

THESIS

CONIFER MICROBIOMES IN THE ERA OF REFORESTATION:
FROM NURSERY ORIGIN TO FIELD OUTPLANTING

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ABSTRACT

CONIFER MICROBIOMES IN THE ERA OF REFORESTATION: FROM NURSERY ORIGIN TO FIELD OUTPLANTING

Increasing wildfire severity and frequency across western North America has created an urgent need for large-scale reforestation. Forest nurseries play a critical role in meeting this demand by producing millions of conifer seedlings annually. Yet seedlings are not transported alone; they carry diverse microbial communities that may influence seedling health, growth, and establishment after planting. These communities may also harbor latent fungal pathogens with the potential to be introduced into recovering post-fire ecosystems. Despite growing interest in plant microbiomes, no studies have used the same cohort of trees to track the trajectory of conifer-associated microbial communities from nursery to field.

This thesis examines microbial community dynamics across the reforestation pipeline from nursery production through early field establishment. Chapter 1 reviews the ecological role of fire in western forests, the history of pathogen movement through plant material, mechanisms of rhizosphere microbiome assembly, microbial dynamics in fire-affected soils, and current knowledge of microbial community dynamics in nursery systems and in outplanted seedlings.

Chapter 2 investigates the influence of nursery and host species on the rhizosphere microbiomes of ponderosa pine (*Pinus ponderosa*) and southwestern white pine (*P. strobiformis*) seedlings grown in two nurseries. Using ITS2 and 16S amplicon sequencing, fungal and bacterial communities associated with nursery seedlings and their potting mix were characterized. Nursery was the dominant driver of microbial community composition, exceeding the influence of host

species and demonstrating that nursery environments leave a distinct microbial signature on seedlings prior to outplanting.

Chapter 3 follows these seedlings after planting within the Calf Canyon-Hermit's Peak burn scar in northern New Mexico. Rhizosphere microbial communities were monitored during the first year of establishment and compared with those in bulk soil and in naturally regenerating ponderosa pine. Results demonstrated substantial turnover of fungal and bacterial communities following outplanting, including recruitment of ectomycorrhizal fungi, increased similarity to resident soil communities, and a decline in the abundance of many nursery-origin microorganisms.

Collectively, this work demonstrates that nursery environments strongly influence the microbial communities that seedlings carry into reforestation sites, but post-fire soils can still function as an important reservoir of microbes that reshape the communities after planting. These findings contribute to a growing understanding of microbiome dynamics in conifer reforestation systems and highlight the importance of continuing to monitor seedlings after planting.

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TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
CHAPTER 1: LITERATURE REVIEW	1
Forest Fire and Changing Fire Regimes	1
This History of Escaping Pathogens.....	3
Assembly of the Conifer Microbiome	5
Burned Soils and Risks for Post-Fire Reforestation	6
Microbiome Applications in Conifer Nurseries and Post-Outplanting Monitoring.....	7
Research Objectives.....	11
REFERENCES	12
CHAPTER 2: FROM THE BEGINNING: HOW NURSERIES SHAPE MICROBIAL COMMUNITIES	18
ABSTRACT.....	18
INTRODUCTION	19
MATERIALS AND METHODS	23
RESULTS.....	32
DISCUSSION	49
CONCLUSIONS.....	54
REFERENCES	55
CHAPTER 3: INTO THE WILD: OUTPLANTING SEEDLINGS INTO POST-FIRE LANDSCAPES.....	67
ABSTRACT.....	67
INTRODUCTION	68
MATERIALS AND METHODS	71
RESULTS.....	77
CONCLUSIONS.....	103
REFERENCES	105
APPENDIX 1: CHAPTER 2	113
APPENDIX 2: CHAPTER 3	139
LIST OF ABBREVIATIONS	167

CHAPTER 1: LITERATURE REVIEW

Forest Fire and Changing Fire Regimes

The history of fire management in the United States is closely tied to the history of war and westward expansion. Following the Civil War, increased migration into the western United States brought miners, timber cutters, and ranchers into what they viewed as a vast and largely unspoiled landscape (Williams, 2000). Railroads rapidly expanded westward due to land grants awarded by Congress, and timber companies, having already heavily exploited eastern forests and those of the Great Lakes region, soon followed. During the period between the Civil War and the World Wars, growing concern over resource depletion and land degradation began to shape new ideas about forest conservation and pushed the federal government toward action (Williams, 2000).

Early conservationists and naturalists, such as Franklin B. Hough, advocated for the preservation of the West's landscapes and for the protection of ordinary forests (Williams, 2000). Based on observations from eastern US forests, Hough warned that timber resources could be exhausted and argued that government intervention was necessary to ensure forest preservation and renewal. Despite increasing awareness of conservation needs, little federal action occurred until the 1891 Public Land Act, which granted the president authority to establish "forest reserves". This legislation, later known as the Forest Reserve Act, led to the creation of the first forest reserve in March of 1891. Subsequent legislation, including the Organic Act of 1897, provided a framework for managing these reserves, and by the early twentieth century, the Federal Forest administration was unified under the U.S. Department of Agriculture with the formal establishment of the Forest Service in 1905 (Williams, 2000).

Although federal policies emphasized protection, they did not eliminate the nation's reliance on timber. Both World Wars required enormous quantities of wood for railroads, ships, and infrastructure (Williams, 2000). This urgent need reshaped how fire was perceived in American forests. Fire became viewed as a threat to national security and economic stability, and suppression was framed as a patriotic duty (Donovan & Brown, 2005; Pyne, 1994). This perspective was reinforced through public campaigns such as Smokey Bear, whose messaging reflected wartime and postwar timber demands (Donovan & Brown, 2005). In 1935, the implementation of the "10 a.m. policy" mandated aggressive suppression of all fires by 10:00 a.m. the day following their report, regardless of fire size or ecological context (Donovan & Brown, 2005). As a result, the idea that fire could serve beneficial ecological roles was increasingly dismissed as dangerous and unpatriotic.

Fire, however, is neither inherently good nor bad; it is a natural ecological process. In many forest ecosystems, fire historically played a key role in regulating understory growth, nutrient cycling, and species composition (Ryan et al., 2013). The systematic elimination of fire has altered forest structure, increased fuel loads, and promoted dense understories (Cooper, 1960), dominated by shade-tolerant species (Ryan et al., 2013). The long-term suppression of fire has fundamentally changed forest ecosystems and contributed to the extreme severity of modern wildfires (Coop et al., 2020; Ryan et al., 2013).

These altered fire regimes have produced more frequent and more severe wildfires across western North America, with millions of hectares of forest lost to high-severity burns in recent decades (Abatzoglou et al., 2019; Dobrowski et al., 2024). Restoring tree cover at the scale of these losses depends on producing large numbers of seedlings in forest nurseries, and recent federal initiatives such as the REPLANT Act have committed to substantial increases in conifer

nursery output to meet national reforestation goals (Balloffet & Dumroese, 2022). Nurseries are a critical part of the reforestation pipeline (Fargione et al., 2021), but the same intensive production conditions that allow nurseries to produce seedlings efficiently also create environments where plant pathogens can establish, multiply, and potentially travel with seedlings into outplanting sites (Charron et al., 2025; Marshall et al., 2021). Understanding how pathogens move through these networks is crucial to the success of contemporary reforestation efforts.

This History of Escaping Pathogens

Pathogens are mobile organisms, capable of spreading through multiple pathways, including air, water, soil, and biological vectors. This capacity for movement is a fundamental component of pathogen survival and persistence, allowing them to exploit new hosts and environments as conditions change. In forest and agricultural systems, pathogen movement is increasingly facilitated by globalization, expanding trade networks, and the large-scale transport of live plant material (Ghelardini et al., 2022; Santini et al., 2018).

The movement of pathogens via live plant material is not a novel phenomenon in forest ecosystems. One of the most devastating examples is Chestnut Blight, caused by the fungal pathogen *Cryphonectria parasitica*. Chestnut Blight was first reported in 1904 on American chestnut (*Castanea dentata*) in New York City, New York. Its subsequent spread led to the functional extinction of the American chestnut across much of its native range, fundamentally altering the ecology of North American forests (Rigling & Prospero, 2018). In more recent history, the movement of wood products has facilitated the introduction of numerous fungal pathogens. Laurel wilt, caused by the fungal pathogen *Harringtonia lauricola* carried by the invasive Redbay ambrosia beetle (*Xyleborus glabratus*), was introduced to the United States in wood products from Asia in the early 2000s and continues to spread through the southern states

(Fraedrich et al., 2007; Mayfield et al., 2019). The spread of Laurel wilt is currently devastating urban and natural forests while threatening commercial avocado production (Mayfield et al., 2019).

Soilborne pathogens are also on the move, with well-documented consequences in both agricultural and forest systems. In agriculture, *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 has spread internationally through contaminated planting material and soils, causing devastating losses in banana production and demonstrating how difficult soilborne pathogens are to contain once established (Dita et al., 2018; IPPC Secretariat., 2023). *Phytophthora* species have been widely distributed through nursery stock and soil movement as well. In Australia, *P. cinnamomi* has caused extensive forest dieback, infecting a broad range of native plant species and persisting in soils for decades, resulting in long-term ecosystem degradation (Hardham & Blackman, 2018). In North America, *Phytophthora* species have been shown to move via nursery-grown oak seedlings used for restoration and landscaping, leading to their widespread establishment in urban forest and wildland ecosystems (Charron et al., 2025; Frankel et al., 2020). Once introduced, these pathogens can persist in soil and plant communities, complicating restoration efforts, increasing disease pressure on susceptible hosts. They are notoriously difficult to control due to their broad host ranges and persistent survival in soil and water (Frankel et al., 2020; Grünwald et al., 2012).

Collectively, these examples demonstrate that invasive pathogens are tightly linked to anthropogenic movement and can fundamentally restructure plant–microbe interactions across ecosystems. Given the unprecedented scale of reforestation needs and the risk of introducing pathogens into new areas, research into these potential threats is increasingly vital to reforestation success.

Assembly of the Conifer Microbiome

Conifers are not passive recipients of microbial communities but active architects of their microbiome. Evidence of phyllosymbiosis, the covariation between host phylogenetic relatedness and microbiome composition, has been demonstrated in both Pinaceae and Cupressaceae when grown in a common soil (Graham et al., 2025). Significant differences were characterized in both bacterial and fungal microbiomes between the two families, suggesting that long evolutionary histories have shaped host-microbiome selection processes in conifers (Graham et al., 2025).

This host-mediated assembly is not uniform across plant compartments; the composition of microbiomes in both the endosphere and rhizosphere is primarily influenced by host genetics, but the rhizosphere is additionally shaped by environmental conditions and soil type (Park et al., 2023; Yu & Hochholdinger, 2018). Plants modulate community composition by releasing targeted exudates that attract beneficial partners and deter pathogens (Graham et al., 2025; Park et al., 2023). Mycorrhizal associations further structure assembly outcomes, with Pinaceae species predominantly recruiting the ectomycorrhizal taxa, *Wilcoxina* and *Rhizopogon* (Graham et al., 2025).

Bulk soils serve as the primary microbial reservoir from which these selective biological processes operate (Park et al., 2023; Xun et al., 2024). As microbiome recruitment plays a vital role in plant health, disturbances that alter soil communities may have important consequences for seedling establishment. In post-fire environments, the disruption of resident microbial communities may weaken the ecological filters that normally govern microbiome assembly, potentially allowing detrimental nursery-origin microorganisms to establish and persist after outplanting.

Burned Soils and Risks for Post-Fire Reforestation

Wildfire changes soil environments, creating conditions that differ radically from unburned systems by altering soil chemistry, transforming physical structure, and disrupting soil fauna (McLauchlan et al., 2020; Pressler et al., 2019). These changes cascade through soil processes, influencing nutrient availability, soil moisture dynamics, and plant–microbe interactions (Certini, 2005; Dove et al., 2022). At the microbial level, these effects can be severe and functionally specific; fire has strong negative effects on soil microbial biomass, abundance, and diversity (Pressler et al., 2019). When fires are high-severity, soil microbial communities can be substantially altered, leading to near-complete losses of ectomycorrhizal fungi and favoring Actinobacteria adapted to post-fire conditions, resulting in pronounced shifts in community composition (Dove et al., 2022; Nelson et al., 2022). Consequently, when conifer seedlings are planted in post-fire landscapes, they encounter a degraded soil environment in which mutualistic fungal partners may be absent. Planting trees in burned landscapes may therefore create novel interactions among recovering native microbial communities, post-fire pyrophilous colonizers, and microorganisms introduced through reforestation activities.

Whether recovering resident microbial communities would reassert dominance over those associated with planted seedlings remains an open question. Evidence from intact soil systems suggests that resident microbes often retain a competitive advantage. Native microorganisms colonize plant roots more successfully and support plant growth more effectively than non-native taxa (Zhou et al., 2024). Likewise, Jiang et al., 2023 demonstrated a "home-field advantage," showing that microbial consortia derived from local soils outperform commercial inoculants. Resident microbiomes also exhibit structural resilience to perturbation. Repeated inoculation studies have shown that introduced microorganisms can temporarily influence community

composition; however, these changes are often short-lived, as resident microbial communities typically reestablish over time (Wang et al., 2021).

Forest nurseries may represent a highly consequential node in this dispersal network, with seedling movement creating pathways for pathogens to transit from nurseries to reforestation sites (Marshall et al., 2021). In post-fire systems, the microbial interactions that normally suppress introduced taxa may be weakened by fire-driven microbe mortality, representing conditions under which an invasive pathogen might succeed (Pressler et al., 2019; Pulido-Chavez et al., 2023). Fire can also shift microbial communities toward “pathobiomes” dominated by opportunistic or pathogenic taxa, with potential consequences for seedling survival and long-term ecosystem recovery (Fitz Axen et al., 2024). Planting seedlings in burned soil carries ecological risks that remain poorly understood; nursery-associated pathogens may escape into post-fire environments where altered soil conditions and reduced microbial competition could favor their establishment.

The risk of planting into burned soil is likely to vary with fire severity, time since disturbance, and the microbial history of the seedlings themselves. Whether planted seedlings subsequently recruit local microbiota or remain dominated by nursery-origin communities that contain pathogens is therefore a critical ecological function. These risks are unlikely to be captured by aboveground restoration metrics alone, signaling that monitoring frameworks should involve below-ground profiling of microbial community dynamics (Tanney et al., 2025).

Microbiome Applications in Conifer Nurseries and Post-Outplanting Monitoring *Sequencing-Based Approaches to Characterizing the Nursery Seedling Microbiome.*

In forest and conservation nurseries, seedlings are produced under controlled conditions that can profoundly shape their root-associated microbial communities. The degree to which

these communities are detected and characterized depends heavily on the tools used. Over the past two decades, a growing body of research has applied molecular and sequencing-based approaches to evaluate the rhizosphere microbiome of seedlings in nursery environments.

Early studies on characterizing microbial communities associated with conifer seedlings relied on DNA-based identification of ectomycorrhizal (ECM) fungi from root tips. In one of the first large-scale molecular surveys of nursery-grown conifers, Iwański et al. (2006) identified ECM fungi colonizing *Pinus sylvestris* seedlings across four bare-root nurseries in Poland. They detected 13 fungal species, including *Wilcoxina mikolae* and *Thelephora terrestris*. Members of these genera are persistent in nurseries but have been shown to potentially decline after outplanting, depending on host and environment (Colpaert, 1999; Iwański et al., 2006).

The transition from Sanger-based identification to high-throughput sequencing marked a significant expansion in the scope of nursery microbiome surveys. Menkis et al., (2016) used pyrosequencing to assess fungal communities in the rhizosphere and potting mix of container-grown Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) seedlings from nine nurseries across Sweden. They demonstrated that the rhizosphere and potting mix communities were compositionally distinct. Furthermore, pathogenic fungi were detected in the roots of asymptomatic seedlings, confirming that visually healthy stocks can carry latent pathogens.

Building on this multi-nursery survey approach, Marčiulynienė et al., (2021) applied PacBio sequencing to assess fungal and oomycete communities in the roots and rhizosphere soil of bare-root seedlings across seven Lithuanian nurseries, encompassing five tree species. Their survey found that coniferous species hosted higher fungal richness than deciduous species, though standard cultivation practices appeared to keep pathogen loads low. The effects of nursery production methods on fungal community assembly were further examined by Baranowska et al.

(2023) who used amplicon sequencing to compare fungal diversity in the roots of six production systems for Silver fir (*Abies alba*) in Poland. They found that different nursery methods produced highly divergent fungal communities; however, mycorrhizal fungi remained the dominant group across all treatments.

While fungal communities have received the most attention, bacterial rhizosphere communities in nursery conifers remain comparatively underexplored. In one of the few studies to address this gap, Garcia-Lemos et al. (2020) utilized amplicon sequencing to track bacterial communities associated with Nordmann fir (*Abies nordmanniana*) from greenhouse to the field nursery. They identified a “core” rhizosphere microbiome community comprising 19.9% of genus-level taxa that was maintained across growing systems. This microbiome included *Bradyrhizobium*, *Burkholderia*, *Flavobacterium*, *Pseudomonas*, and *Rhizobium*, genera known to harbor nitrogen-fixing and plant growth-promoting taxa. The broader community structure differed significantly between greenhouse and field nurseries, suggesting that the immediate production environment influences bacterial communities.

This body of work reveals several patterns. Molecular tools have been applied to nursery seedling microbiomes with increasing sophistication as these tools improve and become more accessible. While the nursery environment clearly shapes community assembly (Baranowska et al., 2023; Garcia-Lemos et al., 2020), the long-term consequences of these nursery-imprinted microbiomes on post-outplanting performance represent a critical gap that future research should address.

Post-Outplanting Monitoring of Rhizosphere Microbiome Shifts

Once nursery-grown seedlings are transplanted to reforestation sites, their root-associated microbial communities face a dramatically different environment, one without fertilizers,

controlled temperatures, or irrigation. Understanding how microbial communities shift after outplanting is critical for predicting seedling establishment success, yet studies that track these transitions remain surprisingly limited and historically focused on inoculated taxa.

Some of the earliest work on post-outplanting microbiome tracking focused on whether nursery-introduced ectomycorrhizal fungi could persist on roots after transplantation (Hilszczańska & Sierota, 2006). Scots pine (*Pinus sylvestris*) seedlings inoculated with *Thelephora terrestris* were outplanted onto post-agricultural land in Poland. The inoculated fungal strain remained detectable on root systems two years after outplanting, demonstrating that nursery-acquired ECM fungi can persist in the field. Another long-term study monitored inoculated *Rhizopogon roseolus* and *R. luteolus* on stone pine (*Pinus pinea*) and Aleppo pine (*P. halepensis*) seedlings for 43 months after outplanting. This study confirmed that the ectomycorrhizal inoculants persisted and can contribute to increased survival; however, the growth benefits diminished over time as non-inoculated controls eventually converged in height with treatments (Parlade et al., 2004).

In 2014, a study in Portugal evaluated the persistence of inoculated ectomycorrhizal fungi and microbial community dynamics in post-fire soils. Inoculated maritime pine (*Pinus pinaster*) seedlings exhibited greater height growth after five years, and four of the seven inoculated taxa (*Laccaria* sp., *Rhizopogon* sp., *Suillus bovinus*, and *Pisolithus* sp.) remained present in roots throughout the study. Root systems were also colonized by additional fungi, including *Inocybe*, *Thelephora*, and *Paxillus*, indicating that inoculated and naturally recruited fungi can coexist during post-fire establishment (Franco et al., 2014).

Collectively, these studies demonstrate that nursery-inoculated ectomycorrhizal fungi may persist for years after outplanting, even as seedlings are exposed to novel environmental

conditions, however this persistence is dependent on the inoculated taxa and host tree. At the same time, root systems remain receptive to colonization by fungi present at reforestation sites, indicating that seedling microbiomes continue to assemble after transplantation. Despite advances in molecular tools over the past two decades, studies tracking these transitions remain limited. To date, few studies have followed microbial communities across both the nursery and outplanting phases, and none have comprehensively characterized both fungal and bacterial microbiomes throughout this transition.

Research Objectives

Given the increasing scale of post-fire reforestation and the complex interactions among fire, soils, pathogens, and plant-associated microbiomes, there is a critical need to better understand microbial dynamics across the reforestation pipeline (Tanney et al., 2025). The overarching goal of this thesis is to evaluate nursery-associated microbial and pathogen dynamics and how they influence the establishment of conifer seedlings planted into a post-fire environment. Our objectives are to (1) characterize the microbial community composition associated with nursery-grown conifer seedlings (ponderosa and southwestern white pines) prior to outplanting, (2) evaluate changes in seedling-associated microbial communities and pathogen occurrence during early establishment following outplanting into a post-fire landscape, and (3) assess the persistence and temporal dynamics of seedling-associated microbial communities and their relationship to seedling growth metrics. We hypothesize that nursery-origin microbes, including potential pathogens, initially present in the rhizosphere of seedlings, will decline in abundance over time as native soil microbes recover and reassert dominance. Consequently, the rhizosphere microbiomes of transplanted seedlings are expected to progressively converge with those of naturally regenerating trees and bulk soil.

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CHAPTER 2: FROM THE BEGINNING: HOW NURSERIES SHAPE MICROBIAL COMMUNITIES

ABSTRACT

Successful reforestation depends on the production of healthy nursery seedlings, yet little is known about how nursery environments shape the microbial communities associated with conifer seedlings prior to outplanting. We investigated the influence of the nursery environment and host species on rhizosphere microbial assembly in two important southwestern United States reforestation species: ponderosa pine (*Pinus ponderosa*) and southwestern white pine (*P. strobiformis*). Seedlings originating from a shared seed source were grown in two different nurseries and sampled alongside their corresponding unused potting mixes. Fungal and bacterial communities were characterized using ITS2 and 16S amplicon sequencing, while fungi associated with symptomatic root tips were assessed through culture-based isolation and Sanger sequencing. The nursery emerged as the dominant factor structuring both fungal and bacterial communities. Across both host species, nursery origin explained 60-64% of the variation in rhizosphere microbiomes, while host-species effects were weaker and depended on the specific nursery context. Differential abundance analyses revealed extensive taxonomic differentiation between nurseries, with more than 50% of the fungal and bacterial amplicon sequence variants significantly differentially abundant between species grown at the two nurseries. Culture-based surveys of the symptomatic root tips revealed seven putative fungal pathogens, including *Fusarium*, an important pathogen in conifer nurseries. Comparisons among the potting mix, rhizosphere, and symptomatic root tips showed that the rhizosphere contained the greatest diversity of fungal taxa, including ectomycorrhizal genera absent from both the potting mix and

symptomatic root tips. Together, these findings demonstrate that nursery production environments leave a strong microbial imprint on conifer seedlings that exceed the influence of host species identity. Recognizing and managing these nursery-imprinted microbial signatures will be essential for producing resilient seedlings for large-scale reforestation efforts.

INTRODUCTION

Forests play a critical role in maintaining ecosystem services by regulating hydrological cycles, reducing erosion, supporting biodiversity, and storing carbon (Aerts & Honnay, 2011; Moomaw et al., 2020; Pan et al., 2011). Yet, large, high-severity wildfires have left extensive areas of forested land with limited capacity for natural regeneration. The re-establishment of tree cover is increasingly necessary to restore forest structure, stabilize soils, and support long-term ecosystem function (Coop et al., 2020; Dobrowski et al., 2024).

As fires occur more frequently and with higher intensity, active tree planting has become a central strategy for post-fire landscape recovery. Federal agencies have identified large backlogs of restoration needs on public lands, particularly in the western United States (Balloffet & Dumroese, 2022; U.S. Department of Agriculture [USDA] Forest Service, 2022). In response, recent policy initiatives have sought to accelerate tree planting efforts and expand nursery production to meet the demand. The REPLANT Act of 2021 directs the United States Department of Agriculture (USDA) Forest Service to address its reforestation backlog within the next decade, prompting the agency to expand nursery capacity and accelerate planting efforts to meet its restoration goals (Balloffet & Dumroese, 2022; USDA Forest Service, 2022). These policies reflect a growing national recognition of the importance of trees for ecological recovery and climate resiliency. However, translating these policy-driven goals into successful outcomes

depends on the effectiveness of the full reforestation pipeline, which begins with nursery production (Fargione et al., 2021).

While large-scale nursery production efforts address immediate needs for forest recovery, the long-term success of these efforts depends on considerations that extend beyond the availability of seedlings (Fargione et al., 2021; Grossnickle, 2012). High-density planting, shared irrigation systems, and the repeated use of containers can facilitate the spread of soilborne, waterborne, and root-associated pathogens (Dobbs et al., 2023; Dumroese & Haase, 2018; Marčiulynas et al., 2020). Pathogens are of particular concern because they may persist as latent or asymptomatic infections within nursery stock, remaining undetected until seedlings experience environmental stress following outplanting (Menkis et al., 2016; Stewart et al., 2021)

Among the most common and economically important nursery pathogens are species within the genera *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Cylindrocladium* (Dobbs et al., 2023; Dumroese et al., 2000; Dumroese & James, 2005; Foster et al., 2020). These genera are ubiquitous in nurseries, and infected seedlings can exhibit symptoms such as necrotic root tips, reduced root mass, chlorosis, or stunted growth (Admas & Kebede, 2024; Dumroese & James, 2005). While some of these fungal and oomycete pathogens are endemic to the United States, others may be invasive and introduced through the movement of plant material (Ghelardini et al., 2022; Marshall et al., 2021). The presence of pathogens in these systems raises concerns about potential biosecurity risks if they enter the field and establish in recovering wildlands (Tanney et al., 2025).

The integration of microbiome tools can better inform early pathogen dynamics and increase our understanding of long-term community structure. Infections are not always evident in above-ground tissues (Charron et al., 2025; Menkis et al., 2016). For example, *Fusarium*

species are frequently associated with chronic root infections that reduce seedling vigor without causing immediate mortality (Admas & Kebede, 2024; Dobbs et al., 2023; Stewart et al., 2012). Seedlings may appear healthy when outplanted, yet carry latent infections that can compromise seedlings during post-planting stress (Charron et al., 2025; Menkis et al., 2016). Investigating early dynamics in the rhizosphere microbiome could shed light on hidden pathogen pressures. Understanding the microbial challenges seedlings face in the nurseries is crucial, given that they are typically outplanted into harsh post-fire environments where water limitations, temperature extremes, and altered soil conditions further challenge their survival and establishment.

Since the long-term success of the seedlings in the field is critical to successful reforestation, it is imperative that nurseries grow healthy and resilient stock. However, nursery conditions are not standardized, and seedlings grown at different production facilities are exposed to diverse environmental regimes that affect growth. In nurseries, substrates used for container propagation often contain peat, bark, perlite, and other components selected for optimal drainage and aeration (Van Gerrewey et al., 2020). In turn, these substrates serve as the primary microbial reservoir for container-grown plants, and their limited diversity can constrain the taxa available for rhizosphere recruitment, fundamentally shaping the trajectory of microbial colonization (Quijia Pillajo et al., 2024; Van Gerrewey et al., 2024). Additionally, variable nursery conditions expose seedlings to microbes that may not be present in their natural habitats (Menkis et al., 2016). These artificial habitats could represent a critical limiting step in the forest restoration pipeline, where long-term plant health is potentially compromised by a lack of beneficial plant-microbe associations (Cram & Dumroese, 2012; Menkis et al., 2016).

The assembly of microbial communities occurs at the earliest stages of plant development, and the community continues to be shaped throughout the lifespan of the host

(Trivedi et al., 2020; Vandenkoornhuyse et al., 2015). Microbial recruitment occurs through multiple pathways, vertical transmission through seeds and vegetative tissues, and horizontal transmission through the surrounding environment (Berg & Raaijmakers, 2018; Vandenkoornhuyse et al., 2015). The rhizosphere, a zone of soil that is adjacent to, and influenced by plant roots, is considered the most complex and intensive zone for interactions between the plant, microorganisms, and its soil substrate (Lynch & De Leij, 2012). The assembly of the rhizosphere microbiome is a foundational part of the plant's life cycle, and specific taxa are actively recruited by the plant during growth (Berendsen et al., 2012; Stewart et al., 2021). In a system where long-term outplanting survival is the ultimate metric of success, early rhizosphere community dynamics deserve in-depth characterization.

Understanding how nursery management practices influence rhizosphere microbiome assembly is critical for developing production strategies that enhance the microbial foundation of conifer seedlings and thus improve success after outplanting. Seedlings carry a microbial legacy of these artificial habitats when outplanted, and little is known about how these communities affect subsequent seedling success or the long-term fate of these microbial assemblages (Garcia-Lemos et al., 2020). To examine how nursery practices influence microbial assembly and dynamics in conifer seedlings, the objectives of this research are to: (1) Compare microbial communities of potting mix used by different nurseries and determine how they affect subsequent assembly in the seedling rhizosphere, (2) determine how the rhizosphere microbial communities differ between two conifer seedlings ponderosa pine and southwestern white pine, grown within the same nursery and across two different nurseries using a shared seed source, and (3) Characterize potentially pathogenic taxa intimately associated with symptomatic seedling root tips.

MATERIALS AND METHODS

Study System and Nursery Production.

Seed Source and Germination Testing. Two species, ponderosa pine (*Pinus ponderosa*; PIPO) and southwestern white pine (*Pinus strobiformis*; PIST) , which are commonly used for reforestation in the southwestern United States (Region 3), were grown in two nurseries between February and April 2024 (U.S. Forest Service, 2024). Seeds were sourced from the Santa Fe National Forest and processed by the seed processing facility at Nursery 1 (N1), and a subset was subsequently shipped to Nursery 2 (N2).

Each nursery planted 500 PIPO (seed source PP10880079) and 500 PIST (seed source SW10220387) seedlings. Both nurseries conducted germination tests prior to sowing. PIPO had a germination rate of 96% and 89% at N1 and N2, respectively, and was sown at two seeds per cell at N1 and sowed with one seed per cell in N2. PIST had a germination rate of 83% and 45% at N1 and N2, respectively, and sown at three seeds per cell and two seeds per cell at N1 and N2, respectively. In N1, seeds were stored at ambient room temperature, then stratified in an aerated water bath for 48 hours. Seeds were then air-dried and sealed in a plastic bag at 35°C for 30 days, which were occasionally opened to prevent mold. At N2, seeds were stored at 10°C until stratification. For stratification, seeds were soaked in water bath for 18 hours and then transferred to a plastic bag stored at 4°C. PIPO and PIST were stored 4°C for 33 and 35 days, respectively and no mold occurred during this time.

Both species were sown on April 11, 2024, into 6 cubic inch cells in Styrofoam blocks at N1, whereas at N2, both PIPO and PIST were sown into plastic tree cone-tainers on March 14, 2024, and February 16, 2024, respectively. The potting mix at N1 consisted of 75% peat, 12.5% vermiculite, and 12.5% perlite by volume, and at N2, consisted of peat moss, vermiculite, and

perlite in a 2:1:1 ratio (by volume). Seedlings at N1 were irrigated manually with a garden hose and partially by overhead irrigation. At N2, both species were initially watered using benchtop sprinklers until transferred to subirrigation tanks on April 2nd, 2024, for PIST and May 13th, 2024, for PIPO. Fertilization occurred at both nurseries per nursery-specific fertilization protocols, and no fungicides were applied to the seedlings.

Nursery Sampling.

Root and Rhizosphere Sample Collection. Seedlings were sampled at 5-9 weeks after germination to assess the microbial communities present in roots. PIPO were sampled at 9 and 5 weeks old at N1 and N2, respectively, whereas at each nursery PIST were sampled at 9 weeks. Twenty seedlings per species per nursery were collected, resulting in 80 total seedlings sampled. For each seedling, rhizosphere (RZ), potting mix (PM), and root systems (RT) were collected for both tagged amplicon sequencing and culture- based fungal isolation. In addition, unused PM, from three sources per nursery with three replicates per source (ca. 18 total samples), was sampled to characterize background microbial communities present in nursery substrates.

For sampling, seedlings were removed from their containers and placed on clean paper plates. The seedling shoot was removed by cutting the stem at the cotyledon scar. The root systems were then held over a sterile weigh boat, and the rhizosphere was gently removed by hand. Then, 2 g of rhizosphere was transferred into a tube containing 4 mL DNA/RNA Shield (Zymo Research, Irvine, CA, USA). The root system was then rinsed with distilled water and placed into a 15 mL tube. From the potting mix sources at both nurseries, 2 g of material were collected and aliquoted into tubes containing 4 mL DNA/RNA Shield (Zymo Research, Irvine, CA, USA). All samples were stored on ice and transported back to the laboratory. Rhizosphere

and potting mix samples were stored at -20°C ; root samples were stored at 4°C until processing.

Laboratory Processing.

Fungal Isolation from Symptomatic Roots. Root systems were rinsed with sterile-distilled water (SDW) and inspected for lesions. Lesions were characterized by tissues with discoloration or necrosis. Five, 6 mm lesions per root system were excised and surface sterilized in 10% bleach then rinsed in SDW for 30 seconds. Lesions were then plated onto $\frac{1}{4}$ -strength potato dextrose agar (PDA; BD Difco™ Dehydrated Culture Media, Franklin Lakes, NJ, USA). Plates were inspected daily for fungal growth, and colonies displaying unique morphologies were subcultured onto $\frac{1}{4}$ PDA amended with streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) and chloramphenicol (MilliporeSigma, St. Louis, MO, USA).

DNA was extracted from fungal cultures by placing mycelia, scraped from the actively growing margin of the culture using a sterile pipette tip, into a tube containing 100 μL of 5% Chelex™ 100 Resin solution (Bio-Rad Laboratories, Hercules, CA, USA). Samples were incubated at 98°C for 20 minutes, then briefly centrifuged, and the resulting supernatant was used directly as the DNA template for PCR amplification.

To speciate the fungal cultures, the internal transcribed spacer (ITS) region was amplified for each extracted DNA using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). PCR amplification consisted of an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 2 minutes. Samples that failed to yield amplifiable DNA after three Chelex extraction attempts were grown in potato dextrose broth (PDB; BD Difco™, Franklin Lakes, NJ, USA) and re-extracted using the OPS

SYNERGY™ 2.0 Plant DNA Extraction Kit (OPS Diagnostics, Lebanon, NJ, USA). Samples with unsuccessful amplification were designated as unknown. Successful PCR products were verified using gel electrophoresis, purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific), and submitted to Eurofins Genomics (Louisville, KY) for Sanger sequencing. The resulting sequences were assigned taxonomy using the naïve Bayesian classifier in DADA2 (assignTaxonomy; Callahan et al., 2016; Wang et al., 2007) against the UNITE database (general FASTA release, dynamic clustering threshold, version 19.02.2025), with a minimum bootstrap confidence of 50.

Sample DNA Extraction. Potting mix and rhizosphere samples were extracted using the ZymoBIOMICS DNA Miniprep Kit, with slight modifications (Zymo Research, Irvine, CA, USA). DNA/RNA Shield was first removed from the samples by adding 500 µL of molecular-grade water, followed by brief vortexing. Samples were then centrifuged at 14,000 RCF for 5 minutes. Once the supernatant was removed, Zymo lysis beads were added directly to the sample. Next, 750 µL ZymoBIOMICS lysis solution was added, and samples were lysed using a Vortex Genie bead beater run at speed 10 for 40 minutes. The resulting DNA was further extracted using the ZymoBIOMICS manufacturer protocol. DNA was quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

PCR Amplification for Microbial Community Sequencing. Bacterial 16S rRNA, fungal ITS2, and oomycete RPS10 loci were amplified in separate PCR reactions. The V4 region of the 16S rRNA gene was amplified to target bacterial communities using primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') (Parada et al., 2016) and 907R (5'-CCGTCAATTCCTTTGAGTTT-3') (Muyzer et al., 1995). The ITS2 region of fungal taxa was amplified using primers ITS3-2024F-A (5'-GCATCGATGAAGAACGCAGC-3') and ITS4-

2409R-A (5'-TCCTCCGCTTATTGATATGC-3') (Walters et al., 2016). Oomycetes were amplified using a nested PCR approach with primers PRV9-F (5'-GTTGGTTAGAGTAAAAGACT-3') and PRV9-R (5'-GTATACTCTAACCAACTGAGT-3') (Martin et al., 2014), followed by primers RPS10-F (5'-GTTGGTTAGAGYARAAGAF-3') and RPS10-R (5'-ATRYYTAGAAAGAYTYGAACT-3') (Burgess et al., 2022; Foster et al., 2022). Each reaction was performed in a 25 µL volume containing 12.5 µL 2× GoTaq Master Mix, 1 µL of each primer (10 µM), 8.5 µL nuclease-free water, and 2 µL template DNA. PCR conditions for 16S and ITS2 consisted of an initial denaturation at 94°C for 3 minutes, followed by 25 cycles of 94°C for 45 seconds, 52°C for 60 seconds, and 72°C for 90 seconds, with a final extension at 72°C for 10 minutes. Nested PCR conditions for oomycetes used PRV9 primers for the first round followed by RPS10 primers in a second PCR as described by (Burgess et al., 2022).

Library Preparation and Sequencing. PCR1 products were visualized on 2% agarose gels to confirm successful amplification. Amplicons were purified using Mag-Bind® RXNPure Plus magnetic beads (Omega Bio-tek, Norcross, GA, USA) at a 0.8× bead-to-sample ratio. Dual-index barcodes and Illumina adapter sequences were added during a second PCR using indexing primers (Integrated DNA Technologies; Coralville, IA, USA). PCR2 reactions were performed in 21.4 µL volumes containing 2× Quantabio ToughMix, indexing primers, nuclease-free water, and purified PCR1 product. PCR2 products were verified on agarose gels and purified using magnetic bead cleanup before sequencing (Galan et al., 2018). Samples were then sent to the Next-Generation Sequencing Core at Colorado State University for pooling, final cleanup, and sequencing using the Illumina MiSeq Platform with 251 bp paired-end sequencing.

Bioinformatic processing

DADA2 and Taxonomy. Demultiplexed paired-end FASTQ files from nursery samples were processed in DADA2 v1.34.0 in R v4.4.1; R Core Team, 2024 for the three amplicons. The ITS2 and 16S amplicons followed the standard DADA2 workflow, with marker-specific modifications detailed below (Callahan et al., 2016). The ITS2 primers were removed with cutadapt v5.2 (Martin, 2011) using 5'-anchored adapters allowing 0–6 leading N's, with reads then filtered at $\text{maxEE} = c(3, 5)$ and $\text{minLen} = 50$. The 16S V4-V5 sequences had heterogeneity spacers and primers occupying fixed leading positions, so the first 25 bp and 26 pb of the forward and reverse reads, respectively, were removed using trimLeft, followed by quality filtering at $\text{maxEE} = c(3, 5)$. Paired reads for the 16S and ITS2 were merged with $\text{minOverlap} = 20$, and $\text{maxMismatch} = 2$.

The RPS10 primers were removed in two stages following Foster et al. (2022). Reads were pre-filtered with DADA2 using filterAndTrim ($\text{maxN} = 0$) to remove reads with ambiguous bases. The heterogeneity spacers introduced variable N-runs at the 5' end that interfered with downstream primer matching. Then, cutadapt was applied with 5'-anchored forward adapters tolerating 0–6 leading N's (including an optional terminal G) and 5'-anchored reverse adapters tolerating 0–4 leading N's, paired with 3' reverse-complement trimming of read-through to catch short fragments ($n = 2$ adapter removals allowed per read). Trimmed reads were then filtered with $\text{maxEE} = c(4, 3)$, $\text{truncQ} = 2$, and $\text{minLen} = 50$, and paired reads were merged with $\text{minOverlap} = 8$ rather than the DADA2 default of 12 because the default value truncated the full-length sequences (Foster et al., 2022).

Taxonomy was assigned using assignTaxonomy (Wang et al., 2007) at the default bootstrap threshold of 50, against reference databases: UNITE v19.02.2025 (dynamic clustering) for ITS2 (Abarenkov et al., 2024), with tryRC = TRUE; SILVA v138.2 for 16S (Quast et al.,

2013), with species-level assignments added via addSpecies; and OomyceteDB release 2 for RPS10 (Foster et al., 2022), with tryRC = TRUE and outputBootstraps = TRUE. For each amplicon, ASV tables, taxonomy, and sample metadata were combined into phyloseq objects (v1.50.0; McMurdie & Holmes, 2013). For the ITS2, 16S, and RPS10, there were 2 mock communities, 8 DNA extraction negative controls, and 6 PCR negative controls, for a total of 114 samples.

Pipeline performance was assessed using ZymoBIOMICS Microbial Community Standards (Zymo Research, Irvine, CA) for the 16S and ITS2 pipelines and Foster Mock2 communities (Foster et al., 2022) for RPS10, which were processed with field samples through the full pipeline. Mock samples were removed prior to phyloseq object construction; negative controls were retained for downstream decontamination. For the 16S dataset, mitochondrial and chloroplast sequences were removed prior to decontamination.

Decontamination and Rarefaction. Contaminant ASVs in the ITS2 and 16S datasets were identified with decontam v1.30.0 (Davis et al., 2018) using the prevalence method (threshold = 0.5) after excluding zero-read negative controls. For RPS10, decontam was replaced with a three-stage quantitative subtractive filter due to the limited reliability of the RPS10 mock recovery. Cells (sample $\times$ ASV) containing > 10 reads were zeroed to remove per-sample sequencing noise. The maximum read count observed across all mock samples for each ASV was also subtracted from the count for each sample, and this was applied to ASVs in the negative controls as well. Since the PS10 mock data did not match the expected results, RPS10 was not carried forward into the downstream diversity, ordination, or differential-abundance analyses performed on the ITS2 and 16S datasets.

The decontaminated ITS2 and 16S datasets were rarefied to 10,000 reads per sample. Rarefaction was repeated 1,000 times without replacement (Cameron et al., 2021; Schloss, 2024). The averaged phyloseq objects served as input for all downstream Alpha- and Beta-diversity analyses.

Statistical Analysis.

Rhizosphere and Potting Mix - Tagged Amplicon Sequencing. To describe how nursery conditions shape microbial communities, three comparisons were made: the potting mixes (PM) used in the two nurseries; the potting mix and seedling rhizospheres (RZ); and the rhizospheres of the two host species, both within and between nurseries. All statistical analyses were conducted in R version 4.5.2 (R Core Team 2025), and Benjamini–Hochberg false discovery rate correction (Benjamini & Hochberg, 1995) was applied to the p-values where multiple comparisons were conducted, with significance of $p < 0.05$.

Shannon’s diversity index, applied to the rarefied data, was used to describe Alpha diversity. Each of our comparisons was tested using two-sided Wilcoxon rank sum tests with effect sizes quantified using Cliff’s delta (Torchiano 2020). To describe the Beta diversity between PM and seedling RZ samples, an overall PERMANOVA was conducted. Community dissimilarity was computed as Bray-Curtis distances (Bray & Curtis, 1957) from the rarefied data. Follow-up pairwise PERMANOVAs, using subsets from the matrix, assessed the community differences in the PM between nurseries, the seedling RZs and the PM, and the seedling RZs within nursery and between nurseries (Anderson, 2001). The overall PERMANOVA and each pairwise comparison were accompanied by a PERMDISP test (Anderson, 2006). Community structure was visualized with Principal Coordinates Analysis (Paradis & Schliep, 2019).

ASV-level differential abundance was tested with MaAsLin2 (Mallick et al., 2021) on the ITS2 and 16S datasets. Samples with fewer than 10,000 reads were excluded, so the same samples were analyzed as in the rarefaction-based analyses, though differential abundance was modeled on raw (non-rarefied) counts. Each comparison was modeled separately as a single two-group comparison. Within each comparison, ASVs were retained if they were present in at least 10% of samples and had at least 3 total reads summed across the comparison's samples. For each retained ASV, MaAsLin2 was fit as $\text{feature} \sim \text{Contrast}$. Counts were total-sum-scaled and log-transformed and modeled with a linear-model analysis method (normalization = "TSS", transform = "LOG", analysis_method = "LM"). For each comparison, the significant ASVs ($q < 0.05$) were ranked by ascending q-value, and per-ASV mean relative abundances were computed. ASVs with zero variance after scaling were dropped. To test the significance of ASV mean relative abundance, the two groups were compared at the sample level using a Wilcoxon rank-sum test. Heatmaps displayed individual samples grouped by treatment; columns were annotated by group, and for fungi, rows were annotated by FungalTraits primary-lifestyle guild (Pölme et al., 2020).

Symptomatic Root Tips - Culture Based. The taxonomy of fungal isolates from symptomatic root tips was determined by comparing their sequences against the UNITE database. To ensure the culture identifications were comparable to the rhizosphere amplicon data, sequences were classified using the same approach applied to the fungal amplicon dataset (Callahan et al., 2016; Wang et al., 2007).

A presence/absence community table was then constructed from the isolates, and the community was summarized at the genus level, and the seedling collected was the unit of observation. A genus was coded as present (1) if at least one isolate was recovered from that

seedling. Isolates that could not be assigned at the genus level were collapsed into a single "Unidentified" category. To describe the diversity of the fungi associated with the symptomatic root tips (SRT), comparisons of seedlings within a nursery and between nurseries were conducted. Alpha diversity and Beta diversity were calculated using the same methods as previously described.

Differential abundance was tested at the genus level using a separate community table built by summing isolate counts per genus per sample and converting to within-sample mean relative abundances. Wilcoxon rank-sum tests compared nurseries, both pooled across hosts and stratified by host species.

Comparisons Between Symptomatic Root Tips and Amplicon.

To compare the genera recovered across sample types, a three-way comparison was conducted among the culture-based SRT community, the RZ amplicon community, and the PM amplicon community. For each sample type, a genus was considered present if it occurred in at least one sample of that sample type; unclassifiable taxa (unidentified, unclassified, Incertae sedis, and genus-level-incomplete assignments) were excluded. Genera were partitioned into the seven regions of a three-set comparison (unique to each compartment, each pairwise overlap, and the intersection of all three) and visualized as a genus-level Venn diagram. Region membership was cross-referenced with FungalTraits primary-lifestyle guild assignments (Pölme et al., 2020).

RESULTS

Amplicon Library Performance and Sequencing Summary.

In total, 98 environmental samples (80 RZ and 18 PM samples) were processed for microbial ITS, 16S, and RPS10 community sequencing, yielding 294 microbiome libraries. All

16S libraries resulted in high-quality sequences. At ITS2, 7 libraries failed sequencing, resulting in 91 samples, and at RPS10, only 32 libraries were successfully sequenced. After filtering, a total of 3,174,140 reads remained for the ITS2, averaging 34,880 reads per sample and ranging from 10,146 – 83,442 reads. For the 16S, 3,250,114 reads remained, averaging 33,164 reads per sample and ranging from 1,566 – 90,552 reads. For the RPS10, 623,355 reads remained, averaging 16,404 reads per sample and ranging from 1 to 129,208 reads. A total of 3,264, 12,428, and 204 ASVs were identified from the ITS2, 16S, and RPS10 regions, respectively. At the 16S, a total of 742 ASVs were identified as mitochondria and 160 ASVs were identified as chloroplasts, and after removal 11,526 ASVs remained. Genus-level assignment was achieved for 65.84% of the fungal ASVs, 44.22% of the bacterial ASVs, and 40.6% of the oomycete ASVs (at bootstrap ≥ 50).

The mock communities were designed to recover eight bacterial genera, two fungal genera, and 24 oomycete species. For the ITS2 and 16S all expected fungal and bacterial genera were recovered. For RPS10, mock recovery was poor, with one of the two mock community replicates (OoMock-RPS10) failing to amplify. In the one amplified mock, we recovered five of the 24 expected species. The pattern of missing and unexpected species was similar across the pipeline parameters experimented with, suggesting that the discrepancy reflects the mock community samples themselves rather than the choice of DADA2 parameters. We therefore restrict RPS10 interpretation to qualitative species identifications.

Following decontamination, both datasets, the ITS2 and 16S, were rarefied to 10,000 reads per sample (Supplemental Figure 2.1). In the fungal (ITS2) dataset, 6 of 91 samples fell below this threshold and were excluded, leaving 85 samples for downstream analyses. In the

bacterial (16S) dataset, 10 of 98 samples fell below the threshold and were excluded, leaving 88 samples.

Nursery Rhizosphere and Potting Mix Communities.

Alpha diversity analyses. The fungal and bacterial Shannon Alpha diversity of the PM between the nurseries was not significant (all $p_FDR > 0.05$). In comparing the seedlings to the PM, the seedlings at N1 had significantly higher fungal and bacterial Shannon diversity and, therefore, were more diverse than the PM (all $\delta < -0.575$, all $p_FDR < 0.041$). However, at N2, the seedlings only had significantly higher bacterial Shannon diversity when compared to the PM ($\delta < -0.984$, all $p_FDR < 0.001$) (Supplemental Table 2.1)

Taking a closer look at the dynamics within nurseries, the N1 PIST had significantly higher fungal Shannon diversity than the N1 PIPO ($\delta = -0.437$, $p_FDR < 0.041$), but no significant differences were observed in Alpha diversity of the bacterial communities. At N2, no difference in Alpha was observed between the PIPO and PIST rhizospheres.

Comparing the seedlings of the same species across nurseries revealed that N2 PIPO and N2 PIST had significantly higher Shannon diversity in the bacterial communities when compared to the N1 PIPO and N1 PIST (all $\delta < -0.765$, all $p_FDR < 0.001$), respectively. No significant differences were observed in the fungal communities across the two nurseries for either species (Figure 2.1; Supplemental Table 2.1).

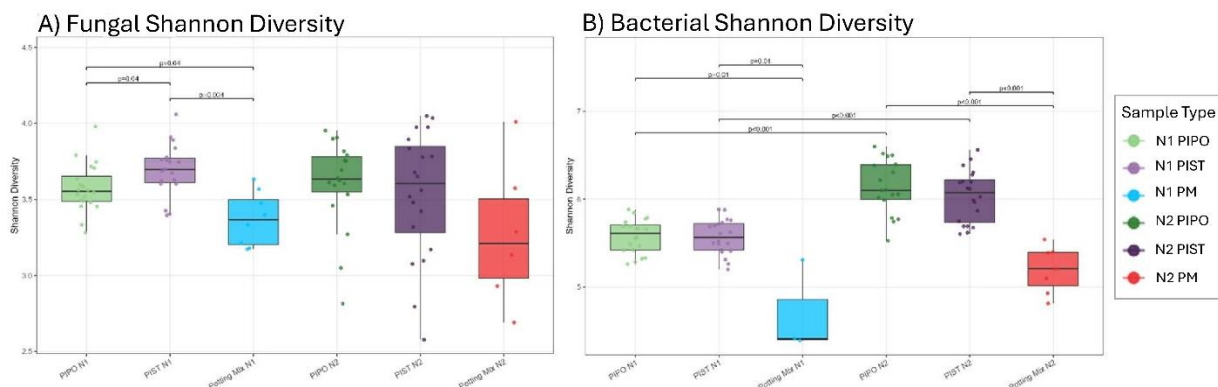


Figure 2.1. Alpha diversity (Shannon index) of (A) fungal (ITS2) and (B) bacterial (16S) rhizosphere communities across two conifer nurseries, N1 and N2. Within each nursery, diversity was characterized for the rhizosphere of *Pinus ponderosa* (PIPO) and *P. strobiformis* (PIST) seedlings and for the unused potting mix (PM) substrate. Box plots show the median (center line), interquartile range (box), and whiskers extending to $1.5 \times$ the interquartile range; overlaid points are individual samples. Brackets indicate pairwise comparisons with associated p -values from Wilcoxon rank-sum tests, corrected for multiple comparisons (FDR); only significant comparisons are shown.

Beta diversity analyses. Tests of Beta diversity across all sample types were significant for both fungal and bacterial communities (ITS2: $R^2 = 0.65$, $p = 0.001$; 16S: $R^2 = 0.66$, $p = 0.001$) and explained two-thirds of the among-sample variance. However, the PERMDISP indicated significant heterogeneity in multivariate dispersion across sample types (fungal: $F = 8.12$, $p = 0.001$; bacterial: $F = 10.94$, $p = 0.001$) (Figure 2.2). To assess the extent of variation among these communities, pairwise PERMANOVA was used to complement the overall PERMANOVA results. All pairwise tests confirmed significant variation in community comparison of the PM between nurseries, the PM and the seedling RZs, and the seedling RZs both within nursery and between nurseries (Supplemental Table 2.2).

Within the nursery, the PIPO and PIST species differed in their RZ communities, but this effect was strongly nursery dependent. N1 seedlings showed larger variation in fungal ($R^2 =$

0.765), and bacterial ($R^2 = 0.571$) communities between N1 PIPO and N1 PIST. This difference between communities was smaller at N2. For the fungal community, the variation between N2 PIPO and N2 PIST was 8.4% ($R^2 = 0.084$), and for the bacterial community, the variation was 6% ($R^2 = 0.060$) (Supplemental Table 2.2).

When comparing the fungal and bacterial communities of the same species grown at different nurseries, nursery consistently explained greater than 59.8% of the variation (PIPO: ITS2: $R^2 = 0.642$; 16S: $R^2 = 0.625$. PIST: ITS2: $R^2 = 0.598$; $R^2 = 0.592$) (Supplemental Table 2.2).

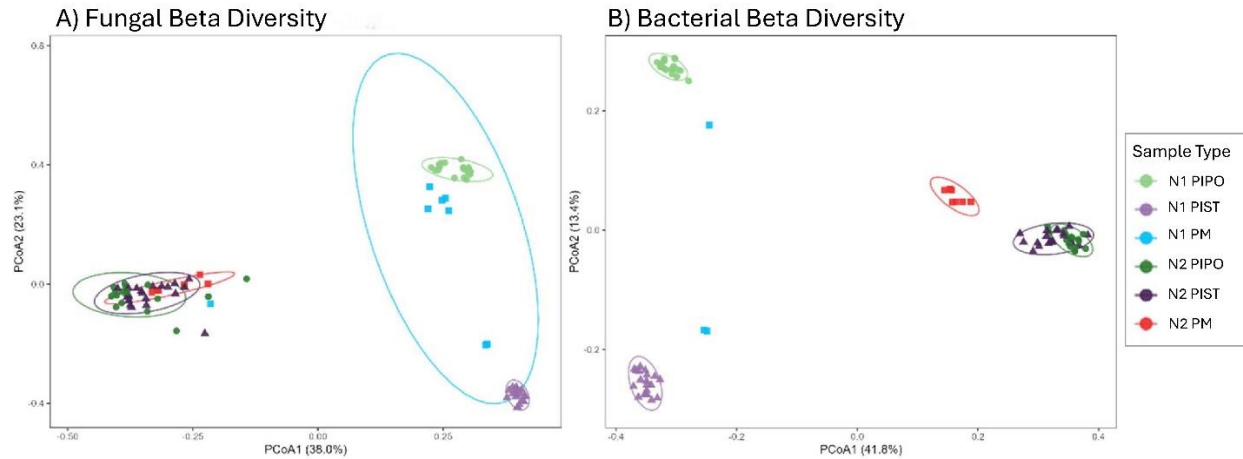


Figure 2.2. Beta diversity of (A) fungal (ITS2) and (B) bacterial (16S) communities across two conifer nurseries, N1 and N2, visualized by principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities. Points represent individual samples colored by sample type: the rhizosphere (RZ) of *Pinus ponderosa* (PIPO) and *P. strobiformis* (PIST) seedlings and the potting mix (PM) within each nursery. Axis labels give the proportion of variance explained by each coordinate. Ellipses denote 95% confidence regions for each group. Community composition differed significantly among sample types in both datasets (PERMANOVA: fungal, $R^2 = 0.65$, $F = 31.17$, $p = 0.001$; bacterial, $R^2 = 0.66$, $F = 31.92$, $p = 0.001$; 999 permutations). Group dispersions were also heterogeneous (PERMDISP: fungal, $F = 8.12$, $p = 0.001$; bacterial, $F = 10.95$, $p = 0.001$).

Differential Abundance of ASVs. Differential abundance analyses were performed to further characterize the taxa present within and among sample types. For the PM comparison

between the nurseries, 15.8% of the fungal ASVs and 4.8% of the bacterial ASVs were significantly differentially abundant (Table 2.1). In the PM, there was one putative fungal pathogen ASV (*Microbotryum*) with higher mean relative abundance in the N2 potting mix (Supplemental Figures 2.2, 2.3).

At both nurseries, seedling RZ had more differentially abundant fungal ASVs than the PM. At N1, the N1 PM - N1 PIST comparison revealed that 31.2% of ASVs were significantly differentially abundant, compared to 12.7% between the N1 PM - N1 PIPO (Table 2.1). The N1 PIPO contained one putative fungal pathogen ASV (*Sporothrix*) with significantly higher mean relative abundance than in the PM. Despite the large number of differentially abundant ASVs between the PM and the N1 PIST, none were putative fungal pathogens (Table 2.1; Supplemental Figures 2.4, 2.5). At N2, the percent of differentially abundant fungal ASVs was smaller (9.4% PIPO, 7.3% PIST), but there were two significantly differentially abundant putative fungal pathogen ASVs (*Microbotryum* and *Ustilago*) in both seedlings that had significantly higher mean relative abundance in the PM (Table 2.1; Supplemental Figures 2.6, 2.7).

For differentially abundant bacterial ASVs between the N1 PM and the N1 seedling RZ, the comparison was limited by sample sizes; however, between the N1 PM - N1 PIPO and N1 PM - N1 PIST, 10.9% and 11.3% of ASVs were significantly differentially abundant (Table 2.1; Supplemental Figures 2.8, 2.9). The total number of bacterial ASVs tested for the same comparison at N2 was almost double that at N1, and the percentage of significantly differentially abundant ASVs was higher as well. For the N2 PM - N2 PIPO, 22.1% of ASVs were significantly differentially abundant, whereas 19.9% were for N2 PM - N2 PIST (Table 2.1; Supplemental Figures 2.10, 2.11).

Within N1, the seedlings of PIPO and PIST harbored very different ASVs. For fungal ASVs, 61.8% were significantly differentially abundant, and for bacteria, 58.8% were significantly differentially abundant (Table 2.1; Supplemental Figures 2.12, 2.13). Of the fungal ASVs, 2 were putative fungal pathogens. *Bauerago* was significantly relatively abundant in N1 PIPO, and *Gjaerumia* was significantly relatively abundant in N1 PIST (Supplemental Figure 2.12). This difference was not present at N2, where the comparison between species recovered the same number of fungal ASVs as in the N1 comparison, but only 5.8% were significantly differentially abundant. No putative fungal pathogens were found between N2 PIPO and N2 PIST, which may indicate that any fungal pathogens present are ubiquitous across hosts (Table 2.1, Supplemental Figure 2.14). Of the 2458 bacterial ASVs tested, 0.1% were significantly differentially abundant between N2 seedlings (Table 2.1; Supplemental Figure 2.15).

By comparing PIPO and PIST grown at the two nurseries, nursery plays a large role in the differentially abundant taxa. In the PIPO, 71.4% and 80.3% of fungal and bacterial ASVs, respectively, were differentially abundant (Table 2.1). Comparing PIST between nurseries revealed a similar pattern, with 74.1% of fungal ASVs and 71.8% of bacterial ASVs significantly differentially abundant between N1 PIST and N2 PIST (Table 2.1).

The putative fungal pathogen ASVs that were enriched between nurseries were similar for PIPO and PIST. Both seedlings had ASVs from *Coleophoma*, *Microbotryum*, and *Platyglœa*, with significantly higher mean relative abundance at N2. The PIPO had an additional ASVs of *Bipolaris* and *Sporothrix*. *Sporothrix* was significantly more abundant in N1 PIPO, and *Bipolaris* in the N2 PIPO. There was one *Bauerago* ASV with no significant difference in mean relative abundance, and one *Bauerago* ASV that was significantly more abundant in the N2 PIPO. For

the PIST grown at different nurseries, ASVs of *Alternaria* and *Gjaerumia* had significantly higher mean relative abundance in the N1 PIST (Figure 2.3).

Table 2.1. Differential abundance of fungal (ITS2) and bacterial (16S) Amplicon Sequence Variants (ASVs) across pairwise contrasts among potting mix (PM) and seedling rhizospheres (RZ) of the two nurseries (N1 and N2). Differential abundance was tested with MaAsLin2 (TSS normalization, log transform, linear model); ASVs with $q < 0.05$ were considered significant, with Benjamini–Hochberg correction applied independently within each contrast. ASVs were retained for testing if present in $\geq 10\%$ of samples and with ≥ 3 total reads across the comparison's samples. Results are organized into four contrast sets: ASVs differentially abundant (i) in the PM between nurseries, (ii) in the PM compared to seedling rhizospheres (RZ) within each nursery, (iii) between the two host species (RT: PIPO, *Pinus ponderosa*; PIST, *P. strobiformis*) within each nursery, and (iv) in each host species between nurseries.

(i) Differential Abundance of ASVs in the PM between nurseries

Locus _A	Contrast ^B	A ^C	B ^D	Sig of tested ^E	%_sig ^F	n_sig_A _G	%_A ^H	n_sig_B ^I	%_B ^J
ITS2	PM - N1 vs N2	N1	N2	31 of 196	15.8	12	19	38.7	61.3
16S	PM - N1 vs N2	N1	N2	95 of 1974	4.8	28	67	29.5	70.5

(ii) Differential Abundance of ASVs in the PM compared to seedling RZ within nursery

Locus _A	Contrast ^B	A ^C	B ^D	Sig of tested ^E	%_sig ^F	n_sig_A _G	%_A ^H	n_sig_B ^I	%_B ^J
ITS2	N1 - PM vs PIPO	PM	PIPO	20 of 157	12.7	7	13	35	65
ITS2	N1 - PM vs PIST	PM	PIST	59 of 189	31.2	30	29	50.8	49.2
ITS2	N2 - PM vs PIPO	PM	PIPO	18 of 192	9.4	11	7	61.1	38.9
ITS2	N2 - PM vs PIST	PM	PIST	14 of 192	7.3	11	3	78.6	21.4
16S	N1 - PM vs PIPO	PM	PIPO	134 of 1234	10.9	45	89	33.6	66.4
16S	N1 - PM vs PIST	PM	PIST	149 of 1319	11.3	53	96	35.6	64.4
16S	N2 - PM vs PIPO	PM	PIPO	500 of 2264	22.1	215	285	43	57
16S	N2 - PM vs PIST	PM	PIST	438 of 2197	19.9	215	223	49.1	50.9

(iii) Differential Abundance of ASVs in the two host RT within nursery

Locus _A	Contrast ^B	A ^C	B ^D	Sig of tested ^E	%_sig ^F	n_sig_A _G	%_A ^H	n_sig_B ^I	%_B ^J
ITS2	N1 - PIPO vs PIST	PIPO	PIST	123 of 199	61.8	55	68	44.7	55.3
ITS2	N2 - PIPO vs PIST	PIPO	PIST	11 of 189	5.8	8	3	72.7	27.3

16S	N1 - PIPO vs PIST	PIPO	PIST	988 of 1680	58.8	491	497	49.7	50.3
16S	N2 - PIPO vs PIST	PIPO	PIST	3 of 2458	0.1	2	1	66.7	33.3

(iv) Differential Abundance of ASVs in the host tree RT between nurseries

Locus A	Contrast ^B	A ^C	B ^D	Sig of tested ^E	%_sig ^F	n_sig_A G	%_A ^H	n_sig_ B ^I	%_B ^J
ITS2	PIPO - N1 vs N2	N1	N2	150 of 210	71.4	73	77	48.7	51.3
ITS2	PIST - N1 vs N2	N1	N2	163 of 220	74.1	93	70	57.1	42.9
16S	PIPO - N1 vs N2	N1	N2	1879 of 2340	80.3	665	1214	35.4	64.6
16S	PIST - N1 vs N2	N1	N2	1680 of 2341	71.8	689	991	41	59

^A ITS2 (fungal) or 16S (bacterial).

^B The two groups compared (A vs B).

^C The reference group.

^D Second group in the comparison.

^E Number of significant ASVs out of the total tested in that comparison.

^F Percentage of tested ASVs that were significant.

^G Number of significant ASVs enriched in group A (negative MaAsLin2 coefficient, i.e., toward the reference group).

^H Percentage of all significant ASVs in that comparison.

^I Number of significant ASVs enriched in group B (positive MaAsLin2 coefficient).

^J Percentage of all significant ASVs in that comparison.



Figure 2.3. FungalTraits Plant-pathogen Amplicon Sequence Variants (ASVs) differentially abundant in rhizospheres (RZ) of *Pinus ponderosa* (A) and *P. strobiformis* (B) between the two nurseries (N1 and N2). Heatmaps show all significant plant-pathogen ASVs (MaAsLin2, TSS normalization, log transform, $q < 0.05$) for the across-nursery contrast in each host species (9 ASVs in *P. ponderosa*, 8 in *P. strobiformis*). Each column is a RZ sample, grouped by source nursery (N1, blue; N2, red); each row is an ASV, labeled by genus and ordered alphabetically. Cell color is the mean relative abundance across samples (row-scaled). Asterisks following a genus label denote ASVs with a significant difference in relative abundance between nurseries (Wilcoxon rank-sum on per-sample relative abundance, Benjamini-Hochberg $q < 0.05$). ASVs sharing a genus name are distinguished by a numeric suffix (e.g., *Bauerago*, *Bauerago 1*).

Fungal communities of symptomatic root tips.

From the same roots used in the rhizosphere tagged-amplicon portion of the study, a total of 365 fungal isolates were cultured from symptomatic root tips. Of these, 313 isolates were assigned to a genus, representing 22 distinct genera. The unidentified isolates were retained and collapsed into a single "Unidentified" group.

Alpha diversity results for Shannon diversity (H') were not significant for species grown within or between nurseries (Supplemental Table 2.3). For Beta diversity, the overall PERMANOVA on Host $\times$ Nursery detected significant community-level differences ($R^2 = 0.078$, $F = 2.13$, $p = 0.006$). Pairwise PERMANOVA revealed small but significant separation of the PIPO and PIST in N2 ($R^2 = 0.051$, $p_FDR = 0.038$), but not in N1. In comparing species grown between the two nurseries, the variation of only the PIPO fungal communities was significant ($R^2 = 0.051$, $p_FDR = 0.014$) (Figure 2.4, Supplemental Table 2.4).

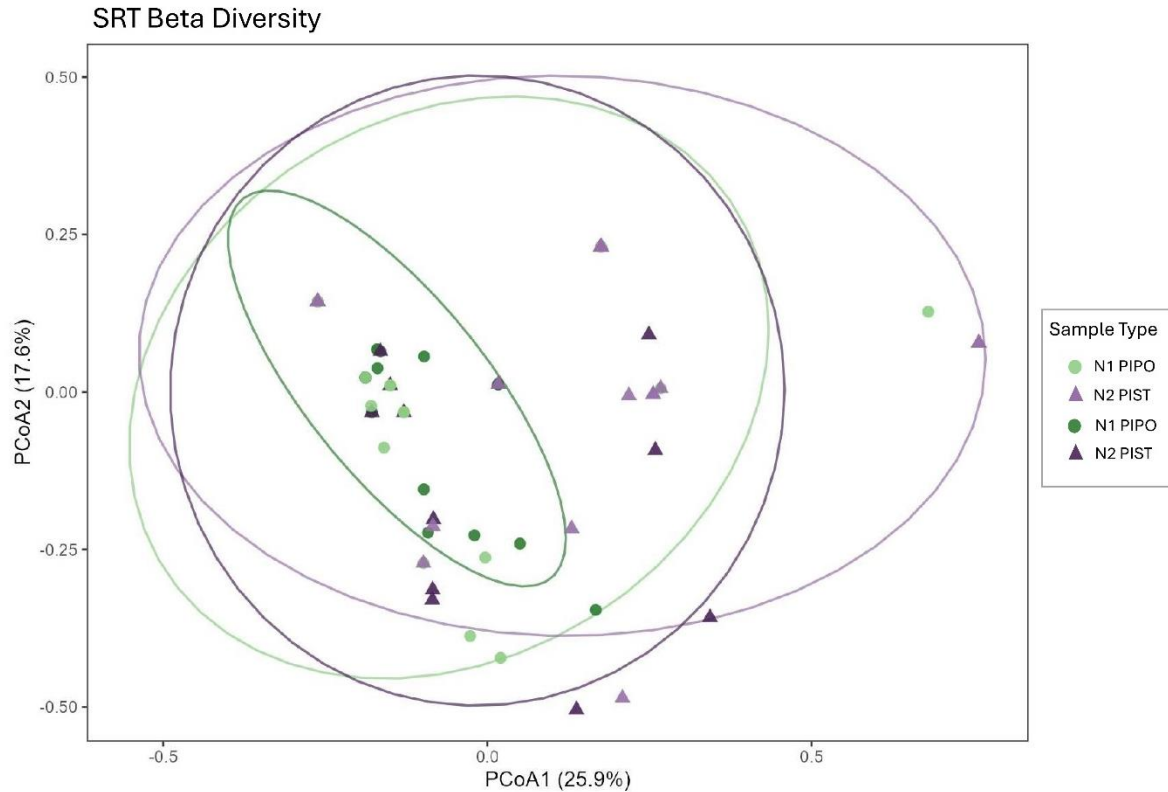


Figure 2.4. Principal coordinates analysis (PCoA) of culture-based fungal communities from symptomatic root tips (SRT). The ordination is based on Bray-Curtis dissimilarities of genus-level presence/absence data across all samples. Each point is a single tree, colored by host species and nursery of origin [greens/circles, *Pinus ponderosa* (PIPO); purples/triangles, *P. strobiformis* (PIST); light, N1; dark, N2]; axis labels give the proportion of variance explained by each coordinate, and ellipses are 95% *t*-distribution confidence ellipses. Community composition differed among the four host $\times$ nursery groups (PERMANOVA: $R^2 = 0.078$, $F = 2.13$, $p = 0.006$; 999 permutations), with homogeneous group dispersions (PERMDISP: $F = 0.25$, $p = 0.87$), indicating the difference reflects group location rather than dispersion. In pairwise comparisons (Benjamini-Hochberg adjusted), composition differed between host species within Nursery 2 ($p_{\text{adj}} = 0.038$) and between nurseries within *P. ponderosa* ($p_{\text{adj}} = 0.014$), but not between host species within Nursery 1 ($p_{\text{adj}} = 0.157$) or between nurseries within *P. strobiformis* ($p_{\text{adj}} = 0.281$).

Relative abundance of SRT fungal taxa. Despite community-level shifts detected by PERMANOVA, no genus differed significantly between nurseries after a Benjamini–Hochberg correction, either when pooled across hosts or when stratified by host (Supplemental Table 2.5). Across the 23 genera detected, *Penicillium* dominated isolate communities in both nurseries (mean relative abundance: N1 = 0.48; N2 = 0.50). There was also a large proportion of

Trichoderma (mean relative abundance: 0.19); however, *Trichoderma* and *Mucor* were noticeably absent from the N2 PIPO seedlings (Figure 2.5).

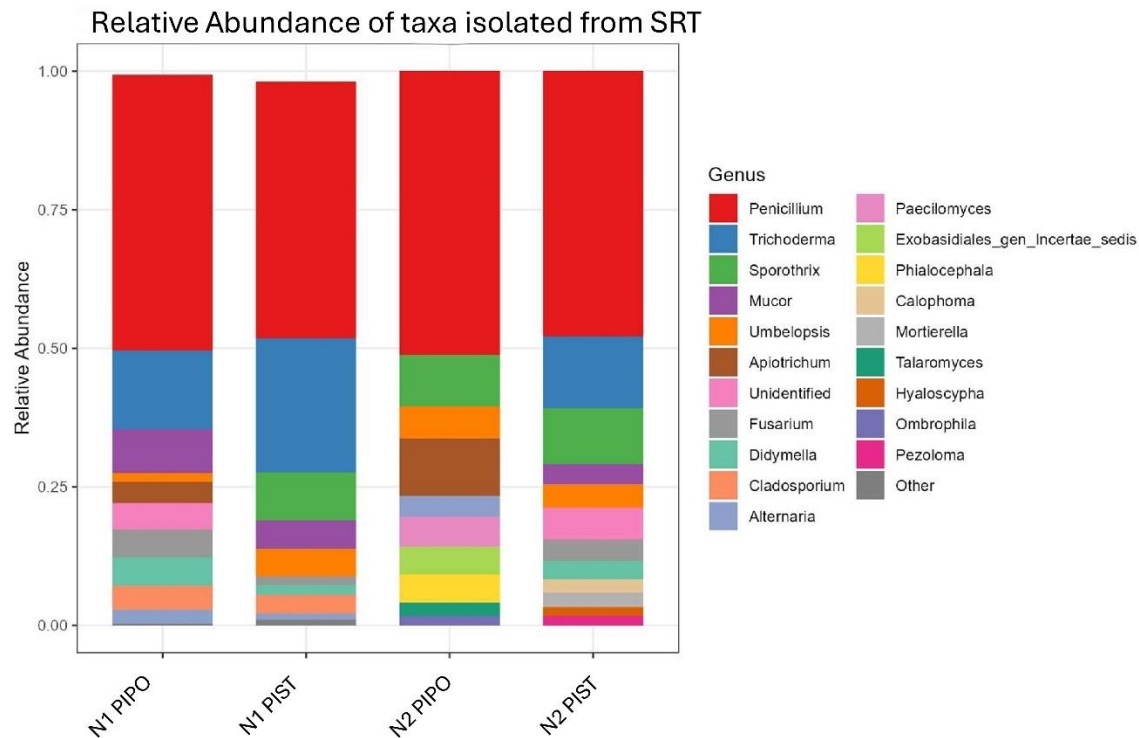


Figure 2.5. Genus-level composition of culture-based fungal communities from symptomatic root tips (SRT), by host species [*Pinus ponderosa* (PIPO); *P. strobiformis* (PIST)] and nursery (N1 and N2). Bars show the mean relative abundance of each genus across trees within each host × nursery (N1 PIPO, N1 PIST, N2 PIPO, N2 PIST). The 20 most abundant genus-level categories (ranked by mean relative abundance) are shown individually, and all others are pooled as "Other". "Unidentified" denotes isolates with no genus-rank assignment (NA) in the reference taxonomy.

Overlap between symptomatic root tips, rhizosphere, and potting mix.

Guild characterization. A total of 352 fungal genera were recovered across the three datasets and assigned to 15 primary-lifestyle guilds. The rhizosphere harbored the largest pool, with 163 genera detected exclusively in the rhizosphere and a further 141 were shared with the PM. The symptomatic root tips (SRT) contained no exclusive genera, sharing only two within the rhizosphere (*Didymella*, *Scytalidium*) and one with the PM (*Curvularia*). Eighteen genera were

common to all three sample types, and 27 genera were found only in the PM. The 18 shared genera were primarily saprotrophs (*Apiotrichum*, *Cladosporium*, *Hyaloscypha*, *Mortierella*, *Mucor*, *Ombrophila*, *Penicillium*, *Phialocephala*, *Talaromyces*, *Umbelopsis*), but *Trichoderma* and several broad-host plant pathogens (*Alternaria*, *Calophoma*, *Fusarium*, *Sporothrix*) were also shared (Table 2.2, Supplemental Table 2.6).

Across the full dataset, saprotrophic guilds were dominant, followed by plant pathogens (44 genera). The rhizosphere–potting-mix shared pool (141 genera) was functionally broad but was primarily made up of saprotrophs (88 genera), plant pathogens (19 genera), and mycoparasites (10 genera) (Figure 2.6, Table 2.2, Supplemental Table 2.6).

Even though seedlings were not inoculated, several ectomycorrhizal genera were detected in the rhizosphere. Overall, N2 seedlings had more ECM taxa than N1 seedlings. *Cortinarius* was present in seedlings of both nurseries at higher levels than any other ECM (3 ASVs from N1 and 22 ASVs from N2). Another notable ECM present in both nurseries was *Suillus* (2 ASVs from N1 and 7 ASVs from N2), whereas *Rhizopogon* ASVs were found only in the N1 PIST. The N2 PM contained four genera, *Alpova*, *Cortinarius*, *Paxillus*, and *Tricholoma*, but the N1 PM only contained *Cortinarius*. Of these, only *Cortinarius* and *Tricholoma* were also detected in seedling rhizospheres (Supplemental Table 2.7).

Venn diagram of genera overlap from three sample types

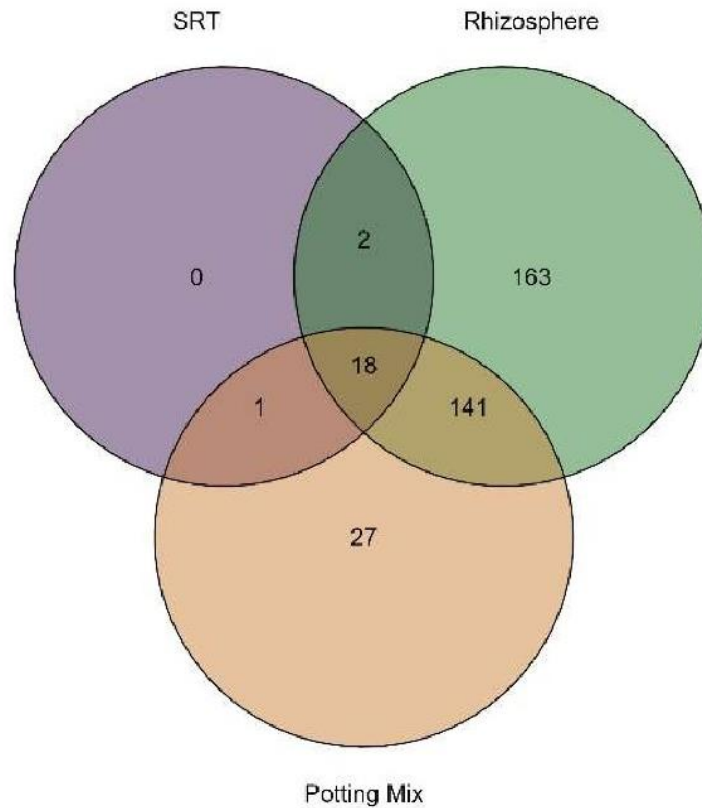


Figure 2.6. A Genus-level Venn diagram that shows the number of fungal genera unique to and shared fungal taxa among sample types: symptomatic root tips (SRT), rhizosphere (RZ), and potting mix (PM). Of 352 genera total, 18 were shared by all three; 163 were unique to the rhizosphere and 27 to the potting mix, while no genera were unique to SRT. SRT genera were recovered by culturing, whereas rhizosphere and potting mix genera were detected by ITS amplicon sequencing, so the lower SRT richness partly reflects the detection method.

Table 2.2. Fungal genera recovered from symptomatic root tips (SRT) by culturing, with their overlap across sample types and primary lifestyle. Each genus is listed by the Figure 2.6 Venn region it occupies: shared by all three sample types (All three), shared by SRT and rhizosphere only (SRT_RZ), or shared by SRT and potting mix only (SRT_PM), together with its primary lifestyle assigned by FungalTraits. All 21 named SRT genera are included; each was also detected in the rhizosphere and/or potting mix, consistent with the absence of SRT-unique genera in Figure 2.6. Isolates not assigned to a genus (Unidentified) were excluded.

<u>Region</u>	<u>Genus</u>	<u>primary lifestyle</u>
<u>All three</u>	<u>Alternaria</u>	<u>plant pathogen</u>
<u>All three</u>	<u>Apiotrichum</u>	<u>soil saprotroph</u>
<u>All three</u>	<u>Calophoma</u>	<u>plant pathogen</u>
<u>All three</u>	<u>Cladosporium</u>	<u>litter saprotroph</u>
<u>All three</u>	<u>Fusarium</u>	<u>plant pathogen</u>
<u>All three</u>	<u>Hyaloscypha</u>	<u>litter saprotroph</u>
<u>All three</u>	<u>Mortierella</u>	<u>soil saprotroph</u>
<u>All three</u>	<u>Mucor</u>	<u>soil saprotroph</u>
<u>All three</u>	<u>Neoleptodontidium</u>	<u>unassigned</u>
<u>All three</u>	<u>Ombrophila</u>	<u>wood saprotroph</u>
<u>All three</u>	<u>Paecilomyces</u>	<u>foliar endophyte</u>
<u>All three</u>	<u>Penicillium</u>	<u>unspecified saprotroph</u>
<u>All three</u>	<u>Pezoloma</u>	<u>root endophyte</u>
<u>All three</u>	<u>Phialocephala</u>	<u>soil saprotroph</u>
<u>All three</u>	<u>Sporothrix</u>	<u>plant pathogen</u>
<u>All three</u>	<u>Talaromyces</u>	<u>unspecified saprotroph</u>
<u>All three</u>	<u>Trichoderma</u>	<u>mycoparasite</u>
<u>All three</u>	<u>Umbelopsis</u>	<u>soil saprotroph</u>
<u>SRT_RZ</u>	<u>Didymella</u>	<u>plant pathogen</u>
<u>SRT_RZ</u>	<u>Scytalidium</u>	<u>wood saprotroph</u>
<u>SRT_PM</u>	<u>Curvularia</u>	<u>plant pathogen</u>

Oomycete detection.

Although the RPS10 dataset was not used for quantitative community analyses, six putative oomycete species were detected in the 13 retained samples, including *Achlya hypogyna*, *Globisporangium aquisylvae*, *G. okanoganense*, *G. rostratiformis*, *Phytophthora cactorum*, and *P. pseudocryptogea*. Of the 13 samples retained, three were of N1 PIPO, one of N1 PIST, five of N2 PIPO, three of N2 PIST, and one of N2 potting mix (Supplemental Table 2.8).

DISCUSSION

This study provides one of the first detailed characterizations of the microbial communities associated with conifer seedlings in operational reforestation nurseries. By examining fungal, bacterial, and oomycete communities across two nurseries growing two conifer species from a shared seed source, we were able to assess that nursery environments and practices shape seedling microbiomes. Three patterns emerged. First, nursery origin was the dominant factor structuring both fungal and bacterial communities, overshadowing host species effects and extending to differences in potting-mix communities. Second, species differences were present but depended on the nursery. Third, culture-based isolation from symptomatic root tips captured taxa not detected through amplicon sequencing, revealing selective associations that sequencing alone would have missed.

Nursery was the dominant driver of variation in both fungal and bacterial communities, with numerous ASVs differing significantly in abundance between nurseries. This nursery effect was consistent across host species, whereas species-level differences varied by nursery. Our results suggest that nursery production conditions exert a stronger and more enduring influence on rhizosphere microbiome composition than host species, aligning with previous work showing that nursery conditions play a central role in microbial community composition (Baranowska et al., 2023). Similar patterns have been reported for ECMs in oak (*Quercus* spp.) (Leski et al., 2010), Scots pine (*Pinus sylvestris*), and Norway spruce (*Picea abies*) (Menkis et al., 2006). Substrate composition is likely a major driver of these patterns, as it is primary driver of microbial assembly in soilless systems (Pot et al., 2022; Quijia Pillajo et al., 2024), often outweighing the effects of host species on rhizosphere communities (Wang et al., 2022). Furthermore, bacterial communities in commercial potting media vary considerably among

products (Al-Sadi et al., 2016). As such, we found that the potting mixes used at the two nurseries had distinct microbial community signatures, suggesting that potting mix may be a strong candidate shaping long-term seedling communities.

However, the rhizosphere consistently harbored taxa not shared with the potting mix, indicating additional microbial assemblages from the environment (Fitzpatrick et al., 2018; Wagner et al., 2016). Seedlings differed significantly in Beta diversity from the potting mix at both nurseries, and at N1, seedling species also differed significantly from each other. This host effect was not as strong at N2 where seedling Beta diversity remained significant; however, the variation between communities was lower, and there were fewer differentially abundant ASVs between the seedling species. These findings indicate that while potting mix contributes to rhizosphere assembly, it does not fully explain the observed community patterns among seedlings. This may indicate that environmental conditions at N2 may have homogenized microbial communities between PIPO and PIST. However, the differences in seedling age may also play a role in these observed patterns as the PIST were about four weeks older than the PIPO. Other aspects of the nursery environment, including irrigation water and greenhouse management practices, may also influence microbial community assembly. N2, located in a high-elevation desert, is a relatively small operation and draws irrigation water from a pond located on the property. In contrast, N1, located in a lower-elevation semi-arid environment, is a larger facility that frequently opens its greenhouses to the surrounding environment and sources irrigation water from multiple locations, including a well connected to a local reservoir. These differences may expose seedlings to distinct environmental microbiome shaped by regional environmental conditions and water sources (Garcia-Lemos et al., 2020).

Beyond these regional and environmental factors, nursery-specific management practices likely further contributed to community differentiation. The two nurseries differed in distinct container types (Styroblocks at N1 vs. plastic cone-tainers at N2), potting mix formulations (75:12.5:12.5 vs. 2:1:1 peat:vermiculite:perlite), irrigation strategies (manual and overhead vs. sub-irrigation), and seed stratification protocols (aerated 35 °C water bath vs. 4 °C cold soak); sowing density, sowing date, and seedling age at sampling. These differences undoubtedly play a role in the communities associated with the rhizosphere, the potting mix, and the symptomatic root tips, but a two-nursery design cannot separate the nursery effect into its component practices. Container type, substrate, irrigation, and stratification are fully confounded with nursery identity, and any of these factors, or some combination of them, could also be driving the observed community differences. This limits the conclusions to descriptive ones; nursery origin matters, but the mechanisms or factors that drive these differences remains an open question.

Ectomycorrhizal fungi (ECM) provide some of the strongest evidence that environmental exposure may contribute to nursery-associated differences in microbial communities. ECM taxa were not intentionally introduced through nursery management, were uncommon in the potting mix, and occurred more frequently in seedlings from N2. Little is known about ECM transmission via seeds, as most seed-associated fungal studies focus on pathogens (Cram & Fraedrich, 2010; Larsson et al., 2024).

Nursery influence extended beyond potentially beneficial symbionts. Differences were also observed among putative fungal pathogens between nurseries, though none have been confirmed to cause disease in conifer seedling roots in this study. These taxa include heterobasidiomycetous fungi known to cause disease on grasses (Aime et al., 2006; Kemler et al., 2006; Malysheva et al., 2021), saprophytes associated with decaying wood (Meng et al.,

2025), endophytes (Zou et al., 2025), foliar pathogens (Larsson et al., 2024), or found in many types of environments with variable lifestyles (Chang et al., 2021; Jankowiak et al., 2023; Pernice et al., 2024)

Culture-based isolation of symptomatic root tips sheds more light on putative pathogen dynamics as seven putative fungal pathogens were recovered. Among the recovered isolates was *Fusarium*, a well-documented genus that causes soilborne root rot, wilt, and damping-off, and is routinely recovered from seedlings (Admas & Kebede, 2024; Dobbs et al., 2023). *Alternaria*, *Calophoma*, *Didymella*, and *Curvularia* are also isolated; although typically associated with foliar and stem lesions, they can act as root pathogens under some conditions (Jayasiri, 2017; Larsson et al., 2023; Marin-Felix et al., 2017; Priwiratama et al., 2024; Sheller et al., 2020). Some putative fungal pathogens recovered are more ambiguous and more commonly associated with a saprotrophic lifestyle (Chang et al., 2021; Jankowiak et al., 2023; Tong et al., 2025). Pathogenicity and virulence assays using these isolates are needed to determine their potential threat to seedlings in nursery systems.

Further investigation of oomycetes is also warranted, particularly given their detection across sample types in this study. However, the RPS10 amplicon sequencing approach used to characterize oomycete communities had several limitations. Mock community recovery was poor, and only 32 of the 98 samples yielded usable read counts, with just 13 retaining more than 1,000 reads. The RPS10 marker is comparatively new, and reference databases and primer optimization are still in development (Foster et al., 2022). Targeted methods such as baiting assays, isolation onto selective media, or genus-specific qPCR could complement this newly developed technique where quantitative oomycete community data are needed.

Even so, the species identifications are worth reporting because oomycetes include some of the most economically important pathogens of conifer seedlings. The detection of oomycetes in nursery samples warrants further investigation, particularly given the presence of putative members of the genera *Phytophthora* and *Pythium*. Six putative oomycete species were identified as *Achlya hypogyna*, *Globisporangium aquisylvae*, *G. okanoganense*, *G. rostratifingens*, *Phytophthora cactorum*, and *P. pseudocryptogea*. *Phytophthora* detections are most notable; *P. cactorum* is a well-documented pathogen with a broad host range that includes conifer seedlings and is a known cause of damping-off and root rot in nursery contexts (Tkaczyk et al., 2016). *Phytophthora pseudocryptogea*, a more recently described species within the *P. cryptogea* complex (Safaiefarahani et al., 2015), has been associated with root and collar rot of woody hosts in nursery and ornamental settings (Aloi et al., 2023). The three *Globisporangium* species (formerly *Pythium* sensu lato; Uzuhashi et al., 2010), belong to a genus that includes both saprotrophs and root pathogens, with several species implicated in damping-off of forest tree seedlings (Lazreg et al., 2016). These findings support other studies that have described the presence of Oomycetes in conifer nurseries (Marčiulynienė et al., 2021).

Overall, these findings are hypothesis-generating and highlight the need for broader investigations into the factors shaping seedling microbiomes during nursery production. Future work should include larger multi-nursery surveys and factorial experiments designed to isolate the effects of specific management practices and environmental conditions. Additional research is also needed to determine the functional significance of the taxa identified here. Isolates recovered from symptomatic root tips should be evaluated in pathogenicity assays to assess their roles in seedling disease. Finally, the detection of oomycetes across sample types suggests that

these organisms warrant further study, particularly given their importance as root pathogens and the current dataset's limitations in resolving their dynamics.

CONCLUSIONS

Comprehensive evaluations of seedling microbiomes in conifer nursery systems remain limited, yet are vital given the recently mandated increases in conifer production for reforestation efforts under the REPLANT Act (Balloffet & Dumroese, 2022). This study represents a first step toward understanding how nursery environments influence conifer seedling microbiomes. Our findings demonstrate that nurseries are not neutral backgrounds: the microbial communities of seedlings are shaped by the nursery in which the seedlings were grown. Whether these nursery-specific microbial signatures persist after outplanting in the field, attenuate over time, or influence seedling survival remains an open question, which is addressed in the following chapter.

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CHAPTER 3: INTO THE WILD: OUTPLANTING SEEDLINGS INTO POST-FIRE LANDSCAPES

ABSTRACT

Successful reforestation depends on the ability of the seedlings to establish and integrate into their new environment. To characterize microbial trajectories during early establishment, seedlings of ponderosa pine (*Pinus ponderosa*) and southwestern white pine (*P. strobiformis*) were outplanted into a post-fire restoration site within the Calf's Canyon-Hermit's Peak burn scar in northern New Mexico. Rhizosphere fungal and bacterial communities were monitored at planting (Time 0), 6 months, and 1 year post-outplanting using ITS2 and 16S amplicon sequencing. Seedling growth, naturally regenerating ponderosa pine, and bulk soil communities were also assessed to evaluate microbial dynamics during establishment. Fungal and bacterial community composition differed significantly at planting, but these differences declined over time as the seedling rhizosphere increasingly resembled the bulk soil and naturally occurring ponderosa pine microbiomes. Differential abundance analyses revealed extensive turnover of rhizosphere taxa, with nursery-origin fungi and bacteria declined over time whereas soil-origin taxa increased in abundance. Soil-origin ectomycorrhizal fungi increased in relative abundance within the first year, while nursery-origin putative fungal pathogens declined. The movement of nursery-origin fungi into the bulk soil was rare, with fewer than 3% of candidate fungal amplicon sequence variants (ASVs) detected outside the rhizosphere. However, two putative nursery-origin fungal pathogens, *Fusarium*, were recovered from bulk soil samples. These findings demonstrate that rhizosphere communities undergo substantial restructuring during the first year following outplanting, nursery-origin ASVs become less abundant, and microbial movement into the bulk soil remains limited. Furthermore, this study highlights the importance of post-

outplanting monitoring and demonstrates that seedlings can successfully recruit native symbiotic taxa even when planted in a post-fire soil.

INTRODUCTION

Forest reestablishment through natural regeneration is highly variable and strongly influenced by fire severity (Davis et al., 2023; Welch et al., 2016). High-severity wildfires can reduce seed viability within the soil seedbank and eliminate mature seed-producing trees (Knapp et al., 2012; Rodman et al., 2020). When wildfires burn as mosaics, they leave unburned patches capable of serving as seed sources (Chapman et al., 2020; Krawchuk et al., 2016). However, high-severity, stand-replacing fires can eliminate these sources entirely, severely hindering natural regeneration (Rodman et al., 2020; Welch et al., 2016). Even where seeds survive, or parent trees persist through fire, seedling establishment is slow and may be constrained by competition from recovering understory vegetation (Shatford et al., 2007; Ursell & Safford, 2022). Consequently, natural regeneration is often insufficient to achieve desired reforestation outcomes. In the face of increasingly frequent and severe wildfires (Abatzoglou et al., 2019; Cunningham et al., 2024), forest managers need to supplement recovery by planting nursery-grown seedlings.

Given the increase in high-severity wildfires and the scale of reforestation mandated by recent policy initiatives (Balloffet & Dumroese, 2022), it is critical to evaluate seedling dynamics from nursery production through long-term field establishment in burned soils. Wildfires substantially alter native microbial communities, and high-severity fires can reduce beneficial ectomycorrhizal fungi while increasing the abundance of some pathogenic fungal taxa (Bowd et al., 2022; Nelson et al., 2022). Fire-induced changes to bacterial communities can also affect key ecosystem processes, including nutrient cycling, decomposition, and soil organic matter turnover

(Bowd et al., 2022; Nelson et al., 2022; Patrick et al., 2024). These altered soils may affect seedling establishment and growth by disrupting plant–microbe interactions critical for nutrient acquisition and stress tolerance. Furthermore, disturbed, post-fire burned soils may be highly susceptible to invasive soil microbes introduced alongside outplanted seedlings.

Nurseries can inadvertently serve as reservoirs for both endemic and non-native pathogens (Charron et al., 2025; Dobbs et al., 2023), which may be transported to restoration sites on infected planting stock or associated growing media (Dobbs et al., 2023; Frankel et al., 2020; Menkis et al., 2016). Once introduced, these pathogens may persist in soils, potentially impacting both the planted seedlings and the greater environment (Frankel et al., 2020; Menkis et al., 2016). Furthermore, pathogens can reduce reforestation success while increasing operational costs associated with seedling production.

The production of a single conifer seedling requires investments in seed collection, propagation, labor, infrastructure, pest management, and transportation (Fargione et al., 2021). When pathogen pressure reduces seedling survival in the nursery or after outplanting, these investments are effectively lost. Given that modern reforestation programs produce and plant millions of seedlings annually, even modest increases in mortality can result in substantial financial losses. Beyond these direct costs, pathogen-associated seedling mortality may delay forest recovery, reduce stand density (Rodman et al., 2020), while repeated planting efforts may be needed to overcome poor establishment in harsh sites (South et al., 2023). These economic costs and environmental concerns highlight the importance of understanding pathogen dynamics within nursery systems and across the reforestation pipeline. Addressing the dynamics of nursery pathogens is therefore not only a matter of seedling health, but a critical component of effective, cost-efficient, and resilient forest restoration.

Despite these substantial risks, post-planting monitoring remains limited across most reforestation efforts (Fargione et al., 2021). Concurrently, a growing body of research has shown that nursery environments, management practices, and host species strongly influence the assembly of the seedling microbiomes (Baranowska et al., 2023; Graham et al., 2025; Menkis et al., 2016), with both beneficial symbionts and latent pathogens commonly detected on planting stock (Charron et al., 2025; Marčiulynienė et al., 2021; Menkis et al., 2016). While our understanding of microbial community assembly during nursery production has improved, far less is known about the fate of these communities after outplanting. The limited studies available suggest that some nursery-associated ectomycorrhizal fungi can persist for years after planting and influence subsequent seedling performance; however, long-term monitoring remains rare and has largely focused on a small number of artificially inoculated symbionts (Franco et al., 2014; Quoreshi et al., 2008). Together, these findings highlight a critical knowledge gap regarding the persistence, turnover, and ecological consequences of nursery-associated microbial communities following outplanting.

To address this knowledge gap, this study examines the fate of nursery-associated microbial communities following outplanting into a post-fire environment. Utilizing two distinct nurseries that grew two conifer species from the same seed source, ponderosa pine (*Pinus ponderosa*, PIPO) and Southwestern white pine (*P. strobiformis*, PIST), we monitored rhizosphere microbiome changes following planting at a site in the Calf's Canyon Hermit Peak burn scar in northern New Mexico. This study specifically evaluated the dynamics within the rhizosphere, the soil environment directly influenced by plant roots and a key site of plant-microbe-soil interactions (Lynch & De Leij, 2012). We aimed to characterize microbial changes occurring from initial planting through the first year of field establishment. Our goals were to (1)

assess the temporal dynamics of seedling rhizosphere microbial communities and evaluate their relationship to seedling growth metrics, (2) characterize how the microbial communities of nursery-grown seedlings and naturally occurring regeneration vary relative to bulk soils over time, and (3) determine the trajectory of nursery-origin microbes, with a specific focus on putative fungal pathogens and mycorrhizae. We hypothesize that nursery-origin microbes, including potential pathogens, would initially establish within the rhizosphere of transplanted seedlings, but would decline in abundance over time as native soil microbes reasserted dominance. Consequently, the rhizosphere microbiomes of transplanted seedlings are expected to progressively converge with those of both naturally regenerating trees and the surrounding bulk soil.

MATERIALS AND METHODS

Study Site and Experimental Design. Trees from Nursery 1 (N1) were removed from their styroblock wells using plastic crafting dowels sterilized with 70% ethanol. Once removed from the block, the trees were placed in individual plastic bags for transport. Trees from Nursery 2 (N2) were grown in plastic cone-tainers and were transported to the site in their cone-tainers. All trees were transported to the field site at the New Mexico State University John T. Harrington Research Nursery in Mora, New Mexico, in an air-conditioned van. At the site, trees were planted in a 1.5-meter grid arranged in 4 blocks with 4 treatment types: N1 PIPO, N1 PIST, N2 PIPO, and N2 PIST. The site, protected from ungulate browsing by a 10-foot deer fence, is actively managed for weeds and rodents by staff at the nursery. Trees were planted in mid-October 2024.

Field Sampling. Sampling was conducted at three time points, Time 0 (October 2024, coinciding with planting), 6 months (April 2025), and 1 year (October 2025). At each timepoint,

80 planted seedlings (20 per treatment) were destructively sampled. Soil cores were collected using a soil auger. Cores were taken at time 0 in locations prior to seedling planting. At subsequent time points, cores were taken between rows and west of the target seedling. Each core was placed into a plastic bag, and between samples, the coring auger was disassembled, rinsed thoroughly with 91% isopropanol, and wiped dry with a paper towel. Seedling and soil samples were stored in a cooler until processed.

Rhizosphere and Biomass Collection. In the laboratory, each tree was placed over a labeled paper plate. Seedling height was measured from the cotyledon scar to the terminal bud, and caliper was measured 1 cm below the cotyledon scar. Excess potting mix and soil were removed from the root system by hand. The seedling was then transferred to a clean plate, and the root system was allowed to dry for approximately 1–3 minutes. Then the rhizosphere was manually removed from roots. The seedling was then set aside on the original plate, and the rhizosphere plate was folded twice to homogenize the material without direct contact. Approximately 2 g of rhizosphere was transferred to a tube containing Zymo DNA/RNA Shield (Zymo Research, Irvine, CA, USA), and the tube was shaken and placed on ice. The shoot and root systems were separated using scissors, placed in labeled paper bags, and stored in a freezer until biomass measurements could be completed. All tools, bench surfaces, and gloves were thoroughly sterilized between samples. For biomass measurements, root and shoot systems were dried at 70°C for 72 hours, and weights were recorded. Soil core samples were homogenized in the laboratory and stored in Zymo DNA/RNA Shield, following the same protocols used for the rhizosphere samples.

Microbial Community DNA Extraction, PCR Amplification, Library Preparation, and Sequencing. DNA extraction, PCR amplification, library preparation, and sequencing followed

the methods described in Ganter (Chapter 2). All three loci, 16S, ITS and RPS10, were amplified in separate 25 μ L PCR reactions and sequenced in separate runs on the Illumina MiSeq platform with 251 bp paired-end sequencing chemistry at the Next Generation Sequencing Core at Colorado State University.

Bioinformatic processing. Demultiplexed paired-end FASTQ files from nursery samples were processed in DADA2 v1.34.0 (Callahan et al., 2016) in R v4.4.1; R Core Team, 2024 for the three amplicon datasets. All analyses followed the standard DADA2 workflow described in chapter 2 with the ITS2 dataset filtered at $\text{maxEE} = c(4, 5)$. The oomycete RPS10 primers were removed in two stages following the workflow recommended by Foster et al. (2022) and followed the same workflow and parameters as in Chapter 2. Taxonomy was assigned using assignTaxonomy and the methods described in Chapter 2. Pipeline performance was assessed using ZymoBIOMICS Microbial Community Standards (Zymo Research, Irvine, CA) for the 16S and ITS2 pipelines and Foster Mock2 communities (Foster et al., 2022) for RPS10, processed alongside field samples through the full pipeline. Mock samples were removed prior to object construction; negative controls were retained for downstream decontamination. For the 16S dataset, mitochondrial and chloroplast sequences were removed prior to decontamination. Decontamination for the 16S, ITS2, and RPS10 followed the same steps as described in Chapter 2. The decontaminated 16S and ITS2 datasets were randomly rarefied without replacement to 15,000 reads per sample (Cameron et al., 2021; Schloss, 2024). The averaged phyloseq objects were used as input to all downstream Alpha- and Beta-diversity analyses.

Statistical analyses. All statistical analyses were performed using R version 4.5.2 (R Core Team, 2025). P-values were adjusted using the Benjamini–Hochberg false discovery rate (Benjamini & Hochberg, 1995); significance was accepted at $p < 0.05$.

Four growth metrics, including height (cm), root collar caliper (mm), root biomass (g), and shoot biomass (g), were measured for each seedling at times 0, 6 months, and 1 year. For each metric, a linear mixed-effects model was fit and tested for group differences at individual sampling points: $\text{metric} \sim \text{Nursery} \times \text{Species} \times \text{Timepoint} + (1 \mid \text{Block})$, with all main effects and interactions as fixed effects and Block as a random intercept. Overall effects were obtained from Type III ANOVA analyses (Fox & Weisberg, 2019). Estimated marginal means were computed with emmeans (Lenth, 2024). Pairwise comparisons were evaluated for samples within nursery (N1 PIPO v. N1 PIST, N2 PIPO v. N2 PIST x time), and between nursery (PIPO N1 v. N2, PIST N1 v. N2 x time).

To assess microbial diversity of the seedlings, Alpha diversity was characterized using Shannon's index computed from the rarefied amplicon sequence variant (ASV)-by-sample tables for fungi (ITS2) and bacteria (16S). Fungal and bacterial Shannon diversity was calculated for the within-nursery group and the between-nursery groups using a linear mixed-effects model and Pairwise comparisons as previously described.

Fungal and bacterial community composition was quantified with the Bray–Curtis index (Bray & Curtis, 1957) computed from the rarefied ASV-by-sample tables. A Bray–Curtis distance matrix was created for each locus and used for an overall PERMANOVA. Pairwise comparisons between the two groups utilized group-specific subsets from the matrix. The overall PERMANOVA (999 permutations; Anderson, 2001) was completed with the model, $\text{Bray-Curtis} \sim \text{Nursery} \times \text{Species} \times \text{Timepoint}$. Permutations were restricted within the planting block via $\text{strata} = \text{Block}$. Homogeneity of multivariate dispersions was tested on the full model using PERMDISP (999 permutations; Anderson, 2006).

Before relating microbial community composition to the growth metrics, we screened for collinearity among the four metrics using pairwise Pearson correlations. Height was largely independent of the other metrics, whereas caliper, root biomass, and shoot biomass were correlated across all time points. The same Bray–Curtis dissimilarity matrix (Bray & Curtis, 1957) used for microbial community analyses was used in these tests. Each growth metric tested was modeled separately. Each metric was analyzed in two ways, pooled by timepoint, and stratified by timepoint. Stratifying by time point was used as a follow-up to the pooled model to determine when this correlation began, or whether it was always present. Community composition was visualized using Principal Coordinate Analysis (PCoA), and the four metrics were fitted as vectors with envfit ($n = 999$).

To test whether each metric explained community variation without confounding variables, and confirm the results of the time point stratified PERMANOVAs, we utilized partial constrained ordination (capscale, also called partial dbRDA / CAP Legendre & Anderson, 1999). For each metric, we fit a separate capscale() model with the metric as a constraint and Nursery, Species, Timepoint, and Block as conditional terms (Bray-Curtis ~ Metric + Condition(Nursery + Species + Timepoint + Block)), so that the variation only reflects what was uniquely explained by the metric.

Alpha and Beta diversity analyses were also conducted to compare the naturally occurring regenerative PIPO (NG PIPO) and soil with our nursery samples (N1 PIPO, N2 PIPO) using the previously described statistical framework.

ASV-level differential abundance was assessed with MaAsLin2 (Mallick et al., 2021) on the post-decontamination, non-rarefied samples. Samples with at least 15,000 reads were retained, so that the samples utilized were the same ones used in Alpha and Beta diversity

analyses. To evaluate how rhizosphere abundances shifted over the first year, analyses were stratified by treatment (nursery $\times$ host species). Within each treatment, Time 0 versus 1 year, was fit as $\log(\text{count}) \sim \text{Timepoint} + (1 \mid \text{Block})$, with Time 0 as reference (positive coefficient = enriched later). All fits used total-sum scaling, log transformation, and a linear model (normalization = "TSS", transform = "LOG", analysis_method = "LM"). Only ASVs present in $\geq 10\%$ of samples and with ≥ 3 total reads were tested. Each ASV was assigned a putative origin (Soil-origin, Nursery-origin, Shared, or Unclassified) based on its Time 0 prevalence at a 10% prevalence threshold. Fungal ASVs were annotated by FungalTraits lifestyle (Pöhlme et al., 2020).

The differential abundance of nursery-origin pathogen and ectomycorrhiza (ECM) ASVs and field-recruited ECM ASVs (FungalTraits annotations) was further investigated within each nursery. A nursery-origin pathogen or ECM ASV was defined as an ASV present in $\geq 10\%$ of that nursery's rhizosphere samples (any timepoint) and in $< 5\%$ of bulk soil samples. A field-recruited ECM ASV was defined as an ECM ASV present in $< 5\%$ of that nursery's Time 0 rhizosphere samples. ASVs significant at $q < 0.05$ were displayed as heatmaps of mean relative abundance per time point, with separate heatmaps for each nursery, stratified by host. The change in mean relative abundance between Times 0 and 1 Year was tested using a Wilcoxon rank-sum test.

To assess the trajectory of seedling-associated microbes and determine whether microbes moved into bulk soils, a microbe “spillover” test was conducted. A “spillover” candidate was defined as an ASV present at the Time 0 rhizosphere but absent from the Time 0 bulk soil, and it was defined as “spilled” if detected in bulk soil at 6 months and/or 1 year. Detection required ≥ 3 reads in ≥ 1 sample at a given time point. The analysis used the decontaminated phyloseq object, restricted to samples with more than 15,000 reads; ASVs lacking a genus assignment were

excluded. Results were summarized as counts per spillover category, visualized as a bar plot at the genus level and annotated with a guild color strip using FungalTraits.

RESULTS

Collection and Bioinformatic processing.

Collection. A total of 402 samples were collected. At time 0 (October 2024), this included 80 rhizosphere samples, 80 bulk soil cores, and 2 rhizosphere samples for naturally occurring PIPO regeneration (NG PIPO). At both 6 months (April 2025) and 1 year post-planting (October 2025), sampling yielded 80 rhizosphere samples, 20 bulk soil cores, and 20 NG PIPO rhizosphere samples per time point.

Amplicon sequencing. For the ITS2 locus, there were three mock communities, 23 DNA extraction negative controls, and 12 PCR negative controls, for a total of 440 samples. Eight field samples returned zero reads and were excluded, leaving 394 successful samples. For the 16S locus, there were three mock communities, 23 DNA extraction negative controls, 17 PCR negative controls, for a total of 445 samples. Two field samples returned zero reads, leaving 400 successful field samples. For the RPS10, there were three mock communities, 23 DNA extraction negative controls, 13 PCR negative controls, for a total of 441 samples. One PCR negative control failed prefiltering, seven samples failed filtering (one field sample, three PCR negative controls, three DNA extraction negative controls), 30 field samples, 14 DNA extractions, one mock sample, and 6 PCR-negative controls returned zero reads, leaving 371 successful field samples for downstream analyses.

Following quality filtering, paired-end merging, and the removal of identical reads and chimeras, a total of 13,641,636 reads remained for the ITS2 locus, averaging 34,711 reads per sample and ranging from 1 – 183,260 reads. For the 16S locus, 13,670,656 reads remained,

which averaged 34,176 reads per sample and ranged from 2,638 – 411,905 reads per sample. For the RPS10 locus, 12,146,415 reads remained, averaging 32,739 reads per sample and ranging from 1 to 228,460 reads. All reported results exclude reads in negative samples and mock communities, and failed samples.

Using the UNITE, SILVA, and OomyceteDB databases, bioinformatic processing identified 16,484 unique fungal ASVs (ITS2), 76,091 unique bacterial ASVs (16S), and 1,621 unique oomycete ASVs (RPS10). All ASVs from the ITS2 were confirmed as fungal organisms. With the 16S, a total of 1,065 ASVs were identified as mitochondria, and 194 reads were identified as chloroplasts, and after removal of these ASVs, a total of 74,832 unique ASVs remained. Genus-level assignment was achieved for 43.3% of the bacterial ASVs, 65.3% of the fungal ASVs, and 51% of the oomycete ASVs (at bootstrap ≥ 50).

Mock Community Results and Rarefaction. For the ITS2 and 16S libraries (three replicates each), all expected control mock genera were successfully recovered. However, mock community recovery for the RPS10 locus was poor, missing 13 expected species and detecting many unexpected taxa. Furthermore, one of three mock replicates failed to amplify. This discrepancy was attributed to systemic issues within the mock samples rather than to DADA2 parameter choices. ASV count tables for the ITS2 and 16S were rarefied to a uniform depth of 15,000 reads per sample (Supplemental Figure 3.1).

Seedling and Microbial Community Patterns and Dynamics

Growth metrics. At the time of planting, seedlings from both nurseries showed variable growth in height, caliper, root biomass, and shoot biomass, with significant changes across all metrics over 1 year in the field. Detailed metric changes and changes over time are reported in

the appendix (Supplemental Tables: 3.1 (height), 3.2 (caliper), 3.3 (root biomass), and 3.4 (shoot biomass)). PIST seedlings were consistently taller regardless of nursery origin, and the most change in height for all seedlings occurred from 6 months to 1 year. Stem caliper size also increased from 6 months to 1 year in both PIST and PIPO seedlings; however, N1 seedlings were taller and possessed thicker stem diameters compared to N2 seedlings. Increases in root and shoot biomass were greatest from 0 to 6 months, and N1 PIPO seedlings had more growth in root and shoot biomass than other seedlings (Figure 3.1).

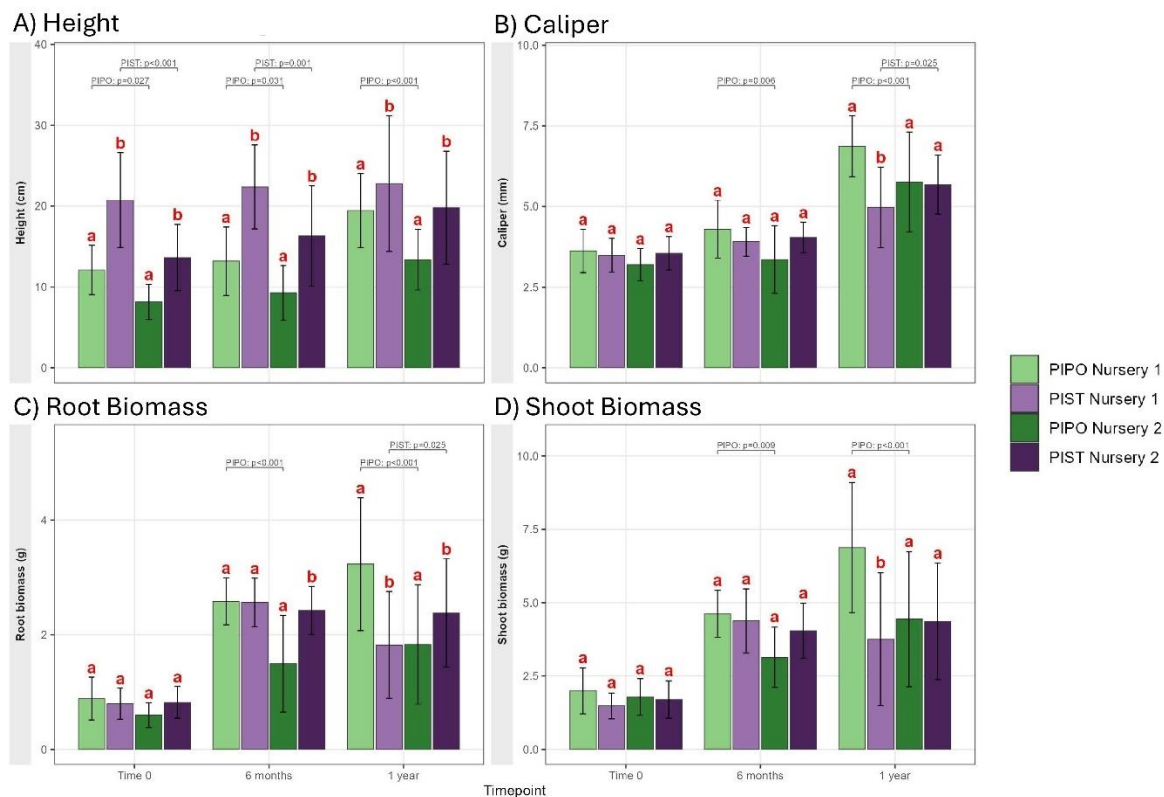


Figure 3.1. Growth metrics of *Pinus ponderosa* (PIPO) and *P. strobiformis* (PIST) seedlings from nurseries 1 (N1) and 2 (N2) at planting (Time 0), 6 months, and 1 year after planting: (A) height (cm), (B) caliper (mm), (C) root biomass (g), and (D) shoot biomass (g). Bars are group means; whiskers are ± 1 SD. Letters denote the PIPO-versus-PIST comparison within each nursery and timepoint, where groups sharing a letter do not differ significantly ($p_{\text{FDR}} \geq 0.05$). Brackets show the N1-versus-N2 comparison within each species; only significant contrasts are drawn, labeled with p_{FDR} .

Alpha and Beta Diversity. Before examining relationships between microbial communities and growth metrics, we first calculated Alpha and Beta diversities of the seedlings. For Alpha diversity (reported as Shannon diversity), the type III ANOVA tests indicated significant main effects of both the source nursery ($p < 0.001$) and sampling timepoint ($p < 0.001$) for fungal and bacterial communities (Figure 3.2). No 2-way or the 3-way interactions were significant for fungal Shannon diversity. However, for the bacteria, the 2-way interactions (Nursery $\times$ Species and Nursery $\times$ Timepoint) were significant (all $p < 0.043$) (Supplemental Tables 3.5, 3.6). Using pairwise comparisons, these changes were further characterized. From time 0 to 1 year, fungal Shannon diversity significantly decreased ($p_{\text{FDR}} < 0.03$), whereas bacterial Shannon diversity significantly increased ($p_{\text{FDR}} < 0.004$) across the study period (Figure 3.2; Supplemental Tables 3.5, 3.6).

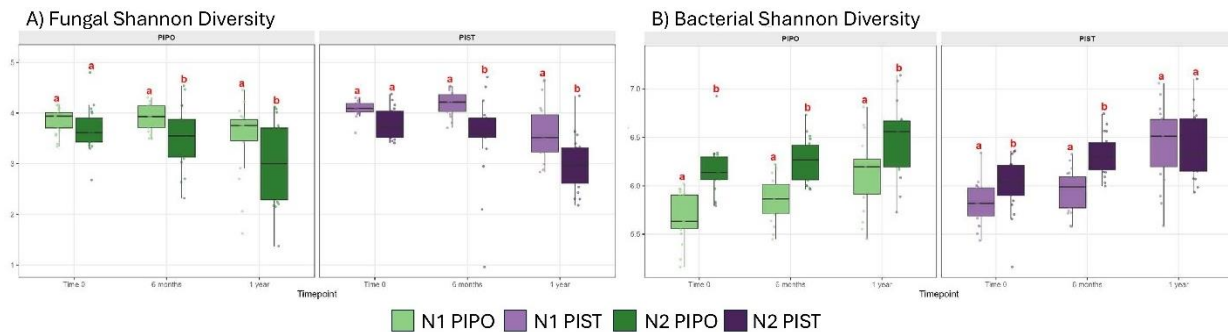


Figure 3.2. Shannon diversity of the rhizosphere across two nurseries and three timepoints (Time 0, 6 months, 1 year), for (A) fungal (ITS2) and (B) bacterial (16S) communities. Within each panel, the same host species, *Pinus ponderosa* (PIPO) and *P. strobiformis* (PIST), are compared between the two nurseries (N1 vs N2) at each time point. Boxplots show the median and interquartile range with individual samples overlaid; colors denote nursery $\times$ species group. Letters reflect pairwise comparisons from a linear mixed model. Within each species $\times$ timepoint, the two nurseries share a letter where they do not differ significantly ($p_{\text{FDR}} \geq 0.05$).

For both loci, the Beta diversity was structured by a significant Nursery $\times$ Species $\times$ Timepoint interaction (fungi: $R^2 = 0.013$, $p < 0.001$; bacteria: $R^2 = 0.012$, $p < 0.001$), indicating that nursery origin and host species jointly influenced community composition as it changed

across time, however multivariate dispersions were not homogeneous (Supplemental Table 3.7). The significant overall interaction motivated further analyses using pairwise comparisons to assess the significance and magnitude of variation between samples and time points. The difference between the communities of seedlings grown in the same nursery and the seedlings grown at different nurseries was all significant, with the magnitude of variation decreasing over time towards convergence (Table 3.1).

Table 3.1. Pairwise PERMANOVA of fungal (ITS2) and bacterial (16S) rhizosphere community composition in outplanted seedlings, by nursery (N1 and N2), host species *Pinus ponderosa* (PIPO) and *P. strobiformis* (PIST), and timepoint (Time 1, 6 months, and 1 year). Two contrast sets are reported per marker: host species within each nursery $\times$ timepoint (PIPO vs PIST) and nursery within each species $\times$ timepoint (N1 vs N2).

ITS2 Pairwise PERMANOVA							
Question ^A		Comparison ^B	Df ^C	R2 ^D	F ^E	p.value ^F	p_FDR ^G
N1	Time 0	PIPO vs PIST	1	0.194	9.163	0.001	0.002
N1	6 months	PIPO vs PIST	1	0.080	2.957	0.002	0.003
N1	1 year	PIPO vs PIST	1	0.064	2.523	0.001	0.002
N2	Time 0	PIPO vs PIST	1	0.080	2.957	0.001	0.002
N2	6 months	PIPO vs PIST	1	0.058	2.032	0.006	0.007
N2	1 year	PIPO vs PIST	1	0.039	1.527	0.021	0.021
PIPO	Time 0	N1 vs N2	1	0.311	16.685	0.001	0.001
PIPO	6 months	N1 vs N2	1	0.254	11.216	0.001	0.001
PIPO	1 year	N1 vs N2	1	0.102	4.187	0.001	0.001
PIST	Time 0	N1 vs N2	1	0.349	18.737	0.001	0.001
PIST	6 months	N1 vs N2	1	0.307	15.049	0.001	0.001
PIST	1 year	N1 vs N2	1	0.096	4.048	0.001	0.001
16S Pairwise PERMANOVA							
Question ^A		Comparison ^B	Df ^C	R2 ^D	F ^E	p.value ^F	p_FDR ^G
N1	Time 0	PIPO vs PIST	1	0.136	5.520	0.001	0.001
N1	6 months	PIPO vs PIST	1	0.080	3.201	0.001	0.001
N1	1 year	PIPO vs PIST	1	0.074	2.868	0.001	0.001
N2	Time 0	PIPO vs PIST	1	0.115	4.549	0.001	0.001
N2	6 months	PIPO vs PIST	1	0.109	4.284	0.001	0.001
N2	1 year	PIPO vs PIST	1	0.044	1.513	0.009	0.009
PIPO	Time 0	N1 vs N2	1	0.368	19.820	0.001	0.001
PIPO	6 months	N1 vs N2	1	0.292	14.430	0.001	0.001
PIPO	1 year	N1 vs N2		0.118	4.568	0.001	0.001
PIST	Time 0	N1 vs N2	1	0.362	20.440	0.001	0.001
PIST	6 months	N1 vs N2	1	0.300	15.860	0.001	0.001
PIST	1 year	N1 vs N2	1	0.125	4.979	0.001	0.001

^A The subset within which the comparison was made (nursery $\times$ timepoint for species contrasts; species $\times$ timepoint for nursery contrasts).

^B Groups compared.

^C Degrees of freedom (between-group).

^D Proportion of variance in community composition explained by the grouping.

^E PERMANOVA pseudo-F statistic.

^F Unadjusted PERMANOVA p-value (Bray–Curtis dissimilarities, 999 permutations).

^G Benjamini-Hochberg–corrected p-value, applied within each marker; values < 0.05 indicate significant compositional differences.

Growth metrics and community composition. To analyze community composition and growth metrics, we first assessed collinearity among the four growth metrics. Height was largely independent of the other metrics, whereas caliper size, root biomass, and shoot biomass were strongly intercorrelated, indicating that these three captured a shared dimension of seedling size rather than independent growth responses (Supplemental Figure 3.2). All four metrics were retained in subsequent analyses, but results for caliper size, root biomass, and shoot biomass should be interpreted with caution due to collinearity.

Seedling height was not significantly associated with differences in the fungal or bacterial communities. Alternatively, caliper, root biomass, and shoot biomass explained a small but significant variation in fungal and bacterial communities ($R^2 = 0.005\text{-}0.009$, $p_{\text{FDR}} < 0.024$). Stratifying by timepoint showed that this relationship was observed at 1 year for fungi ($R^2 = 0.020$ to 0.022 ; all $p_{\text{FDR}} < 0.023$). For bacterial community, significant associations with growth metrics occurred at time 0 for both root and shoot biomasses ($R^2 = 0.022$, 0.021 , both $p_{\text{FDR}} < 0.05$, respectively) and at 1 year for root biomass ($R^2 = 0.024$, $p_{\text{FDR}} = 0.032$) (Supplemental Tables 3.8, 3.9).

When the growth metrics were fitted as vectors onto the fungal and bacterial PCoA, all four metrics aligned significantly with the first two ordination axes (all $p_{\text{FDR}} = 0.001$); caliper size had the strongest relationship (ITS and 16S: $R^2 = 0.40$), followed by height, shoot biomass, and root biomass. The ordinations show all time points, so the axes capture natural seedling growth over time; therefore, these envfit R^2 scores also reflect increases in seedling size with age rather than just an association between growth metrics and community. The timepoint-stratified PERMANOVA remains a more reliable test that shows weaker associations between community structure and differences in growth metrics (Figure 3.3; Supplemental Tables 3.8, 3.9).

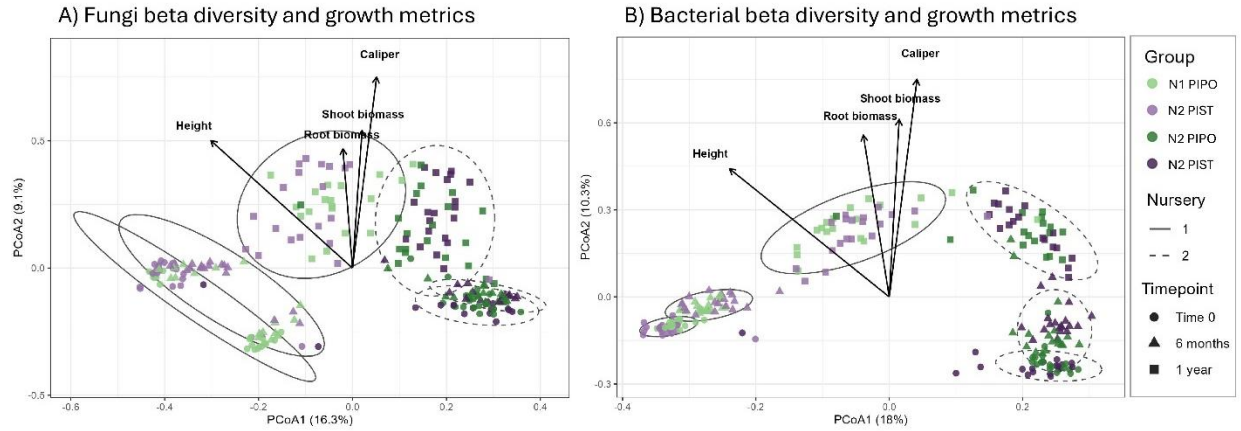


Figure 3.3. Rhizosphere community ordination with growth-metric vectors. Principal coordinates analysis (PCoA) of Bray–Curtis dissimilarities for (A) fungal (ITS2) and (B) bacterial (16S) rhizosphere communities, with the four seedling growth metrics (height, caliper, root biomass, shoot biomass) fit as vectors (envfit). Points are individual samples colored by nursery (N1 and N2) $\times$ species (*Pinus ponderosa* (PIPO) and *P. strobiformis* (PIST)) group; shapes represent timepoints. Grey 95% t-distribution confidence ellipses are drawn per Nursery $\times$ Timepoint, with solid outlines for N1 and dashed for N2. Arrow direction and length indicate each metric's association with the ordination (length proportional to $\sqrt{r^2}$). All four vectors were significant in both communities (envfit, Benjamini-Hochberg $p_FDR \leq 0.001$).

Partial CAP, which removes the variance of nursery, species, timepoint, and block before evaluating each metric, further confirms the findings of the timepoint-stratified PERMANOVA. All four growth metrics explained a small but significant amount of variation in fungal and bacterial community composition. For fungi, each metric then explained between 0.45% and 0.71% of the variation. The bacterial community pattern was slightly stronger, with each metric explaining between 0.49% and 0.87% of the residual variation. In both the fungal and bacterial analyses, root biomass explained the most community variation of any metric, but it was still less than 1% (Table 3.2).

Table 3.2. Partial CAP (partial distance-based redundancy analysis; Legendre & Anderson, 1999) testing whether each seedling growth metric explains rhizosphere community variation beyond the experimental design, for fungal (ITS2) and bacterial (16S) communities. Each metric was fit separately on the Bray–Curtis dissimilarity matrix with Nursery, Species, Timepoint, and Block as conditional (covariate) terms, so the constrained variance reflects each metric's unique contribution after the design is accounted for. Permutation tests used 999 permutations.

ITS2 Partial CAP							
metric	n	cond_var_%	const_var_%	resid_var_%	F	p	q
Height	226	26.268	0.449	73.283	1.330	0.044	0.044
Caliper	226	26.268	0.660	73.072	1.960	0.001	0.002
Root_Biomass	226	26.268	0.709	73.023	2.107	0.001	0.002
Shoot_Biomass	226	26.268	0.562	73.170	1.667	0.007	0.009
16S Partial CAP							
metric	n	cond_var_%	const_var_%	resid_var_%	F	p	q
Height	223	30.445	0.494	69.061	1.530	0.005	0.005
Caliper	223	30.445	0.777	68.778	2.416	0.001	0.001
Root_Biomass	223	30.445	0.873	68.682	2.720	0.001	0.001
Shoot_Biomass	223	30.445	0.727	68.827	2.262	0.001	0.001

^A Seedling growth metric fits as the constraining variable.
^B Number of samples in the analysis.
^C Percentage of total inertia (community variance) attributable to the conditional terms (Nursery, Species, Timepoint, Block); identical across metrics within a marker because the same terms are conditioned out.
^D Percentage of total inertia uniquely explained by the metric, after conditioning.
^E Percentage of total inertia remaining as residual (unexplained); cond, const, and resid sum to 100.
^F Pseudo-F statistic for the constrained (metric) term.
^G Permutation p-value (999 permutations).
^H Benjamini-Hochberg-corrected p-value across the four metrics within each marker; q < 0.05 indicates a significant unique contribution.

Alpha and Beta Diversity of Nursery Seedlings and Soil Samples. To determine whether and to what extent bulk soil is a homogenizing factor, we compared the seedling RZ microbiome with that of the bulk soil. For Alpha diversity, the type III ANOVA of Shannon diversity indicated significant main effects of sample type ($p < 0.001$) and timepoint ($p < 0.003$) for both fungi and bacteria, with bacteria also showing a significant sample type $\times$ timepoint interaction (Supplemental Figure 3.3). For fungi, Shannon diversity of the seedlings decreased significantly over time; however, it was only significantly lower in the bulk soil at 1 year (Supplemental

Figure 3.3b). For the bacteria, the Shannon diversity of seedlings was significantly lower than that of bulk soil at time 0, but both the seedling and bulk soil showed significant increases in Alpha diversity over time (Supplemental Figure 3.3b).

Fungal and bacterial Beta diversities were significant in the overall model. In the pairwise comparison, all communities remained significantly different from each other at all time points, but the extent of variation between groups decreased over time. Variation between fungal communities decreased from 16.8% ($R^2 = 0.168$) at time 0 to 9.3% ($R^2 = 0.093$) at 1 year. This trend was similar in bacteria, with an initial variation of 25.9% ($R^2 = 0.259$) reducing to 9.2% ($R^2 = 0.092$) at 1 year (Supplemental Figure 3.4).

Alpha and Beta diversity of PIPO, NG PIPO, and Soil Samples. For the comparison of seedlings to both the NG PIPO and the bulk soil, only PIPO seedlings were utilized. Furthermore, at time 0, only two naturally occurring PIPO samples were collected at the site; so time 0 was not included. For Shannon diversity among the nursery PIPO, the NG PIPO, and the bulk soil, the type III tests indicated significant main effects of sample type ($p < 0.001$) and timepoint ($p < 0.003$) for both the fungal and bacterial communities, with no significant interaction of sample type $\times$ timepoint (Supplemental Figure 3.5). Pairwise comparisons at 1 year, the bulk soil had significantly higher Shannon diversity than the N2 PIPO (Supplemental Figure 3.5a). For bacterial communities, Shannon diversity across all sample types increased significantly over time ($p_{\text{FDR}} < 0.05$). The bulk soil had a significantly higher Shannon diversity than the N1 PIPO at 6 months and at 1 year (Supplemental Figure 3.5b).

Overall, there were significant differences in the Beta diversity of fungi ($R^2 = 0.05$, $p < 0.001$) and bacteria ($R^2 = 0.06$, $p < 0.001$) of the different sample types (N1 PIPO, N2 PIPO, NG PIPO, and the bulk soil) (Figure 3.4; Supplemental Table 3.10). However, the magnitude of the

variation depended on the time point and nursery. The variation of the fungal and bacterial communities at 6 months between the N2 PIPO and bulk soil/NG PIPO was smaller than the variation between those groups and the N1 PIPO (Figure 3.4; Supplemental Table 3.11). There was also small but significant variation in the fungal and bacterial communities between the bulk soil and the NG PIPO at all timepoints (all $R^2 < 0.09$) (Figure 3.4; Supplemental Table 3.10).

Fungal and bacterial communities of the N1 PIPO are distinct from those of the NG PIPO and soil even after 1 year, but fungal communities do begin to overlap with those of the N2 PIPO (Figure 3.4, Supplemental Table 3.10).

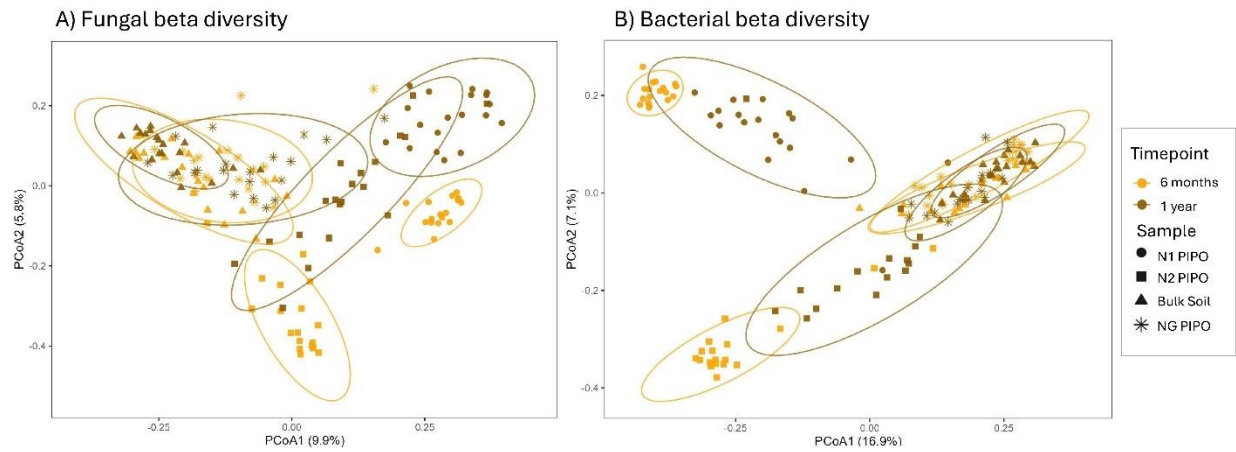
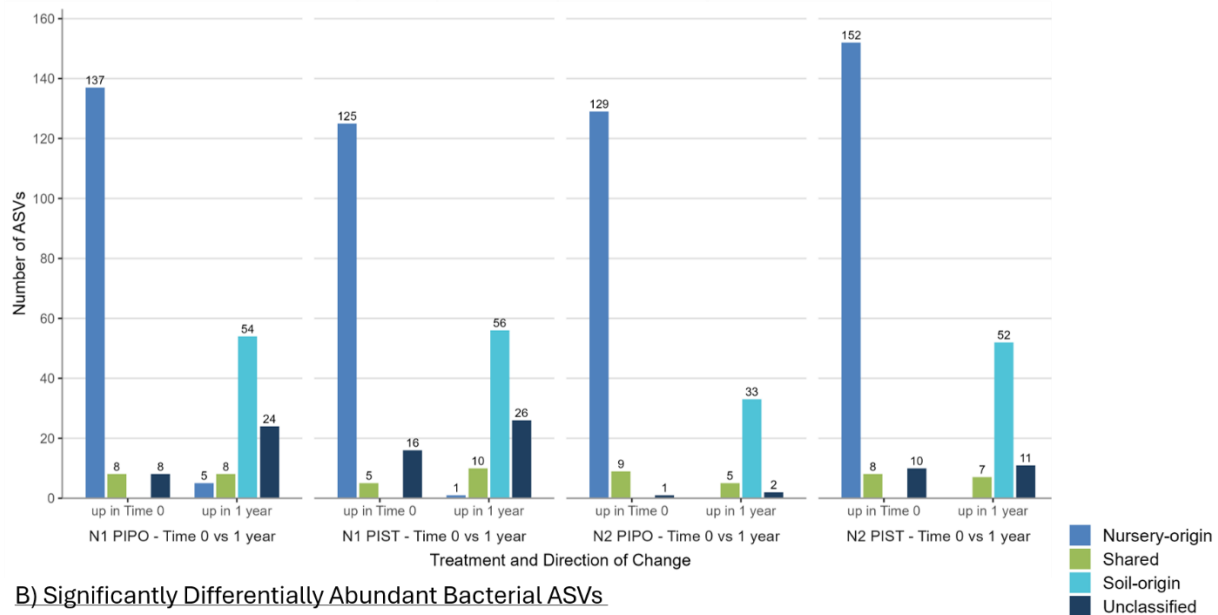


Figure 3.4. Beta diversity of field rhizosphere and soil communities across timepoints, for (A) fungal (ITS2) and (B) bacterial (16S) communities, by principal coordinates analysis (PCoA) with Bray–Curtis dissimilarities. Each point is a single sample; color denotes timepoint (6 months, 1 year) and shape denotes sample type: nursery-planted *Pinus ponderosa* rhizosphere from the two nurseries (N1 / N2 PIPO), bulk soil, and naturally regenerating *P. ponderosa* rhizosphere (NG PIPO). Ellipses are the 95% confidence. Community composition showed a significant sample type $\times$ timepoint interaction in both datasets (PERMANOVA: fungal, $R^2 = 0.052$, $F = 3.28$, $p = 0.001$; bacterial, $R^2 = 0.055$, $F = 3.66$, $p = 0.001$; 999 permutations), indicating that compositional differences among compartments changed between timepoints. Group dispersions were heterogeneous (PERMDISP across the eight sample type $\times$ timepoint groups: fungal, $F = 10.97$, $p = 0.001$; bacterial, $F = 5.53$, $p = 0.001$).

Differential and Relative Abundance. The fungal and bacterial Alpha and Beta diversity differences identified across sample types motivated differential abundance testing and focused on comparisons between N1 PIPO, N1 PIST, N2 PIPO, N2 PIST. Change over time was assessed from time 0 to 1 year.

Over the 1 year period, the percentage of differentially abundant ASVs for fungi ranged from 40% to 51.2% depending on sample type, with N2 PIST showing the greatest change and N2 PIPO the least. Differentially abundant bacterial ASVs ranged from 33.4% in the N1 PIPO to 39.9% in the N2 PIST over the year (Supplemental Table 3.11). At time 0, there were, on average, 136 significantly differentially abundant nursery-origin fungal ASVs and 465 significantly differentially abundant nursery-origin bacterial ASVs. By 1 year, the average number of differentially abundant nursery-origin ASVs had decreased (Fungi: 2 ASVs; Bacteria: 34 ASVs). This decrease is complemented by increases in significantly differentially abundant soil-origin ASVs. At 1 year post-planting, there were, on average, 49 differentially abundant soil-origin fungal ASVs and 280 bacterial ASVs. This large turnover indicates that over 1 year, both the fungal and bacterial communities shifted towards soil-origin microbes, as nursery-origin microbes became less dominant (Figure 3.5).

A) Significantly Differentially Abundant Fungal ASVs



B) Significantly Differentially Abundant Bacterial ASVs

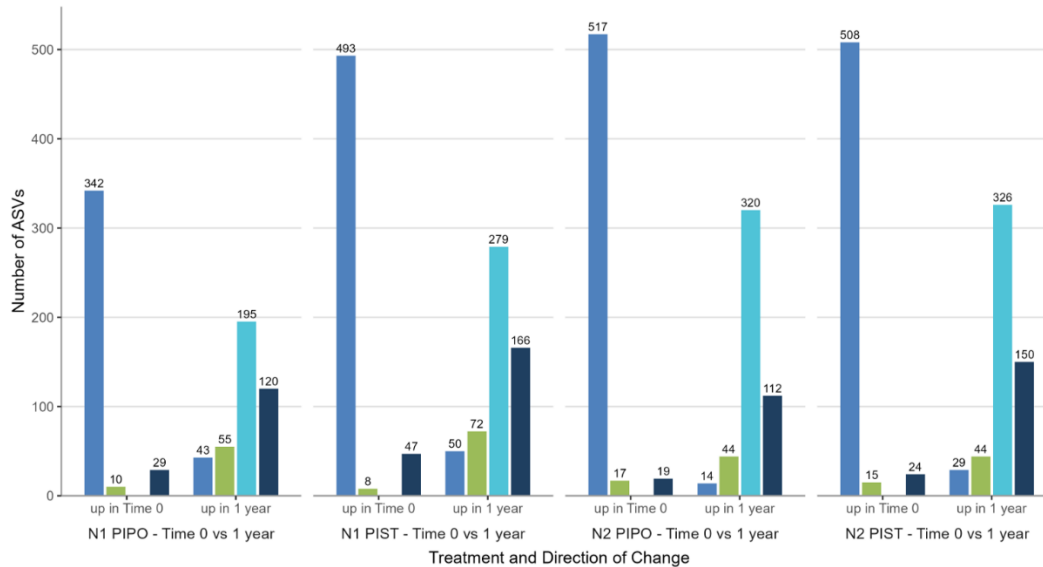
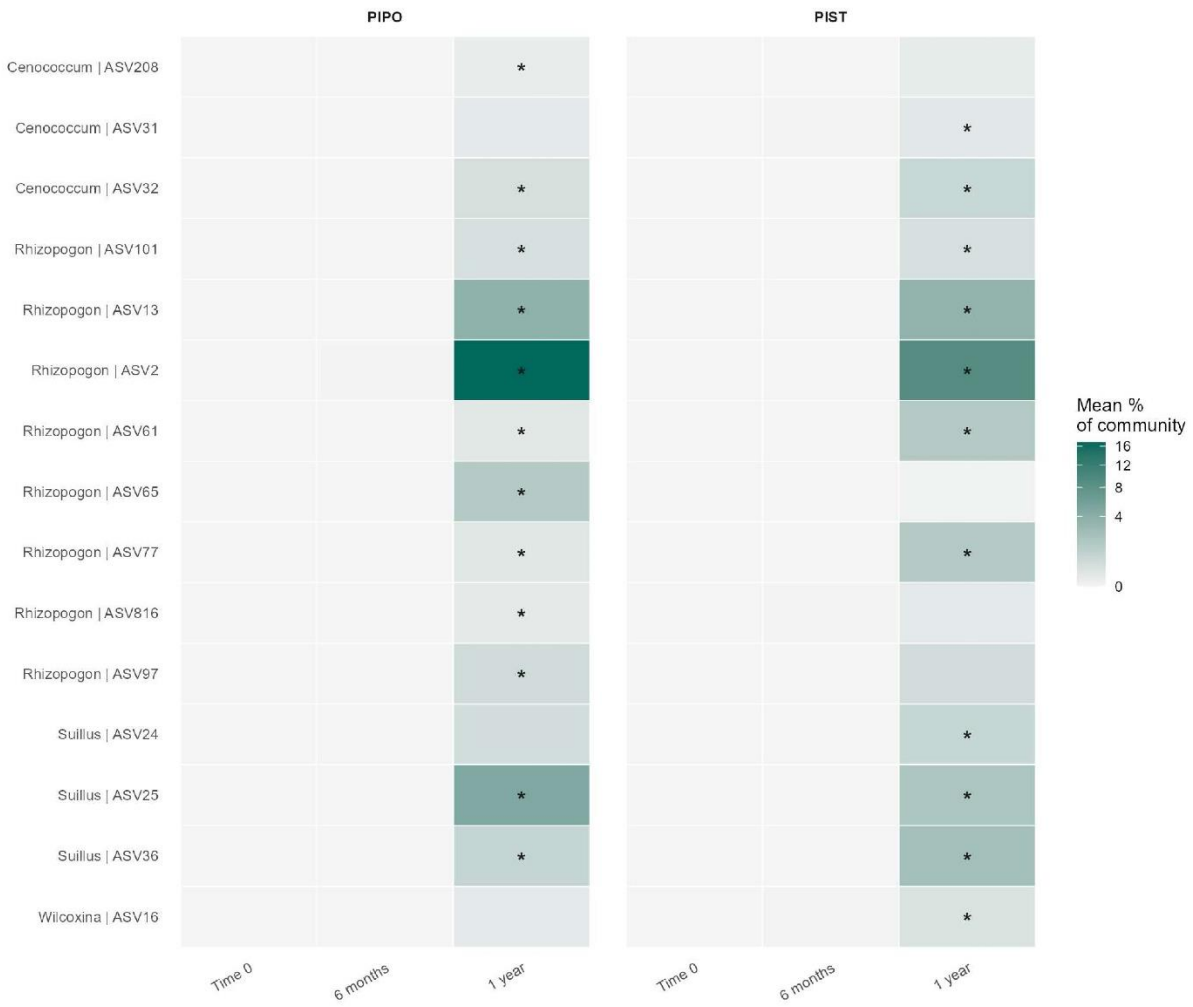


Figure 3.5. Origin classification of differentially abundant rhizosphere Amplicon Sequence Variants (ASVs) in nursery (N1 and N2) seedlings, *Pinus ponderosa* (PIPO) and *P. strobiformis* (PIST) at outplanting (Time 0) and one year later, for (A) fungal (ITS2) and (B) bacterial (16S) communities. ASVs differentially abundant between timepoints (MaAsLin2, Time 0 vs 1 year contrast within each treatment) are partitioned by inferred source: nursery-origin, soil-origin, shared, or unclassified (UC). The Time 0 and 1 year column blocks report the significant ASVs enriched at each time point. *Origin assignment*: Origin was assigned to each ASV once, from Time 0 prevalence (fraction of samples with non-zero detection) in two compartments, Time 0 bulk soil and the Time 0 nursery-planted rhizosphere, using a 10% prevalence threshold in each: soil-origin ($\geq 10\%$ in Time 0 bulk soil, $< 10\%$ in the Time 0 rhizosphere), nursery-origin ($\geq 10\%$ in the Time 0 rhizosphere, $< 10\%$ in bulk soil), shared ($\geq 10\%$ in both), or unclassified (neither).

Differential and Relative Abundance of Fungal Guilds. Several ectomycorrhizal (ECM) taxa were detected in nursery seedlings at time 0, even though the seedlings were not inoculated. The ASVs detected in the seedlings include ASVs of *Rhizopogon* (ASV61, in N1 and N2 seedlings), *Suillus* (ASV25, in N1 and N2 seedlings), and *Pustularia* and *Suillus* (ASV82 and ASV24, respectively, N2 seedlings) (Supplemental Figure 3.6). However, these ASVs were also detected in the soil at time 0, suggesting that although ECM ASVs were present in seedlings when they were planted, they are also broadly present at the site and thus included in subsequent analyses.

Among the seedlings from N1, there were 15 significantly differentially abundant ECM ASVs, including *Cenococcum* (3 ASVs), *Rhizopogon* (8 ASVs), *Suillus* (3 ASVs), and *Wilcoxina* (1 ASV). In the mean relative abundance heat maps, these ASVs were not abundant at time 0 or at 6 months, but their mean relative abundance increased significantly by 1 year (Figure 3.6.A). This increasing trend was also present in the putative ECM taxa associated with N2 seedlings (Figure 3.6.B). There were 16 putative ECM ASVs associated with N2 trees (*Cenococcum* (4 ASVs), *Pustularia* (1 ASV), *Rhizopogon* (8 ASVs), *Suillus* (3 ASVs), all significantly increasing in mean relative abundance as well.

A) N1 - Mean relative abundance of significantly differentially abundant ECM



B) N2 - Mean relative abundance of significantly differentially abundant ECM

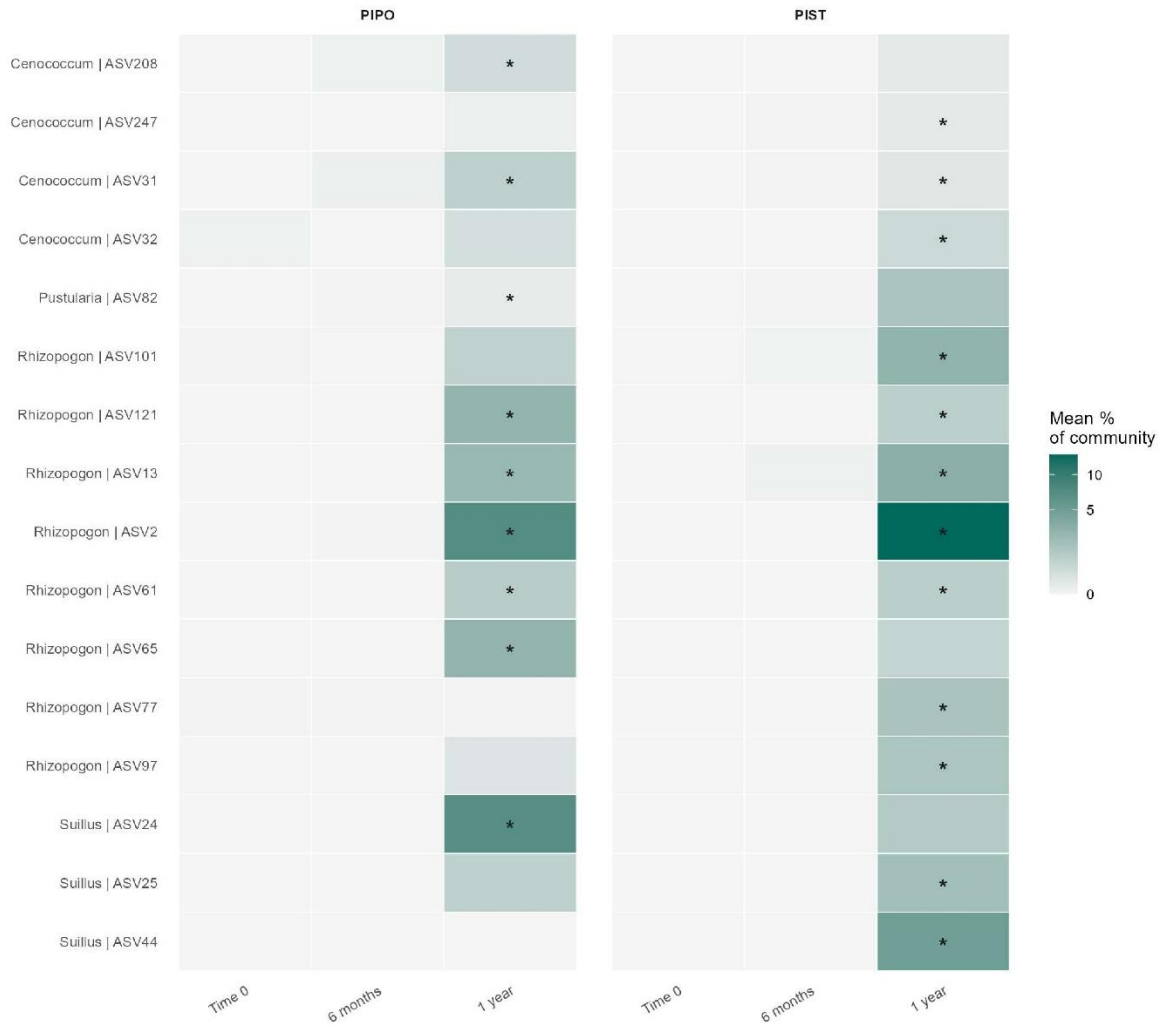
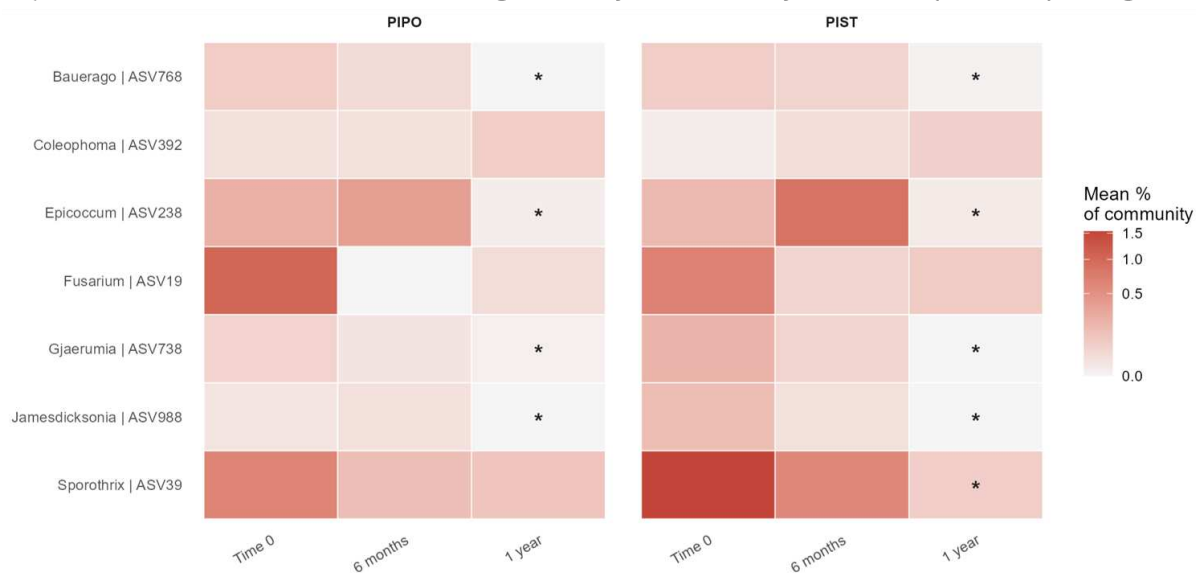


Figure 3.6. Mean relative abundance of significant rhizosphere-recruited FungalTraits-annotated Ectomycorrhizal (ECM) ASVs from Time 0 to 1 year in (A) Nursery 1, PIPO and PIST, and (B) Nursery 2, PIPO and PIST. ASVs were absent (<5% prevalence) from each nursery's Time 0 rhizosphere that were differentially abundant between Time 0 and 1 year (MaAsLin2, within-set $q < 0.05$). Cell color is square-root-scaled mean relative abundance per timepoint; scales are panel-specific (max 16.89% in A, 13.67% in B). A significant Time 0 $\rightarrow$ 1 year change in mean relative abundance is indicated by an * in the 1 year cell of the relevant species facet (Wilcoxon rank-sum, BH $q < 0.05$).

In addition to examining the trajectory of ECMs, putative fungal pathogen dynamics were also further explored. There were seven significantly differentially abundant putative pathogen ASVs from N1. These ASVs were classified to the genera *Bauerago*, *Coleophoma*, *Epicoccum*, *Fusarium*, *Gjaerumia*, *Jamesdicksonia*, and *Sporothrix*. Follow-up mean relative abundance testing indicated that these ASVs accounted for 1.54% of the community, with four of them significantly decreasing in mean relative abundance over time in N1 PIPO and five in N1 PIST. *Epicoccum* increased at 6 months; however, by 1 year, its relative abundance had decreased (Figure 3.7.A). From N2, there were nine significantly differentially abundant putative pathogen ASVs classified into the genera *Bauerago*, *Coleophoma*, *Dactylonectria* (2 ASVs), *Fusarium* (3 ASVs), *Olpidiaster*, and *Sporothrix*. Over 1 year, four ASVs significantly decreased in mean relative abundance in N2 PIPO and five in N2 PIST, at most making up 7.22% of the community. ASVs of *Coleophoma* and *Fusarium* showed slight increases in relative abundance at 6 months but decreased by 1 year (Figure 3.7.B). The decrease in nursery-origin putative pathogens mirrors the decline of all nursery-origin microbes.

A) N1 - Mean relative abundance of significantly differentially abundant putative pathogens



B) N2 - Mean relative abundance of significantly differentially abundant putative pathogens

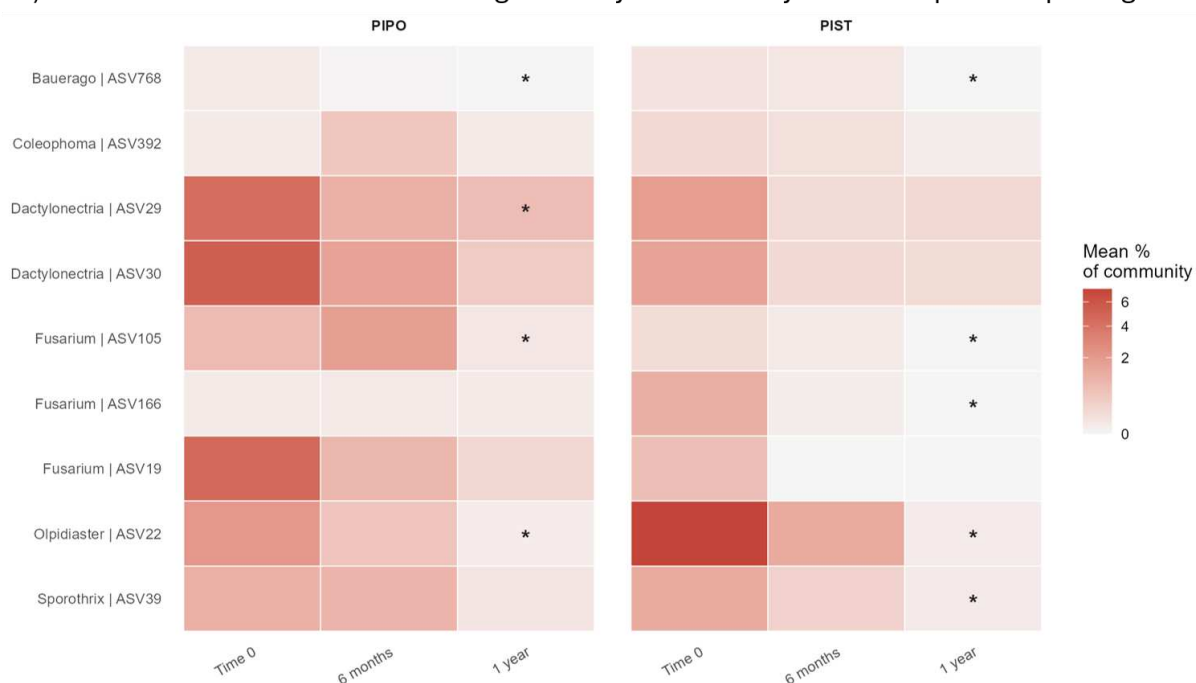


Figure 3.7. Mean relative abundance of significantly differentially abundant nursery-origin putative fungal pathogen ASVs from Time 0 to 1 year in (A) Nursery 1, PIPO and PIST, and (B) Nursery 2, PIPO and PIST. ASVs were FungalTraits-annotated plant pathogens meeting the rhizosphere-associated candidate criteria ($\geq 10\%$ rhizosphere prevalence within the nursery at any timepoint, $< 5\%$ prevalence in bulk soil) that were differentially abundant between Time 0 and 1 year (MaAsLin2, within-set $q < 0.05$). Cell color is square-root-scaled mean relative abundance per timepoint; scales are panel-specific (max 1.54% in A, 7.22% in B). A significant Time 0 $\rightarrow$ 1 year change in mean relative abundance is indicated by an * in the 1 year cell of the relevant species facet (Wilcoxon rank-sum, BH $q < 0.05$).

Analysis of Spillover ASVs. ASV spillover analyses were conducted to further assess the dynamics of fungal ASVs originating from the nurseries and to determine whether some of those ASVs were moving into the bulk soil. Across 1,689 rhizosphere ASVs meeting the spillover-candidate criteria, a large majority (1,649; 97.6%) were never detected in bulk soil samples. 40 candidates spilled into soil, 23 at 6 months and 17 at 1 year, with 4 detected at both time points. Spillover was dominated by non-pathogenic taxa, predominantly saprotrophs, along with mycoparasites and endophytes. Only two of the 40 spillover ASVs, were classified as plant pathogens, both *Fusarium* (ASV799 and ASV835) (Figure 3.8. Supplemental Table 3.12)

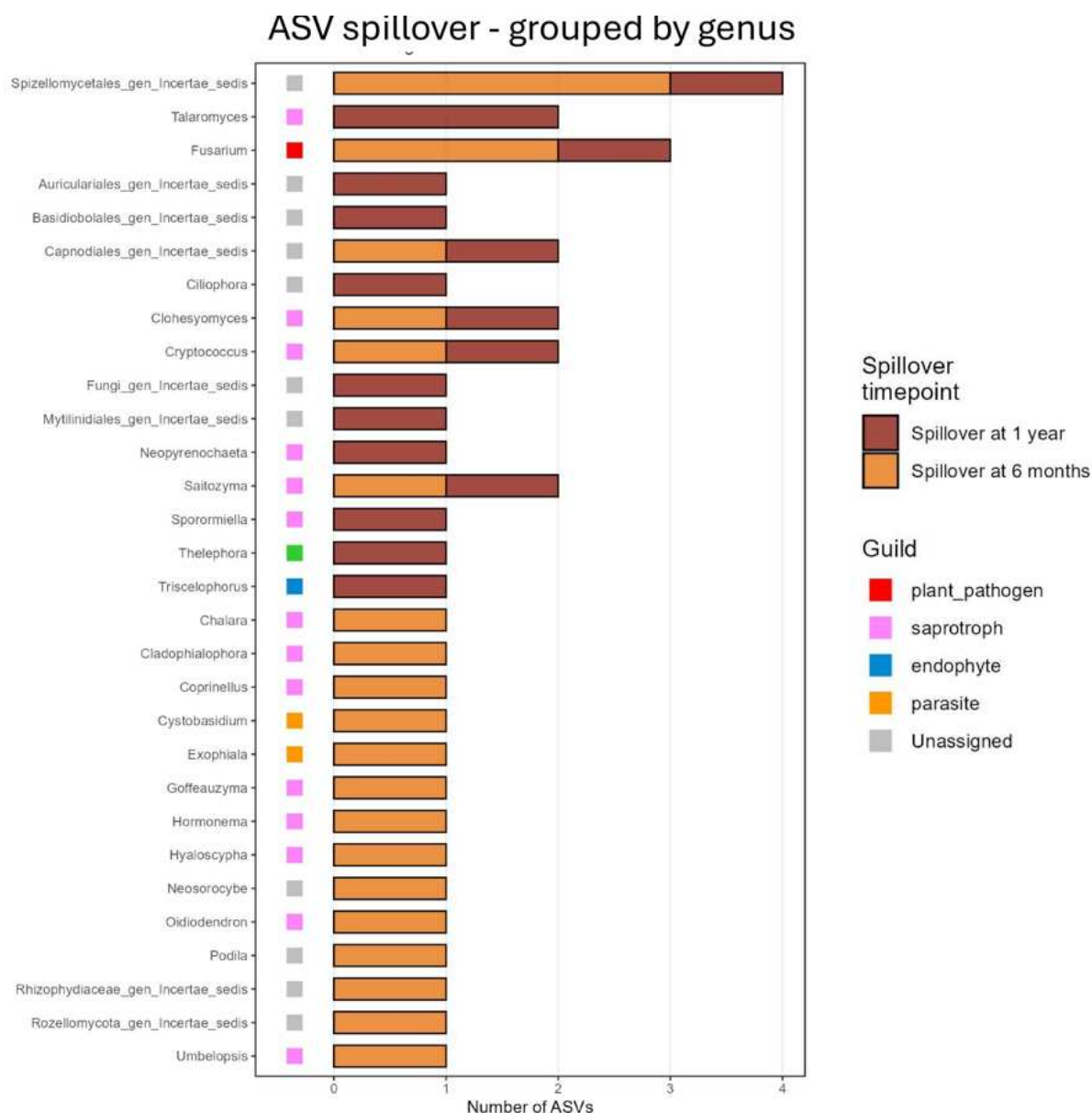


Figure 3.8. Fungal ASV spillover by genus and timepoint. Each bar shows the number of fungal ASVs per genus classified as spillover; ASVs present in the Time 0 rhizosphere but absent from Time 0 bulk soil (≥ 3 reads in ≥ 1 sample) that were subsequently detected in bulk field soil (≥ 3 reads in ≥ 1 sample) at 6 months or 1 year. Bars are stacked and colored by the timepoint at which spillover was first observed (orange = 6 months post-outplanting; maroon = 1 year). The colored square preceding each genus name denotes its primary FungalTraits guild assignment.

Fusarium ASV799, putatively *Fusarium tricinctum*, was detected at all three time points in N1 PIPO, at 6 months with N2 PIPO and N2 PIST, and at 1 year in N2 PIPO. These detections

across nurseries and host species may indicate that this ASV is present in both nurseries but rare in N1 PIST. At 6 months, *Fusarium* ASV799 was also detected in the bulk soil with 129 reads. The sample came from block 3 in the N1 PIST section, which is located on the boundary of the N2 PIST section. This ASV has not been detected in N1 PIST but has been detected in the N2 PIST.

The second spilled *Fusarium* ASV, ASV835 (also putatively *F. tricinctum*), was detected at Time 0 in one N2 PIST seedling in block 2. This ASV was detected at 6 months in two soil samples. The first bulk soil sample came from block 1 in the N2 PIPO section, where, at 6 months, another seedling in the neighboring row also had a positive detection of this ASV. In the bulk soil sample, this ASV had 1,599 reads, and in the seedling rhizosphere sample, it had 44 reads. The second soil sample with this ASV at 6 months and was from block 4 in the N1 PIPO section, on the boundary of N2 PIPO, at 175 reads. The associated seedling sample did not have this ASV detected, nor was it detected in any other seedling in this section of the block or the neighboring section (Supplemental Table 3.13).

Fusarium ASV835 was detected again at 1 year in a bulk soil sample from block 2 (182 reads), in the middle of the N2 PIPO section. The associated seedling sample did not contain this ASV, and at no other time was this ASV detected in block 2. However, this ASV was detected the rhizosphere of two seedlings at 1 year: one N2 PIPO sample (20 reads) in block 3 and an N2 PIST sample (37 reads) in block 4. The detection of *Fusarium* ASV835 only in N2 trees and its presence in a bulk soil sample from blocks with N2 seedlings that also carry this ASV potentially represent a strong case for spillover (Supplemental Table 3.13). However, *Fusarium* ASVs were detected in all samples at all time points, and other *Fusarium tricinctum* ASVs were present in the soil at time 0, 6 months, and 1 year.

Oomycete detection. Although the RPS10 dataset was not used for quantitative community analyses, it nevertheless revealed the widespread presence of oomycetes across sample types and sampling periods (Supplemental Table 3.14). Across all samples, 40 putative oomycete species were identified, including *Achlya hypogyna*, *Aphanomyces cladogamus*, *A. invadans*, *A. laevis*, *Elongisporangium dimorphum*, 18 species of *Globisporangium*, *Phytophthora cactorum*, *P. citricola*, *P. kernoviae*, *P. pini*, *P. pseudocryptogea*, *Pythium apiculatum*, *P. capillosum*, *P. conidiophorum*, *P. diclinum*, *P. junctum*, *P. monospermum*, *P. oligandrum*, *P. oopapillum*, *P. volutum*, *Saprolegnia diclina*, *S. parasitica*, and *Thraustotheca clavata*. These observations suggest that oomycetes are common members of post-fire soil and rhizosphere communities and warrant further investigation.

DISCUSSION

This study monitored conifer seedlings from nursery production through their first year of field establishment within a post-fire landscape, demonstrating that rhizosphere microbiomes undergo restructuring during field integration. Although broad microbial community composition did not correlate with seedling growth, several taxa known to enhance conifer performance were detected in the rhizosphere following outplanting. While nursery-origin strongly influenced initial fungal and bacterial community composition, these nursery signatures weakened over time as seedlings acclimated to the surrounding field (including soil) environment. Concurrently, native ectomycorrhizal fungi were recruited from the bulk soil, nursery-associated putative pathogens declined in relative abundance, and evidence of pathogen spillover into adjacent native soils remained limited. Together, these findings highlight the dynamic nature of seedling microbiome assembly during reforestation and highlight the key role of post-fire soils in shaping microbial community trajectories.

Seedling growth metrics explained only 2% of the overall variation in microbial community composition at any time point. Therefore, while individual seedlings varied in growth characteristics, potentially due to nursery-specific fertilization and watering (?) regimes (Gleason et al., 1990), larger seedlings did not appear to support distinct microbial communities. While few studies have directly examined relationships between conifer seedling growth metrics and whole-community microbiome composition, several general patterns emerge from the literature. Among bacteria, taxa such as *Bacillus* (Moreno-Valencia et al., 2024), *Pseudomonas* (Shishido et al., 1996), and members of the Burkholderiaceae (Puri et al., 2020) are frequently associated with enhanced seedling performance. Among fungi, ectomycorrhizal taxa such as *Suillus* (Ishikawa et al., 2024; Li et al., 2023), *Rhizopogon* (Ishikawa et al., 2024), and *Wilcoxina* (Shishido et al., 1996), are recognized as important partners that support conifer growth and establishment. However, most conifer studies rely on inoculation experiments rather than examining associations between natural microbial communities and seedling performance (Puri et al., 2020; Shishido et al., 1996). This remains an important knowledge gap in understanding microbiome-mediated influences on conifer establishment. Although direct links between microbial community composition and seedling performance could not be established at this early stage in our study, several taxa associated with improved conifer growth and establishment were detected following outplanting. The presence and colonization of seedlings by *Rhizopogon* and *Suillus* demonstrate that viable ectomycorrhizal inoculum persisted within the restoration site despite the documented effects of wildfire (Cairney & Chambers, 1999; Glassman et al., 2016). Recruitment of these symbionts within 1 year is promising, as post-fire fungal communities are often reduced and may require years to recover (Dove et al., 2022; Glassman et al., 2016; Nelson et al., 2022). The presence of *Suillus* is consistent with their well-documented

ecological traits, including fire-resistant spore banks, rapid root colonization, and long-distance dispersal capacity, which make them especially successful in early-successional and post-disturbance environments (Policelli et al., 2019, 2020).

The accumulation of native ECM from the bulk soil also supports the convergence of seedling microbial communities toward the bulk soil. At planting, microbial communities differed significantly between seedlings, with much of this variation attributable to the nursery-origin. However, these nursery signatures diminished over time following outplanting, consistent with studies that have shown that soil bacterial communities follow predictable successional trajectories (Banning et al., 2011). As variation among outplanted seedlings decreased, differences between seedling rhizosphere and bulk-soil communities also declined, indicating progressive convergence toward the surrounding soil microbiome. This pattern suggests that the post-fire soil environment acted as an ecological filter, promoting the assembly of similar microbial communities across seedlings regardless of nursery-origin (Alekkett et al., 2022). Further evidence for soil-driven community assembly comes from the decline in the number of significantly differentially abundant nursery-origin ASVs over time, coupled with an increase in soil-origin ASVs within seedling communities.

However, convergence toward the soil microbiome did not result in seedling rhizosphere communities becoming direct replicas of the surrounding soil (Lance et al., 2020; Walsh et al., 2021). This was observed in the naturally regenerating ponderosa pine (NG PIPO) as their rhizosphere microbiomes were distinct from the bulk-soil community. Furthermore, convergence toward the NG PIPO microbiome was nursery-dependent, where N2 PIPO seedlings had significantly lower community dissimilarity to NG PIPO than N1 PIPO seedlings. This enhanced convergence may reflect that N2 seedlings were grown in the same geographic region as the

restoration site and therefore experienced environmental conditions more similar to those encountered after outplanting. Post-fire soil environment may act as an ecological filter driving community convergence; however, nursery-origin legacies can maintain residual differences between seedling rhizosphere and bulk-soil communities.

A primary objective of this work was to evaluate potential biosecurity risks associated with introducing nursery-grown conifer seedlings into burned landscapes; putative fungal pathogen ASVs of nursery origin received particular attention. Within one year, the relative abundance of nursery-origin putative fungal pathogens declined significantly. Moreover, many of these taxa have limited evidence as pathogens in conifer hosts. Among the taxa detected, *Dactylonectria* and *Fusarium* are the most robustly documented conifer pathogens, frequently associated with root rot and seedling disease within forest nurseries and managed plantations (Dobbs et al., 2023; Marčiulyrienė et al., 2021). Both genera exhibited decreases in relative abundance between baseline planting and one-year post-outplanting. The significant decline of some putative pathogen ASVs does not necessarily reflect changes in the genus as a whole.

To further evaluate the fate of nursery-origin putative pathogens following outplanting, a spillover analysis was conducted to identify fungal ASVs that may have moved from seedlings into the surrounding bulk soil. Most detected spillover taxa were classified as putative saprotrophs or fungi with unassigned ecological guilds. Only two putative fungal pathogen ASVs were detected in the soil, both ITS2 locus identified as *Fusarium tricinctum*. These ASVs were first recovered from bulk soil six months after planting, and one remained detectable after one year. The two *F. tricinctum* ASVs differed in the strength of evidence supporting seedling-to-soil movement. *Fusarium* ASV835 provided the strongest evidence, as it was recovered exclusively

from N2 PIPO seedlings and was subsequently detected in soil samples from several of the same experimental blocks.

The ecological significance of these spillover events is likely limited because *F. tricinctum* was already widespread throughout the study system in both the bulk soil and the NG PIPO, and is common in forest environments (Litovka et al., 2023). *Fusarium tricinctum* has been recovered frequently from woody plants in natural ecosystems, where it often occurs as an endophyte or saprotroph (Litovka et al., 2023). However, members of the *F. tricinctum* species complex are also commonly recovered from conifer seedlings across broad geographic regions and multiple host species (Dobbs et al., 2023). The limited ecological significance inferred for these *F. tricinctum* ASVs should not be generalized to other *Fusarium* taxa or potential fungal pathogens. The consequences of pathogen spillover are likely to depend on the identity, abundance, pathogenicity, and prior distribution of the organism involved.

Despite limited evidence of ecological impact in this case, the detection of nursery-origin *Fusarium* ASVs in surrounding soils demonstrates that fungal movement from planted seedlings to restoration sites may be possible. Pathogens can be latent in the rhizosphere microbiome of seedlings (Charron et al., 2025; Menkis et al., 2016; Stewart et al., 2021), and during a stress event, may become more prominent (Stewart et al., 2021), thereby spilling into the soil. These findings should be interpreted within the context of the restoration site. The arid, high-elevation conditions of this system may constrain the persistence and spread of nursery-associated fungi, potentially reducing the ecological consequences of spillover (Long et al., 2021; Yan et al., 2023). As a result, the limited spillover observed here may not be representative of other restoration environments, particularly those that provide more favorable conditions for pathogen establishment. This spillover result aligns with growing concerns that asymptomatic nursery

stock can serve as vectors for fungal taxa entering forest ecosystems (Charron et al., 2025; Tanney et al., 2025; Zamora-Ballesteros et al., 2019). While most studies of forest pathogens focus on disease management after establishment, understanding the processes that govern pathogen arrival and spread is increasingly recognized as an important research priority (Horta Jung et al., 2025; Paap et al., 2020; Patejuk et al., 2024). Consequently, the spillover framework applied here provides a first step toward evaluating the biosecurity implications of large-scale reforestation efforts.

CONCLUSIONS

Together, these results suggest that nursery environments strongly influence the initial composition of the conifer seedling rhizosphere microbiome, but that these nursery signatures decrease after outplanting as the seedling microbiome interacts with resident soil communities. From a restoration perspective, these findings emphasize that evaluating reforestation success requires simultaneous consideration of both nursery production practices and post-outplanting ecological processes. Nursery practices influence the initial microbial communities seedlings carry into the field, but soil-driven recruitment, turnover, and native symbiotic establishment ultimately determine how these communities change. Dissecting these interactions will be increasingly important as large-scale reforestation efforts expand across fire-affected landscapes.

The seedlings established in this study also provide a unique opportunity to examine longer-term microbiome dynamics during reforestation. Continued monitoring may reveal microbial influences on seedling growth and survival that were not apparent during the first year after planting, while providing insight into the persistence and functional significance of beneficial symbionts and nursery-associated microorganisms. The rapid recruitment of native ectomycorrhizal fungi despite wildfire disturbance highlights the resilience of soil microbial

communities and their potential role in supporting forest recovery. At the same time, the detection of nursery-origin fungi in surrounding soils demonstrates that microbial movement between outplanted seedlings and restoration sites is possible, underscoring the importance of continued evaluation of biosecurity risks associated with large-scale reforestation. Finally, the widespread occurrence of oomycetes across nursery seedlings, naturally regenerating trees, and bulk soils suggests that these understudied organisms may play a larger role in post-fire ecosystem recovery than is currently appreciated and warrant further investigation.

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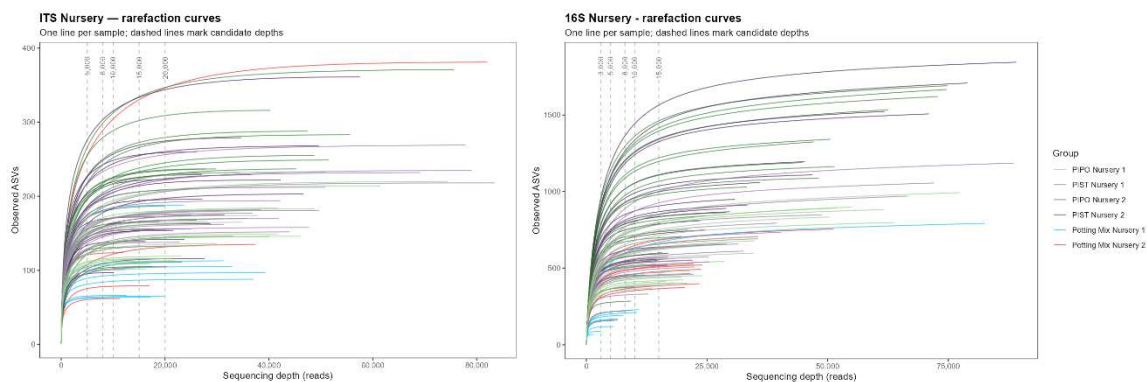
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APPENDIX 1: CHAPTER 2



Supplemental Figure 2.1. Rarefaction curves of observed ASV richness for fungal (ITS, **A**) and bacterial (16S, **B**) nursery communities. Each line is one sample, colored by host species and nursery (PIPO/PIST $\times$ Nursery 1/2) and by potting mix from each nursery; curves plot observed ASVs as a function of sequencing depth. Vertical dashed lines mark candidate rarefaction depths; samples were rarefied to 10,000 reads for downstream richness and diversity analyses, with samples below this depth excluded.

Supplemental Table 2.1. Pairwise comparisons of Shannon diversity among potting mix and seedling rhizospheres across the two nurseries, for fungal (ITS) and bacterial (16S) communities, grouped into four comparison sets.

<u>Shannon diversity of potting mix between nurseries</u>							
Locus ^A	GroupA ^B	n_A ^C	GroupB ^D	n_B ^E	p_raw ^F	p_raw ^G	Cliff's δ^H
ITS	N1 PM	8	N2 PM	6	0.478	0.478	0.250
16S	N1 PM	3	N2 PM	7	0.171	0.171	-0.619
<u>Shannon diversity of potting mix compared to seedling</u>							
Locus ^A	GroupA ^B	n_A ^C	GroupB ^D	n_B ^E	p_raw ^F	p_adj ^G	Cliff's δ^H
ITS	N1 PM	8	N1 PIPO	20	0.021	0.041	-0.575
ITS	N1 PM	8	N1 PIST	19	0.001	0.004	-0.816
ITS	N2 PM	6	N2 PIPO	18	0.102	0.137	-0.463
ITS	N2 PM	6	N2 PIST	20	0.235	0.235	-0.333
16S	N1 PM	3	N1 PIPO	20	0.012	0.012	-0.933
16S	N1 PM	3	N1 PIST	20	0.012	0.012	-0.933
16S	N2 PM	7	N2 PIPO	18	<0.001	<0.001	-0.984
16S	N2 PM	7	N2 PIST	20	<0.001	<0.001	-1.000
<u>Shannon diversity of seedlings within nursery</u>							
Locus ^A	GroupA ^B	n_A ^C	GroupB ^D	n_B ^E	p_raw ^F	p_adj ^G	Cliff's δ^H
ITS	N1 PIPO	20	N1 PIST	19	0.020	0.041	-0.437
ITS	N2 PIPO	18	N2 PIST	20	0.849	0.849	0.039
16S	N1 PIPO	20	N1 PIST	20	0.968	0.968	0.010
16S	N2 PIPO	18	N2 PIST	20	0.260	0.521	0.217
<u>Shannon diversity of seedlings between nurseries</u>							
Locus ^A	GroupA ^B	n_A ^C	GroupB ^D	n_B ^E	p_raw ^F	p_adj ^G	Cliff's δ^H
ITS	N1 PIPO	20	N2 PIPO	18	0.156	0.312	-0.272
ITS	N1 PIST	19	N2 PIST	20	0.407	0.407	0.158
16S	N1 PIPO	20	N2 PIPO	18	<0.001	<0.001	-0.872
16S	N1 PIST	20	N2 PIST	20	<0.001	<0.001	-0.765

^A Amplicon marker (ITS, fungal; 16S, bacterial).

^B The two groups compared.

^C Number of samples in each group.

^F Unadjusted Wilcoxon rank-sum p-value.

^G Benjamini-Hochberg-corrected p-value, applied within each comparison set separately for each locus; the potting-mix-between-nurseries set was a single test and is reported uncorrected ($p_{adj} = p_{raw}$).

^H Rank-based effect size; negative values indicate lower diversity in Group A than in Group B.

Supplemental Table 2.2. Pairwise comparisons of community composition (Beta diversity) among potting mix and seedling rhizospheres across the two nurseries, for fungal (ITS) and bacterial (16S) communities, grouped into four comparison sets.

Beta diversity of potting mix between nurseries								
Locus _A	GroupA ^B	GroupB ^C	Df ^D	R2 ^E	F ^F	p_raw ^G	disp_p_raw ^H	p_raw ^I
ITS	N1 PM	N2 PM	1	0.38 3	7.440	0.003	0.630	0.630
16S	N1 PM	N2 PM	1	0.55 7	10.060	0.008	0.476	0.476
Beta diversity of potting mix compared to seedling								
Locus _A	GroupA ^B	GroupB ^C	Df ^D	R2 ^E	F ^F	p_raw ^G	disp_p_raw ^H	p_adj ^I
ITS	N1 PM	N1 PIPO	1	0.34 3	13.601	0.001	0.019	0.001
ITS	N1 PM	N1 PIST	1	0.47 2	22.371	0.001	0.026	0.001
ITS	N2 PM	N2 PIPO	1	0.14 2	3.652	0.001	0.783	0.001
ITS	N2 PM	N2 PIST	1	0.112	3.033	0.002	0.025	0.002
16S	N1 PM	N1 PIPO	1	0.36 1	11.864	0.002	0.126	0.002
16S	N1 PM	N1 PIST	1	0.33 3	10.499	0.002	0.119	0.002
16S	N2 PM	N2 PIPO	1	0.40 5	15.648	0.001	0.048	0.002
16S	N2 PM	N2 PIST	1	0.34 3	13.066	0.001	0.003	0.002
Beta diversity of seedlings within nursery								
Locus _A	GroupA ^B	GroupB ^C	Df ^D	R2 ^E	F ^F	p_raw ^G	disp_p_raw ^H	p_adj ^I
ITS	N1 PIPO	N1 PIST	1	0.76 5	120.43 1	0.001	0.681	0.001
ITS	N2 PIPO	N2 PIST	1	0.08 4	3.287	0.001	0.044	0.001
16S	N1 PIPO	N1 PIST	1	0.57 1	50.588	0.001	0.341	0.002
16S	N2 PIPO	N2 PIST	1	0.06 0	2.316	0.004	0.089	0.004
Beta diversity of seedlings between nurseries								
Locus _A	GroupA ^B	GroupB ^C	Df ^D	R2 ^E	F ^F	p_raw ^G	disp_p_raw ^H	p_adj ^I
ITS	N1 PIPO	N2 PIPO	1	0.64 2	64.503	0.001	0.001	0.001

ITS	N1 PIST	N2 PIST	1	0.59 8	55.095	0.001	0.001	0.001
16S	N1 PIPO	N2 PIPO	1	0.62 5	60.017	0.001	0.001	0.001
16S	N1 PIST	N2 PIST	1	0.59 2	55.083	0.001	0.001	0.001

^A Amplicon type: ITS (fungal) or 16S (bacterial).

^B First group in the comparison.

^C Second group in the comparison.

^D Degrees of freedom (between-group).

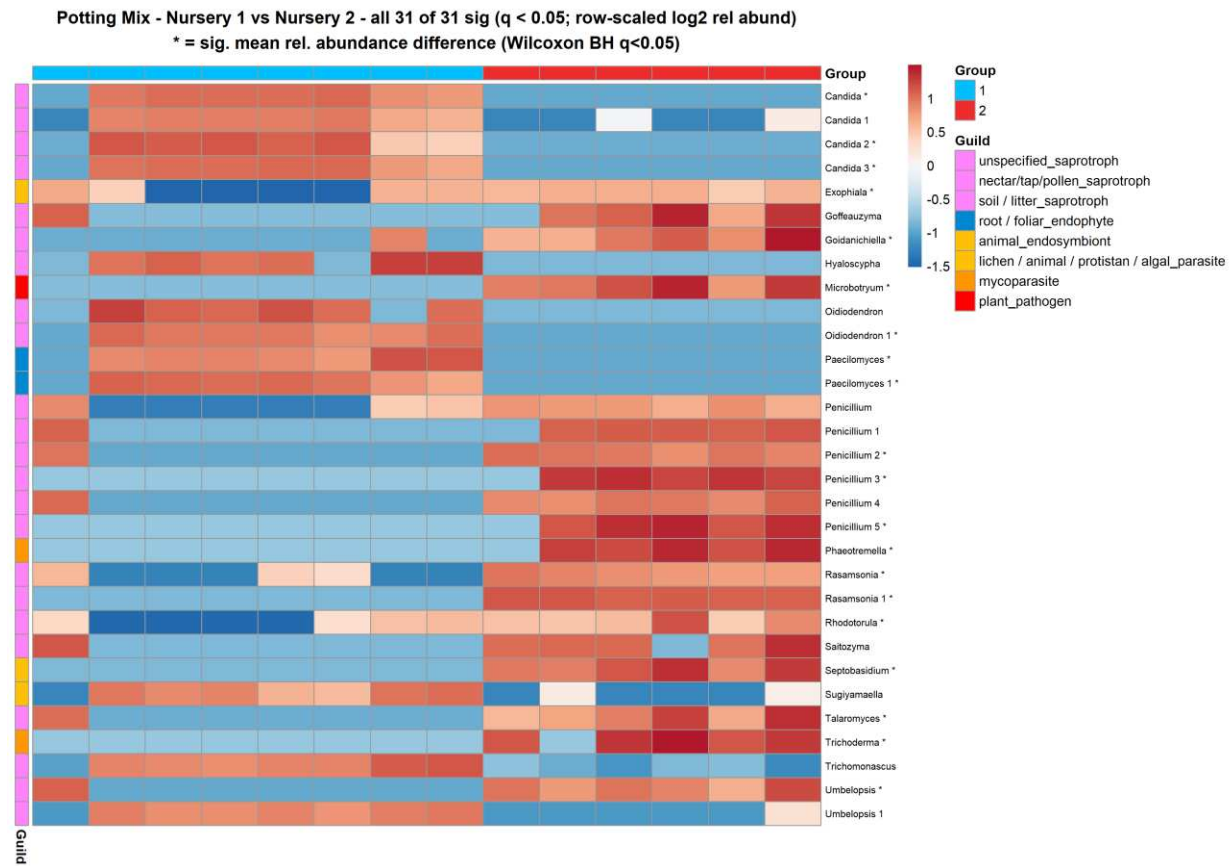
^E Proportion of variance in community composition explained by the grouping (PERMANOVA on Bray-Curtis dissimilarities, 999 permutations).

^F PERMANOVA pseudo-F statistic.

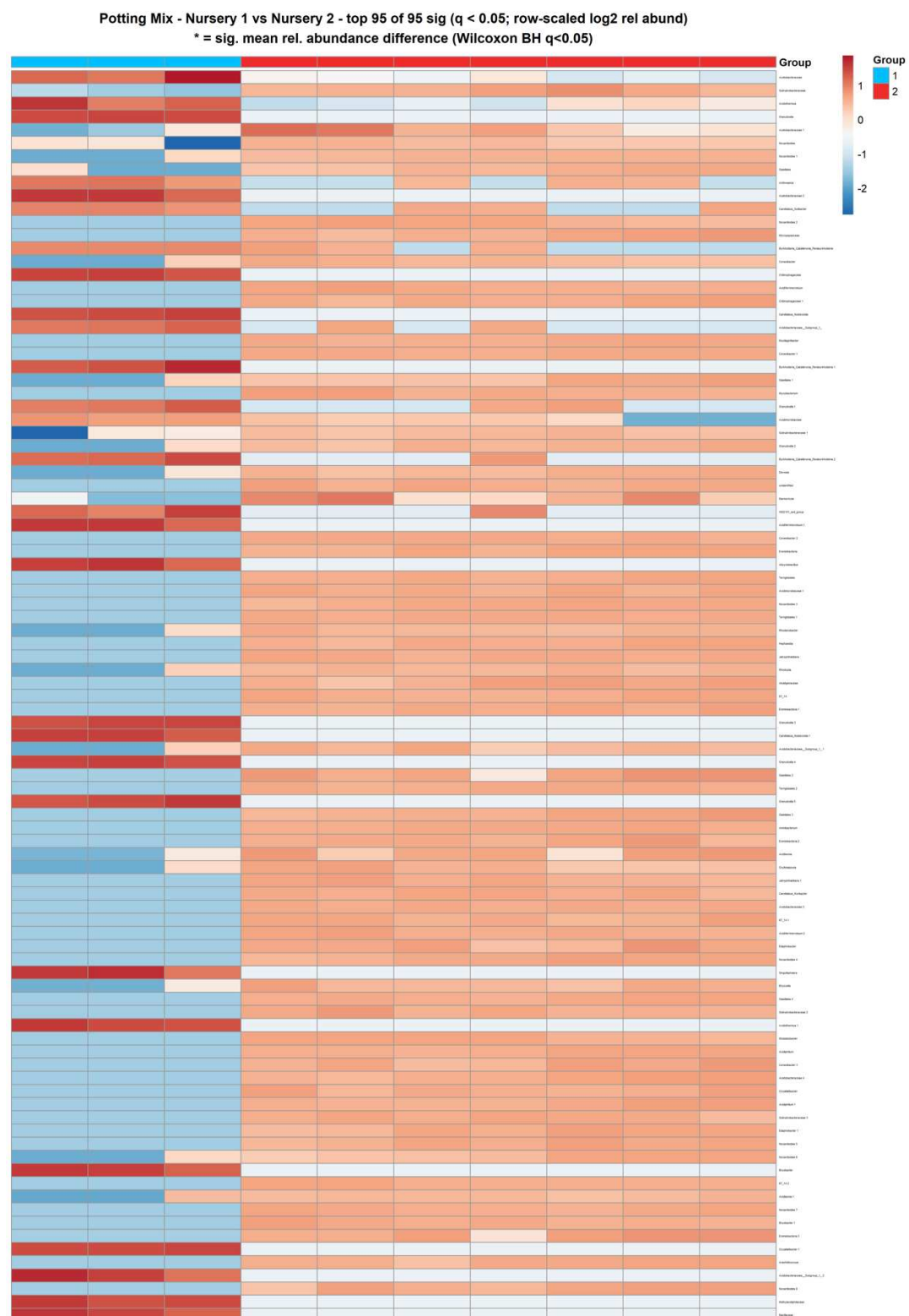
^G Unadjusted PERMANOVA p-value.

^H P-value from a test of multivariate dispersion homogeneity (PERMDISP); values < 0.05 indicate unequal within-group dispersion, so the corresponding PERMANOVA result may reflect dispersion differences in addition to location.

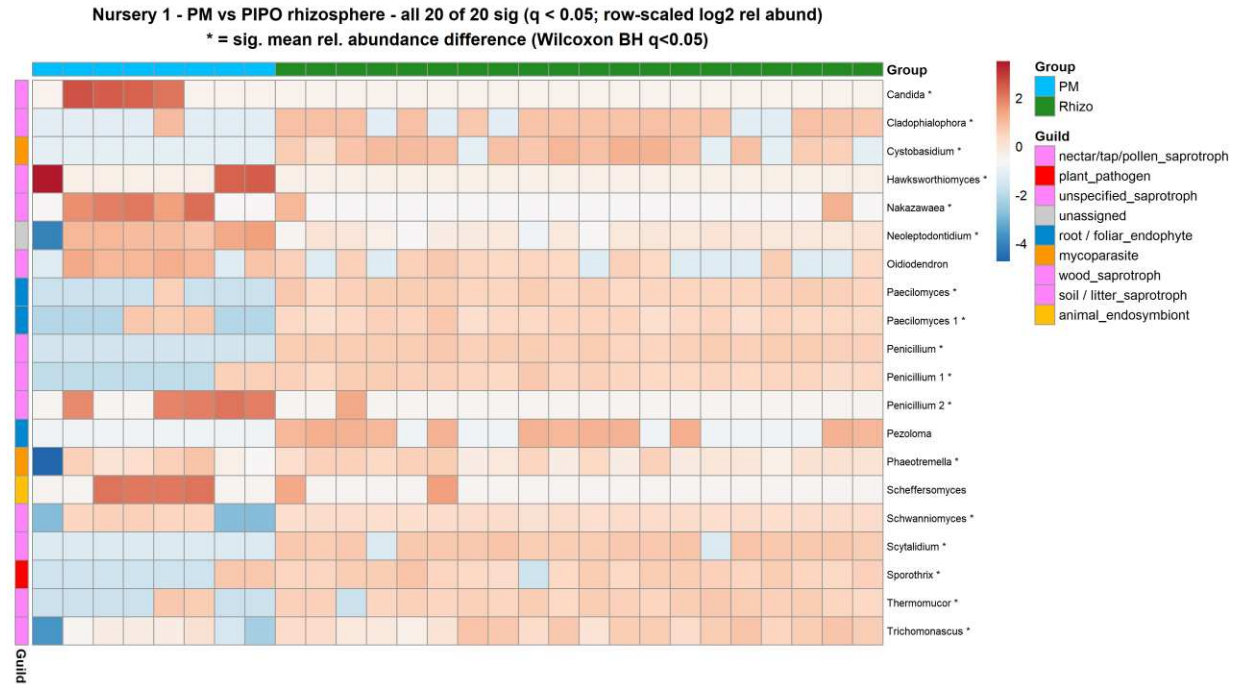
^I Benjamini-Hochberg-corrected PERMANOVA p-value, applied within each comparison set separately for each locus; the potting-mix-between-nurseries set was a single test and is reported uncorrected ($p_{adj} = p_{raw}$).



Supplemental Figure 2.2. Significantly differentially abundant fungal (ITS) ASVs in potting mix between the two nurseries (N1 vs N2). All 31 significant ASVs are shown.

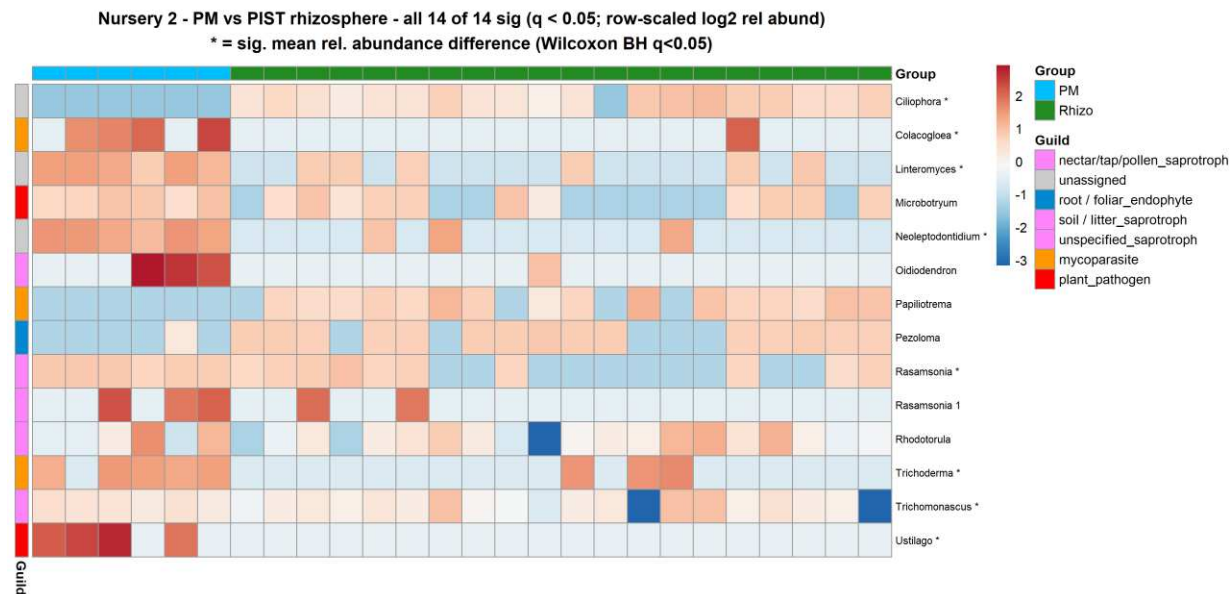


Supplemental Figure 2.3. Significantly differentially abundant bacterial (16S) ASVs in potting mix between the two nurseries (N1 vs N2). All 95 significant ASVs are shown.

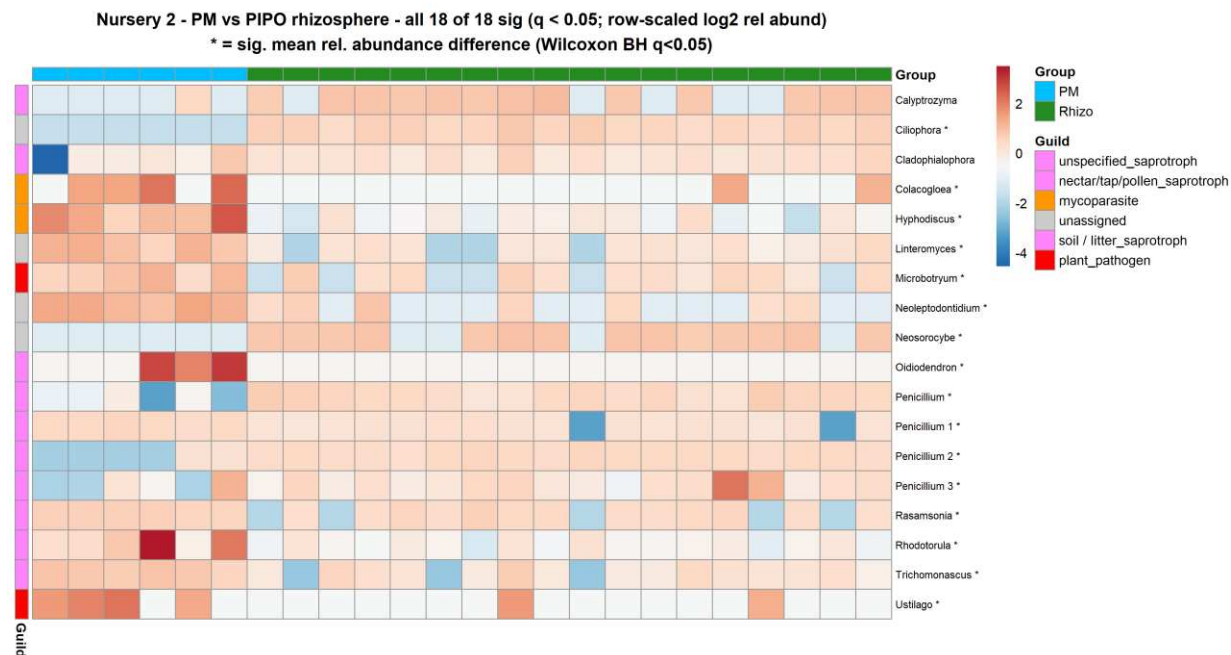


Supplemental Figure 2.4. Significantly differentially abundant fungal (ITS) ASVs in N1 PM and the N1 PIPO. All 20 significant ASVs are shown.

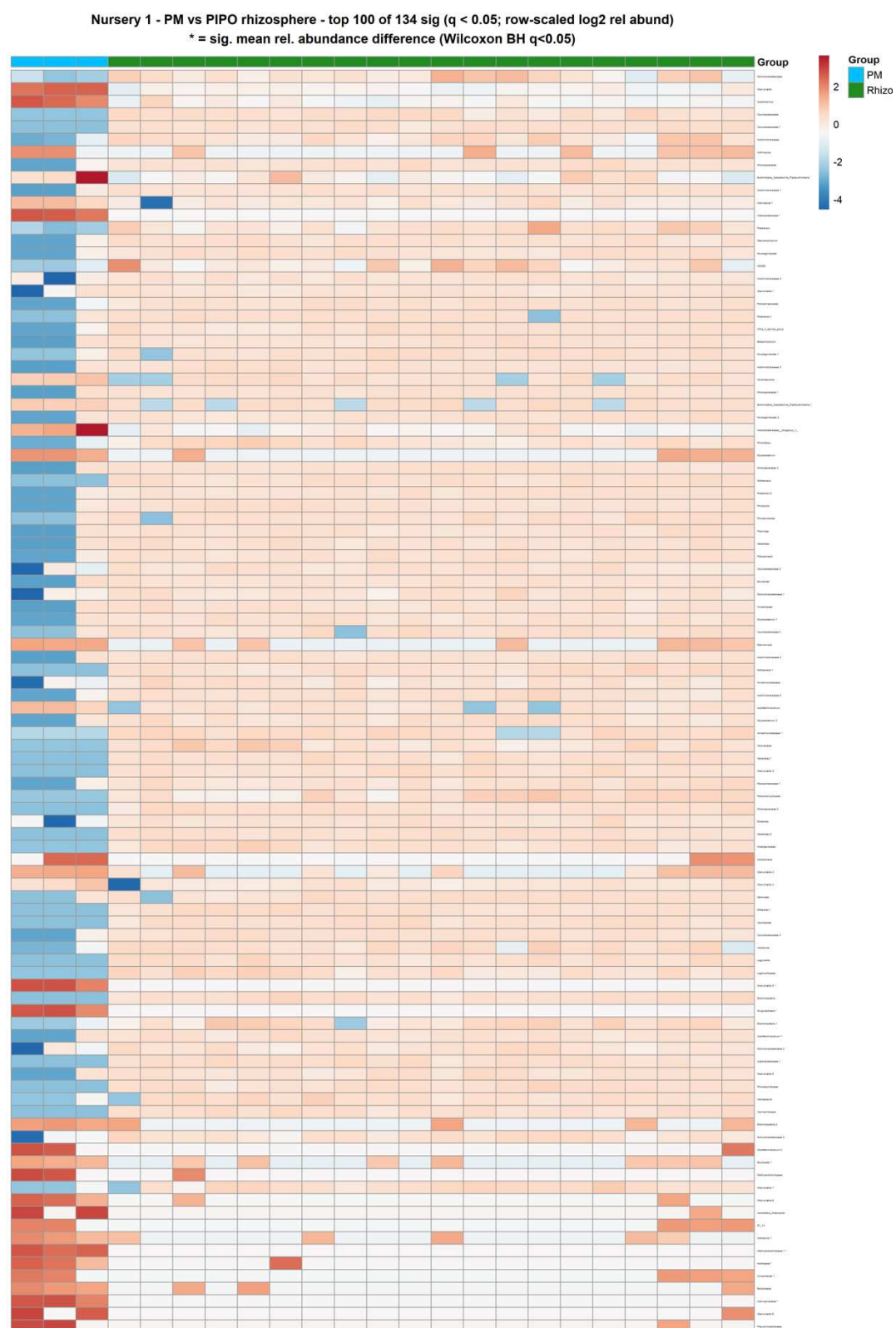
120



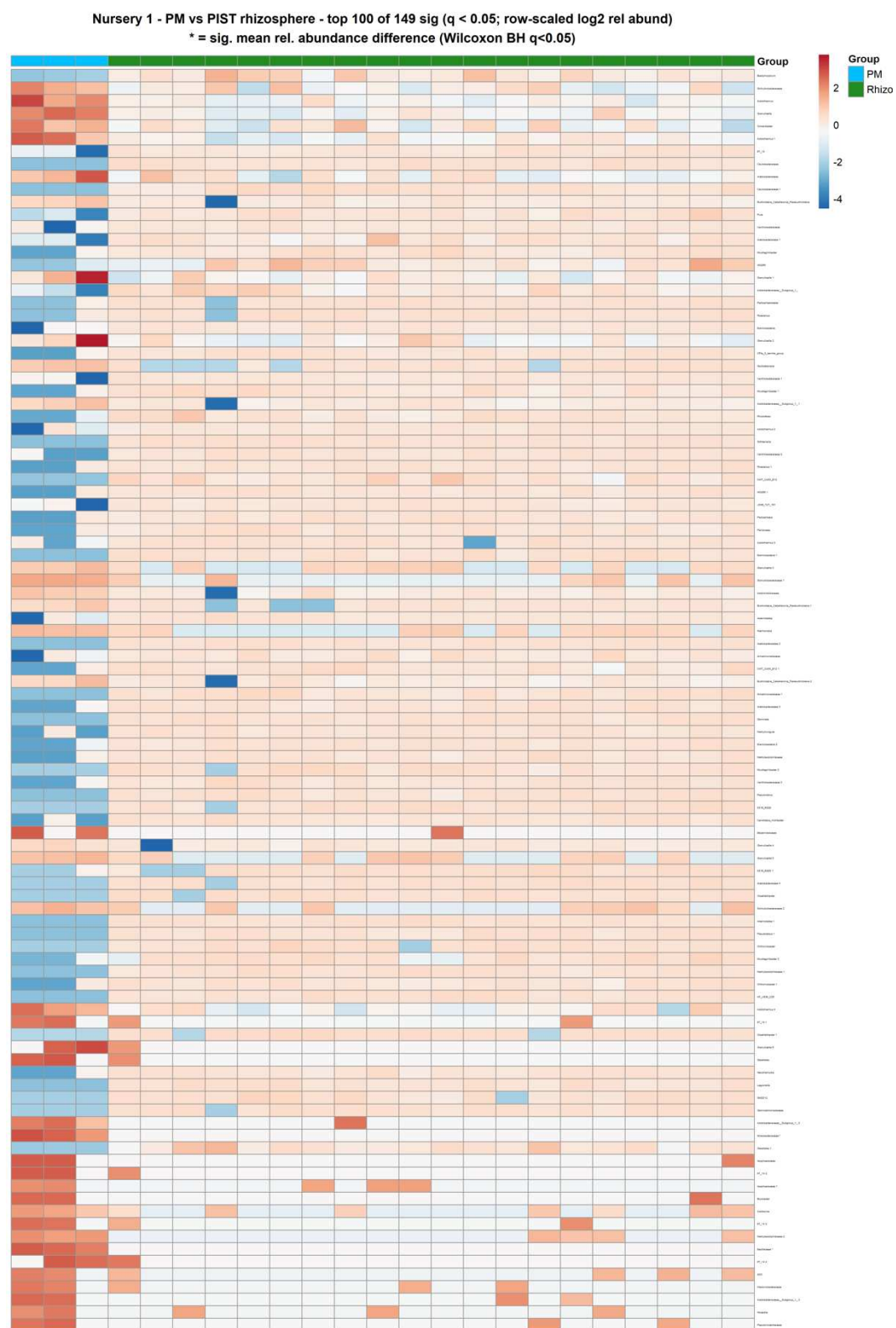
Supplemental Figure 2.6. Significantly differentially abundant fungal (ITS) ASVs in N2 PM and the N2 PIPO. All 18 significant ASVs are shown:-



Supplemental Figure 2.7. Significantly differentially abundant fungal (ITS) ASVs in N2 PM and the N2 PIST. All 14 significant ASVs are shown.



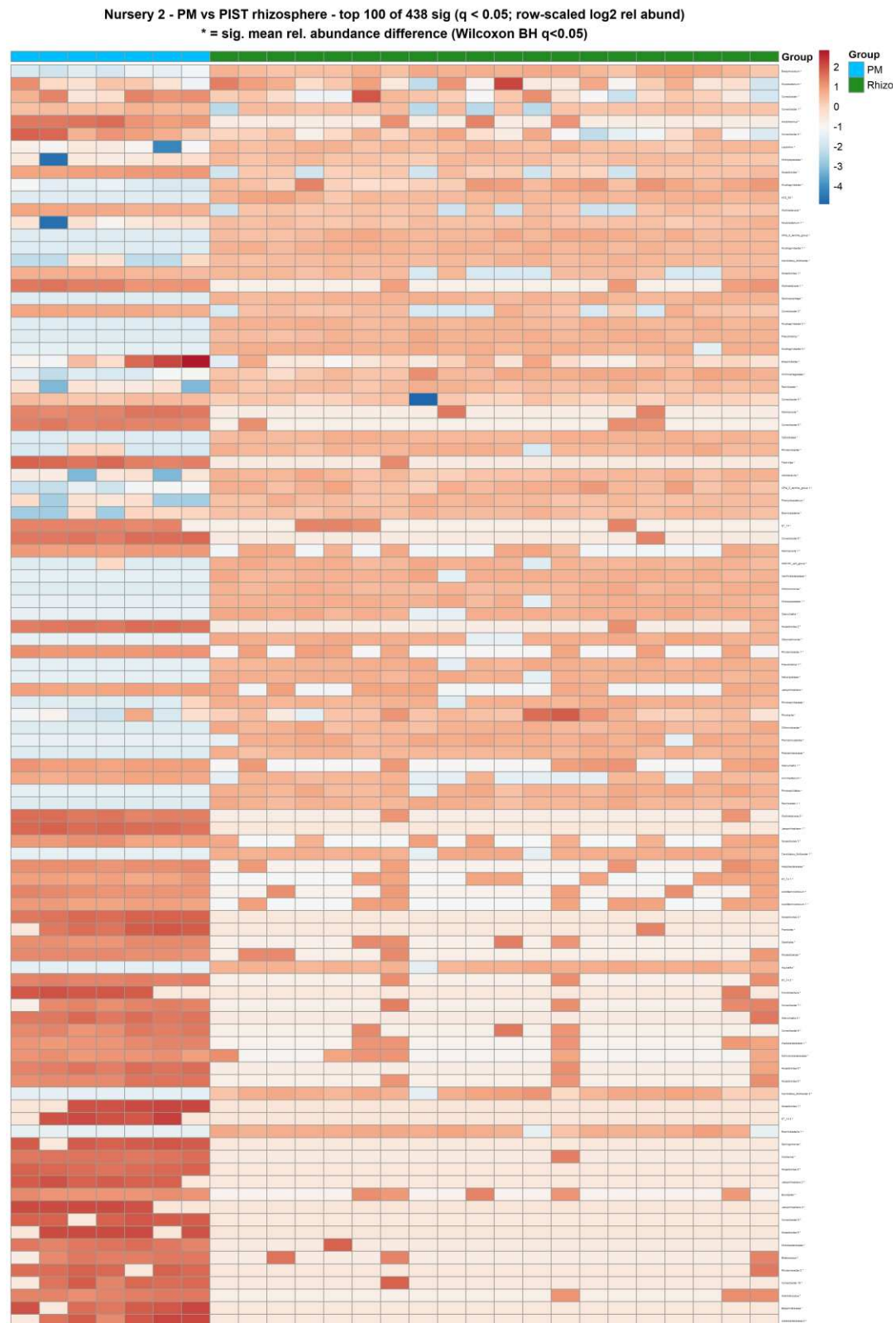
Supplemental Figure 2.8. Significantly differentially abundant bacterial (16S) ASVs in N1 PM and the N1 PIPO. Top 100 of 134 shown.



Supplemental Figure 2.9. Significantly differentially abundant bacterial (16S) ASVs in N1 PM and the N1 PIST. Top 100 of 149 shown.



Supplemental Figure 2.10. Significantly differentially abundant bacterial (16S) ASVs in N2 PM and the N2 PIPO. Top 100 of 500 shown.



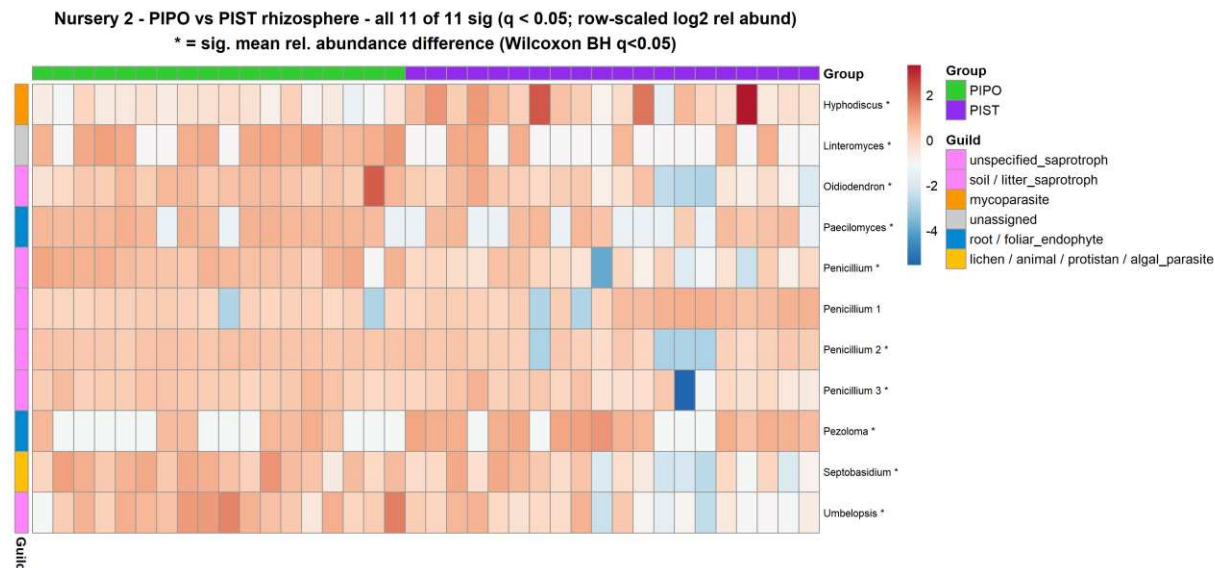
Supplemental Figure 2.11. Significantly differentially abundant bacterial (16S) ASVs in N2 PM and the N2 PIST. Top 100 of 438 shown.



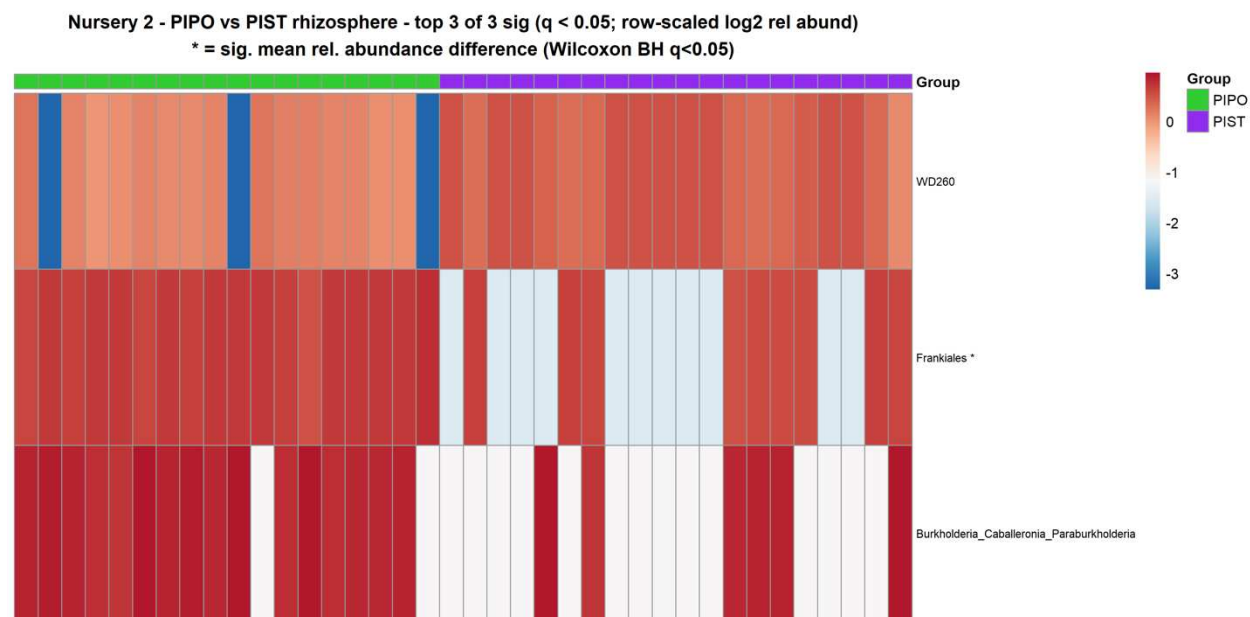
Supplemental Figure 2.12. Significantly differentially abundant fungal (ITS) ASVs in N1 PIPO and the N1 PIST. All 123 ASVs are shown.



Supplemental Figure 2.13. Significantly differentially abundant bacterial (16S) ASVs in N1 PIPO and the N1 PIST. Top 100 of 988 significant ASVs are shown.



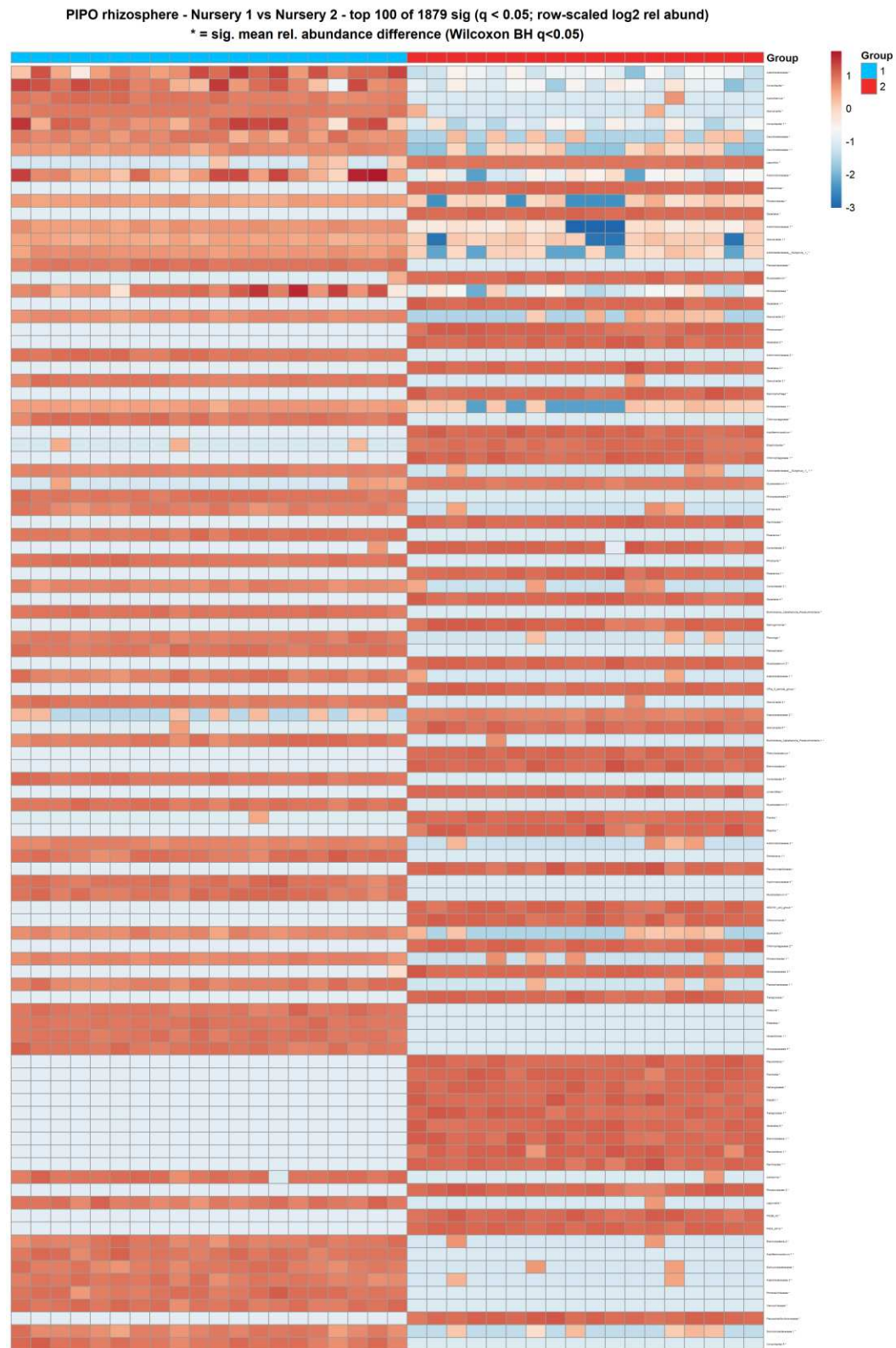
Supplemental Figure 2.14. Significantly differentially abundant fungal (ITS) ASVs in N2 PIPO and the N2 PIST. All 11 ASVs are shown.



Supplemental Figure 2.15. Significantly differentially abundant bacterial (16S) ASVs in N2 PIPO and the N2 PIST. All 3 ASVs are shown.



Supplemental Figure 2.16. Significantly differentially abundant fungal (ITS) ASVs in N1 PIPO and N2 PIPO. All 150 significant ASVs are shown.



Supplemental Figure 2.17. Significantly differentially abundant bacterial (16S) ASVs in N1 PIPO and N2 PIPO. Top 100 of 1879 significant ASVs are shown.



Supplemental Figure 2.18. Significantly differentially abundant fungal (ITS) ASVs in N1 PIST and N2 PIST. All 163 significant ASVs are shown.



Supplemental Figure 2.19. Significantly differentially abundant bacterial (16S) ASVs in N1 PIST and N2 PIST. Top 100 of 1680 significant ASVs are shown.

Supplemental Table 2.3. Pairwise comparisons of Shannon diversity (H') among culture-based fungal communities from symptomatic root tips (SRT), by host species and nursery.

Comparisons cover host species within each nursery and each host species between nurseries.

SRT Wilcoxon Pairwise							
Metric^A	GroupA^B	n_A^C	GroupB^D	n_B^E	p_raw^F	p_adj^G	Cliff'sDelta^H
H'	N1 PIPO	20	N1 PIST	20	0.736	0.832	-0.060
H'	N2 PIPO	20	N2 PIST	20	0.832	0.832	-0.038
H'	N1 PIPO	20	N2 PIPO	20	0.591	0.591	0.090
H'	N1 PIST	20	N2 PIST	20	0.522	0.591	0.113

^A Metric — diversity index (H' , Shannon).

^B GroupA — first group in the comparison.

^C n_A — number of samples in group A.

^D GroupB — second group in the comparison.

^E n_B — number of samples in group B.

^F p_raw — unadjusted Wilcoxon rank-sum p-value.

^G p_adj — Benjamini-Hochberg-corrected p-value, applied within each comparison set.

^H Cliff's δ — rank-based effect size; negative values indicate lower diversity in Group A than in Group B.

Supplemental Table 2.4. Pairwise PERMANOVA comparisons of culture-based fungal community composition from symptomatic root tips (SRT), by host species and nursery.

Comparisons cover host species within each nursery and each host species between nurseries.

These pairwise tests accompany the ordination in Figure 2.4 and follow a significant overall PERMANOVA ($R^2 = 0.078$, $p = 0.006$) with homogeneous group dispersions (PERMDISP $p = 0.87$), supporting a location-based interpretation.

Pairwise PERMANOVA						
GroupA^A	GroupB^B	Df^C	R2^D	F^E	p_raw^F	p_adj^G
N1 PIPO	N1 PIST	1	0.041	1.634	0.157	0.157
N2 PIPO	N2 PIST	1	0.051	2.052	0.019	0.038
N1 PIPO	N2 PIPO	1	0.067	2.711	0.007	0.014
N1 PIST	N2 PIST	1	0.032	1.258	0.281	0.281

^A GroupA — first group in the comparison.

^B GroupB — second group in the comparison.

^C Df — degrees of freedom (between-group).

^D R^2 — proportion of variance in community composition explained by the grouping (PERMANOVA on Bray-Curtis dissimilarities of genus-level presence/absence, 999 permutations).

^E F — PERMANOVA pseudo-F statistic.

^F p_raw — unadjusted PERMANOVA p-value.

^G p_adj — Benjamini-Hochberg-corrected p-value, applied within each comparison set.

Supplemental Table 2.5. Genus-level differential relative abundance of culture-based fungal communities from symptomatic root tips (SRT) between the two nurseries, tested overall (host species pooled) and within each host species. Comparisons used Wilcoxon rank-sum tests on per-tree genus relative abundance. No genus differed significantly between nurseries after correction in either analysis (all $q > 0.05$).

Nursery versus Nursery									
Genus^A	n_ N1^B	n_ N2^C	median_ N1^D	media n_N2^E	mean_ N1^F	mean_N2 ^G	p_ raw^H	log2_fc^I	p_ adj^J
<i>Trichoderma</i>	40	40	0	0	0.192	0.065	0.007	-1.57	0.152
<i>Cladosporium</i>	40	40	0	0	0.038	0.000	0.022	-15.23	0.258
<i>Apiotrichum</i>	40	40	0	0	0.019	0.052	0.235	1.47	0.446
<i>Calophoma</i>	40	40	0	0	0.000	0.013	0.330	13.61	0.446
<i>Curvularia</i>	40	40	0	0	0.005	0.000	0.330	-12.29	0.446
Exobasidiales_gen_ Incertae_sedis	40	40	0	0	0.000	0.025	0.330	14.61	0.446
<i>Hyaloscypha</i>	40	40	0	0	0.000	0.008	0.330	13.02	0.446
<i>Mortierella</i>	40	40	0	0	0.000	0.013	0.330	13.61	0.446
<i>Mucor</i>	40	40	0	0	0.065	0.019	0.079	-1.78	0.446
<i>Neoleptodontidium</i>	40	40	0	0	0.006	0.000	0.330	-12.61	0.446
<i>Ombrophila</i>	40	40	0	0	0.000	0.008	0.330	13.02	0.446
<i>Paecilomyces</i>	40	40	0	0	0.000	0.027	0.082	14.73	0.446
<i>Pezoloma</i>	40	40	0	0	0.000	0.008	0.330	13.02	0.446
<i>Phialocephala</i>	40	40	0	0	0.000	0.025	0.160	14.61	0.446
<i>Scytalidium</i>	40	40	0	0	0.008	0.000	0.330	-13.02	0.446
<i>Sporothrix</i>	40	40	0	0	0.044	0.096	0.311	1.13	0.446
<i>Talaromyces</i>	40	40	0	0	0.000	0.013	0.330	13.61	0.446
<i>Didymella</i>	40	40	0	0	0.034	0.017	0.405	-1.04	0.518
<i>Penicillium</i>	40	40	0.5	0.5	0.480	0.496	0.564	0.05	0.682
<i>Fusarium</i>	40	40	0	0	0.033	0.019	0.638	-0.83	0.734
<i>Umbelopsis</i>	40	40	0	0	0.033	0.050	0.680	0.58	0.745
<i>Alternaria</i>	40	40	0	0	0.019	0.019	1.000	0.00	1.000
Unidentified	40	40	0	0	0.024	0.029	0.975	0.30	1.000
Per Host_Nursery versus Nursery									
Genus^A	n_N1^B	n_N2^C	median_N1^D	median_N2 ^E	p_raw^F	p_adj^G	Host^H		
<i>Trichoderma</i>	20	20	0	0	0.009	0.160	PIPO		
<i>Cladosporium</i>	20	20	0	0	0.081	0.196	PIPO		
<i>Didymella</i>	20	20	0	0	0.081	0.196	PIPO		
<i>Mucor</i>	20	20	0	0	0.040	0.196	PIPO		
<i>Paecilomyces</i>	20	20	0	0	0.081	0.196	PIPO		
<i>Sporothrix</i>	20	20	0	0	0.040	0.196	PIPO		
<i>Unidentified</i>	20	20	0	0	0.081	0.196	PIPO		
<i>Fusarium</i>	20	20	0	0	0.162	0.307	PIPO		
<i>Phialocephala</i>	20	20	0	0	0.162	0.307	PIPO		

<i>Apiotrichum</i>	20	20	0	0	0.212	0.361	PIPO
<i>Curvularia</i>	20	20	0	0	0.342	0.388	PIPO
Exobasidiales_gen_ Incertae_sedis	20	20	0	0	0.342	0.388	PIPO
<i>Ombrophila</i>	20	20	0	0	0.342	0.388	PIPO
<i>Talaromyces</i>	20	20	0	0	0.342	0.388	PIPO
<i>Umbelopsis</i>	20	20	0	0	0.298	0.388	PIPO
<i>Alternaria</i>	20	20	0	0	0.594	0.631	PIPO
<i>Penicillium</i>	20	20	0.5	0.5	0.774	0.774	PIPO
<i>Calophoma</i>	20	20	0	0			PIPO
<i>Hyaloscypha</i>	20	20	0	0			PIPO
<i>Mortierella</i>	20	20	0	0			PIPO
<i>Neoleptodontidium</i>	20	20	0	0			PIPO
<i>Pezoloma</i>	20	20	0	0			PIPO
<i>Scytalidium</i>	20	20	0	0			PIPO
<i>Alternaria</i>	20	20	0	0	0.342	0.547	PIST
<i>Calophoma</i>	20	20	0	0	0.342	0.547	PIST
<i>Cladosporium</i>	20	20	0	0	0.162	0.547	PIST
<i>Hyaloscypha</i>	20	20	0	0	0.342	0.547	PIST
<i>Mortierella</i>	20	20	0	0	0.342	0.547	PIST
<i>Neoleptodontidium</i>	20	20	0	0	0.342	0.547	PIST
<i>Pezoloma</i>	20	20	0	0	0.342	0.547	PIST
<i>Scytalidium</i>	20	20	0	0	0.342	0.547	PIST
<i>Trichoderma</i>	20	20	0.3	0	0.124	0.547	PIST
<i>Unidentified</i>	20	20	0.0	0	0.081	0.547	PIST
<i>Didymella</i>	20	20	0.0	0	0.573	0.748	PIST
<i>Fusarium</i>	20	20	0.0	0	0.574	0.748	PIST
<i>Mucor</i>	20	20	0.0	0	0.655	0.748	PIST
<i>Penicillium</i>	20	20	0.4	0.5	0.610	0.748	PIST
<i>Umbelopsis</i>	20	20	0	0	0.706	0.753	PIST
<i>Sporothrix</i>	20	20	0	0	0.790	0.790	PIST
<i>Apiotrichum</i>	20	20	0	0			PIST
<i>Curvularia</i>	20	20	0	0			PIST
Exobasidiales_gen_ Incertae_sedis	20	20	0	0			PIST
<i>Ombrophila</i>	20	20	0	0			PIST
<i>Paecilomyces</i>	20	20	0	0			PIST
<i>Phialocephala</i>	20	20	0	0			PIST
<i>Talaromyces</i>	20	20	0	0			PIST

^A Genus — fungal genus, or higher-rank placeholder where genus was unresolved (e.g., *Exobasidiales* gen. Incertae sedis);

"Unidentified" denotes isolates the reference taxonomy left unnamed at genus rank, and "Unclassified_Genus" (if present) denotes isolates with a missing genus field.

^{B C} n_N1, n_N2 — number of trees (samples) per nursery.

^{D E} median_N1, median_N2 — median per-tree genus relative abundance (proportion of a tree's genus-level detections) in each nursery.

^{F G} mean_N1, mean_N2 — corresponding means.

^H p_raw — unadjusted Wilcoxon rank-sum p-value (Nursery 1 vs Nursery 2).

^I log2_fc — log2 fold change of mean relative abundance (Nursery 2 ÷ Nursery 1); positive = higher in Nursery 2. Values are computed with a 1×10^{-6} pseudocount, so genera absent from one nursery yield large magnitudes ($|\log2_fc| \approx 12\text{--}15$) that indicate presence/absence rather than a quantified fold change.

^J p_adj — Benjamini-Hochberg-corrected p-value (Overall: across all genera; Per-host: within each host).

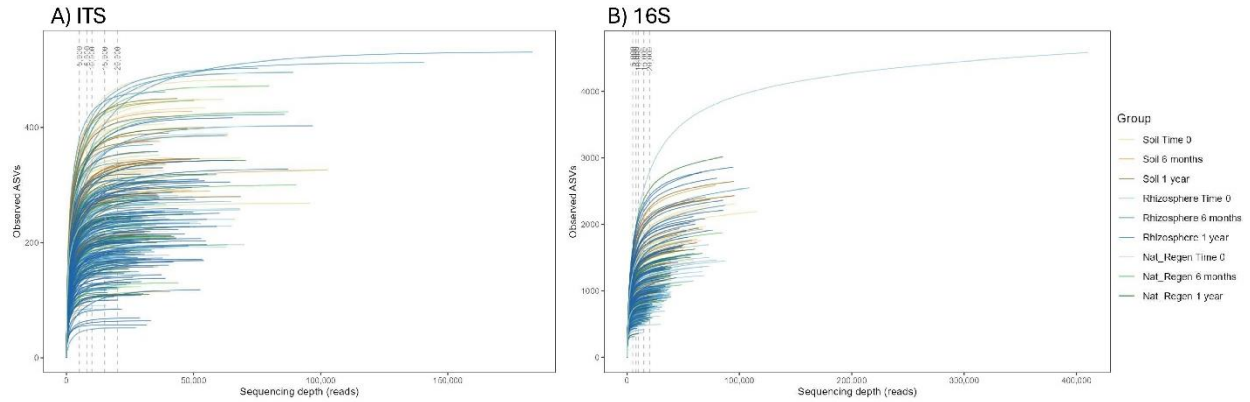
^K Host — host species (PIPO or PIST); blank p_raw and p_adj indicate genera not tested because one or both nurseries had fewer than three trees with that genus ($n < 3$ per nursery required).

Supplemental Table 2.6. Oomycete read counts from RPS10 metabarcoding of nursery samples, by tissue type and nursery. All samples with reads, regardless of whether the reads were successfully assigned with OomyceteDB, are reported. Each row is one sample; columns give the number of reads assigned to each oomycete taxon, reads not assignable to a reference taxon ("Unassigned"), and the per-sample total.

PIPO	PIPO	PIPO	PIPO	PIPO	PIST	PIST	PIPO	PIST	tissue_type
2	2	2	2	1	2	2	1	2	nursery
0	3478	36013	0	0	0	0	0	0	Achlya hypogyna
0	0	0	45322	0	0	0	0	0	Globisporangium aquisylvae
0	0	0	0	25484	0	0	0	0	Globisporangium okanoganense
0	0	0	0	25624	0	0	0	0	Globisporangium rostratifyingens
0	2026	0	0	0	0	0	0	0	Phytophthora cactorum
0	0	0	0	0	0	0	73	0	Phytophthora pseudocryptogea
0	0	0	0	0	0	0	0	0	Pythium volutum
33778	33307	3706	2493	377	78647	79349	81303	129208	Unassigned
33778	38811	39719	47815	51485	78647	79349	81376	129208	total_reads

PIPO	PottingMix	PottingMix	PottingMix	PIPO	PottingMix	PIST	PIPO
1	2	2	2	2	2	1	1
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	287	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	18256
11	0	0	0	0	0	0	0
0	250	493	696	1833	2110	18249	0
11	250	493	983	1833	2110	18249	18256

APPENDIX 2: CHAPTER 3



Supplemental Figure 3.1. Rarefaction curves for the field (A) fungal (ITS) and (B) bacterial (16S) datasets. Each line represents the observed ASV richness of a single sample as a function of sequencing depth, colored by compartment and timepoint: Soil, Rhizosphere, and naturally regenerating ponderosa pine (NG PIPO) at Time 0, 6 months, and 1 year.

Growth metrics

Supplemental Table 3.1.A. Type III tests of fixed terms from a linear mixed-effects model of seedling height (cm).

F ^A	Df ^B	Df.res ^C	Pr(>F) ^D	Term ^E
53.191	1	212	< 0.001	Nursery
94.705	1	212	< 0.001	Species
20.606	2	211	< 0.001	Timepoint
0.295	1	211	0.588	Nursery:Species
0.183	2	211	0.833	Nursery:Timepoint
1.956	2	212	0.144	Species:Timepoint
2.043	2	212	0.132	Nursery:Species:Timepoint

^A F-statistic.

^B Degrees of freedom.

^C Denominator degrees of freedom, estimated using the Kenward–Roger approximation

^D p-value for the Type III test; values below 0.001 reported as "< 0.001."

^E Fixed terms in the model.

Supplemental Table 3.1.B. Estimated marginal means of seedling height (cm) for each nursery of origin × conifer species × timepoint combination, derived from the linear mixed-effects model in Supplemental Table 3.1.A.

Nursery ^A	Species ^B	Timepoint ^C	emmean ^D	SE ^E	df ^F	lower.CL ^G	upper.CL ^H
1	PIPO	Time 0	12.090	1.151	152	9.815	14.365
2	PIPO	Time 0	8.147	1.182	157	5.813	10.482
1	PIST	Time 0	20.740	1.151	152	18.465	23.015
2	PIST	Time 0	13.629	1.255	160	11.152	16.107
1	PIPO	6 months	13.176	1.250	167	10.709	15.644
2	PIPO	6 months	9.261	1.215	162	6.863	11.660
1	PIST	6 months	22.374	1.182	157	20.039	24.708
2	PIST	6 months	16.312	1.251	165	13.841	18.783
1	PIPO	1 year	19.442	1.182	157	17.107	21.777
2	PIPO	1 year	13.380	1.151	152	11.105	15.655
1	PIST	1 year	22.780	1.151	152	20.505	25.055
2	PIST	1 year	19.795	1.151	152	17.520	22.070

^A Source nursery of origin: 1 (N1) or 2 (N2).

^B PIPO, ponderosa pine (*Pinus ponderosa*); PIST, southwestern white pine (*P. strobiformis*).

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Estimated marginal mean.

^E Standard error of the estimated marginal mean.

^F Denominator degrees of freedom, estimated using the Kenward–Roger approximation.

^G Lower limit of the 95% confidence interval.

^H Upper limit of the 95% confidence interval.

Supplemental Table 3.1.C. Pairwise comparisons of seedling height (cm) from the linear mixed-effects model in Supplemental Table 3.1.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
PIPO - PIST	1	Time 0	-8.650	1.628	211	-5.312	< 0.001	< 0.001
PIPO - PIST	2	Time 0	-5.482	1.725	213	-3.179	0.002	0.002
PIPO - PIST	1	6 months	-9.197	1.720	211	-5.348	< 0.001	< 0.001
PIPO - PIST	2	6 months	-7.051	1.745	212	-4.042	< 0.001	< 0.001

PIPO - PIST	1	1 year	-3.338	1.650	211	-2.023	0.044	0.044
PIPO - PIST	2	1 year	-6.415	1.628	211	-3.939	< 0.001	< 0.001
N1 - N2	PIPO	Time 0	3.943	1.650	211	2.389	0.018	0.027
N1 - N2	PIST	Time 0	7.111	1.703	212	4.175	< 0.001	< 0.001
N1 - N2	PIPO	6 months	3.915	1.742	211	2.248	0.026	0.031
N1 - N2	PIST	6 months	6.062	1.721	212	3.522	0.001	0.001
N1 - N2	PIPO	1 year	6.062	1.650	211	3.674	< 0.001	0.001
N1 - N2	PIST	1 year	2.985	1.628	211	1.833	0.068	0.068
See footnotes below.								

Supplemental Table 3.1.D. Pairwise comparisons of seedling height (cm) among timepoints from the linear mixed-effects model in Supplemental Table 3.1.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
Time 0 - 6 months	1	PIPO	-1.086	1.699	211	-0.639	0.523	0.571
Time 0 - 1 year	1	PIPO	-7.352	1.650	211	-4.455	< 0.001	< 0.001
6 months - 1 year	1	PIPO	-6.266	1.720	211	-3.643	< 0.001	0.001
Time 0 - 6 months	2	PIPO	-1.114	1.695	212	-0.657	0.512	0.571
Time 0 - 1 year	2	PIPO	-5.233	1.650	211	-3.171	0.002	0.005
6 months - 1 year	2	PIPO	-4.119	1.674	211	-2.461	0.015	0.035
Time 0 - 6 months	1	PIST	-1.634	1.650	211	-0.990	0.323	0.431
Time 0 - 1 year	1	PIST	-2.040	1.628	211	-1.253	0.212	0.318
6 months - 1 year	1	PIST	-0.406	1.650	211	-0.246	0.806	0.806
Time 0 - 6 months	2	PIST	-2.682	1.771	213	-1.514	0.131	0.225
Time 0 - 1 year	2	PIST	-6.166	1.703	212	-3.621	0.000	0.001
6 months - 1 year	2	PIST	-3.483	1.701	212	-2.048	0.042	0.084

^A Pairwise comparison; the estimate is signed in the direction shown (first level minus second level).

^B Stratum within which each comparison was computed.

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Difference in estimated marginal mean in the direction of the comparison.

^E Standard error of the comparison.

^F Degrees of freedom, estimated using the Kenward–Roger approximation.

^G t-statistic.

^H Unadjusted p-value; values below 0.001 reported as "< 0.001."

^I Benjamini–Hochberg false discovery rate–adjusted p-value (Benjamini & Hochberg 1995), applied within each comparison family separately.

Supplemental Table 3.2.A. Type III tests of fixed terms from a linear mixed-effects model of seedling caliper (mm).

F ^A	Df ^B	Df.res ^C	Pr(>F) ^D	Term ^E
4.904	1	212	0.028	Nursery
4.235	1	212	0.041	Species
154.003	2	211	< 0.001	Timepoint
22.684	1	211	< 0.001	Nursery:Species
0.335	2	211	0.715	Nursery:Timepoint
10.086	2	212	< 0.001	Species:Timepoint
2.707	2	211	0.069	Nursery:Species:Timepoint

^A F-statistic.^B Degrees of freedom.^C Denominator degrees of freedom, estimated using the Kenward–Roger approximation^D p-value for the Type III test; values below 0.001 reported as "< 0.001."^E Fixed terms in the model.

Supplemental Table 3.2.B. Estimated marginal means of seedling caliper (mm) for each nursery of origin × conifer species × timepoint combination, derived from the linear mixed-effects model in Supplemental Table 3.2.A.

Nursery ^A	Species ^B	Timepoint ^C	emmean ^D	SE ^E	df ^F	lower.CL ^G	upper.CL ^H
1	PIPO	Time 0	3.625	0.201	127	3.228	4.022
2	PIPO	Time 0	3.197	0.206	132	2.790	3.605
1	PIST	Time 0	3.489	0.201	127	3.092	3.886
2	PIST	Time 0	3.549	0.218	139	3.118	3.981
1	PIPO	6 months	4.299	0.217	145	3.869	4.729
2	PIPO	6 months	3.359	0.211	138	2.941	3.778
1	PIST	6 months	3.907	0.206	132	3.499	4.314
2	PIST	6 months	4.045	0.218	143	3.614	4.475
1	PIPO	1 year	6.867	0.206	132	6.459	7.274
2	PIPO	1 year	5.758	0.201	127	5.361	6.155
1	PIST	1 year	4.975	0.201	127	4.577	5.372
2	PIST	1 year	5.680	0.201	127	5.282	6.077

^A Source nursery of origin: 1 (N1) or 2 (N2).^B PIPO, ponderosa pine (*Pinus ponderosa*); PIST, southwestern white pine (*P. strobiformis*).^C Time 0 (planting), 6 months, and 1 year after outplanting.^D Estimated marginal mean.^E Standard error of the estimated marginal mean.^F Denominator degrees of freedom, estimated using the Kenward–Roger approximation.^G Lower limit of the 95% confidence interval.^H Upper limit of the 95% confidence interval.

Supplemental Table 3.2.C. Pairwise comparisons of seedling caliper (mm) from the linear mixed-effects model in Supplemental Table 3.2.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p _I _FDR
PIPO - PIST	1	Time 0	0.136	0.280	211	0.485	0.628	0.754
PIPO - PIST	2	Time 0	-0.352	0.297	212	-1.186	0.237	0.355
PIPO - PIST	1	6 months	0.392	0.296	211	1.325	0.187	0.355
PIPO - PIST	2	6 months	-0.685	0.300	212	-2.282	0.024	0.071
PIPO - PIST	1	1 year	1.892	0.284	211	6.659	< 0.001	< 0.001
PIPO - PIST	2	1 year	0.078	0.280	211	0.280	0.780	0.780

N1 - N2	PIPO	Time 0	0.428	0.284	211	1.506	0.134	0.200
N1 - N2	PIST	Time 0	-0.060	0.293	212	-0.206	0.837	0.837
N1 - N2	PIPO	6 months	0.940	0.300	211	3.133	0.002	0.006
N1 - N2	PIST	6 months	-0.138	0.296	212	-0.466	0.642	0.770
N1 - N2	PIPO	1 year	1.109	0.284	211	3.901	< 0.001	0.001
N1 - N2	PIST	1 year	-0.705	0.280	211	-2.514	0.013	0.025
See footnotes below.								

Supplemental Table 3.2.D. Pairwise comparisons of seedling caliper (mm) among timepoints from the linear mixed-effects model in Supplemental Table 3.2.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
Time 0 - 6 months	1	PIPO	-0.674	0.293	211	-2.304	0.022	0.030
Time 0 - 1 year	1	PIPO	-3.242	0.284	211	-11.408	< 0.001	< 0.001
6 months - 1 year	1	PIPO	-2.568	0.296	211	-8.670	< 0.001	< 0.001
Time 0 - 6 months	2	PIPO	-0.162	0.292	211	-0.556	0.579	0.579
Time 0 - 1 year	2	PIPO	-2.561	0.284	211	-9.012	< 0.001	< 0.001
6 months - 1 year	2	PIPO	-2.399	0.288	211	-8.323	< 0.001	< 0.001
Time 0 - 6 months	1	PIST	-0.418	0.284	211	-1.470	0.143	0.156
Time 0 - 1 year	1	PIST	-1.486	0.280	211	-5.298	< 0.001	< 0.001
6 months - 1 year	1	PIST	-1.068	0.284	211	-3.758	< 0.001	< 0.001
Time 0 - 6 months	2	PIST	-0.495	0.305	212	-1.624	0.106	0.127
Time 0 - 1 year	2	PIST	-2.130	0.293	212	-7.264	< 0.001	< 0.001
6 months - 1 year	2	PIST	-1.635	0.293	211	-5.583	< 0.001	< 0.001

^A Pairwise comparison; the estimate is signed in the direction shown (first level minus second level).

^B Stratum within which each comparison was computed.

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Difference in estimated marginal mean in the direction of the comparison.

^E Standard error of the comparison.

^F Degrees of freedom, estimated using the Kenward–Roger approximation.

^G t-statistic.

^H Unadjusted p-value; values below 0.001 reported as "< 0.001."

^I Benjamini–Hochberg false discovery rate-adjusted p-value (Benjamini & Hochberg 1995), applied within each contrast family separately.

Supplemental Table 3.3.A. Type III tests of fixed terms from a linear mixed-effects model of seedling root biomass (g).

F ^A	Df ^B	Df.res ^C	Pr(>F) ^D	Term ^F
17.190	1	211	< 0.001	Nursery
0.118	1	211	0.732	Species
118.123	2	211	< 0.001	Timepoint
33.046	1	211	< 0.001	Nursery:Species
2.215	2	211	0.112	Nursery:Timepoint
7.579	2	211	0.001	Species:Timepoint
6.782	2	211	0.001	Nursery:Species:Timepoint

^A F-statistic.^B Degrees of freedom.^C Denominator degrees of freedom, estimated using the Kenward–Roger approximation^D p-value for the Type III test; values below 0.001 reported as "< 0.001."^E Fixed terms in the model.

Supplemental Table 3.3.B. Estimated marginal means of seedling root biomass (g) for each nursery of origin × conifer species × timepoint combination, derived from the linear mixed-effects model in Supplemental Table 3.3.A.

Nursery ^A	Species ^B	Timepoint ^C	emmean ^D	SE ^E	df ^F	lower.CL ^G	upper.CL ^H
1	PIPO	Time 0	0.886	0.159	117	0.571	1.201
2	PIPO	Time 0	0.601	0.163	123	0.278	0.924
1	PIST	Time 0	0.798	0.159	117	0.483	1.113
2	PIST	Time 0	0.825	0.173	131	0.483	1.167
1	PIPO	6 months	2.581	0.172	135	2.240	2.921
2	PIPO	6 months	1.496	0.167	129	1.164	1.827
1	PIST	6 months	2.564	0.163	123	2.241	2.887
2	PIST	6 months	2.427	0.172	134	2.086	2.768
1	PIPO	1 year	3.228	0.163	123	2.905	3.551
2	PIPO	1 year	1.831	0.159	117	1.516	2.146
1	PIST	1 year	1.822	0.159	117	1.507	2.137
2	PIST	1 year	2.379	0.159	117	2.064	2.694

^A Source nursery of origin: 1 (N1) or 2 (N2).^B PIPO, ponderosa pine (*Pinus ponderosa*); PIST, southwestern white pine (*P. strobiformis*).^C Time 0 (planting), 6 months, and 1 year after outplanting.^D Estimated marginal mean.^E Standard error of the estimated marginal mean.^F Denominator degrees of freedom, estimated using the Kenward–Roger approximation.^G Lower limit of the 95% confidence interval.^H Upper limit of the 95% confidence interval.

Supplemental Table 3.3.C. Pairwise comparisons of seedling root biomass (g) from the linear mixed-effects model in Supplemental Table 3.3.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
PIPO - PIST	1	Time 0	0.088	0.221	211	0.400	0.690	0.827
PIPO - PIST	2	Time 0	-0.224	0.234	212	-0.956	0.340	0.510
PIPO - PIST	1	6 months	0.017	0.233	211	0.072	0.943	0.943
PIPO - PIST	2	6 months	-0.932	0.237	212	-3.936	< 0.001	< 0.001
PIPO - PIST	1	1 year	1.406	0.224	211	6.279	< 0.001	< 0.001
PIPO - PIST	2	1 year	-0.548	0.221	211	-2.480	0.014	0.028
N1 - N2	PIPO	Time 0	0.285	0.224	211	1.274	0.204	0.306

N1 - N2	PIST	Time 0	-0.027	0.231	212	-0.117	0.907	0.907
N1 - N2	PIPO	6 months	1.085	0.236	211	4.591	< 0.001	< 0.001
N1 - N2	PIST	6 months	0.137	0.234	211	0.586	0.559	0.671
N1 - N2	PIPO	1 year	1.397	0.224	211	6.239	< 0.001	< 0.001
N1 - N2	PIST	1 year	-0.557	0.221	211	-2.520	0.012	0.025

See footnotes below.

Supplemental Table 3.3.D. Pairwise comparisons of seedling root biomass (g) among timepoints from the linear mixed-effects model in Supplemental Table 3.3.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
Time 0 - 6 months	1	PIPO	-1.695	0.231	211	-7.350	< 0.001	< 0.001
Time 0 - 1 year	1	PIPO	-2.342	0.224	211	-10.458	< 0.001	< 0.001
6 months - 1 year	1	PIPO	-0.647	0.233	211	-2.773	0.006	0.007
Time 0 - 6 months	2	PIPO	-0.895	0.230	211	-3.889	< 0.001	< 0.001
Time 0 - 1 year	2	PIPO	-1.230	0.224	211	-5.493	< 0.001	< 0.001
6 months - 1 year	2	PIPO	-0.335	0.227	211	-1.476	0.141	0.154
Time 0 - 6 months	1	PIST	-1.766	0.224	211	-7.888	< 0.001	< 0.001
Time 0 - 1 year	1	PIST	-1.024	0.221	211	-4.635	< 0.001	< 0.001
6 months - 1 year	1	PIST	0.742	0.224	211	3.314	0.001	0.001
Time 0 - 6 months	2	PIST	-1.603	0.240	212	-6.669	< 0.001	< 0.001
Time 0 - 1 year	2	PIST	-1.554	0.231	212	-6.726	< 0.001	< 0.001
6 months - 1 year	2	PIST	0.048	0.231	211	0.210	0.834	0.834

^A Pairwise comparison; the estimate is signed in the direction shown (first level minus second level).

^B Stratum within which each comparison was computed.

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Difference in estimated marginal mean in the direction of the comparison.

^E Standard error of the comparison.

^F Degrees of freedom, estimated using the Kenward–Roger approximation.

^G t-statistic.

^H Unadjusted p-value; values below 0.001 reported as "< 0.001."

^I Benjamini–Hochberg false discovery rate–adjusted p-value (Benjamini & Hochberg 1995), applied within each contrast family separately.

Supplemental Table 3.4.A. Type III tests of fixed terms from a linear mixed-effects model of seedling shoot biomass (g).

F ^A	Df ^B	Df.res ^C	Pr(>F) ^D	Term ^F
9.700	1	212	0.002	Nursery
7.236	1	212	0.008	Species
94.377	2	211	< 0.001	Timepoint
15.763	1	211	< 0.001	Nursery:Species
2.486	2	211	0.086	Nursery:Timepoint
8.662	2	212	< 0.001	Species:Timepoint
4.192	2	212	0.016	Nursery:Species:Timepoint

^A F-statistic.^B Degrees of freedom.^C Denominator degrees of freedom, estimated using the Kenward–Roger approximation^D p-value for the Type III test; values below 0.001 reported as "< 0.001."^E Fixed terms in the model.

Supplemental Table 3.4.B. Estimated marginal means of seedling shoot biomass (g) for each nursery of origin × conifer species × timepoint combination, derived from the linear mixed-effects model in Supplemental Table 3.4.A.

Nursery ^A	Species ^B	Timepoint ^C	emmean ^D	SE ^E	df ^F	lower.CL ^G	upper.CL ^H
1	PIPO	Time 0	1.993	0.327	141	1.346	2.639
2	PIPO	Time 0	1.785	0.335	146	1.122	2.448
1	PIST	Time 0	1.481	0.327	141	0.835	2.127
2	PIST	Time 0	1.699	0.356	151	0.996	2.402
1	PIPO	6 months	4.616	0.354	158	3.916	5.316
2	PIPO	6 months	3.139	0.345	152	2.458	3.820
1	PIST	6 months	4.374	0.335	146	3.711	5.036
2	PIST	6 months	4.044	0.355	155	3.343	4.745
1	PIPO	1 year	6.873	0.335	146	6.210	7.536
2	PIPO	1 year	4.435	0.327	141	3.788	5.081
1	PIST	1 year	3.751	0.327	141	3.105	4.397
2	PIST	1 year	4.359	0.327	141	3.713	5.005

^A Source nursery of origin: 1 (N1) or 2 (N2).^B PIPO, ponderosa pine (*Pinus ponderosa*); PIST, southwestern white pine (*P. strobiformis*).^C Time 0 (planting), 6 months, and 1 year after outplanting.^D Estimated marginal mean.^E Standard error of the estimated marginal mean.^F Denominator degrees of freedom, estimated using the Kenward–Roger approximation.^G Lower limit of the 95% confidence interval.^H Upper limit of the 95% confidence interval.

Supplemental Table 3.4.C. Pairwise comparisons of seedling shoot biomass (g) from the linear mixed-effects model in Supplemental Table 3.4.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
PIPO - PIST	1	Time 0	0.511	0.460	211	1.112	0.268	0.535
PIPO - PIST	2	Time 0	0.086	0.487	213	0.176	0.860	0.870
PIPO - PIST	1	6 months	0.243	0.486	211	0.500	0.618	0.870
PIPO - PIST	2	6 months	-0.905	0.493	212	-1.837	0.068	0.203
PIPO - PIST	1	1 year	3.122	0.466	211	6.701	< 0.001	< 0.001
PIPO - PIST	2	1 year	0.076	0.460	211	0.164	0.870	0.870

N1 - N2	PIPO	Time 0	0.207	0.466	211	0.445	0.657	0.657
N1 - N2	PIST	Time 0	-0.218	0.481	212	-0.454	0.651	0.657
N1 - N2	PIPO	6 months	1.477	0.492	211	3.003	0.003	0.009
N1 - N2	PIST	6 months	0.330	0.486	212	0.678	0.498	0.657
N1 - N2	PIPO	1 year	2.439	0.466	211	5.234	< 0.001	< 0.001
N1 - N2	PIST	1 year	-0.608	0.460	211	-1.322	0.188	0.375
See footnotes below.								

Supplemental Table 3.4.D. Pairwise comparisons of seedling shoot biomass (g) among timepoints from the linear mixed-effects model in Supplemental Table 3.4.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
Time 0 - 6 months	1	PIPO	-2.624	0.480	211	-5.468	< 0.001	< 0.001
Time 0 - 1 year	1	PIPO	-4.881	0.466	211	-10.475	< 0.001	< 0.001
6 months - 1 year	1	PIPO	-2.257	0.486	211	-4.648	< 0.001	< 0.001
Time 0 - 6 months	2	PIPO	-1.354	0.479	211	-2.828	0.005	0.007
Time 0 - 1 year	2	PIPO	-2.649	0.466	211	-5.686	< 0.001	< 0.001
6 months - 1 year	2	PIPO	-1.295	0.473	211	-2.741	0.007	0.008
Time 0 - 6 months	1	PIST	-2.892	0.466	211	-6.207	< 0.001	< 0.001
Time 0 - 1 year	1	PIST	-2.270	0.460	211	-4.936	< 0.001	< 0.001
6 months - 1 year	1	PIST	0.623	0.466	211	1.336	0.183	0.200
Time 0 - 6 months	2	PIST	-2.345	0.500	212	-4.688	< 0.001	< 0.001
Time 0 - 1 year	2	PIST	-2.660	0.481	212	-5.531	< 0.001	< 0.001
6 months - 1 year	2	PIST	-0.315	0.480	212	-0.656	0.513	0.513

^A Pairwise comparison; the estimate is signed in the direction shown (first level minus second level).

^B Stratum within which each comparison was computed.

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Difference in estimated marginal mean in the direction of the comparison.

^E Standard error of the comparison.

^F Degrees of freedom, estimated using the Kenward–Roger approximation.

^G t-statistic.

^H Unadjusted p-value; values below 0.001 reported as "< 0.001."

^I Benjamini–Hochberg false discovery rate–adjusted p-value (Benjamini & Hochberg 1995), applied within each contrast family separately.

Alpha and Beta diversity – Nursery Seedlings

Supplemental Table 3.5.A. Type III tests of fixed terms from a linear mixed-effects model of fungal Shannon diversity

F ^A	Df ^B	Df.res ^C	Pr(>F) ^D	Term ^E
42.299	1	211	< 0.001	Nursery
3.211	1	211	0.075	Species
27.052	2	211	< 0.001	Timepoint
1.467	1	211	0.227	Nursery:Species
2.344	2	211	0.098	Nursery:Timepoint
0.205	2	211	0.815	Species:Timepoint
0.214	2	211	0.807	Nursery:Species:Timepoint

^A F-statistic.

^B Degrees of freedom.

^C Denominator degrees of freedom, estimated using the Kenward–Roger approximation

^D p-value for the Type III test; values below 0.001 reported as "< 0.001."

^E Fixed terms in the model.

Supplemental Table 3.5.B. Estimated marginal means of fungal Shannon diversity for each nursery of origin × conifer species × timepoint combination, derived from the linear mixed-effects model in Supplemental Table 3.5.A.

Nursery ^A	Species ^B	Timepoint ^C	emmean ^D	SE ^E	df ^F	lower.CL ^G	upper.CL ^H
1	PIPO	Time 0	3.854	0.121	93	3.61	4.10
2	PIPO	Time 0	3.660	0.124	98	3.41	3.91
1	PIST	Time 0	4.085	0.121	93	3.84	4.33
2	PIST	Time 0	3.791	0.131	107	3.53	4.05
1	PIPO	6 months	3.912	0.131	110	3.65	4.17
2	PIPO	6 months	3.527	0.127	104	3.27	3.78
1	PIST	6 months	4.184	0.124	98	3.94	4.43
2	PIST	6 months	3.499	0.131	109	3.24	3.76
1	PIPO	1 year	3.509	0.124	98	3.26	3.76
2	PIPO	1 year	2.977	0.121	93	2.74	3.22
1	PIST	1 year	3.636	0.121	93	3.39	3.88
2	PIST	1 year	2.996	0.121	93	2.76	3.24

^A Source nursery of origin: 1 (N1) or 2 (N2).

^B PIPO, ponderosa pine (*Pinus ponderosa*); PIST, southwestern white pine (*P. strobiformis*).

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Estimated marginal mean.

^E Standard error of the estimated marginal mean.

^F Denominator degrees of freedom, estimated using the Kenward–Roger approximation.

^G Lower limit of the 95% confidence interval.

^H Upper limit of the 95% confidence interval.

Supplemental Table 3.5.C. Pairwise comparisons of fungal Shannon diversity from the linear mixed-effects model in Supplemental Table 3.5.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR _i
PIPO - PIST	1	Time 0	-0.231	0.166	211	-1.393	0.165	0.495
PIPO - PIST	2	Time 0	-0.131	0.175	212	-0.748	0.456	0.683
PIPO - PIST	1	6 months	-0.272	0.175	211	-1.556	0.121	0.495
PIPO - PIST	2	6 months	0.028	0.177	212	0.160	0.873	0.908
PIPO - PIST	1	1 year	-0.127	0.168	211	-0.755	0.451	0.683

PIPO - PIST	2	1 year	-0.019	0.166	211	-0.116	0.908	0.908
N1 - N2	PIPO	Time 0	0.194	0.168	211	1.158	0.248	0.248
N1 - N2	PIST	Time 0	0.294	0.173	212	1.697	0.091	0.109
N1 - N2	PIPO	6 months	0.384	0.177	211	2.169	0.031	0.047
N1 - N2	PIST	6 months	0.685	0.175	211	3.912	< 0.001	< 0.001
N1 - N2	PIPO	1 year	0.532	0.168	211	3.167	0.002	0.004
N1 - N2	PIST	1 year	0.639	0.166	211	3.858	< 0.001	< 0.001
See footnotes below.								

Supplemental Table 3.5.D. Pairwise comparisons of fungal Shannon diversity among timepoints from the linear mixed-effects model in Supplemental Table 3.5.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
Time 0 - 6 months	1	PIPO	-0.057	0.173	211	-0.332	0.740	0.740
Time 0 - 1 year	1	PIPO	0.345	0.168	211	2.057	0.041	0.061
6 months - 1 year	1	PIPO	0.403	0.175	211	2.302	0.022	0.038
Time 0 - 6 months	2	PIPO	0.133	0.172	211	0.769	0.443	0.531
Time 0 - 1 year	2	PIPO	0.683	0.168	211	4.066	< 0.001	< 0.001
6 months - 1 year	2	PIPO	0.550	0.170	211	3.230	0.001	0.004
Time 0 - 6 months	1	PIST	-0.099	0.168	211	-0.589	0.557	0.607
Time 0 - 1 year	1	PIST	0.450	0.166	211	2.713	0.007	0.014
6 months - 1 year	1	PIST	0.548	0.168	211	3.266	0.001	0.004
Time 0 - 6 months	2	PIST	0.292	0.180	212	1.621	0.106	0.142
Time 0 - 1 year	2	PIST	0.795	0.173	212	4.586	< 0.001	< 0.001
6 months - 1 year	2	PIST	0.503	0.173	211	2.904	0.004	0.010

^A Pairwise comparison; the estimate is signed in the direction shown (first level minus second level).

^B Stratum within which each comparison was computed.

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Difference in estimated marginal mean in the direction of the comparison.

^E Standard error of the comparison.

^F Degrees of freedom, estimated using the Kenward–Roger approximation.

^G t-statistic.

^H Unadjusted p-value; values below 0.001 reported as "< 0.001."

^I Benjamini–Hochberg false discovery rate–adjusted p-value (Benjamini & Hochberg 1995), applied within each contrast family separately.

Supplemental Table 3.6.A. Type III tests of fixed terms from a linear mixed-effects model of bacterial Shannon diversity

F ^A	Df ^B	Df.res ^C	Pr(>F) ^D	Term ^F
57.082	1	208	< 0.001	Nursery
2.698	1	208	0.102	Species
43.072	2	208	< 0.001	Timepoint
8.035	1	208	0.005	Nursery:Species
3.184	2	209	0.043	Nursery:Timepoint
0.908	2	208	0.405	Species:Timepoint
1.829	2	208	0.163	Nursery:Species:Timepoint

^A F-statistic.^B Degrees of freedom.^C Denominator degrees of freedom, estimated using the Kenward–Roger approximation^D p-value for the Type III test; values below 0.001 reported as "< 0.001."^E Fixed terms in the model.

Supplemental Table 3.6.B. Estimated marginal means of bacterial Shannon diversity for each nursery of origin × conifer species × timepoint combination, derived from the linear mixed-effects model in Supplemental Table 3.6.A.

Nursery ^A	Species ^B	Timepoint ^C	emmean ^D	SE ^E	df ^F	lower.CL ^G	upper.CL ^H
1	PIPO	Time 0	5.681	0.068	159	5.546	5.816
2	PIPO	Time 0	6.155	0.068	159	6.020	6.290
1	PIST	Time 0	5.815	0.066	154	5.684	5.946
2	PIST	Time 0	6.011	0.066	154	5.880	6.142
1	PIPO	6 months	5.862	0.066	154	5.731	5.993
2	PIPO	6 months	6.257	0.068	159	6.122	6.391
1	PIST	6 months	5.944	0.065	150	5.816	6.072
2	PIST	6 months	6.322	0.066	154	6.191	6.453
1	PIPO	1 year	6.125	0.066	154	5.994	6.256
2	PIPO	1 year	6.465	0.070	164	6.326	6.603
1	PIST	1 year	6.430	0.066	154	6.299	6.561
2	PIST	1 year	6.405	0.068	159	6.270	6.540

^A Source nursery of origin: 1 (N1) or 2 (N2).^B PIPO, ponderosa pine (*Pinus ponderosa*); PIST, southwestern white pine (*P. strobiformis*).^C Time 0 (planting), 6 months, and 1 year after outplanting.^D Estimated marginal mean.^E Standard error of the estimated marginal mean.^F Denominator degrees of freedom, estimated using the Kenward–Roger approximation.^G Lower limit of the 95% confidence interval.^H Upper limit of the 95% confidence interval.

Supplemental Table 3.6.C. Pairwise comparisons of bacterial Shannon diversity from the linear mixed-effects model in Supplemental Table 3.6.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p _{FDR}
PIPO - PIST	1	Time 0	-0.134	0.095	208	-1.407	0.161	0.322
PIPO - PIST	2	Time 0	0.144	0.095	209	1.512	0.132	0.322
PIPO - PIST	1	6 months	-0.082	0.093	208	-0.886	0.377	0.542
PIPO - PIST	2	6 months	-0.065	0.095	208	-0.683	0.495	0.542
PIPO - PIST	1	1 year	-0.305	0.094	208	-3.247	0.001	0.008
PIPO - PIST	2	1 year	0.060	0.098	209	0.611	0.542	0.542

N1 - N2	PIPO	Time 0	-0.474	0.096	208	-4.916	< 0.001	< 0.001
N1 - N2	PIST	Time 0	-0.196	0.094	208	-2.091	0.038	0.045
N1 - N2	PIPO	6 months	-0.395	0.095	209	-4.148	< 0.001	< 0.001
N1 - N2	PIST	6 months	-0.378	0.093	208	-4.077	< 0.001	< 0.001
N1 - N2	PIPO	1 year	-0.339	0.097	209	-3.515	0.001	0.001
N1 - N2	PIST	1 year	0.025	0.095	209	0.263	0.793	0.793
See footnotes below.								

Supplemental Table 3.6.D. Pairwise comparisons of bacterial Shannon diversity among timepoints from the linear mixed-effects model in Supplemental Table 3.6.A.

Comparison ^A	Nursery ^B	Timepoint ^C	estimate ^D	SE ^E	df ^F	t.ratio ^G	p.value ^H	p_FDR ^I
Time 0 - 6 months	1	PIPO	-0.181	0.095	209	-1.901	0.059	0.078
Time 0 - 1 year	1	PIPO	-0.444	0.095	209	-4.667	0.000	0.000
6 months - 1 year	1	PIPO	-0.263	0.094	208	-2.807	0.005	0.009
Time 0 - 6 months	2	PIPO	-0.102	0.096	208	-1.054	0.293	0.320
Time 0 - 1 year	2	PIPO	-0.310	0.098	209	-3.165	0.002	0.004
6 months - 1 year	2	PIPO	-0.208	0.098	208	-2.128	0.035	0.052
Time 0 - 6 months	1	PIST	-0.129	0.093	208	-1.394	0.165	0.198
Time 0 - 1 year	1	PIST	-0.615	0.094	208	-6.554	< 0.001	< 0.001
6 months - 1 year	1	PIST	-0.486	0.093	208	-5.246	< 0.001	< 0.001
Time 0 - 6 months	2	PIST	-0.310	0.094	208	-3.308	0.001	0.003
Time 0 - 1 year	2	PIST	-0.394	0.095	209	-4.138	< 0.001	< 0.001
6 months - 1 year	2	PIST	-0.083	0.095	209	-0.875	0.383	0.383

^A Pairwise comparison; the estimate is signed in the direction shown (first level minus second level).

^B Stratum within which each comparison was computed.

^C Time 0 (planting), 6 months, and 1 year after outplanting.

^D Difference in estimated marginal mean in the direction of the comparison.

^E Standard error of the comparison.

^F Degrees of freedom, estimated using the Kenward–Roger approximation.

^G t-statistic.

^H Unadjusted p-value; values below 0.001 reported as "< 0.001."

^I Benjamini–Hochberg false discovery rate–adjusted p-value (Benjamini & Hochberg 1995), applied within each contrast family separately.

Supplemental Table 3.7.A. Marginal (Type III analog) permutational multivariate analysis of variance (PERMANOVA) of field rhizosphere community composition for the fungal (ITS) and bacterial (16S) datasets, fit as nursery × species × timepoint on Bray–Curtis dissimilarities with permutations constrained within planting block.

ITS Overall PERMANOVA					
Term ^A	Df ^B	SumOfSqs ^C	R2 ^D	F ^E	Pr(>F) ^F
Nursery:Species:Timepoint	2	1.118	0.013	1.999	0.001
Residual	214	59.848	0.670		
Total	225	89.369	1		
16S Overall PERMANOVA					
Term	Df	SumOfSqs	R2	F	Pr(>F)
Nursery:Species:Timepoint	2	0.842	0.012	1.954	0.002
Residual	211	45.443	0.631		
Total	222	71.990	1		

^A Model term. Residual, within-group variation; Total, total variation in the dissimilarity matrix.

^B Degrees of freedom.

^C Sum of squares of the dissimilarities attributable to the term.

^D Proportion of the total compositional variance attributable to the term.

^E Pseudo-F statistic.

^F Permutation-based p-value (999 permutations, constrained within planting block); values reported to three decimal places.

Supplemental Table 3.7.B. Multivariate homogeneity of group dispersions (PERMDISP) for the fungal (ITS) and bacterial (16S) field rhizosphere datasets, testing dispersion among the 12 nursery × species × timepoint groups on Bray–Curtis dissimilarities.

ITS PERMDISP							
Scope ^A	Term ^B	Df ^C	Sum Sq ^D	Mean Sq ^E	F ^F	N.Perm ^G	Pr(>F) ^H
ITS Overall PERMANOVA	Groups	11	0.879	0.080	9.065	999	0.001
ITS Overall PERMANOVA	Residuals	214	1.887	0.009			
16S PERMDISP							
Scope ^A	Term ^B	Df ^C	Sum Sq ^D	Mean Sq ^E	F ^F	N.Perm ^G	Pr(>F) ^H
16S Overall PERMANOVA	Groups	11	0.404	0.037	9.003	999	0.001
16S Overall PERMANOVA	Residuals	211	0.861	0.004			

^A Dataset and analysis to which the dispersion test corresponds.

^B Source of variation: Groups, among the 12 nursery × species × timepoint groups; Residuals, within groups.

^C Degrees of freedom (Groups Df = 11, i.e., 12 groups – 1).

^D Sum of squares of the distances to group centroids.

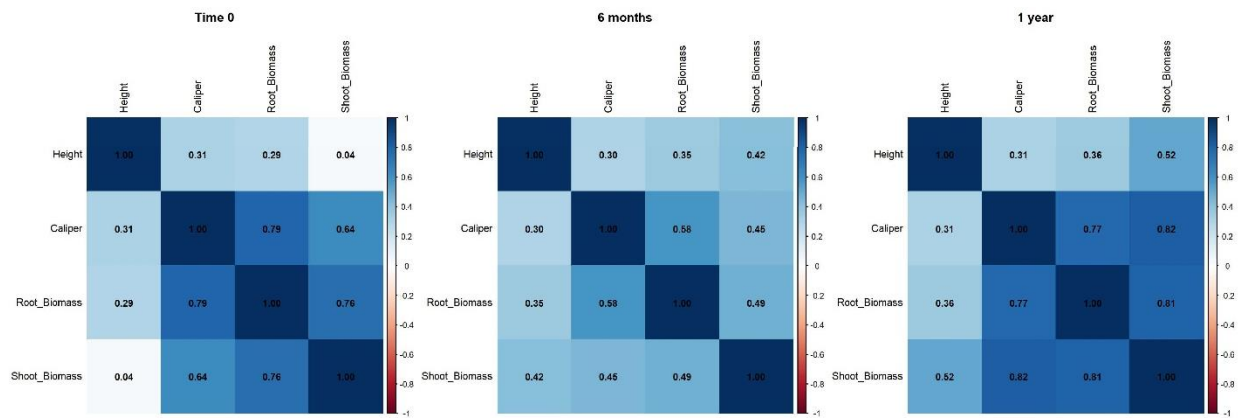
^E Mean square (Sum Sq / Df).

^F F statistic testing homogeneity of multivariate dispersions among groups.

^G Number of permutations.

^H Permutation-based p-value. Not defined for the Residuals row.

Seedling Growth Metrics and Microbial Community Composition



Supplemental Figure 3.2. Pairwise Pearson correlations among the four seedling growth metrics (height, caliper, root biomass, shoot biomass), computed separately within each timepoint (Time 0, 6 months, 1 year) on planted PIPO and PIST rhizosphere samples. Cells are colored by correlation coefficient (scale at right, -1 to 1) with the coefficient printed in each cell.

Supplemental Table 3.8.A. Stratified per-timepoint PERMANOVA testing whether each seedling growth metric explains rhizosphere fungal (ITS) community composition within each timepoint, after accounting for nursery and species. Each metric was tested as the marginal term in a separate model (community ~ Nursery + Species + metric) on Bray–Curtis dissimilarities, with permutations constrained within the planting block.

Timepoint ^{tA}	Metric ^B	n ^C	R2 ^D	F ^E	p ^F	p_FDR ^G
Time 0	Height	76	0.008	0.888	0.497	0.663
Time 0	Caliper	76	0.009	0.916	0.469	0.663
Time 0	Root_Biomass	76	0.007	0.758	0.664	0.664
Time 0	Shoot_Biomass	76	0.012	1.222	0.237	0.663
6 months	Height	71	0.016	1.488	0.112	0.149
6 months	Caliper	71	0.019	1.791	0.043	0.149
6 months	Root_Biomass	71	0.016	1.465	0.102	0.149
6 months	Shoot_Biomass	71	0.011	1.017	0.36	0.360
1 year	Height	79	0.014	1.195	0.156	0.156
1 year	Caliper	79	0.021	1.814	0.014	0.023
1 year	Root_Biomass	79	0.022	1.839	0.007	0.023
1 year	Shoot_Biomass	79	0.020	1.700	0.017	0.023

^A Timepoint at which the stratified test was run: Time 0 (planting), 6 months, or 1 year after outplanting. Nursery and species are retained as covariates within each timepoint; the timepoint itself is constant within each stratum and so is not a model term.

^B Growth metric tested as the marginal term: Height (cm), Caliper (mm), Root_Biomass (g), Shoot_Biomass (g).

^C Number of rhizosphere samples in the timepoint stratum.

^D Proportion of compositional variance attributable to the metric, as its unique (marginal) contribution after nursery and species.

^E Pseudo-*F* statistic.

^F Permutation-based *p*-value (999 permutations, constrained within planting block).

^G Benjamini–Hochberg false discovery rate–adjusted *p*-value, corrected within each timepoint separately

Supplemental Table 3.8.B. Pooled PERMANOVA testing whether each seedling growth metric explains rhizosphere fungal (ITS) community composition across all timepoints, after accounting for nursery, species, and timepoint. Each metric was tested as the marginal term in a separate model (community ~ Nursery + Species + Timepoint + metric) on Bray–Curtis dissimilarities, with permutations constrained within planting block.

Metric ^A	R2 ^B	F ^C	p ^D	p_FDR ^E
Height	0.005	1.355	0.085	0.085
Caliper	0.007	2.008	0.012	0.024
Root_Biomass	0.007	2.115	0.011	0.024
Shoot_Biomass	0.006	1.738	0.025	0.033

^A Growth metric tested as the marginal term: Height (cm), Caliper (mm), Root_Biomass (g), Shoot_Biomass (g).

^B Proportion of compositional variance attributable to the metric, as its unique (marginal) contribution after nursery, species, and timepoint.

^C Pseudo-*F* statistic.

^D Permutation-based *p*-value (999 permutations, constrained within planting block).

^E Benjamini–Hochberg false discovery rate–adjusted *p*-value, each metric separately.

Supplemental Table 3.8.C. Envfit of seedling growth metrics onto the two-dimensional principal coordinates analysis (PCoA) ordination of rhizosphere fungal (ITS) Bray–Curtis dissimilarities.

Metric ^A	R2 ^B	p ^C	p_FD R ^D
Height	0.240	0.001	0.001
Caliper	0.397	0.001	0.001

Root_Biomass	0.155	0.001	0.001
Shoot_Biomass	0.206	0.001	0.001

^A Growth metric fit as a vector: Height (cm), Caliper (mm), Root_Biomass (g), Shoot_Biomass (g).

^B Squared correlation coefficient (r^2) of the fitted vector with the two-dimensional PCoA configuration; quantifies how strongly the metric aligns with the ordination.

^C Permutation-based p -value (999 permutations).

^D Benjamini–Hochberg false discovery rate–adjusted p -value, each metric separately.

Supplemental Table 3.8.D. Multivariate homogeneity of group dispersions (PERMDISP) for the rhizosphere fungal (ITS) dataset, tested separately for each design factor (nursery, species, timepoint) on Bray–Curtis dissimilarities. Significance was assessed by permutation.

Grouping ^A	F ^B	p ^C
Nursery	17.408	0.001
Species	3.525	0.071
Timepoint	2.895	0.056

^A Design factor whose groups were tested for homogeneity of multivariate dispersion: Nursery (N1 vs N2), Species (PIPO vs PIST), or Timepoint (Time 0, 6 months, 1 year).

^B F statistic testing homogeneity of multivariate dispersions among the groups of that factor.

^C Permutation-based p -value (999 permutations).

Supplemental Table 3.9.A. Stratified per-timepoint PERMANOVA testing whether each seedling growth metric explains rhizosphere bacterial (16S) community composition within each timepoint, after accounting for nursery and species. Each metric was tested as the marginal term in a separate model (community ~ Nursery + Species + metric) on Bray–Curtis dissimilarities, with permutations constrained within the planting block.

Timepoint ^A	Metric ^B	n ^C	R2 ^D	F ^E	p ^F	p_FDR ^G
Time 0	Height	74	0.009	1.036	0.292	0.292
Time 0	Caliper	74	0.014	1.561	0.094	0.125
Time 0	Root_Biomass	74	0.022	2.465	0.023	0.050
Time 0	Shoot_Biomass	74	0.021	2.285	0.025	0.050
6 months	Height	76	0.015	1.601	0.065	0.069
6 months	Caliper	76	0.017	1.780	0.020	0.069
6 months	Root_Biomass	76	0.016	1.694	0.046	0.069
6 months	Shoot_Biomass	76	0.016	1.616	0.069	0.069
1 year	Height	73	0.013	1.001	0.420	0.420
1 year	Caliper	73	0.018	1.412	0.058	0.116
1 year	Root_Biomass	73	0.024	1.903	0.008	0.032
1 year	Shoot_Biomass	73	0.017	1.323	0.089	0.119

^A Timepoint at which the stratified test was run: Time 0 (planting), 6 months, or 1 year after outplanting. Nursery and species are retained as covariates within each timepoint; the timepoint itself is constant within each stratum and so is not a model term.

^B Growth metric tested as the marginal term: Height (cm), Caliper (mm), Root_Biomass (g), Shoot_Biomass (g).

^C Number of rhizosphere samples in the timepoint stratum.

^D Proportion of compositional variance attributable to the metric, as its unique (marginal) contribution after nursery and species.

^E Pseudo-*F* statistic.

^F Permutation-based *p*-value (999 permutations, constrained within planting block).

^G Benjamini–Hochberg false discovery rate–adjusted *p*-value, corrected within each timepoint separately

Supplemental Table 3.9.B. Pooled PERMANOVA testing whether each seedling growth metric explains rhizosphere bacterial (16S) community composition across all timepoints, after accounting for nursery, species, and timepoint. Each metric was tested as the marginal term in a separate model (community ~ Nursery + Species + Timepoint + metric) on Bray–Curtis dissimilarities, with permutations constrained within planting block.

Metric ^A	R2 ^B	F ^C	p ^D	p_FDR ^E
Height	0.005	1.539488562	0.059	0.059
Caliper	0.008	2.456508596	0.003	0.004
Root_Biomass	0.009	2.834736783	0.001	0.004
Shoot_Biomass	0.008	2.313983304	0.003	0.004

^A Growth metric tested as the marginal term: Height (cm), Caliper (mm), Root_Biomass (g), Shoot_Biomass (g).

^B Proportion of compositional variance attributable to the metric, as its unique (marginal) contribution after nursery, species, and timepoint.

^C Pseudo-*F* statistic.

^D Permutation-based *p*-value (999 permutations, constrained within planting block).

^E Benjamini–Hochberg false discovery rate–adjusted *p*-value, each metric separately.

Supplemental Table 3.9.C. Envfit of seedling growth metrics onto the two-dimensional principal coordinates analysis (PCoA) ordination of rhizosphere bacterial (16S) Bray–Curtis dissimilarities.

Metric ^A	R2 ^B	p ^C	p_FDR ^D
Height	0.181	0.001	0.001
Caliper	0.404	0.001	0.001
Root_Biomass	0.223	0.001	0.001

Shoot_Biomass	0.268	0.001	0.001
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^A Growth metric fit as a vector: Height (cm), Caliper (mm), Root_Biomass (g), Shoot_Biomass (g).

^B Squared correlation coefficient (r^2) of the fitted vector with the two-dimensional PCoA configuration; quantifies how strongly the metric aligns with the ordination.

^C Permutation-based p -value (999 permutations).

^D Benjamini–Hochberg false discovery rate–adjusted p -value, each metric separately.

Supplemental Table 3.9.D. Multivariate homogeneity of group dispersions (PERMDISP) for the rhizosphere bacterial (16S) dataset, tested separately for each design factor (nursery, species, timepoint) on Bray–Curtis dissimilarities. Significance was assessed by permutation.

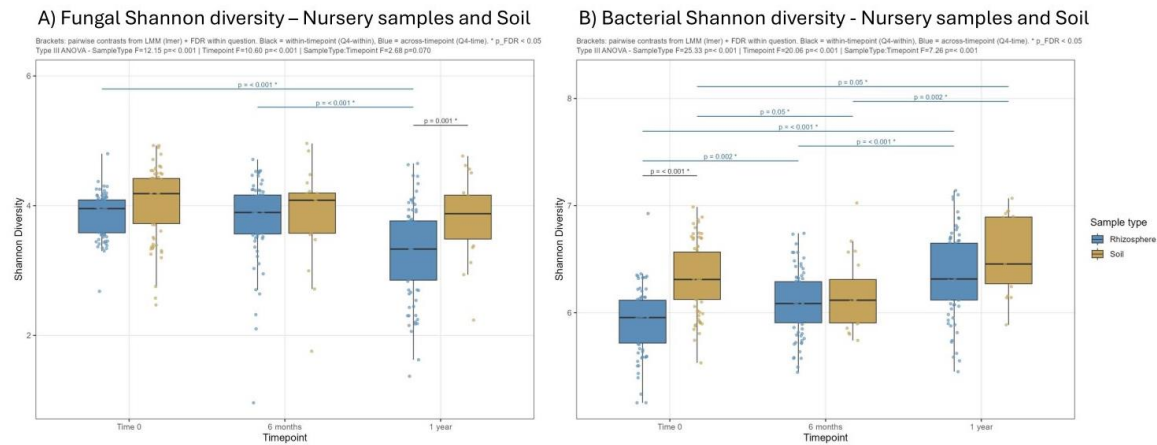
Grouping ^A	F ^B	p ^C
Nursery	0.013	0.915
Species	3.997	0.051
Timepoint	5.045	0.007

^A Design factor whose groups were tested for homogeneity of multivariate dispersion: Nursery (N1 vs N2), Species (PIPO vs PIST), or Timepoint (Time 0, 6 months, 1 year).

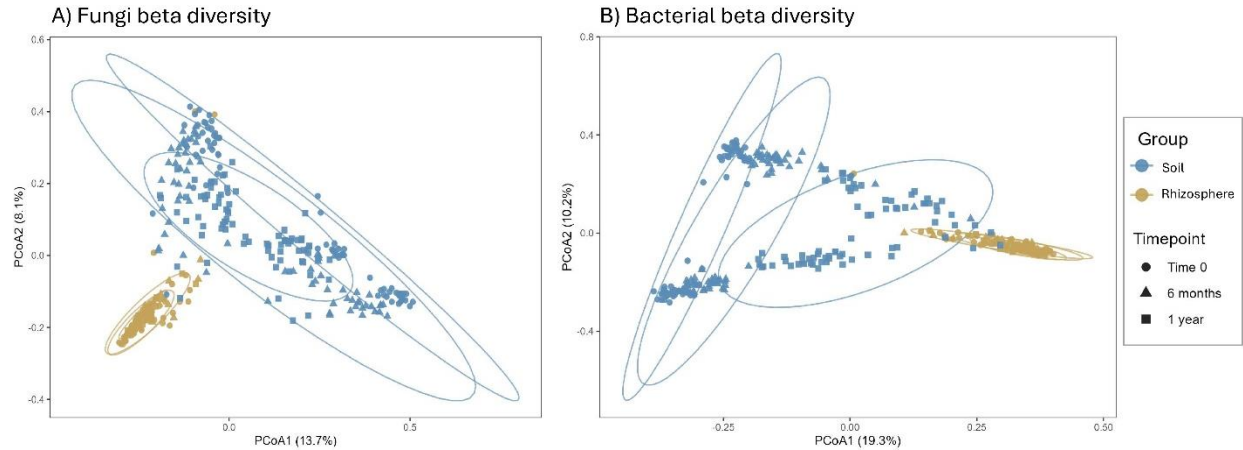
^B F statistic testing homogeneity of multivariate dispersions among the groups of that factor.

^C Permutation-based p -value (999 permutations).

Alpha and Beta Diversity of Nursery Seedlings and the Bulk Soil

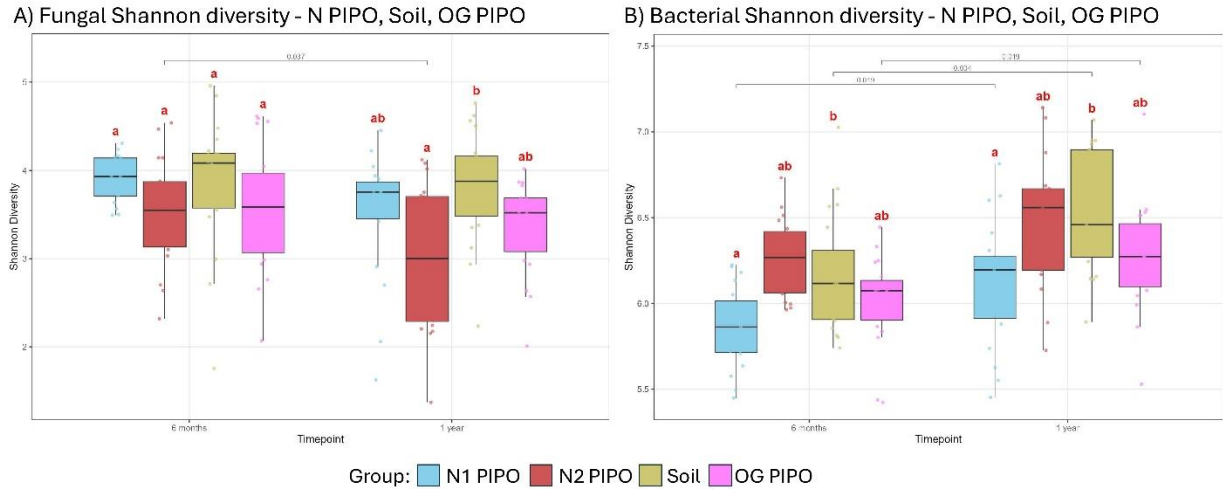


Supplemental Figure 3.3. Shannon diversity of (A) fungal (ITS) and (B) bacterial (16S) communities in the rhizosphere of planted nursery seedlings ("Nursery samples") versus bulk soil, across three timepoints (Time 0, 6 months, 1 year). Boxes show the median and interquartile range; whiskers extend to the most extreme values within $1.5 \times$ IQR; points are individual samples. Fill denotes sample type (Rhizosphere, blue; Soil, gold). Brackets mark pairwise contrasts from a linear mixed-effects model (lmer) with Benjamini–Hochberg FDR correction applied within each contrast family: black brackets, rhizosphere vs soil within a timepoint; blue brackets, contrasts across timepoints within a sample type. Asterisks denote $p_{\text{FDR}} < 0.05$.



Supplemental Figure 3.4. Beta diversity of field rhizosphere and soil communities across three time points, for (A) fungal (ITS) and (B) bacterial (16S) communities, by principal coordinates analysis (PCoA) of Bray–Curtis dissimilarities. Each point is one sample; color denotes sample type (rhizosphere of planted nursery seedlings and bulk soil) and shape denotes time point (Time 0, 6 months, 1 year). Ellipses are 95% confidence. Community composition showed a significant sample type $\times$ timepoint interaction in both datasets (PERMANOVA: fungal, $R^2 = 0.024$, $F = 4.84$, $p = 0.001$; bacterial, $R^2 = 0.023$, $F = 4.96$, $p = 0.001$; 999 permutations), indicating that compositional differences between compartments changed across timepoints. Group dispersions were heterogeneous (PERMDISP across the six sample type $\times$ timepoint groups: fungal, $F = 7.13$, $p = 0.001$; bacterial, $F = 4.40$, $p = 0.002$), so differences partly reflect dispersion as well as location. Within each timepoint, rhizosphere and soil composition differed significantly in both datasets (pairwise PERMANOVA, 999 permutations, all $p_FDR = 0.001$; fungal, $R^2 = 0.168$, 0.087, and 0.093 with $F = 29.09$, 8.44, and 9.95; bacterial, $R^2 = 0.259$, 0.145, and 0.092 with $F = 51.33$, 15.99, and 9.09, for Time 0, 6 months, and 1 year respectively), with the strongest separation at planting.

Alpha and Beta Diversity of Nursery Seedlings, NG PIPO, and the Bulk Soil



Supplemental Figure 3.5. Shannon diversity of field (A) fungal (ITS) and (B) bacterial (16S) communities across four sample groups at 6 months and 1 year after outplanting: nursery-planted *Pinus ponderosa* rhizosphere from Nursery 1 (N1 PIPO) and Nursery 2 (N2 PIPO), bulk soil, and naturally regenerating *P. ponderosa* rhizosphere (NG PIPO). Boxes show the median and interquartile range, whiskers extend to $1.5 \times \text{IQR}$, and points are individual samples. In both datasets, Shannon diversity varied significantly among groups and between timepoints, with no significant group $\times$ timepoint interaction (linear mixed-effects models, Type III tests: fungal — Group, $F_{3,123} = 6.27$, $p = 0.001$; Timepoint, $F = 8.99$, $p = 0.003$; bacterial — Group, $F = 11.28$, $p < 0.001$; Timepoint, $F = 26.64$, $p < 0.001$). Fungal and bacterial diversity moved in opposite directions over the year — fungal declining (most notably N2 PIPO rhizosphere, below bulk soil at 1 year, $p_{\text{FDR}} = 0.001$) and bacterial increasing (with N1 PIPO rhizosphere below bulk soil at both timepoints, $p_{\text{FDR}} \leq 0.014$). Pairwise contrasts were Benjamini–Hochberg FDR-corrected within question.

Supplemental Table 3.10. Pairwise PERMANOVA comparing community composition among the four sample groups — nursery-planted *Pinus ponderosa* rhizosphere from Nursery 1 (N1 PIPO) and Nursery 2 (N2 PIPO), bulk soil, and naturally regenerating *P. ponderosa* rhizosphere (NG PIPO) — within each timepoint (6 months, 1 year), for the fungal (ITS) and bacterial (16S) field datasets, on Bray–Curtis dissimilarities.

ITS Pairwise PERMANOVA					
Comparison ^A	Df ^B	R2 ^C	F ^D	p.value ^E	p_FDR ^F
6 months: N1 PIPO vs Soil	1	0.225	10.187	0.001	0.001
6 months: N1 PIPO vs NG PIPO	1	0.206	9.059	0.001	0.001
6 months: N2 PIPO vs Soil	1	0.156	6.649	0.001	0.001
6 months: N2 PIPO vs NG PIPO	1	0.150	6.364	0.001	0.001
6 months: Soil vs NG PIPO	1	0.059	2.366	0.001	0.001
1 year: N1 PIPO vs Soil	1	0.224	10.670	0.001	0.001
1 year: N1 PIPO vs NG PIPO	1	0.150	6.507	0.001	0.001
1 year: N2 PIPO vs Soil	1	0.138	6.107	0.001	0.001
1 year: N2 PIPO vs NG PIPO	1	0.078	3.215	0.001	0.001
1 year: Soil vs NG PIPO	1	0.074	3.055	0.001	0.001
16S Pairwise PERMANOVA					
Comparison ^A	Df ^B	R2 ^C	F ^D	p.value ^E	p_FDR ^F
6 months: N1 PIPO vs Soil	1	0.316	17.113	0.001	0.001
6 months: N1 PIPO vs NG PIPO	1	0.305	15.765	0.001	0.001
6 months: N2 PIPO vs Soil	1	0.263	12.822	0.001	0.001
6 months: N2 PIPO vs NG PIPO	1	0.262	12.419	0.001	0.001
6 months: Soil vs NG PIPO	1	0.046	1.801	0.025	0.025
1 year: N1 PIPO vs Soil	1	0.203	9.185	0.001	0.001
1 year: N1 PIPO vs NG PIPO	1	0.163	7.006	0.001	0.001
1 year: N2 PIPO vs Soil	1	0.133	5.204	0.001	0.001
1 year: N2 PIPO vs NG PIPO	1	0.089	3.327	0.001	0.001
1 year: Soil vs NG PIPO	1	0.056	2.155	0.002	0.002

^A Pairwise comparison between two sample groups within the indicated timepoint. N1 PIPO and N2 PIPO, nursery-planted *P. ponderosa* rhizosphere from Nursery 1 and Nursery 2; Soil, bulk soil; NG PIPO, naturally regenerating *P. ponderosa* rhizosphere.

^B Degrees of freedom for the group term.

^C Proportion of compositional variance attributable to group within that comparison.

^D Pseudo-*F* statistic.

^E Permutation-based *p*-value (999 permutations)

^F Benjamini–Hochberg false discovery rate-adjusted *p*-value

Supplemental Table 3.11. Summary of differential abundance testing between timepoints (Time 0 vs 1 year) for nursery-planted rhizosphere communities, by treatment (N1/N2 × PIPO/PIST), for fungal (ITS) and bacterial (16S) communities. For each treatment, the table reports the number of ASVs significant at MaAsLin2 $q < 0.05$ among those tested, the corresponding percentage, and the split of significant ASVs by enrichment direction (Time 0 vs 1 year). Significant ASV counts pair with the origin classification in Table 3.3.

Locus ^A	treatment ^B	comparison ^C	sig of tested ^D	pct_sig ^E	Total sig Time 0 ^F	Total sig 1 year ^G
ITS	N1 PIPO	Time 0 vs 1 year	244 of 523	46.7	153	91
16S	N1 PIPO	Time 0 vs 1 year	794 of 2376	33.4	381	413
ITS	N1 PIST	Time 0 vs 1 year	239 of 539	44.3	146	93
16S	N1 PIST	Time 0 vs 1 year	1115 of 2848	39.2	548	567
ITS	N2 PIPO	Time 0 vs 1 year	179 of 448	40	139	40
16S	N2 PIPO	Time 0 vs 1 year	1043 of 2980	35	553	490
ITS	N2 PIST	Time 0 vs 1 year	240 of 469	51.2	170	70
16S	N2 PIST	Time 0 vs 1 year	1096 of 2745	39.9	547	549

^A Locus — amplicon: ITS2 (fungal) or 16S (bacterial).

^B Treatment — N1/N2 × PIPO/PIST.

^C Comparison — the MaAsLin2 contrast tested: Time 0 vs 1 year, within each treatment.

^D Number of ASVs significant at $q < 0.05$, out of the number tested after MaAsLin2 prevalence/abundance filtering ([CHECK: filter thresholds]).

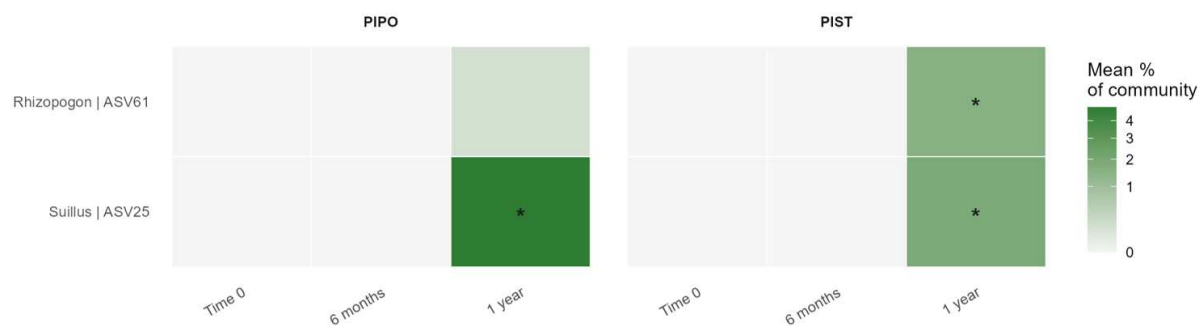
^E Percentage of tested ASVs found significant (significant ÷ tested × 100).

^F Significant ASVs enriched at Time 0.

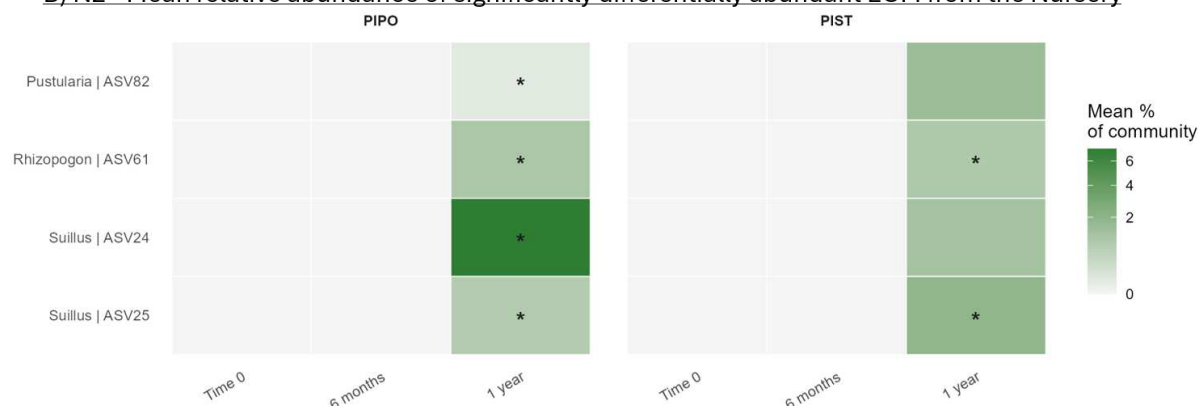
^G Significant ASVs enriched at 1 year.

Columns ^C and ^G sum to the numerator of ^D.

A) N1 - Mean relative abundance of significantly differentially abundant ECM from the Nursery



B) N2 - Mean relative abundance of significantly differentially abundant ECM from the Nursery



Supplemental Figure 3.6. Mean relative abundance of significantly differentially abundant nursery origin-ECM ASVs from Time 0 to 1 year in (A) Nursery 1, PIPO and PIST and (B) Nursery 2, PIPO and PIST. ASVs were FungalTraits-annotated as ECM ($\geq 10\%$ rhizosphere prevalence within nursery, $< 5\%$ in soil, ≥ 3 reads) and were differentially abundant between Time 0 and 1 year (MaAsLin2, within-set $q < 0.05$). Cell color is square-root-scaled mean relative abundance per timepoint; scales are panel-specific (max 4.85% in A, 7.14% in B). Significance in mean relative abundance change between time 0 and 1 year is indicated by an *

Supplemental Table 3.12.A. Spillover of nursery-associated fungi into field soil. Among named ASVs detected in the Time 0 rhizosphere but absent from Time 0 bulk soil (the candidate pool of seedling-associated taxa, n = 1,307), the table reports how many were subsequently detected in field soil at 6 months or 1 year versus never detected in soil. An ASV was counted as detected in a sample group if it had ≥ 3 reads in at least one sample of that group.

Category	Total ASVs	Percent
Never in soil	1649	97.9
Spillover at 6 months	17	1
Spillover at 1 year	23	1.4

Supplemental Table 3.13.B. Identity of the nursery-associated ASVs detected in field soil after planting (from Supplemental Table 3.14.A), by spillover timepoint, with genus guild assignment.

ASV	Category	Genus	Guild
ASV1562	6 months	Capnodiales_gen_Incertae_sedis	
ASV1698	6 months	Chalara	saprotroph
ASV6589	6 months	Cladophialophora	saprotroph
ASV4270	6 months	Clohesyomyces	saprotroph
ASV9819	6 months	Coprinellus	saprotroph
ASV3893	6 months	Cryptococcus	saprotroph
ASV4132	6 months	Cystobasidium	mycoparasite
ASV578	6 months	Exophiala	parasite
ASV799	6 months	Fusarium	plant pathogen
ASV835	6 months	Fusarium	plant pathogen
ASV3662	6 months	Goffeauzyma	saprotroph
ASV4145	6 months	Hormonema	saprotroph
ASV291	6 months	Hyaloscypha	saprotroph
ASV4674	6 months	Neosorocybe	
ASV3724	6 months	Oidiodendron	saprotroph
ASV900	6 months	Podila	
ASV10343	6 months	Rhizophydiaceae_gen_Incertae_sedis	
ASV8485	6 months	Rozellomycota_gen_Incertae_sedis	
ASV2872	6 months	Saitozyma	saprotroph
ASV2755	6 months	Spizellomycetales_gen_Incertae_sedis	
ASV7495	6 months	Spizellomycetales_gen_Incertae_sedis	
ASV11798	6 months	Spizellomycetales_gen_Incertae_sedis	
ASV2038	6 months	Umbelopsis	saprotroph
ASV5242	1 year	Auriculariales_gen_Incertae_sedis	
ASV3603	1 year	Basidiobolales_gen_Incertae_sedis	
ASV4010	1 year	Capnodiales_gen_Incertae_sedis	
ASV10432	1 year	Ciliophora	
ASV4270	1 year	Clohesyomyces	saprotroph
ASV3893	1 year	Cryptococcus	saprotroph
ASV7238	1 year	Fungi_gen_Incertae_sedis	
ASV835	1 year	Fusarium	plant pathogen
ASV5049	1 year	Mytilinidiales_gen_Incertae_sedis	
ASV4960	1 year	Neopyrenochaeta	saprotroph

ASV2872	1 year	Saitozyma	saprotroph
ASV2755	1 year	Spizellomycetales_gen_Incertae_sedis	
ASV4958	1 year	Sporormiella	saprotroph
ASV1248	1 year	Talaromyces	saprotroph
ASV5395	1 year	Talaromyces	saprotroph
ASV47	1 year	Thelephora	ectomycorrhizal
ASV2731	1 year	Triscelophorus	endophyte

Supplemental Table 3.13. Per-sample detection of the two spillover *Fusarium* ASVs (ASV799, ASV835) across rhizosphere and bulk-soil compartments at each timepoint. Rows list every sample in which the ASV met the detection threshold (≥ 3 reads), with read count, timepoint, and the originating sample's treatment and block. These records show the spatial relationships between Time 0 rhizosphere carriers and the soil samples in which each ASV was later detected.

ASV	Type	Reads	Timepoint	Sample
ASV799	Rhizosphere	82	Time 0	N1 PIPO - Block 1
ASV799	Rhizosphere	108	Time 0	N1 PIPO - Block 2
ASV799	Rhizosphere	33	Time 0	N1 PIPO - Block 2
ASV799	Rhizosphere	386	Time 0	N1 PIPO - Block 3
ASV799	Rhizosphere	151	Time 0	N1 PIPO - Block 4
ASV799	Rhizosphere	169	6 months	N1 PIPO - Block 1
ASV799	Rhizosphere	96	6 months	N2 PIST - Block 1
ASV799	Rhizosphere	594	6 months	N2 PIPO - Block 1
ASV799	Rhizosphere	156	6 months	N1 PIPO - Block 2
ASV799	Rhizosphere	289	1 year	N2 PIPO - Block 1
ASV799	Rhizosphere	16	1 year	N1 PIPO - Block 3
ASV799	Soil	129	6 months	Block 3 - TRT N1PIST
ASV835	Rhizosphere	17	Time 0	N2 PIST - Block 2
ASV835	Rhizosphere	44	6 months	N2 PIPO - Block 1
ASV835	Soil	1599	6 months	Block 1 - TRT N2 PIPO
ASV835	Soil	175	6 months	Block 4 - TRT N1 PIPO
ASV835	Rhizosphere	20	1 year	N2 PIPO - Block 3
ASV835	Rhizosphere	37	1 year	N2 PIST - Block 4
ASV835	Soil	182	1 year	Block 2 - TRT N2 PIPO

Supplemental Table 3.14. Number of samples that came back with more than 1,000 oomycete (RPS10) reads by compartment and time point.	
Sample Type	Number of Samples
<u>Time 0</u>	
NG PIPO	1
N1	10
N2	40
Bulk Soil	38
<u>6 months</u>	
NG PIPO	13
N1	37
N2	40
Bulk Soil	18
<u>1 year</u>	
NG PIPO	12
N1	37
N2	35
Bulk Soil	6

LIST OF ABBREVIATIONS

N1 — Nursery 1

N2 — Nursery 2

PIPO — *Pinus ponderosa*

PIST — *Pinus strobiformis*

PM — potting mix

RT — root tips

RZ — rhizosphere

SDW — sterile distilled water

SRT — symptomatic root tips