6*(2). https://doi.org/10.1007/BF00226585

Callaway, R. M., & Walker, L. R. (1997). Competition and Facilitation: A Synthetic Approach to Interactions in Plant Communities. *Ecology*, *78*(7), 1958. https://doi.org/10.2307/2265936

Canarini, A., Fuchslueger, L., Schnecker, J., Metze, D., Nelson, D. B., Kahmen, A., Watzka, M., Pötsch, E. M., Schaumberger, A., Bahn, M., & Richter, A. (2024). Soil fungi remain active and invest in storage compounds during drought independent of future climate conditions. *Nature Communications*, *15*(1), 10410. https://doi.org/10.1038/s41467-024-54537-y

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R. D., Wardle, D. A., & Lindahl, B. D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, *340*(6127). https://doi.org/10.1126/science.1231923

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The <scp>M</scp> icrobial <scp>E</scp> fficiency- <scp>M</scp> atrix <scp>S</scp> tabilization (<scp>MEMS</scp>) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/gcb.12113

Deniau, M., Jung, V., le Lann, C., Kellner, H., Béchade, B., Morra, T., & Prinzing, A. (2018). Janzen–Connell patterns can be induced by fungal-driven decomposition and offset by ectomycorrhizal fungi accumulated under a closely related canopy. *Functional Ecology*, *32*(3), 785–798. https://doi.org/10.1111/1365-2435.13003

Drexhage, M., Chauvière, M., Colin, F., & Nielsen, C. N. (1999). Development of structural root architecture and allometry of *Quercus petraea*. *Canadian Journal of Forest Research*, *29*(5), 600–608. https://doi.org/10.1139/x99-027

Evelin, H., Kapoor, R., & Giri, B. (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress: A review. In *Annals of Botany* (Vol. 104, Issue 7). https://doi.org/10.1093/aob/mcp251
Fletcher, R. A., Callaway, R. M., & Atwater, D. Z. (2016). An exotic invasive plant selects for increased competitive tolerance, but not competitive suppression, in a native grass. *Oecologia*, *181*(2), 499–505. https://doi.org/10.1007/s00442-016-3584-7

Frey, S. D., Lee, J., Melillo, J. M., & Six, J. (2013). The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change*, *3*(4), 395–398. https://doi.org/10.1038/nclimate1796

Fuchs, Z., Vacek, Z., Vacek, S., Černý, J., Cukor, J., Šimůnek, V., Gallo, J., & Hájek, V. (2025). Growth Responses of European Beech (Fagus sylvatica L.) and Oriental Beech (Fagus orientalis Lipsky) Along an Elevation Gradient Under Global Climate Change. *Forests*, *16*(4), 655. https://doi.org/10.3390/f16040655

Fukami, T. (2015). Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

Haase, J., Castagneyrol, B., Cornelissen, J. H. C., Ghazoul, J., Kattge, J., Koricheva, J., Scherer-Lorenzen, M., Morath, S., & Jactel, H. (2015). Contrasting effects of tree diversity on young tree growth and resistance to insect herbivores across three biodiversity experiments. *Oikos*, *124*(12), 1674–1685. https://doi.org/10.1111/oik.02090

Heděnec, P., Zheng, H., Pessanha Siqueira, D., Lin, Q., Peng, Y., Kappel Schmidt, I., Guldberg Frøslev, T., Kjøller, R., Rousk, J., & Vesterdal, L. (2023). Tree species traits and mycorrhizal association shape soil microbial communities via litter quality and species mediated soil properties. *Forest Ecology and Management*, *527*, 120608. https://doi.org/10.1016/j.foreco.2022.120608

Herzog, C., Peter, M., Pritsch, K., Günthardt-Goerg, M. S., & Egli, S. (2013). Drought and air warming affects abundance and exoenzyme profiles of *Cenococcum geophilum* associated with *Quercus robur* , *Q. petraea* and *Q. pubescens*. *Plant Biology*, *15*(s1), 230–237. https://doi.org/10.1111/j.1438-8677.2012.00614.x

Hobbie, E. A., & Colpaert, J. v. (2003). Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytologist*, *157*(1), 115–126. https://doi.org/10.1046/j.1469-8137.2003.00657.x

Hobbie, E. A., & Högberg, P. (2012). Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. *New Phytologist*, *196*(2), 367–382. https://doi.org/10.1111/j.1469-8137.2012.04300.x

Högberg, P., Näsholm, T., Franklin, O., & Högberg, M. N. (2017). Tamm Review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *Forest Ecology and Management*, *403*, 161–185. https://doi.org/10.1016/j.foreco.2017.04.045

I. Thompson, B. Mackey, S. McNulty, & A. Mosseler. (2009). Forest Resilience, Biodiversity, and Climate Change: a synthesis of the biodiversity/resilience/stability relationship in forest ecosystems. *Secretariat of the Convention on Biological Diversity*, *43*, 1–67.
Janowski, D., & Nara, K. (2021). Unique host effect of Tilia japonica on ectomycorrhizal fungal communities independent of the tree's dominance: A rare example of a generalist host? *Global Ecology and Conservation*, *31*, e01863.
https://doi.org/10.1016/j.gecco.2021.e01863

Janzen, D. H. (1970). Herbivores and the Number of Tree Species in Tropical Forests. *The American Naturalist*, *104*(940), 501–528. https://doi.org/10.1086/282687

Jonsson, L. M., Nilsson, M., Wardle, D. A., & Zackrisson, O. (2001). Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *Oikos*, *93*(3), 353–364. https://doi.org/10.1034/j.1600-0706.2001.930301.x

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, *417*(6884), 67–70. https://doi.org/10.1038/417067a

Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., & Leinweber, P. (2008). Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. In *Journal of Plant Nutrition and Soil Science* (Vol. 171, Issue 1). https://doi.org/10.1002/jpln.200700048

Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., Canbäck, B., Choi, C., Cichocki, N., Clum, A., Colpaert, J., Copeland, A., Costa, M. D., Doré, J., Floudas, D., Gay, G., Girlanda, M.,

Henrissat, B., Herrmann, S., … Martin, F. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, *47*(4), 410–415. https://doi.org/10.1038/ng.3223

Koide, R. T., Fernandez, C., & Malcolm, G. (2014). Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist*, *201*(2), 433–439. https://doi.org/10.1111/nph.12538

Kurz, W. A., Stinson, G., Rampley, G. J., Dymond, C. C., & Neilson, E. T. (2008). Risk of natural disturbances makes future contribution of Canada's forests to the global carbon cycle highly uncertain. *Proceedings of the National Academy of Sciences*, *105*(5), 1551–1555. https://doi.org/10.1073/pnas.0708133105

Köhler, J., Yang, N., Pena, R., Raghavan, V., Polle, A., & Meier, I. C. (2018). Ectomycorrhizal fungal diversity increases phosphorus uptake efficiency of European beech. *New Phytologist*, *220*(4), 1200–1210. https://doi.org/10.1111/nph.15208

Langenbruch, C., Helfrich, M., & Flessa, H. (2012). Effects of beech (Fagus sylvatica), ash (Fraxinus excelsior) and lime (Tilia spec.) on soil chemical properties in a mixed deciduous forest. *Plant and Soil*, *352*(1–2), 389–403. https://doi.org/10.1007/s11104-011-1004-7

Leuschner, C. (2020). Drought response of European beech (Fagus sylvatica L.)—A review. *Perspectives in Plant Ecology, Evolution and Systematics*, *47*, 125576. https://doi.org/10.1016/j.ppees.2020.125576

Li, M., Wang, H., Zhao, X., Feng, W., Ding, G., & Quan, W. (2023). Effect of Ectomycorrhizal Fungi on the Drought Resistance of Pinus massoniana Seedlings. *Journal of Fungi*, *9*(4), 471. https://doi.org/10.3390/jof9040471

Li, Z., Liu, X., Zhang, M., & Xing, F. (2022). Plant Diversity and Fungal Richness Regulate the Changes in Soil Multifunctionality in a Semi-Arid Grassland. *Biology*, *11*(6), 870. https://doi.org/10.3390/biology11060870

Ligot, G., Balandier, P., Fayolle, A., Lejeune, P., & Claessens, H. (2013). Height competition between Quercus petraea and Fagus sylvatica natural regeneration in mixed and uneven-aged stands. *Forest Ecology and Management*, *304*, 391–398. https://doi.org/10.1016/j.foreco.2013.05.050

Liu, Y., Yu, S., Xie, Z., & Staehelin, C. (2012). Analysis of a negative plant–soil feedback in a subtropical monsoon forest. *Journal of Ecology*, *100*(4), 1019–1028. https://doi.org/10.1111/j.1365-2745.2012.01953.x

Mangan, S. A., Schnitzer, S. A., Herre, E. A., MacK, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010). Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature*, *466*(7307). https://doi.org/10.1038/nature09273

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H. M., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A. H. M., & Raaijmakers, J. M. (2011). Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria. *Science*, *332*(6033), 1097–1100. https://doi.org/10.1126/science.1203980

Morin, C., Samson, J., & Dessureault, M. (1999). Protection of black spruce seedlings against Cylindrocladium root rot with ectomycorrhizal fungi. *Canadian Journal of Botany*, *77*(1), 169–174. https://doi.org/10.1139/b99-025

Nash, J., Laushman, R., & Schadt, C. (2020). Ectomycorrhizal fungal diversity interacts with soil nutrients to predict plant growth despite weak plant-soil feedbacks. *Plant and Soil*, *453*(1–2), 445–458. https://doi.org/10.1007/s11104-020-04616-y

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., Phillips, O. L., Shvidenko, A., Lewis, S. L., Canadell, J. G., Ciais, P., Jackson, R. B., Pacala, S. W., McGuire, A. D., Piao, S., Rautiainen, A., Sitch, S., & Hayes, D. (2011). A Large and Persistent Carbon Sink in the World's Forests. *Science*, *333*(6045), 988–993. https://doi.org/10.1126/science.1201609

Patoine, G., Thakur, M. P., Friese, J., Nock, C., Hönig, L., Haase, J., Scherer-Lorenzen, M., & Eisenhauer, N. (2017). Plant litter functional diversity effects on litter mass loss depend on the macro-detritivore community. *Pedobiologia*, *65*, 29–42. https://doi.org/10.1016/j.pedobi.2017.07.003

Peay, K. G., Kennedy, P. G., & Talbot, J. M. (2016). Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology*, *14*(7), 434–447. https://doi.org/10.1038/nrmicro.2016.59

Pena, R., Tejedor, J., Zeller, B., Dannenmann, M., & Polle, A. (2013). Interspecific temporal and spatial differences in the acquisition of litter-derived nitrogen by ectomycorrhizal fungal assemblages. *New Phytologist*, *199*(2), 520–528. https://doi.org/10.1111/nph.12272

Peñuelas, J., Lloret, F., & Montoya, R. (2001). Severe Drought Effects on Mediterranean Woody Flora in Spain. *Forest Science*, *47*(2), 214–218. https://doi.org/10.1093/forestscience/47.2.214
PERRY, D. A., MARGOLIS, H., CHOQUETTE, C., MOLINA, R., & TRAPPE, J. M. (1989). Ectomycorrhizal mediation of competition between coniferous tree species. *New Phytologist*, *112*(4), 501–511. https://doi.org/10.1111/j.1469-8137.1989.tb00344.x

Perry, D. A., Meyer, M. M., Egeland, D., Rose, S. L., & Pilz, D. (1982). Seedling growth and mycorrhizal formation in clearcut and adjacent, undisturbed soils in montana: A green-house bioassay. *Forest Ecology and Management*, *4*(3), 261–273. https://doi.org/10.1016/0378-1127(82)90004-4

Petritan, I. C., Marzano, R., Petritan, A. M., & Lingua, E. (2014). Overstory succession in a mixed Quercus petraea–Fagus sylvatica old growth forest revealed through the spatial pattern of competition and mortality. *Forest Ecology and Management*, *326*, 9–17. https://doi.org/10.1016/j.foreco.2014.04.017

Philippot, L., Raaijmakers, J. M., Lemanceau, P., & van der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology*, *11*(11), 789–799. https://doi.org/10.1038/nrmicro3109

Pretzsch, H., Bielak, K., Block, J., Bruchwald, A., Dieler, J., Ehrhart, H.-P., Kohnle, U., Nagel, J., Spellmann, H., Zasada, M., & Zingg, A. (2013). Productivity of mixed versus pure stands of oak (Quercus petraea (Matt.) Liebl. and Quercus robur L.) and European beech (Fagus sylvatica L.) along an ecological gradient. *European Journal of Forest Research*, *132*(2), 263–280. https://doi.org/10.1007/s10342-012-0673-y

Radoglou, K., Dobrowolska, D., Spyroglou, G., & Valeriu-Norocel, N. (2009). A review on the ecology and silviculture of limes: (Tilia cordata Mill., Tilia platyphyllos Scop, and Tilia tomentosa Moench.) in Europe. *BODENKULTUR -WIEN AND MUNCHEN*, *3*, 9–20.

Read, D. J., & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist*, *157*(3), 475–492. https://doi.org/10.1046/j.1469-8137.2003.00704.x

Ruess, R. W., Swanson, M. M., Kielland, K., McFarland, J. W., Olson, K. D., & Taylor, D. L. (2019). Phosphorus Mobilizing Enzymes of Alnus-Associated Ectomycorrhizal Fungi in an Alaskan Boreal Floodplain. *Forests*, *10*(7), 554. https://doi.org/10.3390/f10070554

Schneider, T., Keiblinger, K. M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., & Riedel, K. (2012). Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *The ISME Journal*, *6*(9), 1749–1762. https://doi.org/10.1038/ismej.2012.11

Schuler, L. J., Bugmann, H., & Snell, R. S. (2017). From monocultures to mixed-species forests: is tree diversity key for providing ecosystem services at the landscape scale? *Landscape Ecology*, *32*(7), 1499–1516. https://doi.org/10.1007/s10980-016-0422-6

Schulz, H., Härtling, S., & Stange, C. F. (2011). Species-specific differences in nitrogen uptake and utilization by six European tree species. *Journal of Plant Nutrition and Soil Science*, *174*(1), 28–37. https://doi.org/10.1002/jpln.201000004

Simard, S. W., Beiler, K. J., Bingham, M. A., Deslippe, J. R., Philip, L. J., & Teste, F. P. (2012). Mycorrhizal networks: Mechanisms, ecology and modelling. In *Fungal Biology Reviews* (Vol. 26, Issue 1). https://doi.org/10.1016/j.fbr.2012.01.001

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., Ruiz, L. V., Vasco-Palacios, A. M., Thu, P. Q., Suija, A., Smith, M. E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., … Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213). https://doi.org/10.1126/science.1256688

Tedersoo, L., Pärtel, K., Jairus, T., Gates, G., Põldmaa, K., & Tamm, H. (2009). Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales*. *Environmental Microbiology*, *11*(12), 3166–3178. https://doi.org/10.1111/j.1462-2920.2009.02020.x

van der Heijden, M. G. A., Bardgett, R. D., & van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. In *Ecology Letters* (Vol. 11, Issue 3). https://doi.org/10.1111/j.1461-0248.2007.01139.x

van der Heijden, M. G. A., Martin, F. M., Selosse, M., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, *205*(4), 1406–1423. https://doi.org/10.1111/nph.13288

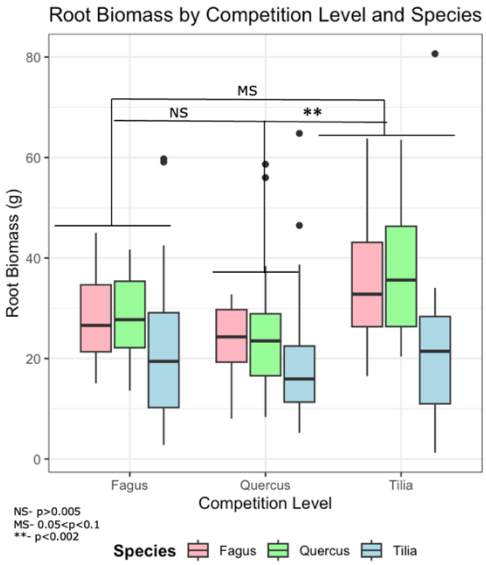
Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, *111*(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111

Westerling, A. L., Hidalgo, H. G., Cayan, D. R., & Swetnam, T. W. (2006). Warming and Earlier Spring Increase Western U.S. Forest Wildfire Activity. *Science*, *313*(5789), 940–943. https://doi.org/10.1126/science.1128834

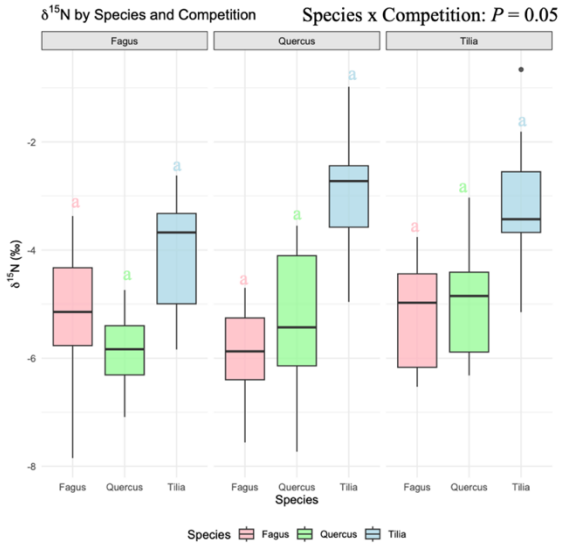
Wu, B.-W., Gao, C., Chen, L., Buscot, F., Goldmann, K., Purahong, W., Ji, N.-N., Wang, Y.-L., Lü, P.-P., Li, X.-C., & Guo, L.-D. (2018). Host Phylogeny Is a Major Determinant of Fagaceae-Associated Ectomycorrhizal Fungal Community Assembly at a Regional Scale. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02409

Železnik, P., Westergren, M., Božič, G., Eler, K., Bajc, M., Helmisaari, H.-S., Horvath, A., & Kraigher, H. (2019). Root growth dynamics of three beech (Fagus sylvatica L.) provenances. *Forest Ecology and Management*, *431*, 35–43. https://doi.org/10.1016/j.foreco.2018.06.024

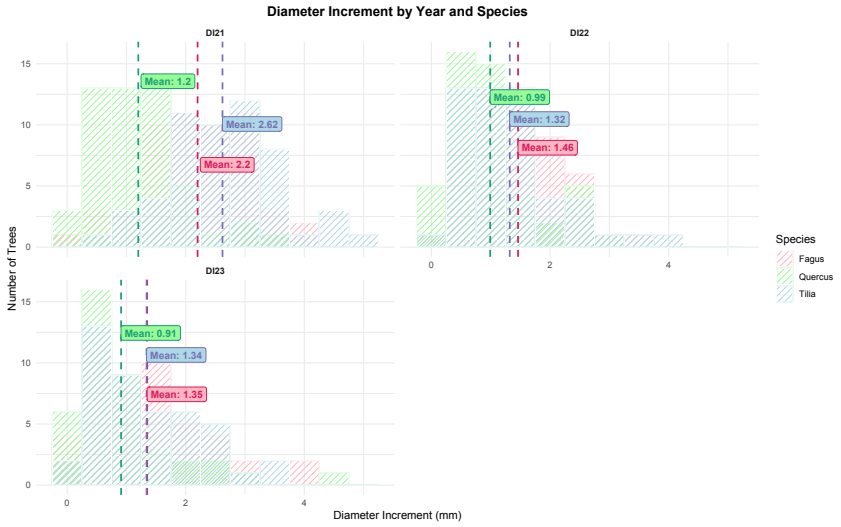
Supplementary material



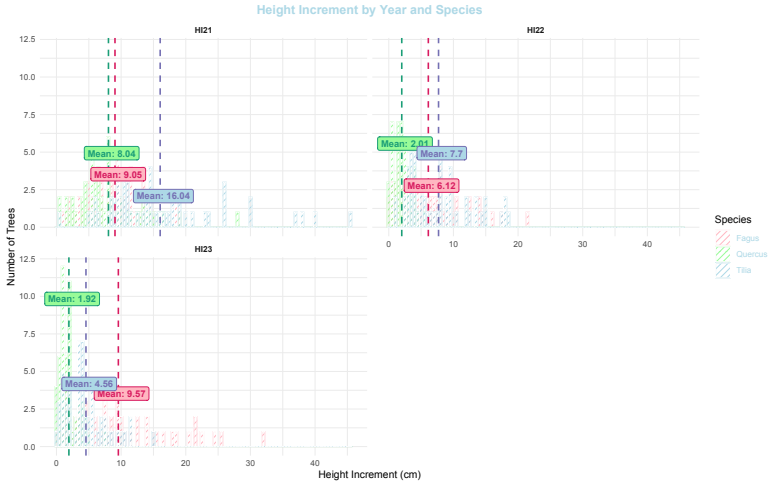
<u>Supplementary Figure 1.</u> Root biomass (g) response by competition level and species. On the y-axis the root biomass in g is shown, while on the x-axis the competition of each species for each competition is illustrated. The graph shows statistically significant the effect of Tilia as competition.



<u>Supplementary Figure 2.</u> $\delta^{15}N$ ratio by species and competition.
In this graph on the y-axis, we have the $\delta^{15}N$ and on the x – axis the competition. Statistical analysis showed that species and competition are significant p=0.05. The $\delta^{15}N$ of each species was compared for the different competitions but no significant difference was found (illustrated by the letter a). Notable is the enriched ratio of $\delta^{15}N$ for Tilia, an indicator of ECM associations.

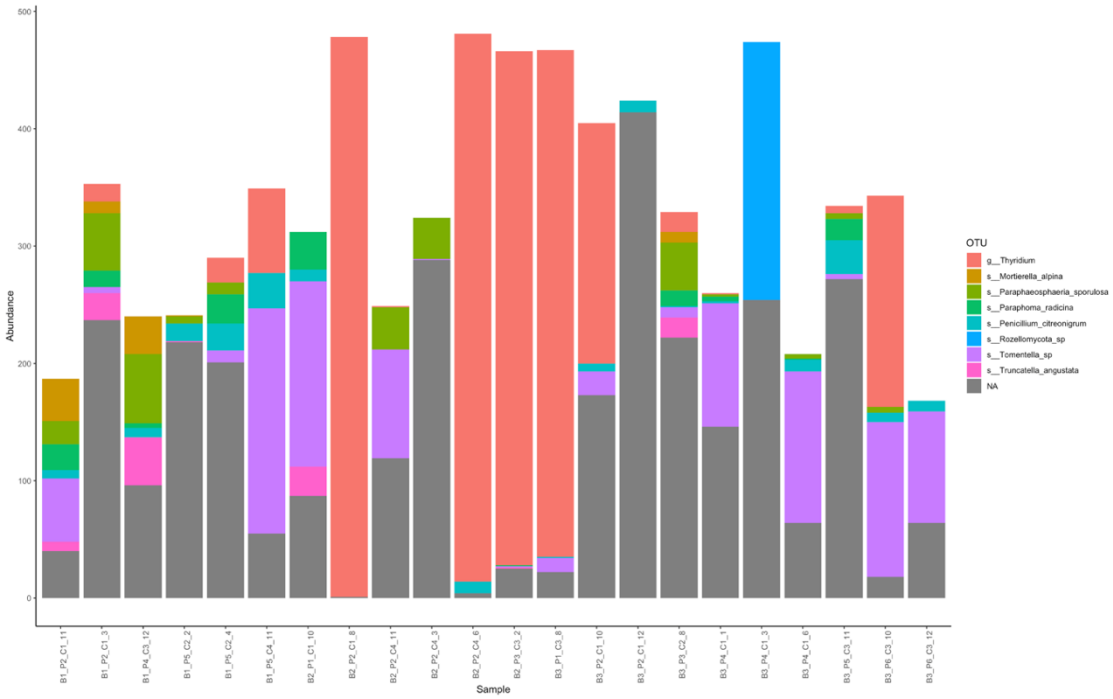


<u>Supplementary figure 3.</u> Diameter increment per year and species.
On the graph the number of trees is shown on the y- axis and the diameter increment on the x – axis. The mean increment for the trees per species and per years 2020-2021,2021-2022,2023-2024 were calculated.

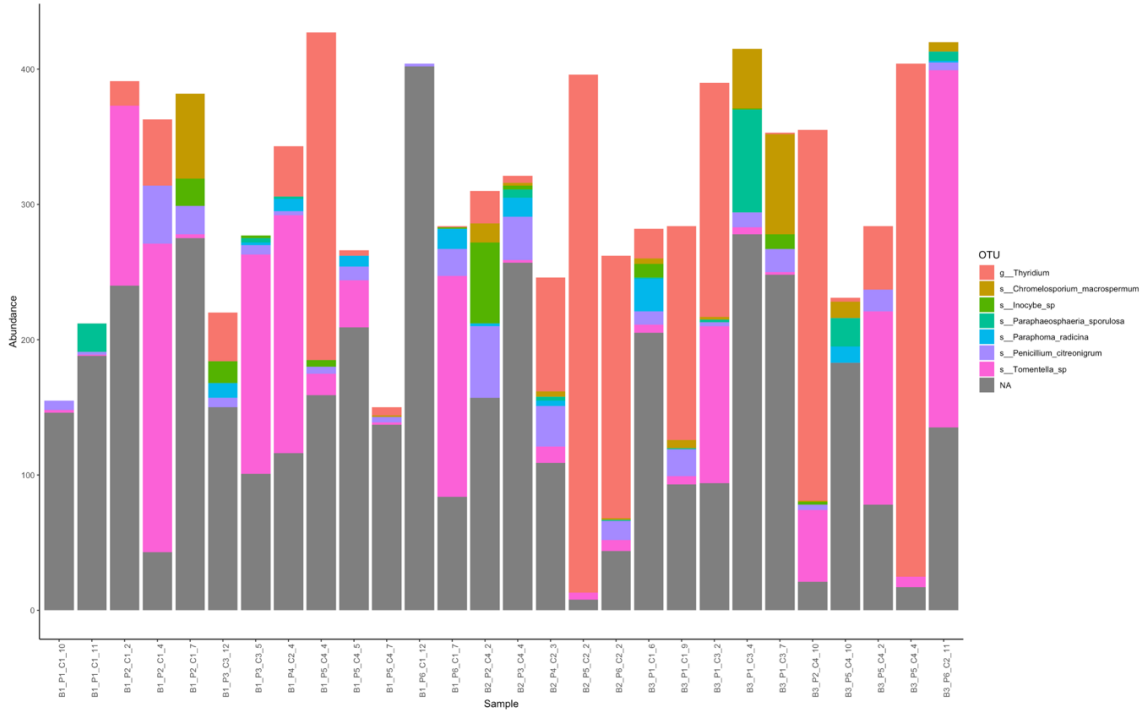


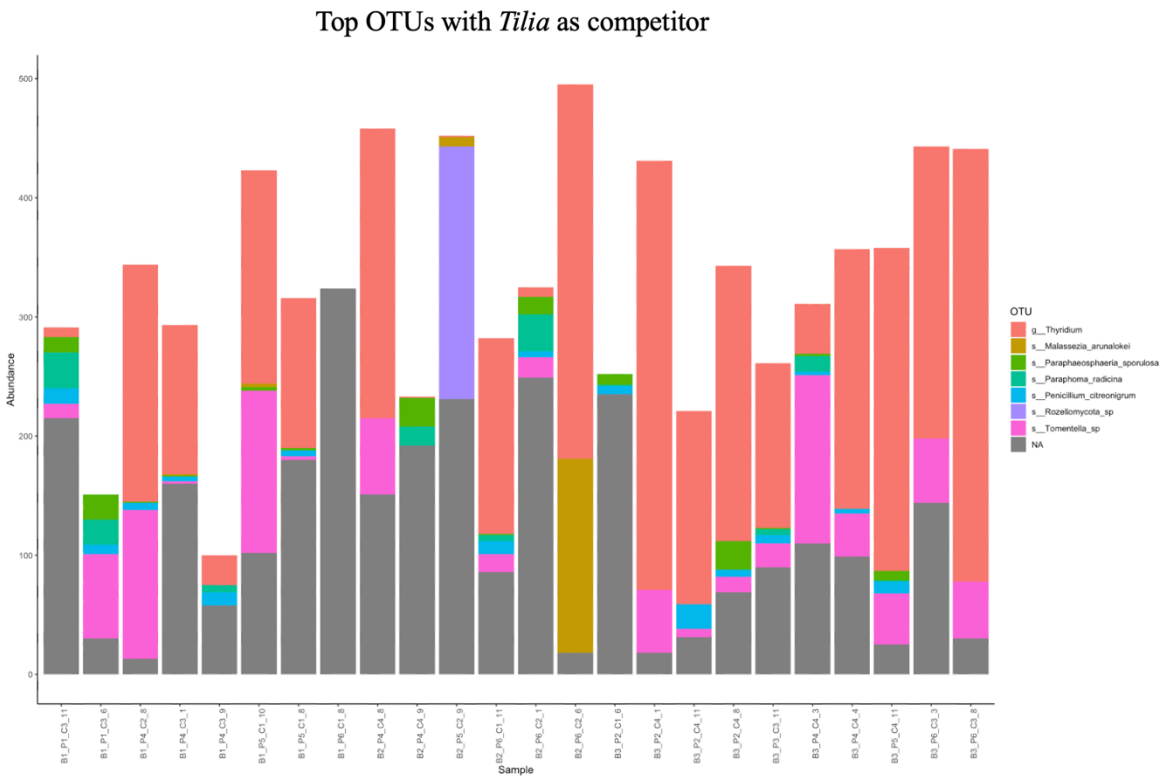
<u>Supplementary figure 4.</u> Height increment per year and species.
On the graph the number of trees is shown on the y- axis and the height increment on the x – axis. The mean height increment for the trees per species and per years 2020-2021,2021-2022,2023-2024 were calculated from the obtained measurements. Its color corresponds to the different tree species.

Top OTUs with *Quercus* as competitor

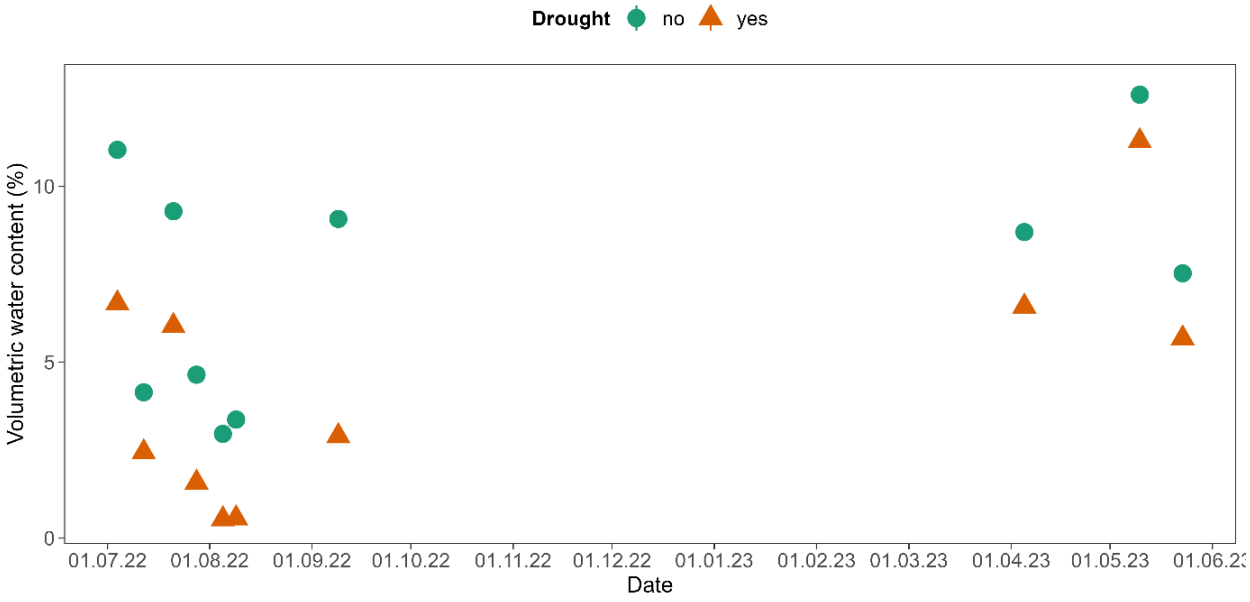


Top OTUs with *Fagus* as competitor





<u>Supplementary figure 5.</u> Top OUT matches for each tree species as a competitor. In each graph for each tree species the top OTU matches and their relative abundances in each sample are shown. The most common fungi found in all the different competitor scenarios are *Thyridium* a saprotrophic fungus and *Tomentella* an ectomycorrhizal fungus.



<u>Supplementary figure 6.</u> Soil moisture content during the experiment.
Measurements of the volumetric water content were taken during the experiment. On the y-axis is the water content (%) and x-axis the date of sampling. Different signs are given for the different treatments (drought or ambient rainfall).