Ecosystem Legacies of Invasive Pines with Exotic Grasses and Shrubs
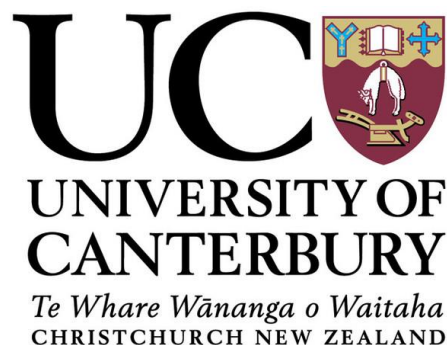
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General Abstract

The aim of my thesis was to investigate the connection between invasive plants and the resident plant community via soil modification. Pines were chosen as the primary model invasive species for my project due to the dramatic transformations associated with pine invasions and local significance in Aotearoa | New Zealand.

I began my research by leveraging soils from a previous experiment which involved various plant communities grown in the same steam sterilised soil. This provided known soil legacies to test various legacy factors associated with the previous plant communities and how those would affect the growth of future plants. For chapter two, I grew locally-relevant invasive species from three different functional groups, *Pinus contorta* (tree), *Cytisus scoparius* (shrub), and *Holcus lanatus* (grass) in a greenhouse experiment with these legacy soils. I found that legacies with a high proportion of exotic plants or with the presence of pine resulted in plants with the largest biomass. A potential biological mechanism was investigated by scoring nodules on *Cytisus* and mycorrhization on *Pinus*, which did not show a measurable effect across treatments. There were significant trends on fungal DNA sequences from pine seedling root samples, including showing that a pine legacy decreased the fungal community diversity while increasing pine seedling growth.

Although the soil legacies for chapter two included invasive pine species in the legacy community, at most it was two seedlings in each growth phase, which might not have the same impact as soil from established adult trees or from various degrees of invasion. To address this issue, I sampled soil from along a pine invasional gradient, as measured by pine dominance. Also, I tested whether plant responses to the legacy soils differed if plants were grown individually or in communities; a closer representation of the natural system. This greenhouse study involved a community pot per sampling plot as well as an individual pot for each species represented in the community assay. An increasing pine legacy was found to be beneficial to most plants, but in a community context any benefit to native plants was obscured by the strong competitive fitness of many exotic species.

Chapters two and three indicated that there was a biological effect in pine legacy soils, and that there was a reciprocal positive interaction between grasses and pines. Fungal endophytes were chosen as a biological indicator as they are associated with their host

plant's health and also will be affected by changes in resident soil. I collected grass roots (both exotic and native species) from paired plots (a pine invasion and a nearby uninvaded grassland). The endophytic fungal communities from exotic grasses and pine invasion sites had a greater abundance of potential pathogens, while sharing many generalists. This indicates a potential for spill over from exotic to native grasses, and that pine invasion soils might be a reservoir for pathogens. Exotic grass species might be better equipped to deal with these pathogens due to previous experience, compared to a lack of past interactions with native grass species, particularly if natives are under competitive stress.

By demonstrating the effects of invasive pine legacy and various community legacy effects, my research could be helpful to land managers looking to control invasive pine spread. My thesis showed a pine soil legacy can be particularly beneficial to other exotic invasive species, which, in turn, can facilitate future invasions.

Acknowledgements

Ngā mihi nui! | Thank you very much!

Authorship Disclosure

In Chapter 2, I used soil from a large, multi-year mesocosm experiment designed and conducted in 2016 by Dr. Lauren Waller, Dr. Warwick Allen, and colleagues (Waller et al. 2020) including two members of my supervisory committee. I used this soil to provide a known soil legacy to test exotic plant species' growth responses. I designed and ran the experiment detailed in Chapter 2 (with guidance from my supervisory committee); only the starting material was created by others.

Chapters 3 and 4 were designed and conducted by me (with guidance from my supervisory committee).

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Chapter 1

General Introduction

As some terms can have multiple meanings, I define here the definitions I will use throughout my thesis.

Glossary (sources for definition)

Invasive: (Blackburn et al. 2011)

A species that is introduced as a result of human activity and dispersing, surviving, and reproducing at locations remote from the area where it was first introduced, with self-sustaining populations in the wild.

Competition: (Welden and Slauson 1986)

The reduction in fitness of one organism as a result of the use, defence, or sequestering of resource items by another organism.

Dominance: (United Nations 1997)

Exertion of a major controlling influence of one or more species upon all other species by virtue of their number, size, productivity or related activities (*in my case growth rate/biomass)*

Endophyte:

A fungus found living within plant tissue of an asymptomatic plant (including mutualists, commensals, and parasites) (Hardoim et al. 2015, Compant et al. 2016, Shearin et al. 2018). Note that this differs from some definitions (Rodriguez et al. 2009, Saikkonen et al. 2010a) which include only mutualists.

Facilitation: (Stachowicz 2001)

Positive interactions between organisms that benefit at least one of the participants and cause harm to neither.

Legacy/Soil legacy:(Kostenko and Bezemer 2020)

Biotic and abiotic conditions in the soil created or altered by specific plant species or plant communities, this can affect other plants that grow later in this soil in a form of plant-soil-feedback.

Plant-soil feedback: (Bennett and Klironomos 2019)

When plants alter soil properties that differentially influence the performance of seedlings of conspecifics compared with heterospecifics, with consequent effects on plant populations and communities, plant can be present or no longer actively modifying soil, as in the case of legacies.

Overview

A major theme of ecology is that many things are interconnected within an ecosystem. These interactions occur at all scales, including in and on living organisms. For example, all species from plants to animals have associated microorganisms and, in many cases, these are essential to the health of the organism (Nunez et al. 2009, Hacquard et al. 2015, Hayward et al. 2015). Much of the research has focused on how smaller organisms like bacteria and fungi affect their host organism, showing that these connections are complex and not only bottom-up, because the host can also affect the associated smaller organisms (Berg and Smalla 2009, Coyte et al. 2015, Moran and Sloan 2015). With this in mind, any changes to an ecosystem could create a cascade; affecting all organisms within the community.

Invasive Species Background

Invasive plant species are an increasingly dominant component of ecosystems in Aotearoa | New Zealand and globally. New Zealand has more than 1,798 naturalised vascular plant species (Brandt et al. 2021), more than the total number of native species. According to the Global Invasive Species Database (Pagad et al. 2015), IUCN SSC Invasive Species Specialist Group), 177 of these are classified as invasive. The direct impacts of such species can include changes in water flow (Le Maitre 2004), susceptibility to wildfires (Brooks et al. 2004), nutrient cycles (Ehrenfeld 2003, Zhang et al. 2019), diversity of native plant communities (Ehrenfeld 2010, Vilà et al. 2011), associated fauna (Dehlin et al. 2008, Peralta et al. 2019), and even to local animal behaviour (Stewart et al. 2021).

Much work has been done on all stages of invasion (Blackburn et al. 2011) and the direct impacts associated with those invasions (Ehrenfeld 2003, Vilà et al. 2011, Reynolds et al. 2017), but few experiments include multiple interactions among invasive plant species or indirect effects of invasion. Indirect effects of invasive plants might be expected to occur via modification of microbial communities. For example, Creed et al. (2022) showed responses of native symbionts to invasion on both their native hosts as well as the potential for the invader

to become a host. Also, Steel et al. (2022) showed that invasive pines could be a reservoir for fungal pathogens in New Zealand.

Plant-Fungal interactions

All plants interact with a variety of microbial organisms, and often the closest relationships are with fungi. Plant-associated fungi range from mutualists like ectomycorrhizal (ECM) fungi (Amaranthus and Perry 1994), arbuscular mycorrhizal (AM) fungi (Lekberg and Koide 2005), or endophytes (Mayerhofer et al. 2013, Gange et al. 2019), to deadly parasites (Kamiya et al. 2014) or pathogens, often those affecting food crops (Dean et al. 2012) The role of fungal endophytes as potential "bodyguards" (*sensu* Gange et al. 2019) of plants, in particular protection from herbivores (Gange et al. 2019) and pathogens (Pérez et al. 2013), has been a long standing and ongoing area of research (Siegel et al. 1987, Schardl 1996, Faeth 2002). Põlme et al. (2018) reviewed existing literature of plant-host preferences and found little differences among host identity and fungal network properties, except with orchid and ericoid mycorrhizal fungi.

While much research has been focused on tight plant-fungal partnerships, less attention has been given to fungal generalists (Saikkonen et al. 2010b). Several plant species with typically distinct guilds of mycological associates have been shown to share fungi, even endophytes which live within the plant itself (Toju et al. 2018, Wang et al. 2021, Maciá-Vicente and Popa 2022). This sharing of fungi also extends between native and exotic plant species. For example, Visscher et al. (2021) found that native and exotic grasses from a New Zealand grassland had comparable diversity and abundance of foliar endophytes, but native grass species hosted more single-host fungi. Similarly, Bunn et al. (2015) showed that arbuscular mycorrhizal fungal (AMF) abundance was similar between native and invasive species, although invasive species were hosts to different AMF fungal communities in 78% of studies included in the meta analysis. Plant-fungal interactions could be the source of many apparent invasive plant species associated changes.

Long-term Impacts of Invasion & Legacy Effects

When invasive plant species modify soils and microbial communities, they can have lasting impacts even following their removal (Grove et al. 2012, Dickie et al. 2014b, Reynolds et al. 2017). This is termed the 'soil legacy'. Research on soil legacies is relatively recent, with Bartelt-Ryser et al. (2005) to be among the first to detach it from a more traditional plant-soil-feedback model, although they described it as "soil carry-over effects" from a previous plant community. Kardol et al. (2007) were one of the first to actively use the term "legacy" within a

successional framework in which plant community dynamics at any stage can impact the soil influences from past communities. Much of the initial work focused on agriculture, in terms of a cultivation legacy, and best practices for farming (Cramer et al. 2008). The focus on agriculture continues to be a dominant theme with recent work on ancient human movement and cultivation, and how this could have contributed to plant diversity patterns in the diverse ecosystem of the Amazon (Montoya et al. 2020).

Restoring the native ecosystem becomes difficult when soil legacies have lasting impacts, requiring a substantial investment of time and or resources (Blackburn et al. 2011, Mason et al. 2017, Sapsford et al. 2020). Many invading plant species become "transformers" as described by Pyšek et al. (2004) which specifically refers to invasive plant species that modify the ecosystem in ways that are difficult to reverse. These effects can have long residence times within the soil and continue to affect a plant community even after the removal of the invasive plant (Kostenko and Bezemer 2020).

Pine Invasion Background

One group of invasive plant species which has been the focus of large-scale management and invasion research is the Pinaceae family and in particular pine trees (*Pinus*) (Dickie et al. 2014a, Nuñez et al. 2017, Sapsford et al. 2020, Dickie et al. 2022). Pines have a wide range of successional niches and an overall level of adaptability and hardiness which aids invasion (Richardson et al. 1994, Grotkopp et al. 2002, Grivet et al. 2017).

Most pine invasions occur in the Southern Hemisphere, where there are no native Pinaceae; with the exception of *Pinus merkusii,* the Sumatran pine, which barely ranges across the equator (Cooling 1968, Keeley 2012, Procheş et al. 2012). Their distinct mutualisms lead to different microbial communities in the soil which in turn contribute to differences in nutrient cycling (Bever et al. 2010, Cheeke et al. 2017, Lin et al. 2018)*.*

Initial plantings of pines in the Southern Hemisphere failed to thrive (Nunez et al. 2009, Hayward et al. 2015). However, later introductions with pine-associated soil proved successful. In addition, initial invasions only occurred in the vicinity of successful pine plantations (Nunez et al. 2009, Hayward et al. 2015) These observations have now been mostly attributed to the presence of ectomycorrhizal (ECM) fungal associations, previously lacking in southern soils, such as in South Africa (Richardson et al. 1994), Argentina (Nunez et al. 2009), Chile (Hayward et al. 2015), and New Zealand (Dickie et al. 2010). This and that it may only take a few target fungal species to facilitate a pine invasion (Hayward et al. 2015,

Policelli et al. 2019) demonstrate a tight connection between pines and fungi that live in roots and soil, which reinforces the need for research on legacy influences and plant-soil feedback in respect to pine invasion.

Pine trees are particularly invasive in New Zealand, leading to numerous environmental changes: extending from a loss of biodiversity to changes in the microbiome (Dickie et al. 2014b, Taylor et al. 2016, Sapsford et al. 2020, Dickie et al. 2022). Work by Leduc and Rowden (2018) even suggests introduced pine pollen is driving novel nutrient dynamics in nearby ocean-trenches. On the land, pines are rapidly turning grassland into forest (Sapsford et al. 2020). Surveys published by Howell et al. (2016) estimated 7.3 million ha (approximately 30% of New Zealand), is susceptible to invasion by pines and other conifers, and at current spread of 5–6% per year, this area could be invaded in less than 30 years (Gawith et al. 2020). Areas with a pine-invasion history are often re-invaded by pine or become dominated by exotic grasses (Edwards et al. 2020, Sapsford et al. 2020, Dickie et al. 2022).

Thesis Aims

My thesis aims to study the plant-soil-feedbacks and soil legacies associated with pines. My research investigates the connection between existing plant communities and soil legacies from past communities. These dynamics were tested in respect to differing legacies (for example along a pine density gradient and a plant community with a mixture of native and exotic species) and a variety of plant communities (with a range of functional groupings and phylogenetic relatedness as well as native and exotic species) as well as looking at grass root fungal endophytes along pine invasion edges.

The thesis objectives were addressed in three major experiments:

Chapter 2

I tested the hypothesis that soil from different plant communities provokes different growth responses in different plant species. I hypothesized that plant community modifications that led to a higher nutrient availability would lead to larger seedling biomass, as would greater abundance of mutualists and lower abundance of pathogens. I used a greenhouse study to determine the responses of three exotic plant species to varying experimentally-created legacies by growing them in soil with a variety of known legacies (including pine presence, varying levels of exotic dominance, and grass dominance, amongst other factors). Response was measured by the resultant seedlings' final biomass. This research also included next-

generation sequencing of pine seedling root tips to determine the responses of pine-associated fungal communities to the various legacy factors.

Chapter 3

Findings from Chapter two combined with field site observations indicated potential relevance of invasive pine legacy within a community context growth response, as well as potential dominance or density-dependent pine legacy effects. I therefore measured the responses of plant communities and single plant species to increasing pine dominance legacy, with the hypothesis that increases in pine dominance will lead to increased competitive advantage of exotic over native species and to grasses dominating over shrubs. A second hypothesis is that the community response will be different from that of plants grown individually, as the stress of competition might be a stronger force than legacy effects. The experiment used soil taken along a pine invasional gradient. It was used to grow a representative community of exotic and native grasses and shrubs in a greenhouse. The plants were grown individually and in a mixed species community to allow for competition and a better comparison to environmental conditions as competition is inherent in the environment, but not always included in experimental designs.

Chapter 4

During the research presented in the previous chapters and in the field, I noted a connection between pine legacy and grass growth rate or dominance. To explore the potential presence of a biological driver, I investigated the fungal endophyte community within grasses as they can impact plant health and can be transferred amongst a plant community as well as also remaining in the soil as a legacy effect. I tested the hypothesis that native and exotic grasses host different endophytic fungal communities and that these change in the presence of pine trees. This involved collecting grass roots from pine invasion sites and nearby un-invaded grasslands and then using culturing and Sanger sequencing of fungal isolates to determine endophyte community composition.

Figure 1: Thesis Overview Diagram

Brown rectangle symbolizes the soil through which the plants interact, coloured arrows indicate connections between plants and communities through the soil (per experimental chapter: Chapter 2= blue, Chapter 3 = green, Chapter 4 = yellow).

Chapter 2

Community Legacies of Exotic Plants as Drivers of Invasion and Re-invasion

Abstract

- Past plant communities leave a legacy in the soil that affects future plant communities, and may contribute to invasional meltdown or re-invasion. However, direct tests and measurement of legacy effects has been difficult to date.

- Here I used soil with various experimentally created legacy effects (for example: varying pine presence, proportion of exotic plants, proportion of grasses, proportion of nitrogen fixing plants, and a variety of mycorrhizal associations) to grow exotic invasive plants (*Pinus contorta*, *Cytisus scoparius,* and *Holcus lanatus*)*,* to test how plant community legacy factors affect their growth, including assessment of symbionts (nodulation or ectomycorrhizal infection and fungal communities).

- *Pinus* seedlings grew largest when in soil with previous pine presence and a greater exotic legacy, and also showed lower fungal diversity on root tips associated with previous pine presence. Generally the fungal community was one of three major types: dominated by *Wilcoxina,* dominated by *Inocybe,* or highly diverse. A pine legacy also boosted growth of *Cytisus scoparius*. There was no difference in nodulation or percent ectomycorrhizal infection across the different legacy treatments.

- This experiment includes legacies on a community scale rather than only a select few plant species. Understanding legacy effects is particularly relevant with invasive species and restoration, as knowing how legacies drive future plant communities can help inform management practices and prevent subsequent invasions.

Introduction

Plant species composition can influence the structure of subsequent communities; this is known as a legacy effect (Jordan et al. 2008, Van der Putten et al. 2013, Reynolds et al. 2017). Knowledge of the previous plant community is particularly relevant when involving invasive species, as the legacy effect can persist after death or removal of the legacy-creating plants,

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and invasive plants are often targeted for removal by managers. A wide variety of techniques are used to remove invasive plants, many of which are costly and highly labour intensive (Mason et al. 2017, Nuñez et al. 2017). This can make removal particularly problematic if plants reinvade or cause further issues, such as resulting in succession of other undesirable species. A soil legacy that remains post-removal could have long-lasting effects that promote a potentially unappealing outcome, such as reinvasion by the original invasive species, or dominance of the community by other exotic plant species.

Plant invasions generally increase overall plant community biomass though reduce local diversity (Vilà et al. 2011). Legacies can be chemical, biological, or even physical. In many cases with invasive plant species, the biological components can have the most significant impact (Heinen et al. 2020), possibly due to the co-evolution of mutualists-host-pathogens relationships (Rafaluk-Mohr et al. 2022). Moreover, biological and chemical legacies can be interdependent, as when nutrients are limiting, mutualists may be more important or pathogens more damaging; in which case, chemical legacies could have a more visible impact (Nuske et al. 2021).

Exotic plants often have different below-ground associated fungal and bacterial communities than native plants (Zobel and Öpik 2014, Bowen et al. 2017, Dickie et al. 2017). This difference can bestow an advantage on exotic plants that could share mutualistic associations among one another (McLeod et al. 2016, Dickie et al. 2017) or have previous exposure to shared pathogens and thus greater potential for resistance (Jordan et al. 2008). These potential benefits to exotics within an invasive-dominated ecosystem can facilitate other invasive species in what has been termed an invasional meltdown or a secondary invasion (Simberloff and Von Holle 1999, Richardson et al. 2000, Pyšek et al. 2004).

There is an overall lack of manipulative experiments involving invasive plant communities and soil legacies. Of the 199 studies included in a meta-analysis on plant invasion impacts by (Vilà et al. (2011), only 14% involved manipulative experiments (e.g. removals and additions); most data were from observational field studies. Field research can lead to results with confounding variables that cannot be extricated from intended interactions. This is a particular issue in plant invasions, as invasive plants tend to invade disturbed and high nutrient sites. As such, invasive plants have also been hypothesised to be simply a passenger and not the actual driving force of environmental change (O'Leary et al. 2018). Thus, greenhouse experiments allow researchers to address this uncertainty about causality.

Greenhouse studies allow for many choices: one crucial option is which plants to include. While an active pine invasion is dominated by pine, there are often several key species that tend to co-occur with pine and thrive post-pine removal, such as the leguminous shrub Scotch broom (*Cytisus scoparius*) (Torres et al. 2021, Fernández-Guisuraga et al. 2022) and various grasses (Dickie et al. 2017, Damasceno et al. 2018). Ecological connections between these plants are not well understood, particularly as they have different major fungal associations: pines are ectomycorrhizal (ECM) and brooms are arbuscular mycorrhizal (AM), as are most grasses.

Here I aimed to study the impacts of legacy soils from known plant communities on multiple invasive plants. The soil originated from a two growth phase experiment that used unique plant communities grown in the same initial steam-sterilized soil (Waller et al. 2020) allowing me to test how a variety of plant community characteristics affect plant growth, and thus invasion potential, without many confounding factors. For test plants, I chose representatives from different functional groups and with different fungal associates: *Pinus contorta* (Lodgepole pine, an ECM tree), *Cytisus scoparius* (Scotch broom, an arbuscular mycorrhizal (AM), rhizobia-associated, N-fixing shrub), and *Holcus lanatus* (Yorkshire fog-grass, an AM grass).

Hypotheses

I specifically looked for evidence of two types of responses associated with legacy effects:
(1) driven by changes in nutrient availability due to previous community modifications, and (2) driven by increasing populations of shared mutualists or potential pathogens due to previous plant communities.

These two types of legacy effects led to my hypotheses that higher nutrient availability would lead to larger biomass, as would higher levels of mutualists and lower levels of pathogens.

Methods

Legacy Establishment Mesocosms (Waller et al. 2020)

Mesocosms were grown with a range of percent exotics within the communities ranging from 0-100% in five even increments (0, 25%, 50%, 75%, and 100%), with four communities at each level of exotic dominance. Within these plant communities, two different pine species (*P. radiata* and *P. contorta*) were included. Each was sole pine in two different communities (a 75% exotic and a 100% exotic) and both were co-planted in 3 communities (25%, 75%, 100% exotic). There were 2 other ECM species included in 7 communities as well *(Leptospermum scoparium* and *Alnus glutinosa)*, though never co-occurring, though each co-occurred with

pine once. There was also a range of exotic grasses from 0-50% total plant community; with one community at 50%, one at 37.5%, three at 25%, and five at 12.5%. Percent of the nitrogen-fixing plant community was from 0-50%; one at 50%, three at 37.5%, three at 25%, and eight at 12.5%. Each community was replicated 9 times with a $2 \times 2 \times 2$ factorial combination of herbivores (presence/absence), invasion by broom seedlings (presence/absence) and soil treatment (home/away, home meaning the same community legacy was re-planted into a home soil). (Waller et al. 2020). This allowed me to test whether legacies left by different exotic plant species influenced the growth of subsequent species.

The home/away soil treatment created a further level of legacy. During a conditioning phase, soil was exposed to all 8 plant species from each community in single-species pots. These "phase 1" soils were then mixed in the mesocosm and planted with either the same eight species (home soil) or soil from a different community (away soil). For considering pine legacies, we treated these as phase 1 legacies (9 months treatment, 14 months earlier), phase 2 legacies (14 month immediate treatment), or phase 1 and 2 legacies (23 total months with the same species). The community legacy establishment period could range from a minimum of 9 months if only in the first growth phase, or up to 23 months, if the community was the same for both phases. For more information see the experimental processing diagram (Chapter 2 Appendix, Figure 3).

Harvest

I harvested mesocosm soils soon after plants were harvested (1-3 days after plant removal) aseptically using trowels that had been sterilized in a 0.1% sodium hypochlorite solution for 10 minutes and then rinsed in clean tap water. Harvesting all 180 soils at the same time was not possible, so each was stored at 4°C for 1-4 week(s) until all soils had been harvested; with the minimum storage being 5 days and max being 4 weeks. There was likely some loss in fungal and bacterial diversity, and potentially other issues associated with storage; however, conditions are well within standards set by ISO 18400-102:2017 "Soil Quality –Sampling" (Standardization 2017) and timelines for microbial community stabilization (Lauber et al. 2010) and as samples were processed randomly any storage conditions would affect all groups evenly.

Total carbon and nitrogen in soil were analysed by Hill Laboratories, Christchurch NZ, using a Dumas type combustion with an Elementar Vario-Max CN Elemental Analyzer (Waller et al. 2020). Nutrients measured include available nitrogen in kg/hectare (AN), anaerobically mineralisable nitrogen (AMN), organic matter (OM) and total carbon (tC).

Greenhouse Experiment

Seed Germination

Seed trays were soaked in a 0.1% sodium hypochlorite solution for 10 minutes to remove potential external sources of biological inoculum and then rinsed in clean tap water. Trays were then filled with a steam-sterilized 1:1, perlite:vermiculite mixture. Then I planted approximately 5x required seed at a depth of twice seed size and then dampened with clean tap water. Pine seeds were stored at 4°C for 2-3 weeks for cold stratification, then grown at ambient temperature. Broom seeds were scarified with hot water, then germinated at room temperature. *Holcus* used in the experiment was direct-seeded into pots due to overgrowth in prepared seed trays and inability to safely transfer seedlings.

Planting and Growth Period

I soaked pots in a 0.1% sodium hypochlorite solution for 10 minutes then rinsed before filling with soil from the mesocosm. Pots were set on 0.1% sodium hypochlorite washed and rinsed rocks as a base to prevent cross-contamination from drainage of nearby pots. Pot placement was randomized via R (Chapter 2 Appendix, Figure 1). Plants were watered on alternate days. The glasshouse was maintained at an ambient temperature of 20-25 °C with a natural light regime.

I planted 2 seedlings from each species per soil, to minimize transplant mortality, and randomly thinned to 1 seedling at 1 month. I direct seeded *Holcus* using 5 planting spots 3-5 (Chapter 2 Appendix, Figure 2), with the aim to assist in identifying target seedlings, then randomly thinned to 1 plant at 1 month. I weeded all plants weekly to promote the growth of the target species. Early during this process, many other grasses sprouted. These confounded the weeding process with the *Holcus* seedlings, leading to accidental weeding of *Holcus* in favour of plants that grew from seeds already present in the soil. Sections from each grass sample were frozen for future DNA identification.

Above-ground growth was reviewed at three and six months with direct height measurements, check-ins weekly, and observations documented. After reviewing growth, I harvested at six months, as plants were out-growing pots and appeared to begin suffering (leaves yellowing and/or falling off).

Harvest and Final Measurements

I measured plant height and collected small leaf punches from grasses for future analysis; stored at -80 ˚C until extraction. All other above-ground biomass was dried in paper bags at 50 °C and checked daily for mass loss, with final measurements made after four days when samples displayed constant mass as compared to previous day. Samples were weighed in small batches to minimize rehydration and resulting increase in mass.

All roots were gently washed to remove soil and other foreign material, and then I froze roots for future processing, a maximum of three days at -20 ˚C.

Holcus roots were dried at 50 °C for eight days, when their mass reached stability. I weighed these in small batches to avoid rehydration.

Broom roots were processed in the same way except before drying, I scored their nodulation using the methods outlined in Yates et al. (2016). After scoring, the roots were bisected, and placed half into ethanol for future microscopy work and used the other half for dry mass.

Pine roots were floated in de-gassed water in a large Petri dish under a dissecting scope and percent mycorrhizal infection was documented visually using grid-line intercept methods (Giovannetti and Mosse 1980). I used un-stained live roots, as after scoring samples were taken for DNA analysis and staining would likely compromise the samples. The method itself involved using 140 mm diameter Petri dishes overlaid with a cm-gridded glass disc also 140 mm diameter and counting each apparent mycorrhizal root and non-mycorrhizal root that intersected the grid. Then calculating the percent infection based on infected vs total scored roots.

I also took samples for fungal DNA analysis from pine roots. A single pooled sample was taken from each seedling, which included 3 x 2 cm sections of fine roots with at least 10 ectomycorrhizal (ECM) tips each and 10 more randomly selected ECM tips to ensure a variety of morphotypes and fair representation of the root system. I sampled each root system twice; providing an archive tube and working tube. After taking DNA samples, the roots were bisected, half into ethanol for future microscopy work and half for dry mass. I compared pre-versus post-DNA sampling mass and found mass lost due to sampling was negligible (less than 0.005 g).

Molecular Methods

I extracted DNA from pooled pine root tips using the Extract-N-Amp™ Plant PCR Kit by Sigma-Aldrich. Primers, ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used for PCR amplification of the Internal Transcribed Spacer (ITS) region. PCR was performed as follows: denaturation of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 57 °C and 30 s at 72 °C, with a final step at 72 °C for 7 min (and held at 4 °C). All PCRs were completed with negative controls, with positive control in initial primer testing. DNA from a soil sample collected from a site with 98% pine coverage was used as a positive control during methods development. To confirm amplification, I performed agarose gel electrophoresis with the PCR products, using a 1% agarose gel stained with RedSafeTM (iNtRON). No PCR products were seen in negative controls and samples that showed poor amplification were rerun, positive controls were strongly positive. After gel electrophoresis verification, products were cleaned, pooled and sent for Illumina MiSeq, 2 × 250 bp sequencing at the Otago Genomics Facility at the University of Otago.

I merged the reads using USEARCH v11.0.667 (Edgar 2010). I then removed sequences less than 200 bp and those with more than one expected error using VSEARCH 2.10.4 (Rognes et al. 2016). In order to improve overall analysis, and to account for common PCR and sequencing issues, any sequences occurring only once or twice were removed (Dickie 2010, Leray and Knowlton 2017). The remaining sequences were clustered to 97% similarity threshold. OTUs were identified using BLAST v2.5.0+ (Altschul et al. 1997) and the UNITE public database (Nilsson et al. 2019). I removed OTUs which were not within the kingdom Fungi and all OTUs which had a < 200 bp match to any known species. Extraction buffer blanks, as well as negative controls were sequenced and OTUs which were found within these controls (0.38% of OTUs) were also removed from further analysis.

To look at functional grouping I used FUNGuild (Nguyen et al. 2016) to match my BLAST-identified OTUs to guilds. In keeping with my hypothesis I focused on pathotrophs (potential pathogens) and symbiotrophs (ectomycorrhizal fungi in particular), following definitions of "pathotroph" and "symbiotroph" from Nguyen et al. 2016.

Statistical Analysis

All analysis was performed in R (R Core Team 2020). To quantify the effects of the soil legacy from various plant community characteristics on resulting seedling biomass, I used linear mixed-effect models via lmer function of the R package "lme4 (v1.121)" (Bates et al. 2014) accounting for the plant community from the mesocosm as a random effect within the model. Before final testing, data was visually checked for basic normality, by plotting residuals versus

fitted to check for heteroscedasticity, as well as a Q-Q plot and density plot, to see if the residuals follow a normal distribution (yielding a plot with a roughly 45-degree straight line for Q-Q, and bell curve for density), see Chapter 2 Appendix Figure 4. I applied the command "update" for model simplification to determine which response variables best predicted the observed changes in seedling biomass based on analysis of variance of the original versus updated model (Crawley 2007). Following Crawley (2007), third-order interactions (or higher) were only considered significant when $p < 0.01$, while main effects and second-order interactions were considered significant at $p < 0.05$. Model simplification steps are shown in Chapter 2 Appendix Table 1. Biomass of each of the three species was analysed separately. Considering the bi-modal nature of the pine seedling results which might violate assumptions of normality, I also ran a rank-transformation on the data (a non-parametric approach for complex models (Conover and Iman 1981)), which yielded an identical best model with significance that was not qualitatively different (p-value for best model increased by 0.02).

Due to the interrelatedness of nutrients measured, I decided to run a Principal Component Analysis (PCA) on the nutrient data and use the major axes as predictors in the mixed effect models. Components were explored using scree plots and explained variance per component to determine those useful for modelling and to access correlation to components, results in Chapter 2 Appendix Figure 5 & Table 2.

The community sequence data from pine seedling root tips was analysed using the R package "vegan" (Oksanen et al. 2013). I used a permutational multivariate analysis of variance (PERMANOVA) using "adonis2" within the vegan package to compare the fungal communities based on the legacy data, as well as connections to seedling biomass. I also used heatmap (R function of same name) with clustering (clusterh in R) to visualize the communities. To look for patterns within the fungal community a Principal Coordinates Analysis (PCoA) was used with R package "vegan" (Oksanen et al. 2013) and base R commands. Components were explored using scree plots and explained variance per component, and correlation between components and data, results in Chapter 2 Appendix Figure 6 & Table 3.

To look for interactions between predictors I used a series of related mixed-effects models between 3 major groups: plant community legacy factors from original mesocosm study (pine presence/absence from phase 1, pine numbers from phase 2, proportion of nitrogen fixers, proportion exotic plants, functional groups, and other non-pine ectomycorrhizal/ECM plants), chemical nutrient measurements from the soil at harvest as the dominate PCA axes, and

fungal communities from pine root tips (50% or more *Wilcoxina*, 50% or more *Inocybe*, or other), Chapter 2 Appendix Table 1.

Results

Legacy Factor Effects on Seedling Biomass

Pine presence in either the first or second phase led to larger seedlings in all species when used as the sole predictor using lmer in R (pine biomass ~ legacy, $F = 9.9$, $p = 0.007$; broom biomass ~ legacy, $F = 5.3$, $p = 0.039$; *Holcus* biomass ~legacy, $F = 7.8$, $p = 0.028$; Figures 1-3 respectively).

The pine soil legacy became less of a driver when other plant community legacy factors and nutrients were included (Table 1). The plant community factors included were proportion of exotic, presence of ECM plants other than pine, pine presence phase 1, number of pines in phase 2. The pine legacy was divided into phases to be able to see potential differences between a past presence and the amount in a recent legacy. The proportion of exotic plant soil legacy still drove the variation in pine biomass (Figure 4), and pine and other ectomycorrhizal legacy drove broom biomass. No model significantly explained variation in *Holcus* seedling biomass, though a pine soil legacy effect (phase 1 legacy) was trending towards significance ($p = 0.078$).
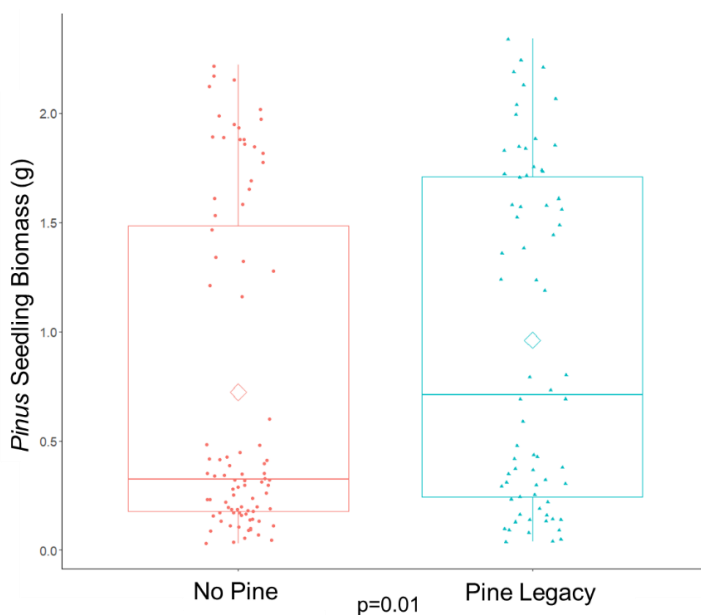
Figure 1: Pine seedling biomass: red circles are for seedlings grown in soil without a pine legacy, green triangles are for seedlings grown in soil with a pine legacy. Diamonds indicate means.
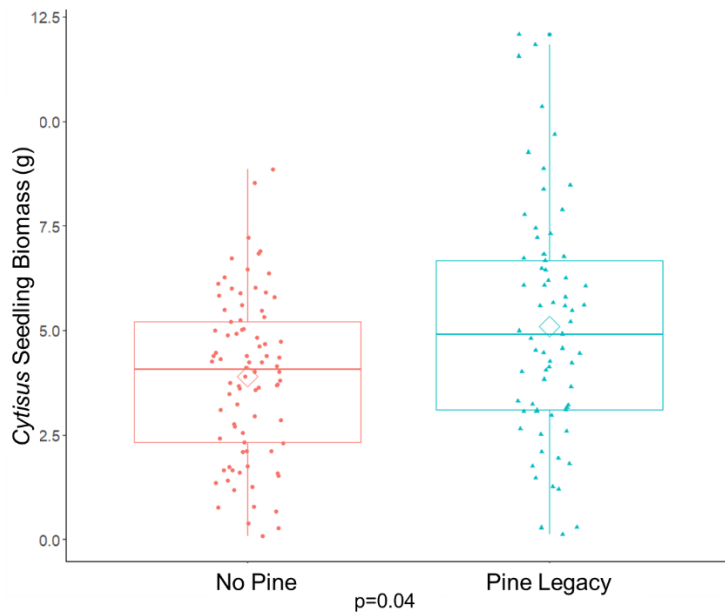
Figure 2: Broom seedling biomass: red circles are for seedlings grown in soil without a pine legacy, green triangles are for seedlings grown in soil with a pine legacy. Diamonds indicate means.
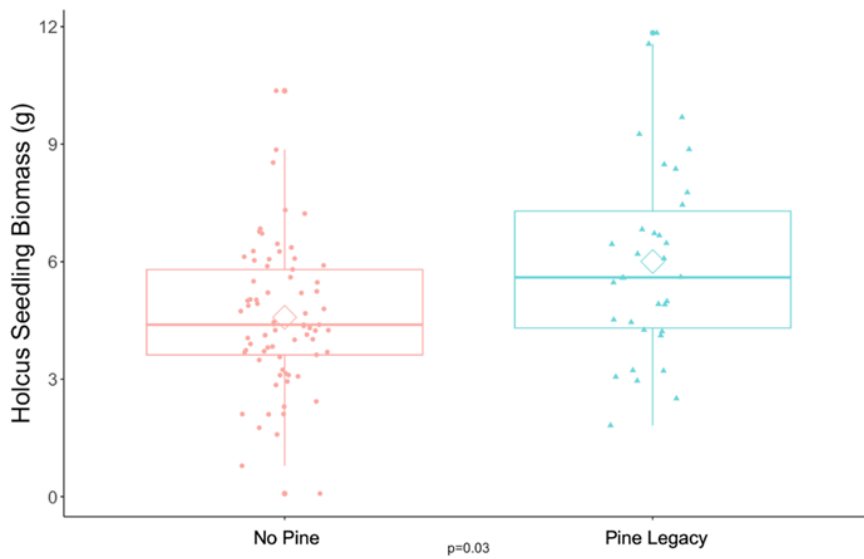
Figure 3: *Holcus* seedling biomass: red circles are for seedlings grown in soil without a pine legacy, green triangles are for seedlings grown in soil with a pine legacy. Diamonds indicate means.
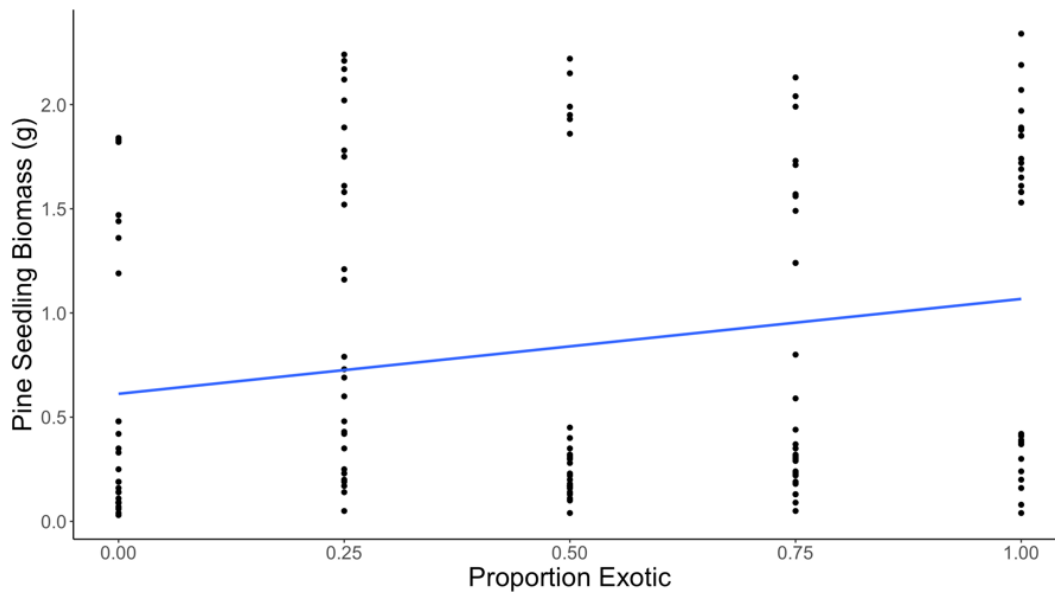
Figure 4: Pine seedling biomass by proportion exotic legacy.

Soil Nutrient Effects on Seedling Biomass

The PCA on the soil nutrients collapses into 2 main axes: pca1; associated with carbon measurements such as organic matter and total carbon, as well as with nitrogen measurements such as available nitrogen in kg/hectare (AN), anaerobically mineralisable nitrogen (AMN), total nitrogen (tN) and pca2 also associated with carbon and nitrogen measurements (Figure 5).

Soil nutrient measurements only showed significant effects on final biomass for pine seedling biomass when tested with legacy factors, though only axis 2 (associated with most closely with AMN, see Chapter 2 Appendix: Figure 5 & Table 2 for correlations and contributions to axes as well as ordination component exploration) remained in the model, and paired with proportion exotic legacy (Table 1). All factors were further explored for pine seedlings by the addition of fungal community data, as seen in later analysis.
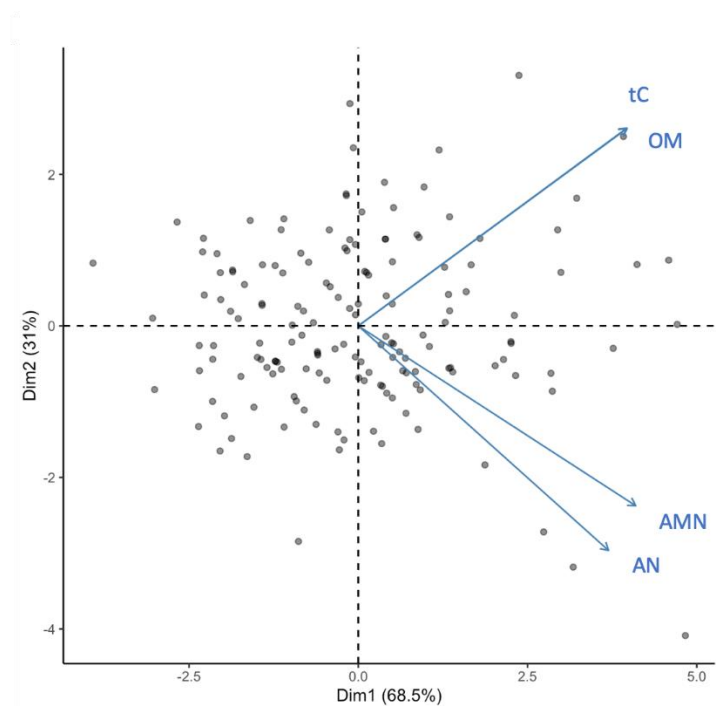
Figure 5: PCA from nutrients: metrics associated with Nitrogen (AMN, AN) which are similar and those associated with Carbon (tC, OM) which are completely overlapping.

Table 1: Coefficients from seedling biomass mixed effects models with soil legacy factors and soil nutrient PCA axes, p-values indicated by symbols ***< 0.001, **<0.01 , * <0.05, ◇<0.1

Species	Pine Phase 1	Pine Phase 2	Exotic	Other ECM	Soil Nutrient Axis 2	Pine Phase 1 : Other ECM
Pine	●	●	0.51 *	●	0.13 ***	●
Broom	-0.30 *	0.62**		-0.94 ◇	●	2.74 ***
Holcus	0.61 ◇	●		●	●	●
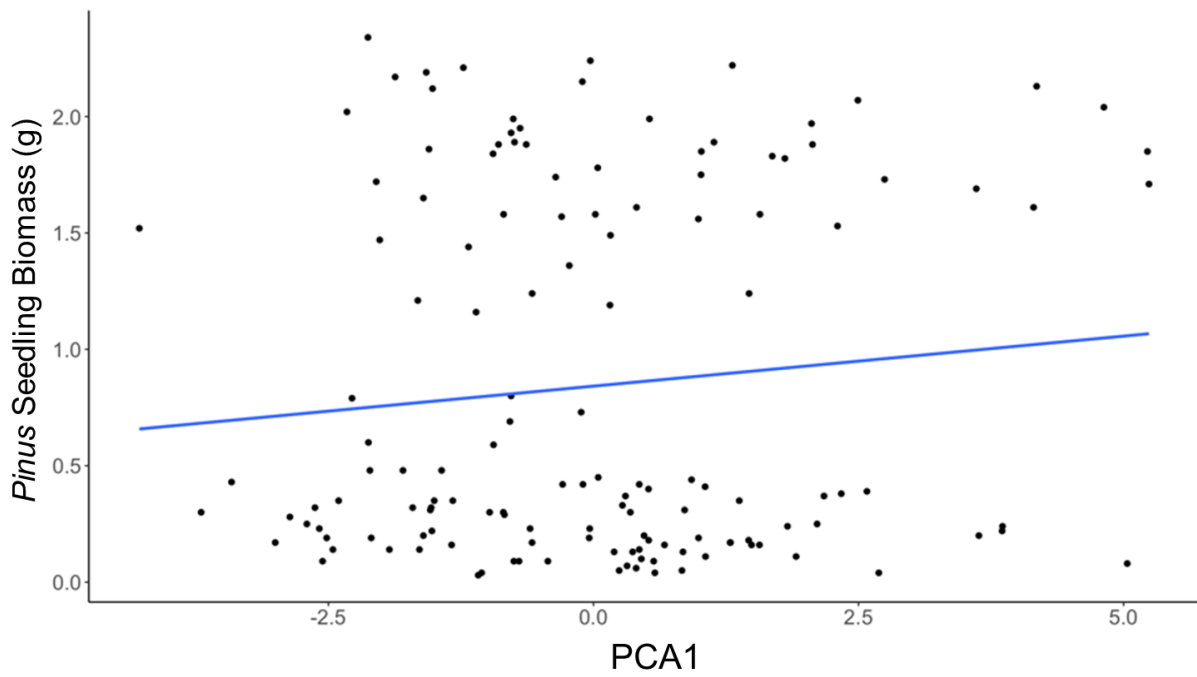
Figure 6: Pine seedling biomass by PCA axis 1, $R^2 = 0.01$
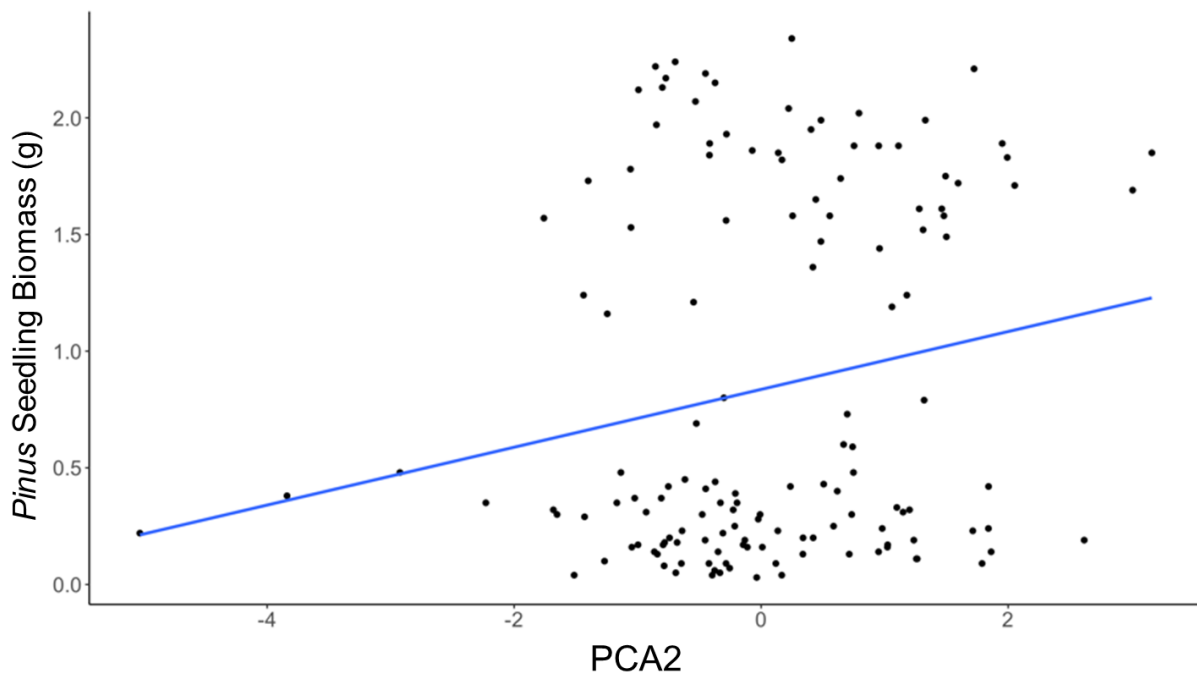
Figure 7: Pine seedling biomass by PCA axis 2, $R^2 = 0.04$

No Trend of Mutualists (Pine or Broom) with Seedling Biomass

Soil legacy effects on seedling biomass were not reflected by increased mutualist interactions for either pine or broom as seen in scoring of visible infection.

The percent infection of pine roots showed no trends among samples with any legacy factors, as all pine seedlings showed high rates of infection, ranging from 56-98%.

Similarly, I found no relationship between legacy factors and nodulation. There was a range in nodules from 3-80 total, and nodule scores from 1-8, using methods from Yates et al. (2016).

Fungal Communities Differ Across Pine Roots and Affect Pine Seedling Biomass

The OTU grouping resulted in 1,572 unique OTUs, with an average of 78 OTUs per seedling root sample, and of these 20% were identified by FUNGuild as ECM species.

While there was no difference in the degree of visible ectomycorrhizal infection of pine seedlings with legacies, there were strong effects on the composition of fungi.

There was a significant difference between communities from a pine legacy soil versus those without any pine as found by PERMANOVA, adonis2 in R, ($p = 0.008$ $R^2 = 0.03$). Further, within ECM identified OTUs there was also a significant difference between pine legacy groups, Figure 8.

Fungal Communities were found to be of three major types as seen in ordination (PCoA) (using pcoa function in R based on calculations by Gower 1966) Figure 9. The first four axes explained 23.35%, 14.8%, 7.96%, and 6.31% of the variance respectively, with the remaining axes explaining less than 5% of the variance each. See Chapter 2 Appendix: Figure 6 and Table 3 for correlations and contributions to axes and general ordination component information. The pattern that emerged showed three major community groups: those dominated by *Wilcoxina*, those dominated by *Inocbye* or those not dominated by either of these OTUs. See Chapter 2 Appendix, Table 4 for the 50 most abundant OTUs.

This trend is also seen in the heat map of ECM species Figure 10. The top most abundant OTUs (*Wilcoxina* and *Inocbye*) each tend to occur as the dominant member of the community or barely at all, but there is not a cohesive grouping of these within the dendrograms produced. These three major community types are well distributed across treatments with no significant trends with any measured soil legacy factor.
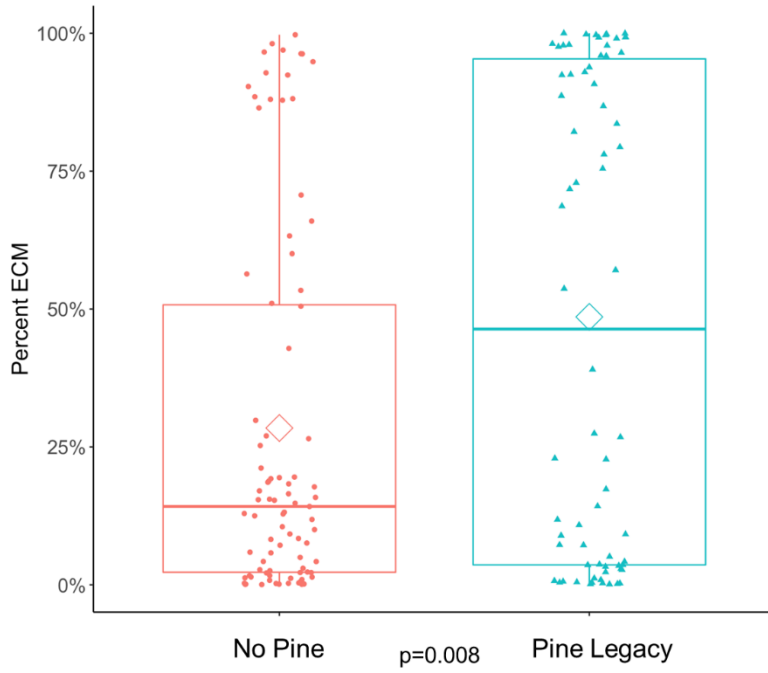
Figure 8: Percent ECM OTUs from seedlings grown in soil without a pine legacy, green triangles are for seedlings grown in soil with a pine legacy. Diamonds indicate means
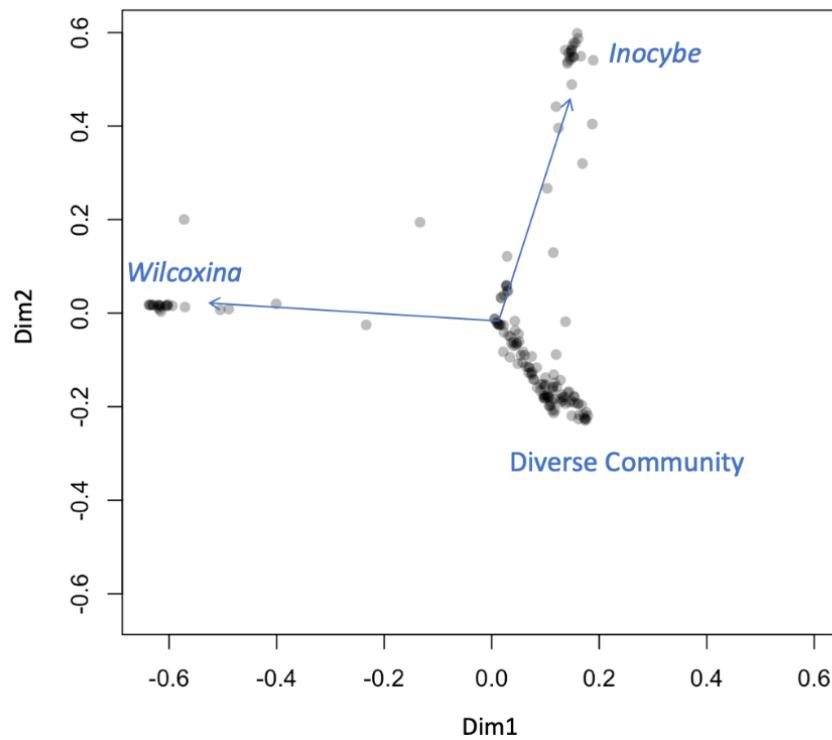

Figure 9: PCoA on fungal community data, points are individual samples, two major groups were dominated by Wilcoxina or Inocybe respectively, and the middle section are considered the diverse community with no dominant species.
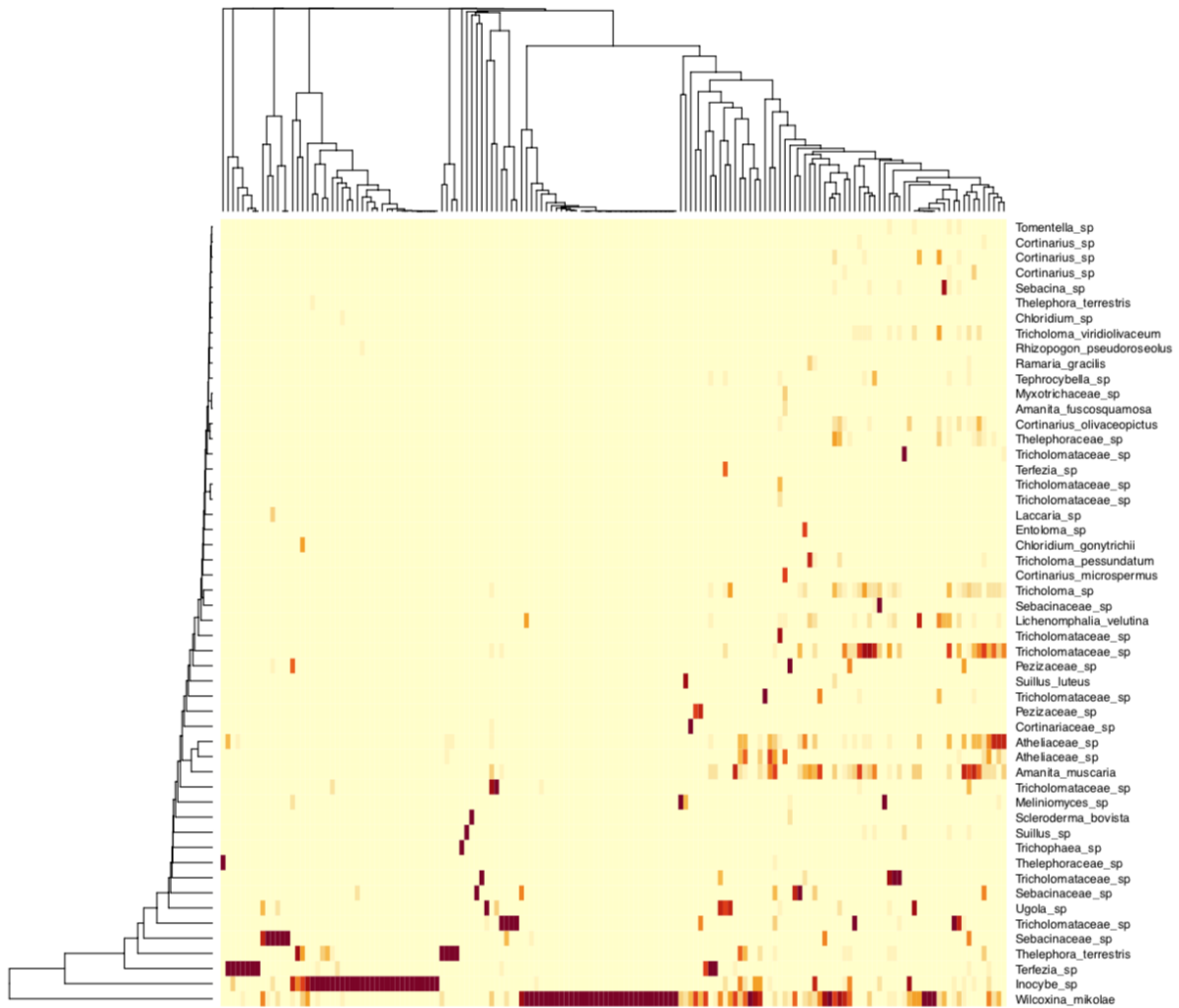
Figure 10: Heat map of ECM species, grass sample on x-axis, species match to OTU on y-axis, warmer colours indicate greater abundance.

With the addition of the fungal data from pine root tips, I expanded upon previous models and the connections between the predictors using a series of linear mixed effects with plant community held as a random effect. These are listed in Table 2 and summarized in Figure 11, also see Chapter 2 Appendix Table 1 for details on the model simplification process used to find the best model.

Response	Pine Phase 1	Pine Phase 2	N-fixers	Exotic	Grass	Soil Axis 1	Soil Axis 2	Wilcoxina Dominated	Inocybe Dominated	Shannon Diversity	Pine Phase 1: Pine Phase 2	Pine Phase 2: N-fixers	Pine Phase 2: Exotic	Pine Phase 2: Grass
Soil Nutrient Axis 1	0.011	-4.540 **	-2.651 *	1.706 *	-1.910	•	•	•	•	•	2.480 *	-41.558 **	30.364 **	-31.884 **
Soil Nutrient Axis 2	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Wilcoxina Dominated	•	•	•	•	-0.513 **	-0.038 *	•	•	•	•	•	•	•	•
Inocybe Dominated	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Shannon Diversity	-0.616 *	-0.356 *	•	•	•	•	•	•	•	•	•	•	•	•
Seedling Biomass	•	•	•	0.492 *	•	•	0.111 *	•	•	-0.104 **				

Table 2: Coefficients from pine seedling biomass mixed effects models with soil legacy factors, soil nutrient PCA axes, and fungal community measurements. P-values indicated by symbols **<0.01 and * <0.05, no symbol with a number indicates not significant, but kept in the model as involved in an interaction, ● indicates a non-significant term dropped from the final model, grey cells indicate predictors that were not relevant for that response variable
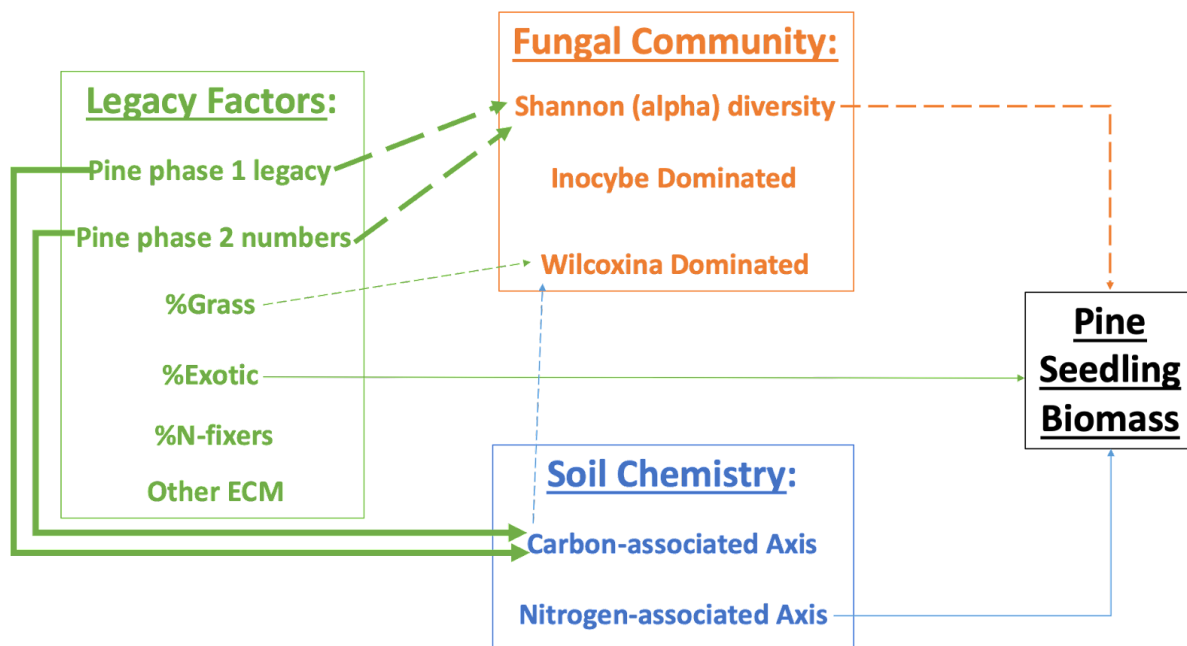
Figure 11: Summary figure from linear mixed effects model's significant effects displayed in Table 2, with plant community as a random effect. Dashed lines indicate negative coefficients; Arrow thickness scaled to R^2 value.

Discussion

Overall, my research shows that a short-term invasion could leave a legacy that affects the growth of future plants. In particular, a pine and exotic-dominated soil legacy led to greater exotic biomass. This could mean that having a pine or other exotic invasion could facilitate a future invasion, even if the invasive plants were removed; their exotic-boosting legacy could remain in the soil.

Nutrients Alone did not Drive Biomass

I did not see the expected change in nutrient availability driving biomass in my seedlings. The nutrients in my soils could not by themselves explain the results without including some biological contribution from the previous plant community. This indicates that there is something beyond solely nutrient modification that created the growth response observed. While the nitrogen-associated PCA axis did affect pine seedling biomass there were no significant soil legacy drivers for this axis, though nitrogen-fixer legacy was trending toward a positive impact with a p-value of 0.07.

Limited Shared Symbionts as Legacy Effects

My expectation that established plants would alter the symbiotic interactions of seedlings was partially supported. Total mycorrhizal infection of pine seedlings was high in all cases, and there was also no trend with rhizobia nodulation and plant community. However, pine legacies negatively impacted the Shannon diversity of fungi, suggesting pine-associated fungi dominated the fungal community. This could be a potential mechanism for the pine legacy benefits on future pine seedlings, as the community could remain dominated by pine-associated fungi.

Fungal Community Patterns from Pine Seedlings

The fungal sequences obtained from the pine root tips showed three major patterns; a community dominated by *Wilcoxina*, a community dominated by *Inocybe*, and a diverse community. Unsurprisingly, both of these have been found to be ectomycorrhizal in past studies (Ivory and Pearce 1991, Seress et al. 2016). These two groups were not well predicted by any of the plant community legacies, except for a pine legacy lowering diversity. While lower fungal community diversity was associated with larger pine seedlings, there was not a relationship with either *Wilcoxina* or *Inocybe* dominated communities and pine seedling size, even though both fungal species are commonly associated with pine (Hayward et al. 2015, Policelli et al. 2019). The fact that these communities had no connection might not be surprising given research that some ECM can be present though not really contributing to the plant (Dickie et al 2002).

Future Directions

A legacy issue not often addressed is the residence time required to create a legacy. A single introduction event has been shown to have impacts on plant communities lasting decades (Wubs et al. 2019), though older legacies have been shown to have greater impacts (Ke et al. 2021). In my experiment the introduction was a soil inoculum and seed source, but what about a novel plant community, how long does it take to leave its mark? Phase one and phase two pine presence both contribute separately to the effect on carbon and fungal diversity. The fact that phase one pine presence is not simply replaced by phase two infers that pine legacy effects might not be simply cumulative, and that even short-term, older legacies can persist in their influence.

Limitations

Some factors that were relevant in the experiment that provided my starting soil were not significant as legacy factors in my experiment such as, the presence of herbivores and invasion by broom seedlings into the mesocosm. The potential legacies from these factors

either did not persist or were overshadowed by other factors. The *Holcus* results were likely compromised by the fact that over-half of the grass seedlings were not actually *Holcus*, but grew from seeds resident in the legacy soil.

Overall Conclusion

A plant community legacy is complicated but even a short-term legacy can affect future plant communities. This legacy is mediated, and sometimes obscured by both chemical and microbial and or fungal effects. These legacy effects should be considered when planning removal of invasive plants, in particular pines or exotic grasses as their soil legacy might encourage re-invasion of pine or increases in other exotic plants such as broom.

Chapter 3

Invasive Pine Legacy as a Driver in Plant Community

Abstract

- Invasive plants can affect the plant community they invade even after they are gone, as their legacy can remain in the soil. It is not known how this invasive legacy will change the growth of various plants, if the extent of invasion changes the legacy, and if these effects would differ within a community context or across plant functional groups. Most soil-feedback and legacy soil experiments only consider a few species and often these are grown only in isolation, thus missing the competition that would exist in natural community settings.

- I field-sampled soil from along a pine invasional gradient to test how variable intensities of pine dominance affected plant community outcomes in both community and single-species assays. Experimental species chosen are evenly represented by exotic and native, including both grasses and shrubs.

- When grown in single species pots, all plant species appeared to benefit from increased pine presence. The pine legacy effect was obscured by exotic dominance in competition when plants were grown together in a community, particularly at low to moderate levels of pine. Grasses also dominated in community assays.

- This work shows that grass dominance following pine invasion could be driven by soil legacies, and amplified in competition, suggesting a need for caution in applying single-species results to a plant community response.

Introduction

When plants establish themselves outside of their native range, they often disrupt the ecosystem they invade. These invaders can change many things, including plant community dynamics, and these changes can persist even after the invaders are no longer living or present. Stahlheber et al. (2015) showed trees in a savannah left a plant-community-altering legacy that endured beyond the life of the tree, such that the original plant community (before

the trees were established) did not return. Potential direct effects include physical mechanisms such as preventing natural erosion cycles or creating shade (Blackburn et al. 2011, Vilà et al. 2011) and alterations to nutrient cycling (Ehrenfeld 2010, Peltzer et al. 2010), but effects can also manifest from the introduction of new microbial organisms via co-invasion (Reinhart and Callaway 2006, Dickie et al. 2014b, Lekberg et al. 2018).

An invasive plant with biotic associations (e.g. with fungi and bacteria) that differ from co-located native plants can create biological legacies in terms of microbial community shifts as well as chemical effects due to plant-specific and associated microbial processing of available nutrients (Zobel and Öpik 2014, Dickie et al. 2017). These modifications can facilitate other invasive species in what has been termed a secondary invasion or in some cases an invasional meltdown (Simberloff and Von Holle 1999, Richardson et al. 2000, Pyšek et al. 2004). Exotic plants that naturalize or become invasive can cause enduring biotic changes in the resident microbial community, as well as associated abiotic effects, that combined often facilitate these or other exotics to outcompete natives in future invasions or re-invasions (Wardle et al. 2004, McLeod et al. 2016, Dickie et al. 2017). A plant invasion exists because the invader has greater competitive success compared to the resident plant community. While there is a wealth of research on competition, this can easily be oversimplified in respect to competitive outcomes at the plant community scale (Wilcox et al. 2018). When plant competition is addressed, it is usually in single pairwise assays, or only measuring responses in respect to a specific target species (Aschehoug and Callaway 2015, Levine et al. 2017, Lekberg et al. 2018). This narrow focus can allow potentially larger, more complex interactions to be missed.

The greater the invasion, in terms of biomass and spatial extent, the greater the impact on the environment (Diez et al. 2010, Vilà et al. 2011, Dickie et al. 2014b). These influences could be limited at low densities and more pronounced at higher densities, potentially reaching a point where the exotic plant effects lead to a dramatically different environment (Diez et al. 2010, Vilà et al. 2011, Dickie et al. 2014b, Sapsford et al. 2020). Different components of the ecosystem can respond differently to increases in the biomass or density of the invasive species, so there is potential for both invasion effects to change through time and for different components of the system to be driving legacies at different stages. For example, a short lived, rapid turnover native could be supplanted more quickly by an invader than a long lived tree species that is affected more gradually as replacements fail to come through the canopy. For example, Abreu & Durigan (2011) found a few native shrubs and trees persisted in a Brazilian

savannah invaded by *Pinus elliottii* and exotic African grasses, but all native herbaceous plants were competitively excluded.

When the invader is a tree and the introduced environment is not a forest, the risk of a dramatic change increases. As in the Brazilian savannah (Abreu & Durigan 2011), it is also the case in New Zealand, where invasive pines are rapidly turning grasslands into pine forest (Gawith et al. 2020, Sapsford et al. 2020). Removal and control of these pines is costly and often leads to invasion by other exotic species (generally grasses and shrubs), or re-invasion of pine (Richardson et al. 1994, Dickie et al. 2014b, Taylor et al. 2016, Nuñez et al. 2017). In New Zealand's pine invasions, grasses, generally exotic grasses, have been found to dominate post-pine removal in both greenhouse experiments and field observations (Dickie et al. 2014b, Taylor et al. 2016, Dickie et al. 2017, Wardle and Peltzer 2017). *Pinus contorta* has been found to significantly impact other plants, even within its native range (Taylor et al. 2016). However, the effects were found to be more pronounced and in lower densities within the introduced region (Taylor et al. 2016). The mechanisms behind these effects are not fully understood.

In many cases, restoration to the plant community pre-invasion is the preferred goal after an invasive species is removed, not simply a new-normal without the targeted invader. Many studies research restoration by planting natives to observe fitness and overall success, but do not include exotics in their experiments (Dickie et al. 2014b, Stuble and Young 2020) or only include observations about exotic presence (Abreu & Durigan 2011). However, as it is nearly impossible to remove exotic seed sources, especially those resident in the soil, when present in invaded areas, including a representative mix of native and exotics could provide a more realistic model of the environment.

A major interface for plant competition or facilitation is the soil, with plant-soil feedbacks and legacies as a leading area of research (Van der Putten et al. 2013, Driscoll and Strong 2018). Often the main finding is that plants experience negative feedback from plants similar to themselves (Kulmatiski et al. 2008), however this is not always the case, particularly with plants that have obligate mutualists, such as ectomycorrhizal fungi (Martin et al. 2016). Generally the strongest competitors would likely be of the same functional group and phylogenetically similar. Burns and Strauss (2011) found conspecific plant competition to dominate in the field and confamilial competition to be more pronounced in potted experiments. However, all species chosen by Burns and Strauss (2011) were relatively similar in functional grouping. Verdú et al. (2012) reviewed 31 different studies in terms of phylogenetic relatedness versus functional guild for nursing potential in restoration efforts.

They found when both plants share the same functional group, the most facilitation was shown at greater phylogenetic distance.

Understanding plant interactions with invasion impacts is crucial to restoration and management. Previous research has shown links between invasions and changes in plant response, such as favouring plants from similar provenance or certain functional groups; though rarely in a community context similar to that of the invaded environment and not including varying levels of invasion. My research addresses these gaps within pine invasions in New Zealand by measuring plant responses and including plant community responses. This work was structured around testing the following hypotheses.

The first hypothesis seeks to address the lack of knowledge about pine legacy effects in terms of level of invasion (pine density or dominance) effects on the plant community, by provenance and functional group. This serves to test field observations that exotics and grasses tend to thrive after pine invasion.

Hypothesis 1

Increases in pine dominance will lead to legacies that increase the competitive advantage of exotic plants over native species and that grasses will dominate over shrubs.

The second hypothesis investigates the importance of using a community response, as opposed to classic single species tests. For experiments to be more ecologically relevant to an in situ plant community, it is useful to consider studying the community in a community context; not just pairwise or for one plant, but in a multi-species assay with competition.

Hypothesis 2

Testing growth responses of a community will yield a fundamentally different results than from single species assays.

Methods

Experimental Design

I took soil from 15 plots along a pine invasion (mostly *Pinus nigra*) gradient near Mt. Barker, in Canterbury, New Zealand. These plots were chosen to include various levels of invasive pine dominance, from essentially zero to complete canopy cover. Eight plant species were

planted in soil from each site, both together in a community pot (including all eight) and separately in eight single species pots. These were grown until resource-limited as determined by visible signs of stress with no other apparent cause; stress indicators used were yellowing/browning and/or withering leaves, root-bound as seen by roots at pot surface or through bottom drainage holes.

I selected plant species based on dominant species in early succession, and to represent different functional groups of natives and exotics. Grasses and shrubs were chosen as these are generally the first colonizers post-disturbance, which occurs due to invasive plant removal (Buckley et al. 2007, Young et al. 2015, Williams et al. 2017). I selected two native grasses *Poa cita* and *Festuca actae,* two exotic grasses *Dactylis glomerata* and *Festuca arundinacea*, two nitrogen-fixing shrubs *Sophora microphylla* (native) and *Cytisus scoparius* (exotic), and two non-nitrogen-fixing shrubs *Veronica hulkeana* (native) and *Rosa rugosa* (exotic).

The species used for this experiment were based on field data from the soil sampling sites shared by Manaaki Whenua on dominant plant species and functional groups to ensure the experiment would be relevant, information on plants that have previously been successful in greenhouse experiments (Waller et al. 2020), and seed availability at the time of experiment

The choices for grass species were further informed by phylogenetic relatedness (Lloyd et al. 2007). The shrub phylogenetic data came from Potter et al. (2007) and Lewis (2005), and Peters et al. (2010) (for the *Fabaceae*) (Table 1).

Table 1: Plants chosen for chapter three experiment

Plant	Provenance	Functional group	Family/SubFamily (for grasses)
Poa cita	Native	Grass	*Poaceae/Pooideae*
Festuca actae	Native	Grass	*Poaceae/Pooideae*
Dactylis glomerata	Exotic	Grass	*Poaceae/Pooideae*
Festuca arundinacea	Exotic	Grass	*Poaceae/Pooideae*
Veronica hulkeana	Native	Shrub	*Plantaginaceae*
Rosa rugosa	Exotic	Shrub	*Rosaceae*
Sophora microphylla	Native	Shrub/Tree	*Fabaceae*
Cytisus scoparius	Exotic	Shrub	*Fabaceae*

Soil Collection

Sample sites were chosen from existing Manaaki Whenua 20m x 20m pine field study plots at Mt. Barker, as previous surveys measured pine dominance, could be used to plan my soil collection along a known invasion gradient. Representative plots from each available invasion level were randomly chosen. To obtain plots free from any pine, three plots were placed across a fence line into a grazed but otherwise similar grassland (Figure 1).

Figure 1: A: Map of plot sites (the bare patches are from pine control experiments and were not used in my experiment), B: Images of plots from along the pine dominance gradient.

I ran a power analysis to determine the number of soil sources required to address my hypothesis to ensure that I had sufficient sample size (Ellis 2010). The Ellis 2010 paper was

chosen as comparable to my sites and my data from previous studies had not yet been completed to sufficient levels to be useful at the time of the analysis. 15 plots was suggested via this a priori power analysis using r^2 values from Weigelt and Jolliffe (2003) and Lekberg et al. (2018). From each plot, I collected soils aseptically using trowels that had been sterilized in a 0.1% sodium hypochlorite solution for 10 minutes and then rinsed from each plot. I pooled soil from just outside the midline on each edge of the square plot (apx 13-14 L per hole) from surface to approximately 15-30 cm depth (to include the topsoil and the majority of the biologically active layers) and homogenized these for a representative soil (50-56 L total volume).

Seed Germination (four-six weeks before start of experiment)

Seed trays were soaked in a 0.1% sodium hypochlorite solution for 10 minutes to remove potential external sources of biological inoculum and then rinsed in clean tap water. Trays were then filled with a 1:1 steam-sterilized perlite:vermiculate mixture. Approximately five times seed required to produce adequate plants for experiments (two per plot per treatment) was sown at a depth two times seed size and then dampened with clean tap water. Seeds were treated according to distributor's instructions, including cold stratification and scarification as needed. I was unable to germinate *Rosa* seed even trying various cold strata and scarification techniques, so I used propagation via fresh cuttings starting in perlite:vermiculate trays.

Experiment Set-up

Soil samples were homogenized per plot and mixed with steam-sterilized sand in a 1:1 mixture. Sand was added to aid drainage during the experiment and to lower the amount of soil required from each plot, as plots are shared-use and the scale of my experiment required large volumes which could affect others' work.

Sand was steam-sterilized at 5 hrs at approximately 85°C, then stirred with a clean rake (rake soaked in a 0.1% sodium hypochlorite solution for 10 mins, then rinsed with clean tap water before each use), and then another 5 hrs at approximately 85°C. To mix soils with the clean sand, I used a 0.5% sodium hypochlorite solution spray, followed by a clean tap water spray, to clean a new cement mixer (never used for cement or otherwise). The mixer was run until samples were well-mixed (3-5 minutes), and cleaned with sodium hypochlorite spray and clean water between soils.

<u>*Competition Community*</u>

Large (75 L) planting pots were used for the community treatment to allow for plants to establish and then compete. Before planting each pot was soaked in a 0.1% sodium hypochlorite solution for 10 mins and rinsed with clean water before use.

In each pot I planted two seedlings from each species, to minimize the impact of transplantation mortality on my study, and then randomly thinned to one seedling at one month. To allow for even interactions, in particular the likely benefits from being near a nitrogen-fixing plant species, similar niched species were planted across from each other in a dial-pattern (see below planting diagram): native grasses *Poa cita* and *Festuca actae,* exotic grasses *Dactylis glomerata* and *Festuca arundinacea*, nitrogen-fixing shrubs *Sophora microphylla* and *Cytisus scoparius*, and non-nitrogen-fixing shrubs *Veronica hulkeana* and *Rosa rugosa* (Figure 2).
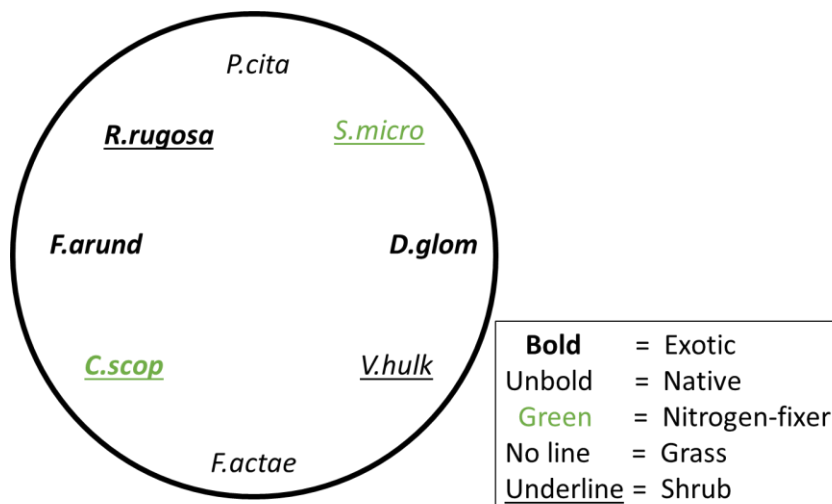
Figure 2: Community pot planting diagram. Functional group indicated by shrubs underlined, provenance by exotic species in bold, nitrogen fixing species in green

Single Species Assay

Single pots were smaller in volume (800 mL) due to space constraints and the earlier mentioned requirement to not remove too much soil from around the experimental plots. I used the same cleaning procedure as with the community pots and the same pine-gradient soil mixture. Pot location was randomized using R, and pots placed on 0.1% sodium hypochlorite washed and rinsed rocks as a base to prevent cross-contamination from drainage of nearby pots.

Experiment Maintenance and Harvest

Foliar growth was reviewed at two, four, and six months via digital camera and direct height measurements. Plants were watered on alternate days. The glasshouse was maintained at an ambient temperature of 20-25°C with a natural light regime.

Plants were harvested at 185 days for single pots and 227 days for community pots. Single pots were harvested earlier than the community pots as the pots were smaller and plants were root-bound and visually suffering (yellowing, losing leaves) earlier. Community pots were allowed to grow for longer to intensify competition and increase the amount of interactions amongst individuals. The growth times were meant to capture growth before plants completely lost exponential growth as resources became limited.

All above-ground biomass was dried in paper bags at 50 °C checked daily for mass loss, with final measurements made after 4-6 days when the sample displayed constant mass as compared to the previous day. Samples were weighed in small batches to minimize rehydration and resulting increase in mass.

To account for a potential abiotic mechanism for pine dominance effects, several soil properties were determined from air-dried samples from each representative plot. Total Carbon and Nitrogen were measured using a LECO CNS-2000 thermal combustion furnace, available Phosphorus via flow injection analysis colorimetry after sequential 1:10 Bray 2 (ammonium fluoride / hydrochloric acid) extraction, and pH was determined using an electronic probe at 1:2.5 weight per volume.

Statistical Analysis

To standardize my results amongst treatments (community or single pots), I log transformed biomass divided by growth time to have a relative growth rate for all plants. Both treatments were grown to an apparent resource limitation, with single pots reaching that threshold earlier.

Pine dominance is defined by the stand basal area, and further log + 1 transformed to reduce variance and to allow for the zeros in basal area from grasslands.

Stand basal area measures the surface area occupied by each tree stem summed per plot = $\frac{\sum_{i=1}^{n} g_i}{a}$ **where** $g_i = \frac{\pi}{4} \left(\frac{d_i}{100} \right)^2$ with i=1…n; n as the number of pine tree diameters in a plot, g_i is the corresponding basal area (m^2 ha^{-1}) of a pine tree, a=area, in my case 400 m^2,

d_i being diameter at breast height (cm), as measured by official Manaka Whenua vegetation surveys, formula from Siipilehto (1999).

All analyses were performed using R version 4.0.2 (R Core Team 2020). To quantify the effects of soil from various pine dominance levels on plant biomass, while taking into account differing plant species, and whether grown in a community or in a single pot, I used linear mixed-effect models via the R package "lme4 (v1.121)" (Bates et al. 2014). I set pine dominance, plant provenance, functional group, and competition treatment (single pot vs. community) as fixed effects, with sample plot and plant species as random effects. Before confirming best model to analyse data, the data was visually checked for assumptions of normality, by plotting residuals versus fitted to check for heteroscedasticity, as well as a Q-Q plot and density plot, to see if the residuals follow a normal distribution (yielding a plot with a roughly 45-degree straight line for Q-Q, and bell curve for density), Chapter 3 Appendix Figure 1.

To obtain the best model, terms and interactions were removed as appropriate via model simplification (Crawley 2007), see Chapter 3 Appendix Table 2. P-values for mixed effects models were calculated via the "lmerTest" package (Kuznetsova et al. 2017) and R^2 metrics (marginal R^2 and conditional R^2) calculated using package "MuMIn" (Nakagawa and Schielzeth 2013). The marginal R^2 represents the variance explained by the fixed terms only and the conditional R^2 includes variance from both the random and fixed terms.

Most plants successfully grew throughout the experiment, though there were two species where this was not always the case. *Veronica* plants were often quite stunted, and died in one instance. *Rosa* plants died in 8 of the 30 pots. The *Rosa* deaths created a strong bias in analyses. In order to deal with this, all *Rosa* data were dropped from models and ordination.

To look for community response patterns across plots and treatments, as well as each species contributions to these apparent trends, I used a redundancy analysis (RDA) in R package "vegan" (Oksanen et al. 2013) to analyse the growth rate data by plot as driven by pine dominance and competition treatment. As there was not a true community for single pots, the growth rates were combined per plot into a predicted community composition without competition from each plot. This predicted community was used to compare responses in the mixed community/competition pots per plot. Components for ordination and correlations between data and components shown in Chapter 3 Appendix Figure 2 & Table 1.

To test whether the trends observed could be solely attributed to abiotic soil properties (pH, carbon, nitrogen, and phosphorus), these values and all potential interactions were added to the best model, and removed as appropriate via model simplification as done previously.

Results

Growth of both the exotic and native species groups increased with increasing pine dominance (Figure 3), and was best predicted by a model including provenance (exotic, native), treatment (community, single), and functional group (shrub, grass) (Table 2). Exotics grew larger than native when in competition, but there was less of a difference when each was grown without competition (Figure 3). While pine dominance had a positive trend on the growth of all plant species, the greatest increase in growth rate was for native plant species grown in competition (3-way interaction of provenance, competition treatment and pine dominance; $F_{1, 208} = 5.53$, $p = 0.02$, marginal $R^2 = 0.55$, conditional $R^2 = 0.86$; Figure 3, Table 2).
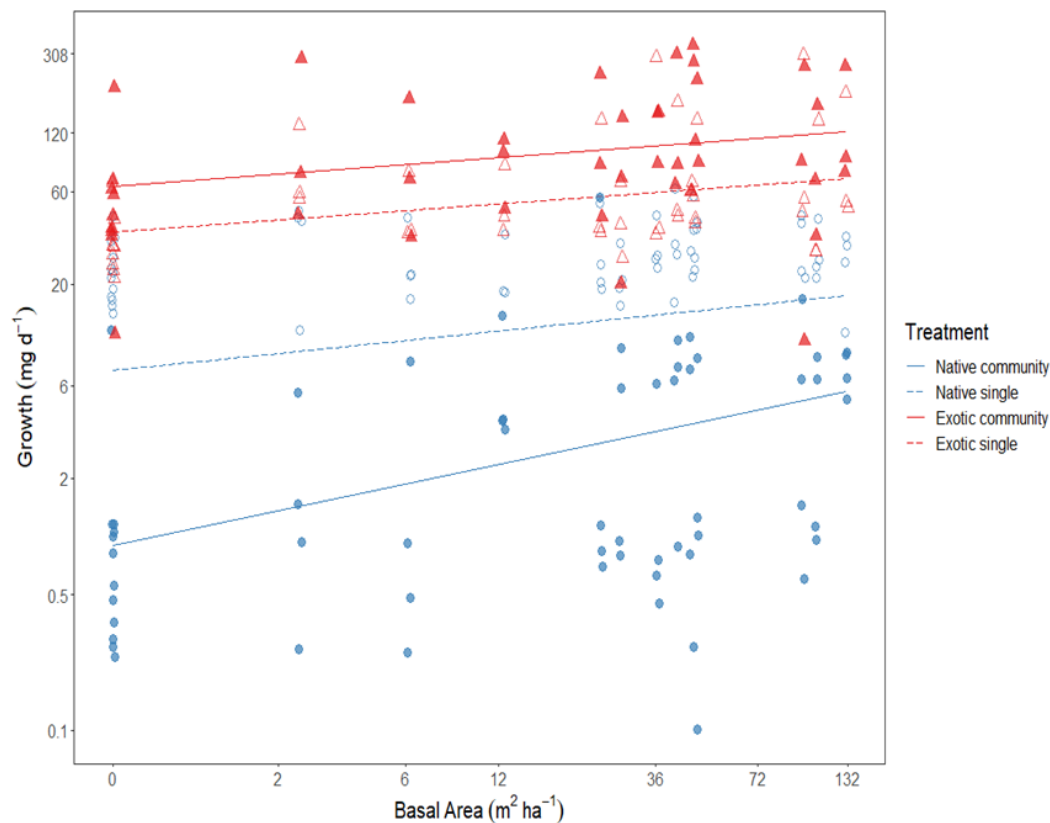
Figure 3: Effects of treatment and provenance on growth rate as a function of pine basal area, based on model coefficients, Actual samples plotted as blue circles for natives, red triangles for exotics, filled in for community pots and empty for single

Table 2: model output: Growth Rate (mg d-1) ~ Pine Dominance (log (basal area +1)) * Provenance (exotic or native) * Competition (single or community) + Functional Group (grass or shrub) + Functional Group : Competition + (1|Plot) + (1|Species), Non-significant terms includes if part of a higher interaction. Model simplification steps in Chapter 3 Appendix Table 2.

	F-value	**p-value**
Pine Dominance	23.87	< 0.0001
Provenance	4.33	0.088
Competition	68.54	< 0.0001
Functional Group	1.86	0.231
Pine Dominance : Provenance	0.753	0.387
Pine Dominance : Competition	5.64	0.019
Provenance : Competition	95.02	< 0.0001
Competition : Functional Group	25.57	< 0.0001
Pine Dominance : Provenance : Competition	5.41	0.0212
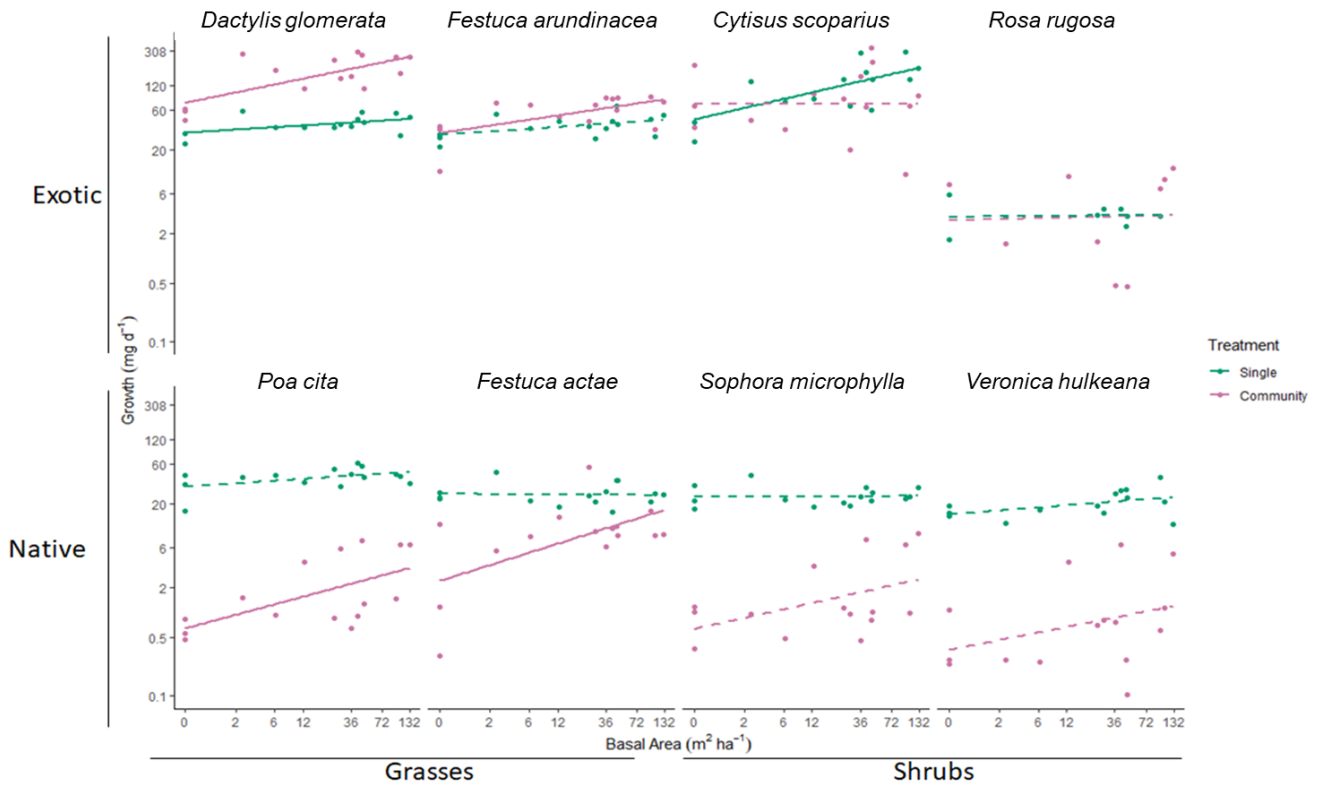
Figure 4: Graph with all species growth by community vs single growth rate across pine dominance, separated into groups by treatment and provenance, solid lines show significant relationships (p ≤ 0.05)
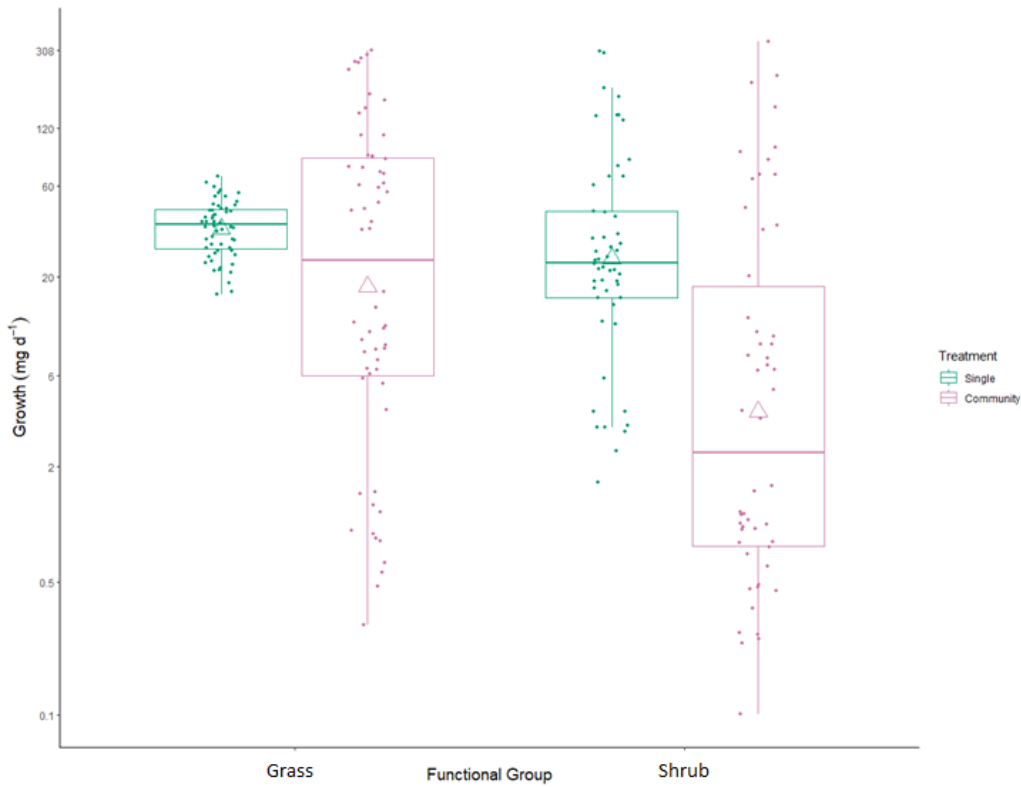
Figure 5: Graph with growth by functional groups (grass & shrub), Δ = mean per group by treatment across all soils.

When analysed individually and by competition, each plant species experienced some amount of increase with increasing pine dominance (Figure 4). For single-species pots these trends were statistically significant only for *Cytisus* and *Dactylis*; (F_1 = 14.56, $p < 0.01$ and F_1= 4.76, $p = 0.05$, respectively) though *Festuca arundinacea* showed a similar tendency, F_1 = 4.03, $p = 0.06$. In community assays, only the grasses showed significant increases with increasing pine dominance (*Dactylis*: F_1 = 13.78, $p < 0.01$, *Festuca actae*: F_1= 6.94, $p = 0.02$, *Festuca arundinacea*: F_1 = 7.53, $p = 0.02$, and *Poa cita*: F_1= 7.40, $p = 0.02$).

The grasses generally out-performed the shrubs in most cases, with *Cytisus* being the only shrub that was comparable to the exotic grasses in all cases, and even larger than native grasses in competition. The average growth rate for both functional groups decreased in competition, but the shrubs, as a group, were more greatly impacted (Figure 5). This trend was likely driven by the slow growth of native plant species, particularly when in competition (Figures 3 & 4).

Native plant contributions to the overall community are less with competition compared to what would be expected based on when they are grown alone (indicating that competition further depresses their slow growth rate). Pine dominance effects are small compared to this effect but generally promote exotic species over native. (Figure 6). This is seen in the RDA where the native species have the largest species effect on the position of each site, and all in the same direction. A permutation test (ANOVA) was run on the RDA to test the significance of the RDA constraints. This showed the importance of competition, F_1 = 39.64, $p < 0.01$. This is also visible in the RDA examining single and community treatment groups separately.
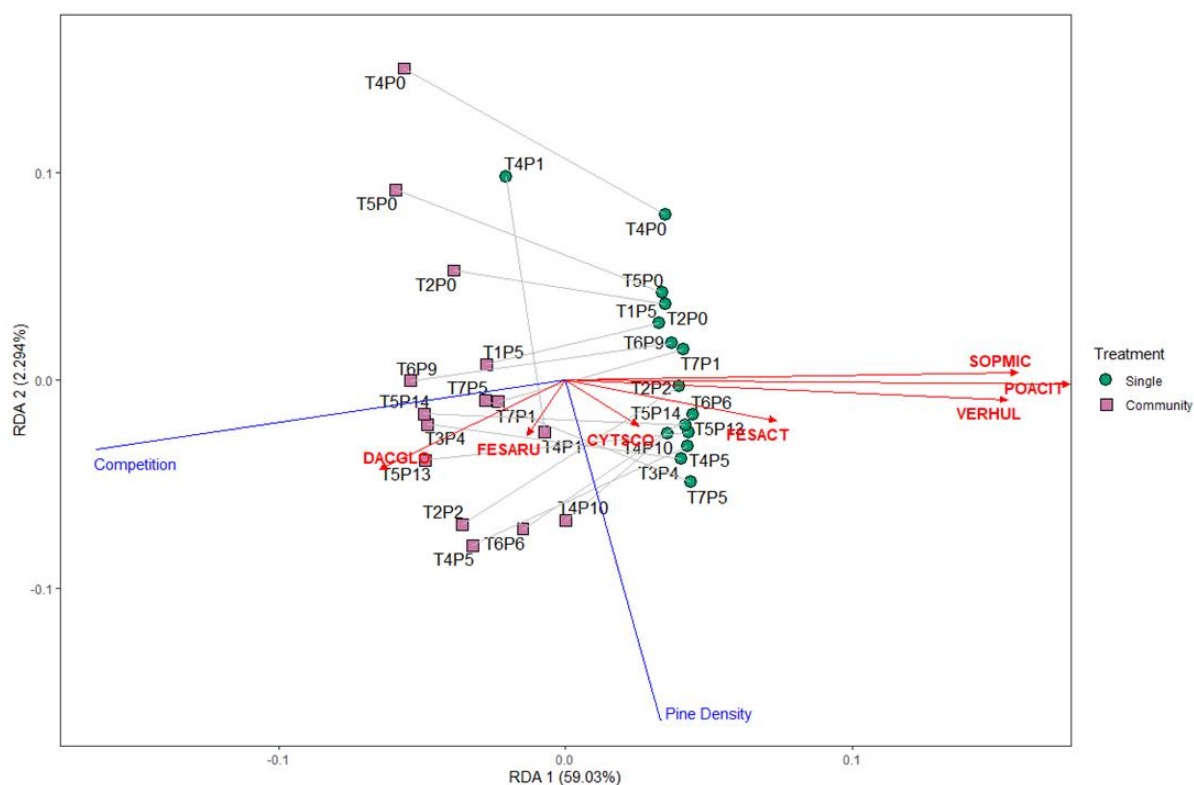
Figure 6: RDA for both treatments, red vectors represent species driving the trends, blue lines are predictor impacts, grey lines connect soils from the same plot.

Competition dramatically changes the plant community, but not the overall response to pine dominance as in the RDA, the community vs single pots create similar patterns amongst the soil sample sites; ordered comparably from top to bottom, as shown by the grey connecting lines on figure 3. All the grassland sites group together, at the top of the RDA, with an ANOVA run on the RDA for pine dominance showing a significant effect, F_1= 3.17, p = 0.04. PERMANOVA also showed competition and pine dominance to be significant for driving the scatter seen in the ordination, F_1 = 67.61, $p < 0.01$ and F_1= 5.64, p = 0.01, respectively.

Plant growth could not be explained by abiotic soil properties alone. Further, soil properties were not a substitute for pine dominance, as all these predictors fell out of models during simplification except for soil properties (pH and total carbon) that had significant interactions with competition. The contribution of pH and carbon to growth was minimal compared to the best model without abiotic soil properties (overall model increase of 0.009 in marginal R^2 and 0.005 conditional R^2 as compared to best model that does not contain soil nutrient data).

All soil properties measured are displayed in Figure 7 against the basal area to look for trends with pine dominance. With increasing pine dominance: pH decreases significantly (F_1 = 61.78,

$p < 0.01$), while phosphorus and carbon increase ($F_1 = 28.45$, $p < 0.01$ and $F_1 = 13.76$, $p < 0.01$ respectively), nitrogen slightly decreases, but the trend was not significant ($F_1 = 1.39$, $p = 0.24$). In all cases the R^2 values were generally low, with the exception of phosphorus (pH: 0.19, C: 0.05, N: 0.01, P: 0.55), so basal area alone is not a sufficient predictor of these properties even though some are significantly related to pine dominance, with the notable exception of phosphorus.
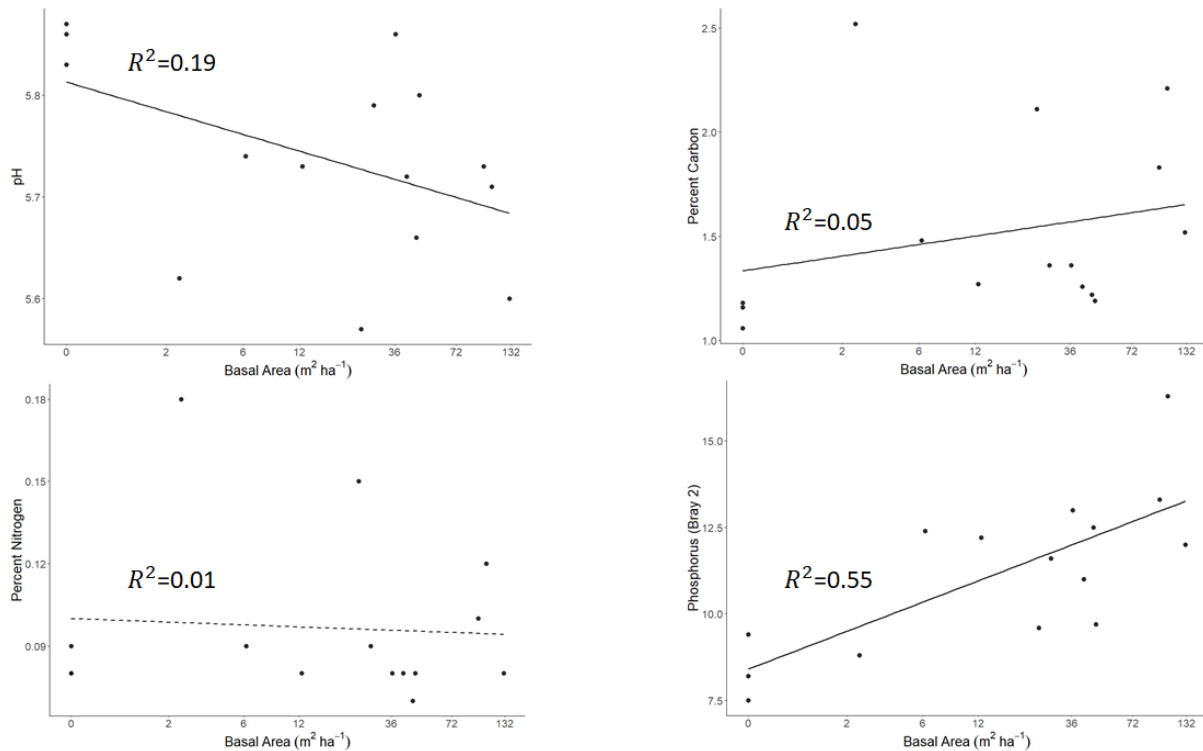
Figure 7: Soil abiotic properties data with pine dominance: solid lines indicates significant relationships. R^2 displayed per graph.

Discussion

Plant invasions affect the ecosystem and the future plant community after invasive pine removal. My work has shown that an increasing pine legacy as measured by previous pine dominance in soil legacy increases plant growth. I have shown this trend in classic single species pot experiments and multi-species community pots that allow for competition, often not done in research, although excluding competition could lead to results that are less applicable to natural settings. The growth rates were large in most exotic species in single pots and for both exotic and native grasses growing in a community.

Plant Community Response (Provenance)

Invaders by definition are strong growers who tend to supersede the resident native plants (Allen et al. 2020, Hulme 2020, Waller et al. 2020). Exotic species were found to show the most positive response after a pine invasion or in soils with a pine legacy (Dickie et al. 2014b, Waller et al. 2020). In my results, exotic plants outgrew native plants when grown in a community, though not always the case in comparable single pots. Nonetheless, the increase of growth with increasing pine dominance was not unique to exotic plants. The higher baseline growth rate for exotics was such that changes associated with increasing pine dominance did not greatly increase growth rates as compared to native plant growth. All exotic plants (except *Rosa*) outgrew native plants of the same functional group, as seen in community/competition and single species assays. All the exotic plants in my experiment are also invasive, as are many exotic species in New Zealand. A recent survey (Brandt et al. 2021) of exotic and native plant species in New Zealand found naturalized exotic species tend to be less woody overall, like grasses and herbaceous plants. Brandt et al. (2021) indicated the invasive nature of grasses often had to do with the shorter-lived and faster-growing lifestyle as compared to many woody plants.

Plant Community Response (Functional Group)

As predicted by my hypothesis, grasses increased in growth rate along the pine dominance gradient, and this trend was more significant in competition. This grass-dominance was at times obscured by the exotic competitive advantage as native grasses were greatly outperformed by exotic grasses and in some cases by the exotic shrub, *Cytisus*. Past bioassays have indicated a linkage between pines and grass success, (Dickie et al. 2014b), though these included few grass species, and no exotic grasses or shrubs. Generally grasses are the first plants to colonize disturbed soil, which is often the situation post removal of invasives like pines, (Buckley et al. 2007, Young et al. 2015, Williams et al. 2017). My research shows that a pine legacy could reinforce this early grass colonization and resulting dominance. Field observations indicate that after a longer period grasses could be shaded, and thus outcompeted by successful shrubs that can co-establish with grasses, like *Cytisus* (Young et al. 2015, Allen et al. 2020).

Treatment Response (Competition)

While competition was indicated by my models to be a major driving force behind the results, the overall trends with pine dominance remained similar amongst single and community/competition treatments. Although at a species level, individuals responded

differently per site in single versus community treatment in terms of relative growth rate (with natives having a depressed growth rate in competition, and exotics having a slight increase to neutral in competition). Growing plants in a community changed their overall growth rates, which seems intuitive, but with relatively few studies using a community competition response, it is difficult to determine if this is universally true (Stahlheber et al. 2015, Lekberg et al. 2018, Waller et al. 2020). However, competition is something that need to be considered when using plant response as an indicator, especially if looking to compare to the environment outside of experimental conditions.

There are a wide range of soil legacy and plant-soil-feedback studies, most of these are pairwise or single pots, thus missing the competition interactions found in a community. Including competition made the effect of pine dominance on plant growth generally more apparent, particularly when compared the responses from either extreme of the density gradient, as seen in the significant interactions in my mixed effects model. My work has shown that results from single plant studies might not directly translate to those same species as part of a mixed community, as seen in the species graphs (Figure 2). This limits applicability of data from single pot experiments (Dickie et al. 2014b, Levine et al. 2017, Lekberg et al. 2018), as in my experiment, competition was a much stronger predictor of outcomes than pine dominance, and in some cases changes the overall trend (as with *Cytisus* in community).

Implications for Future Management

It was unexpected that native plants in competition had a strong growth response with a pine legacy generally, even when not statistically significant and overshadowed by the competition effects. Potentially, the increasingly positive effects of soil conditions associated with pine dominance helped native plants offset the negative effects of competition at higher densities. This could be due to the increase in phosphorus availability along the pine dominance gradient (Saggar et al. 1998, Chen et al. 2002, Liu et al. 2018) (Figure 7). While there was not a large change in phosphorus overall, the difference is more apparent when comparing any mid-high pine site with the three grasslands sites, where the native plant growth was generally the lowest, particularly when in competition (Saggar et al. 1998, Dickie et al. 2014b).

This effect with pine legacy and competition could be useful in future research, applied or otherwise. As a general aside, in restoration, we could include different competitors than those in my experiment, like additional native plants or less dominant (less-invasive) exotic plants that might be able to compete with the fast-growing exotic species while lowering the competitive pressure on native species. In this way, competition within pine legacy soil might

provide a benefit to the community as a whole, and not be overbalanced by strong invasive competitors. This is similar to the idea behind successful restoration efforts using "nursery species", such as gorse (Barker 2008), to aid native plants to establish.

My research has reinforced common concerns about exotic invasive plants overtaking native ecosystems, in particular exotic grasses and brooms (*Cytisus)*, as these dominated the community pots. If land is destined to be pasture after a pine invasion, the only controls needed would be preventing bush over-growth and pine-reestablishment, which could be achieved by appropriate grazing (Dickie et al. 2022, García-Díaz et al. 2022). If restoration is desired, measures should be considered to minimize exotic plant presence and seed sources of these strong invasives, as my experiment re-affirms, once they arrive they will likely outcompete natives that attempt to co-establish.

Long-term Trajectories

Based on my experiment some longer-term potential trajectories could be hypothesized. If pines were successfully removed and no other control measures taken, most likely invasive grasses, like *Dactylis glomerata* and *Festuca arundinacea,* will dominate the ecosystem, as well as invasive shrubs like *Cytisus scoparius*. Though grasses would likely be first, *Cytisus* has the potential to overgrow these in time, as it has been noted to invade native grasslands and areas near and post-pine invasion (Allen et al. 2020). The novelty in my research comes from the fact that some natives do benefit from a pine legacy, and even native grasses benefit in competition, but the effect of competition overwhelms that. Contrary to my hypothesis, it does not appear as though a pine legacy suppresses natives or preferentially enhances exotics, simply that exotics outgrow natives regardless of any pine legacy associated benefit.

Future Directions

The benefit granted to plants post-pine cannot be exclusively attributed to common abiotic factors as measured here. Past research has implied that an increase in available phosphorus or changes in pH associated with pine growth might account for pine legacy associated growth of various plants (Saggar et al. 1998, Dickie et al. 2014b). While these trends were found in my experiment, they did not supplant pine dominance as a predictor, nor add quantitatively to model significance when included. This indicates a potentially more important biotic driver associated with a pine legacy that increases plant growth. More research needs to be done to elucidate this mechanism. Potential candidates are pine-associated fungi and microbiota, or perhaps even larger organisms such as nematodes, which have been shown to vary across a pine invasion (Dehlin et al. 2008, Peralta et al. 2019).

Overall Conclusion

A pine invasion can boost growth rate in many plant species, both exotic and native, but effects can be overshadowed by competitive abilities of many exotic plant species. Grasses in particular tend to respond to a pine legacy, perhaps due to the generally fast-growing nature of grasses compared to shrubs.

Chapter 4

Impacts of Invasive Pines on Grass Root Endophytes

Abstract

- Research and field observations have shown an association between grasses (particularly exotic grasses) and pine invasions in New Zealand, but the mechanism is unknown. Given that these pines co-invade with fungi, the mechanism could be biological. Fungi tend to interaction closely with most plants, to the point that most plants have fungal endophytes within their tissues. These endophytes could be transferred from pines to grasses or the endogenous endophytic community of grasses could be indirectly impacted by pine presence via the surrounding soil.

- I field-sampled four native and four exotic grass species from 10 paired plots within pine invasions and in nearby non-invaded grasslands. Grass root sections were surface-sterilized and cultured for endophytic fungi. Isolates from these cultures were grouped by visual morphotyping and RFLP profile to lessen redundant sequencing efforts. Representatives of each of these gruopings were analysed using Sanger sequencing.

- The root fungal endophyte community richness and composition differed amongst exotic and native grasses. It was also different in grasses growing within a pine invasion and those living in a grassland, but there was no significant interaction between the two groupings (invasion status and provenance). Further investigation of the highest relative abundance OTUs showed significant trends with either provenance or invasion status, but rarely both. OTUs associated with pine invasions and exotic plants tended to be pathogens, whereas most OTUs associated with grasslands and natives were considered symbiotrophs or saprotrophs.

- These results imply a potential for pathogen spill over or accumulation of pathogens within an invasion and this can be exacerbated by co-invasion or presence of other exotic species.

Introduction

All macro-organisms have associated microorganisms and fungi; these associations range from mutualism (Nunez et al. 2009, Hardoim et al. 2015) to varying levels of pathogenicity (Kamiya et al. 2014, Hacquard et al. 2017). Understanding these associations helps to comprehend the greater ecosystem interactions. In plants, these interactions are often connected to plant health and responses to environmental changes. There are microbial interactions associated with all stages of plant invasion and with all components of the invaded plant community (Blackburn et al. 2011, Zenni et al. 2014). A highly influential group of these microbiota are endophytic bacteria and fungi that are found within the plant tissue and can affect plant health and fitness (Pérez et al. 2016, Põlme et al. 2018, Suryanarayanan and Shaanker 2021). Many plant species, in particular grasses, maintain close relationships with endophytic fungi. These can range from generalist interactions to those that are highly specific to individual plant species (Shipunov et al. 2008, Saikkonen et al. 2010b). These endophytes often provide a variety of beneficial effects like decreasing herbivory pressure and mediating environmental stress (often in terms of drought, heat, or salinity) (Cheplick et al. 2009, Rodriguez et al. 2009, Afkhami et al. 2014), as well as protection against pathogens (Faeth 2002, Cheplick et al. 2009, Rodriguez et al. 2009, Pérez et al. 2013).

Endophytes can influence the viable growth range of both exotic and native plant species. For example Afkhami et al. (2014), whose work compared the range expansion of similar plant species with and without a specific beneficial endophyte (*Epichloë*) across environmental gradients (salinity and moisture), showed mutualistic fungal endophytes reduce drought stress and expand the range of their grass host *Bromus laevipes* with an approximately 20% increase in viable growth area. Aschehoug et al. (2012) showed evidence of increased competitive ability in the forb *Centaurea stoebe,* compared to plant species from its native range in Europe with different fungal communities. These findings suggest that specific endophytic communities can aid plant invasion.

After invasive pine removal, grasses, and in particular exotic grasses, tend to dominate the plant community (Dickie et al. 2014b, Sapsford et al. 2020). As exotic and native grasses in New Zealand share foliar fungi (Visscher et al. 2021), this has wide-ranging implications, including host jumping of fungal pathogens and mutualists. Little work has been done in New Zealand on fungal associations in wild grasses, except in terms of arbuscular mycorrhizal fungi (AMF) infection and diversity within coastal dune ecosystems (Johansen et al. 2015). While most grasses have associations with AMF, pines are ectomycorrhizal and unlikely to directly interact with AMF. Põlme et al. (2018) notes that many mycorrhizal fungi are linked to host-

identities and AMF tend to associate with specific plants. Although much research has focused on host-specific endophytes, many endophytes are actually generalists (Spear and Broders 2021, Xing et al. 2022). This indicates that even plant species that are very distantly related, like pines and grasses, might be capable of sharing endophytes. Invasive pines can change the overall fungal community and may be pathogen reservoirs (Sapsford et al. 2020, Steel et al. 2022). The microbial community disruption of invasives might not be the only disruption caused by invasion. Dassen et al. (2021) showed physical disruption of the AMF network tends to increase plant biomass and thus aid in competitive fitness, and as invasive species disrupt many ecosystems processes the disruption or restructuring of mycorrhizal networks is a potential mechanism.

In many cases of pine invasion research, or of invasive plants in general, only the native plant species' response is considered, often with an eye to restoration or loss of native ecosystems (Dickie et al. 2017). However, because pine invasions result in increased abundance of exotic grasses in the affected areas, it is important to include exotic grasses in studies looking at pine invasion effects. This is aligned with comments by (Saikkonen et al. 2010a) who also calls for a "wider and wilder range of plant-endophyte" sampling and focusing on multispecies interactions; this is still lacking in contemporary literature. My results from Chapter 2 indicated that responses by individual species are not always indicative of what happens when plants are grown together and that including both exotic and native plants can change the results.

It is not known why exotic grasses thrive after pine invasions but given the impacts that pines and pine legacies have on fungal communities and the strong interactions between grasses and their endophytes, there could be answers in the grass fungal endophyte community. I hypothesized that individual grass species have different endophytic communities within pine invasions than in nearby uninvaded grasslands due to the changes pines make in soil and the fungal community, and that this differs between native and exotic host grasses, based on host specificity.

To test this hypothesis, I selected four native and four exotic grass species commonly found at pine invasion fronts. These were collected from 10 paired plots (pine invaded and nearby grassland). Their roots were collected, surface-sterilized and plated for culturing. Culturing allowed for definitive proof that the fungi were alive and within the roots. Due to large numbers of culture isolates, I visually morphotyped all isolates and a coarse genotyping approach using Restriction Fragment Length Polymorphism (RFLP) to identify putative species (OTUs). A limited number of samples of each of these were subsequently sequenced to determine their

taxonomic identity. Sequences were grouped per plant. These groups were regarded as representative of the endophytic community from each plant and analysed by provenance (exotic or native) and by invasion status or plot type (pine or grassland).

Methods

Field Sampling

10 sample sites with 2 paired plots per site were chosen along pine invasion fronts in and along the Clarence River valley in Molesworth Station near Hanmer Springs (Figure 1).

Each plot has 2 paired sampling plots, one primarily a grassland and one with a pine presence. Within each paired plot, four native and four exotic grasses were targeted for collection (*Poa colensoi, Festuca nova-zelandiae, Chionochloa flavescens, Chionochloa rubra, Agrostis capillaris, Anthoxanthum odoratum, Festuca rubra, Dactylis glomerata*). These species were chosen because they are the most abundant grass species in the region, based on previous Manaaki Whenua | Landcare Research surveys (D. A. Peltzer & K. Orwin, unpublished data).
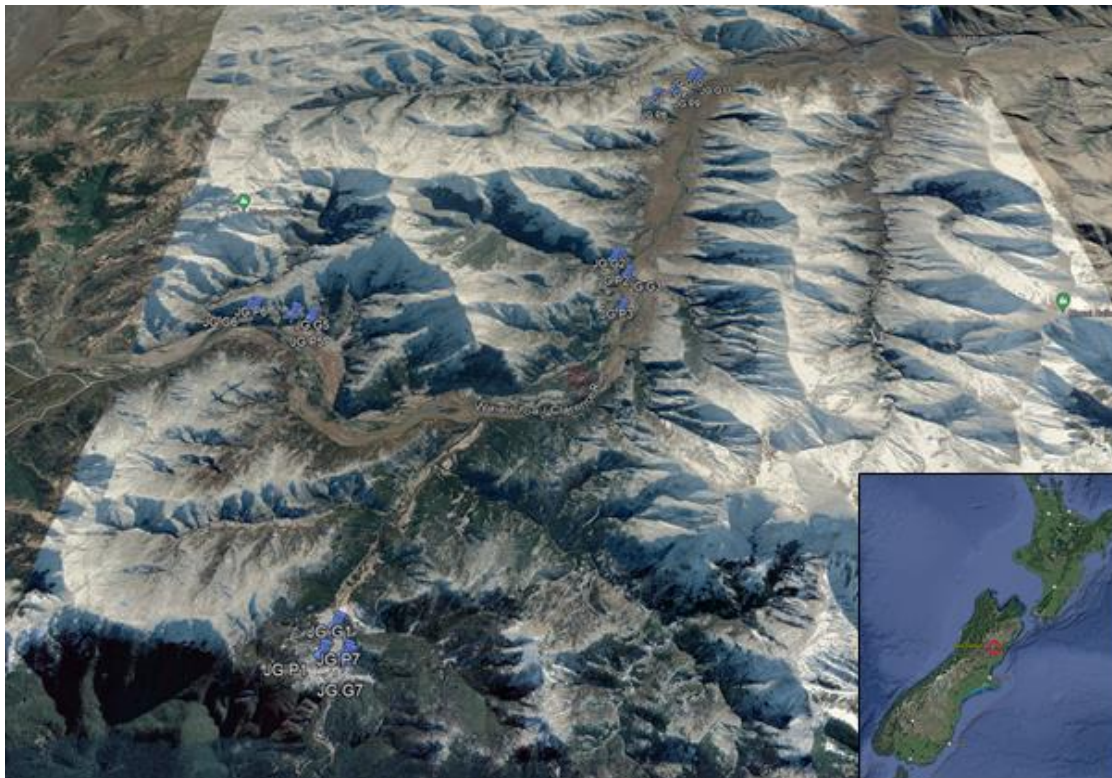
Figure 1: Google Earth image with plots as blue pins, subset for site location

Plot Site/Sample Selection

All plots were 20 x 20 m in size. Metadata collected at plot level included pine percent cover, nitrogen fixing plant species presence (including *Cytisus scoparius*, *Trifolium* spp., *Ulex europaeus*, *Discaria toumatou*) and percent cover, overall percent cover by functional group (grass, tussock grass, forb, shrub, tree), and rough estimate of exotic plant species by percent cover.

Grassland plots were at least 40 m from any established pine (1 m or more in height) and at least 10 m from any visible pine seedling. Paired pine plots had at least 10% pine cover within the 20 x 20 m sampling area.

Two individual grass plants of each of up to eight species were sampled in each plot (those in bold were present and collected at every site, others collected when present), (n=number of total individuals sampled):

Native: ***Poa colensoi (n=40)***, ***Festuca nova-zelandiae (n=40)***, *Chionochloa flavescens* (n=28)*, Chionochloa rubra (n=16)*
Exotic: ***Agrostis capillaris (n=40)***, ***Anthoxanthum odoratum (n=40)***, *Festuca rubra* (n=32)*, Dactylis glomerata* (n=32)

After the grasses were identified (by a Manaaki Whenua botanist) and GPS coordinates were taken, each individual was photographed, and observations were made about appearance, in particular noting any signs of disease. I used cleaned (10 min in 0.1% sodium hypochlorite) trowels to remove sufficient roots to fill a 305 x 440 mm sample bag.

All roots were kept cool (2 - 3°C) in clean plastic bags until processing (within 24 - 96 hours of collection). After washing in clean tap water to remove soil, each root sample was sub-sectioned into 15 lengths of root from various regions of the root sample, with a total of approximately 15-20 cm analysed for each. Root sections were handled using clean nitrile gloves (surface-disinfected with 70% ethanol) before sectioning, though after sectioning roots only touched with forceps sterilized 10 min in 1 % sodium hypochlorite, followed by three rinses in sterile autoclaved water.

Roots sections per plant were placed in histology cassettes, and surface-sterilized following Day et al. (2016) (washed in tap water, excess water removed via shaking, surface-disinfected

in 70 % ethanol for 3 min, then by 10 min in 1% sodium hypochlorite, three rinses in sterile autoclaved water, again shaking off excess water, and finally drying with autoclaved paper towels).

Culturing

Four 2 cm pieces of surface-sterilized roots were embedded per plate of acidified potato dextrose agar with 0.5 g l⁻streptomycin (PDA+S), as per Day et al. (2016). Three plates were generated from each individual plant root system.

I created a control plate for each plot per species with a random coin flip to choose a paired plot. These were made by smearing one processed root section across a PDA+S plate and then sealing the plate after discarding the root sample. This was done to determine if the surface sterilization procedure was effective.

All plates were incubated at ambient temperature (approximately 20˚C) until growth was visible (2 - 5 days). Cultures were subcultured as needed to obtain individual colonies.

Morphotyping of Cultures

Due to the large number of visible cultures, and the high likelihood that different but morphologically similar ones represent the same species, I used a morphotyping approach to reduce the number of samples that had to be sequenced. Morphotypes were grouped using visual characteristics not any specific pre-existing descriptions and erred on the side over over-splitting when unsure. Photos were taken of the top and bottom of each plate to document the morphology of each morphotype. To confirm that colonies belonging to the same morphotype are also genetically similar and therefore most likely belong to the same species (or at least a close relative), I subsequently genotyped cultures using restriction fragment length polymorphism (RFLP) analysis as described below.

DNA Extraction and PCR Amplification

DNA extraction was completed using the REDExtract-N-Amp™ PCR Kit by Sigma-Aldrich on a representative of each morphotype per plant. Primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used for PCR amplification of the Internal Transcribed Spacer (ITS) region. PCR was performed as follows: denaturation of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 57°C and 30 s at 72°C, with a final step at 72°C for 7 min (and held at 4°C). All PCRs were completed with negative controls with positive controls in initial primer testing. DNA from a soil sample collected from a site with 98% pine coverage was used

as a positive control during methods development. To confirm amplification, I performed agarose gel electrophoresis with the PCR product, using a 1% agarose gel stained with RedSafeTM (iNtRON). No PCR products were seen in negative controls and samples that showed poor amplification were rerun, positive controls all amplified strongly.

Restriction Fragment Length Polymorphism (RFLP) and Sequencing

PCR products of all large morphotype groups (those with more than 10 individuals) were genotyped using RFLP, with PCR products of smaller groups going directly to sequencing. The RFLP enzymes I used were Hinf1 and Dpn2 (in separate reactions), from New England BioLabs (Ipswich, Massachusetts, United States) (Dickie and FitzJohn 2007, Barroetaveña et al. 2010).

I used 1.5 µL of 1X CutSmart® Buffer, 0.25 µL of each restriction enzyme per reaction, 4 µL of PCR product, and 9.25 µL of PCR grade water, for a total reaction volume of 15 µL. Samples were incubated overnight at 37°C. The digests were run on 2.5% Invitrogen™ UltraPure™ low melting point agarose with RedSafe™ dye in a 0.5 X TBE buffer for 70 minutes at 100 V and 500 mA. I loaded 8 µL of the digestion product into each lane.

Gels were visualized using the Uvidoc HD2 UV photo machine and band size was estimated by comparison to 5 µL of low molecular weight DNA ladder (25 bp to 766 bp, New England BioLabs). Patterns of band sizes were analysed with GelAnalyzer (http://www.gelanalyzer.com/) and further sub-grouped as necessary again, erring on the side of splitting when unsure.

A subset of samples (three when possible) of each RFLP morphotype group was sent for Sanger sequencing at Macrogen Inc., Korea, using ITS4 as the sequencing primer. For morphotype groups with 10 or fewer individual cultures, all cultures were sequenced.

Statistical Analysis

All statistical analysis was performed using R (R Core Team 2020). First, I trimmed sequences to remove low quality base pairs at both ends following Steel et al. (2022), then grouped sequences at 97% similarity into OTUs.

The data was analysed by compiling OTUs per grass sample, and then using the R package "vegan" (Oksanen et al. 2013) to explore these communities. I used "adonis2" a permutational multivariate analysis of variance (PERMANOVA) to compare the fungal community richness based on a linear mixed effect (lmer) model with predictors of grass provenance (exotic or

native) and plot pair (within the pine invasion or nearby grassland), keeping as random effect paired-plot nested within plot, and grass species. Lmer models were run via the R package "lme4 (v1.121)" (Bates et al. 2014). Before further testing, data was visually checked for basic normality, by plotting residuals versus fitted to check for heteroscedasticity, as well as a Q-Q plot and density plot, to see if the residuals follow a normal distribution (yielding a plot with a roughly 45-degree straight line for Q-Q, and bell curve for density), Chapter 4 Appendix Figure 2. I used the "update" command for model simplification to determine which response variables best predicted the observed changes in OTUs. I created heatmaps (R function "heatmap") and clustering (clusterh in R) to further visualize the OTUs. To investigate the fungal community via ordination, I ran a Principal Coordinates Analysis (PCoA) with R package "vegan" (Oksanen et al. 2013) and base R command cmdscale (R Core Team 2020). Components were explored using scree plots and explained variance per component to determine those useful for modelling, results in Chapter 4 Appendix Figure 1 & Table 1.

I used blastn (Altschul et al. 1990) (available at http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the *NCBI* searcher (http://www.ncbi.nlm.nih.gov/) to identify each OTU. To determine functional groups, I used FUNGuild (Nguyen et al. 2016) to match OTUs to guilds and trophic modes. For more detail, I also took a closer look at some of the most abundant OTUs, including those that occurred at least 3 times on a grass sample, and more than 12 times total. These were analysed with the same mixed effect model used to test the communities. I ran a generalized linear mixed effect (glmer) from R package glmmTMB (Brooks et al. 2017) to allow for a negative-inflated binomial distribution (Aldo et al. 2010). All OTUs were processed by functional guild and trophic mode derived from FUNGuild and then modelled by functional guild using the same model predictors, with glmer via "lme4 (v1.121)" with a binomial distribution. To check overall abundance patterns across grass samples, I ran the same model on the number of cultures obtained from each grass plant. For all models, I used the R command "update" to perform model simplification, determining which response variables best predicted the observed changes based on analysis of variance (ANOVA) of the original versus updated model (Crawley 2007), Chapter 4 Appendix Table 2.

Results

A total of 2,244 cultures were obtained and identified (1,495 by direct sequencing, 350 by RFLP matching to sequenced cultures, and 399 by morphological matching to sequenced cultures). There were an average of 10.54 cultures per plant. The OTU grouping resulted in 380 unique OTUs, with an average of 7.45 OTUs per plant. Evenness in OTUs were

compared amongst treatments, with no significant difference found. There was also little to no growth on all control plates; indicating effective surface-sterilization.

To better understand the diversity in the fungal community, I looked at OTU richness (Figure 2). Richness was tested via mixed effects models, leaving only plot type and species as predictors via a significant interaction term after model simplification (final model: richness ~ Plot.Type + Species + Plot.Type:Species + (1 | Plot/Plot.Type) + (1 | Species)) (ANOVA, Plot.Type : Species, F = 2.78, p = 0.009). This indicated that the observed differences in endophyte richness are best explained by differences in richness between different species and between grassland and pine-invaded plots. Whether grasses were native or exotic was a less important factor in determining endophyte richness
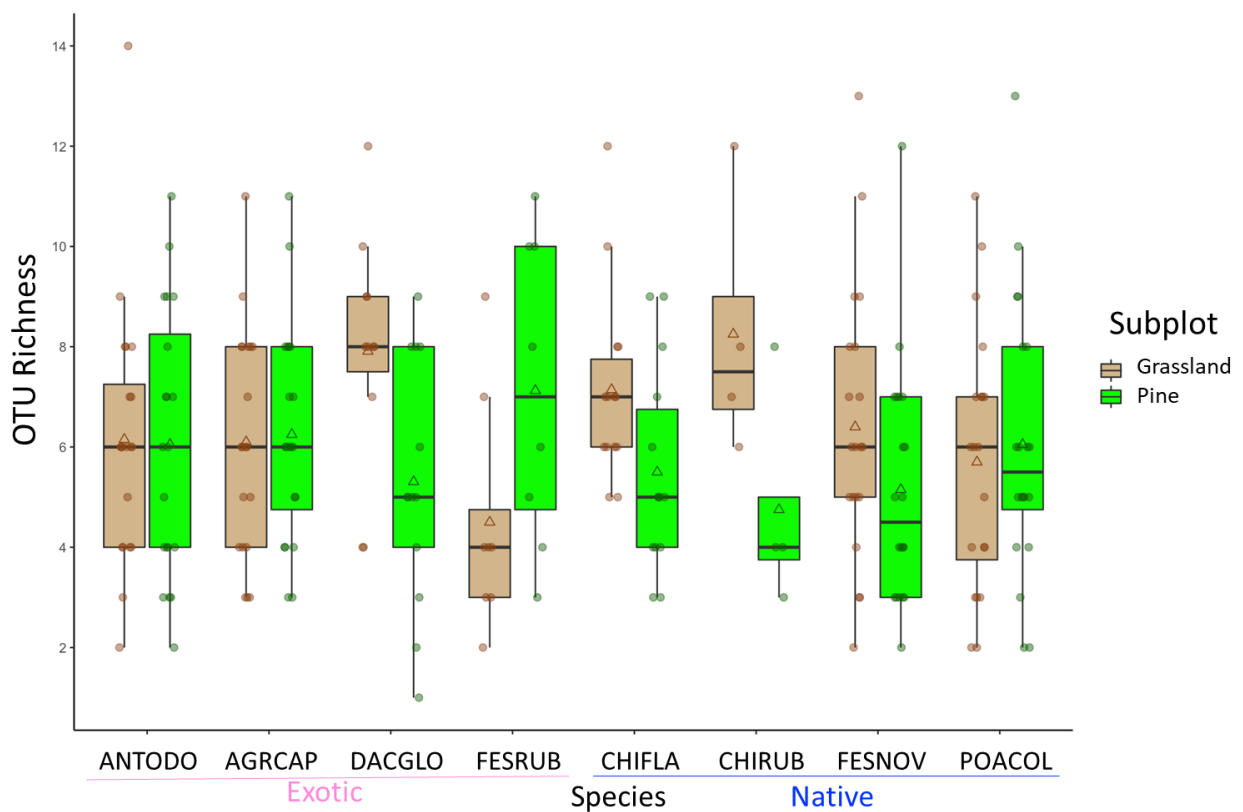
Figure 2: OTU richness per subplot by grass species, points indicate data points, triangles are the averages per species/subplot

There was a significant effect of pine invasion status (PERMANOVA; F = 3.53, p = 0.001) and species provenance (PERMANOVA; F = 4.96, p = 0.001) on OTU community composition from grass roots, but no significant interaction (Figure 3).

I used Principal Coordinate Analysis (PCoA), to visualize the diversity in the fungal root endophyte community. The first three axes explained 27.38%, 19.64%, and 8.56% of the variance respectively, with the remaining axes explaining less than 5% of the variance each. The ordination (Figure 3) and heat maps (Figures 4 & 5) show *Pyrenophora* to be the main driver of the exotic effect, while *Pezicula ericae* could drive the pine invasion effect.
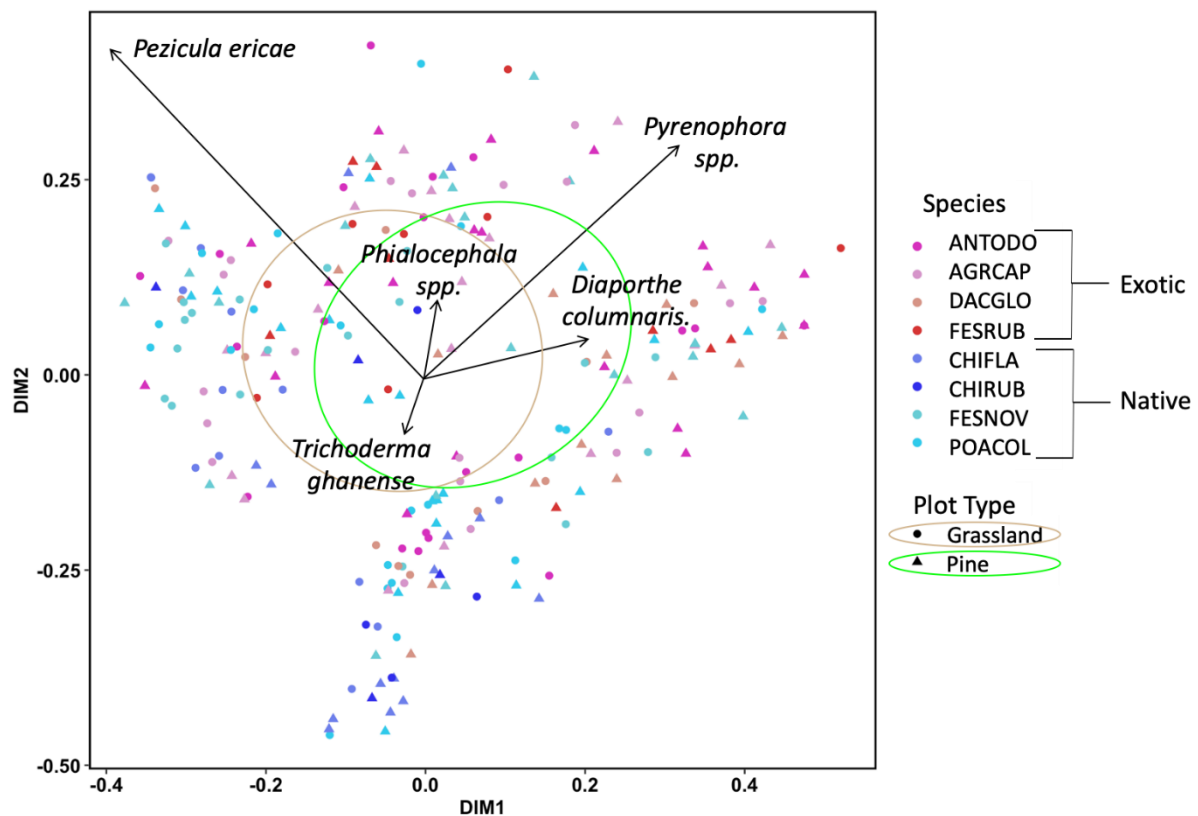
Figure 3: PCoA of fungal community composition in each grass sample. The 5 species drivers with the largest effects on community composition are shown as vectors. Exotic species are in warm colours (reds/pinks) and native in cool colours (blues/greens).

Of the top 26 most abundant OTUs analysed (i.e. those that occurred at least 3 times on any grass and occurred more than 12 times total), 14 were significantly influenced by either or both of the predictors (Table 1, Figures 4 & 5). Models did not find significant trends on OTU abundance per grass sample, indicating that the distribution of OTUs was fairly uniform across groups.
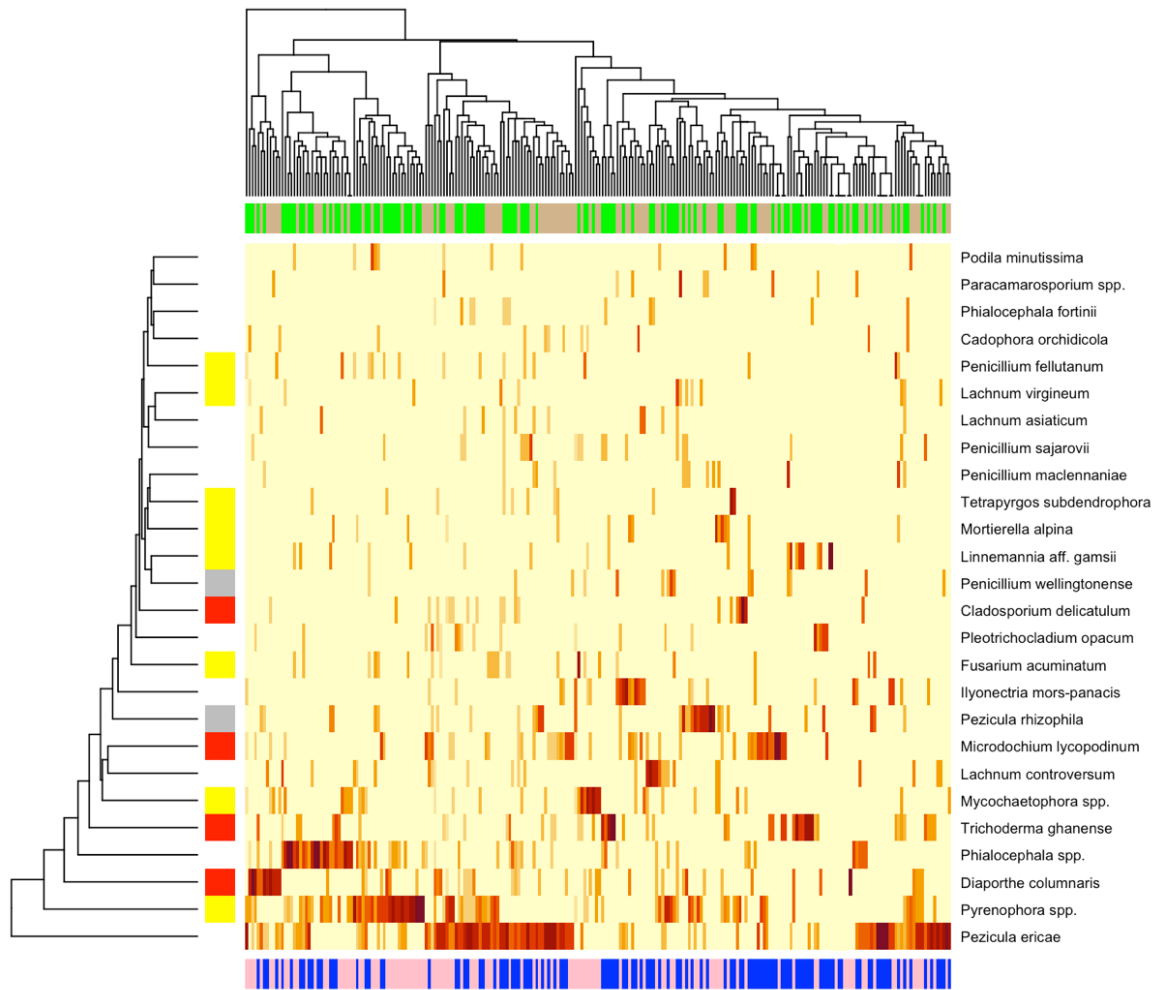
Figure 4: Heat map from most abundant OTUs, warmer colours indicated greater abundance. Individual grass samples along the x-axis. Invasion status (top bar) indicates tan for samples from grassland plots, green for samples from pine invaded plots. Provenance (bottom bar) indicates pink for exotic species, blue for native species. Significance (left bar) indicates white for no significant effect, yellow indicates significant effect of provenance, red indicates a significant effect of plot type (invasion status), and grey indicates where both are significant or the interaction term is significant (Table 1)

Table 1: Individual fungal OTUs, showing mean count when present (mean counts per plant 9.53), total frequency (out of 2,244 cultures total), provenance, and model coefficients for individual fungal OTUs showing significant responses to provenance, treatment, and their interaction.

Fungal identity	Mean Count (when present)	Total Frequency	Provenance (Native)	Pine invasion	Provenance * Pine
OTU2:	1.51	149	-0.83*	--	--

Pyrenophora spp.					
OTU79: *Diaporthe columnaris*	1.36	87	--	0.67*	--
OTU3: *Trichoderma ghanense*	1.30	65	--	1.30***	--
OTU31: *Microdochium lycopodinum*	1.18	60	--	-1.55***	--
OTU49: *Pezicula rhizophila*	1.32	49	-0.65*	-0.81**	--
OTU4: *Mycochaetophora spp.*	1.30	48	-2.07***	--	--
OTU75: *Fusarium acuminatum*	1.13	26	-0.95*	--	--
OTU46: *Cladosporium delicatulum*	1.10	22	--	1.33*	--
OTU110: *Linnemannia aff. gamsii*	1.05	21	0.95*	--	--
OTU54: *Penicillium fellutanum*	1.12	19	-1.41*	--	--
OTU53: *Mortierella alpina*	1.13	17	-1.80*	--	--

OTU21: *Penicillium wellingtonense*	1.07	16	3.58*	1.57	-3.34*
OTU82: *Lachnum virgine*	1.15	15	2.31*	--	--
OTU5: *Tetrapyrgos subdendrophora*	1.18	13	2.13**	--	--
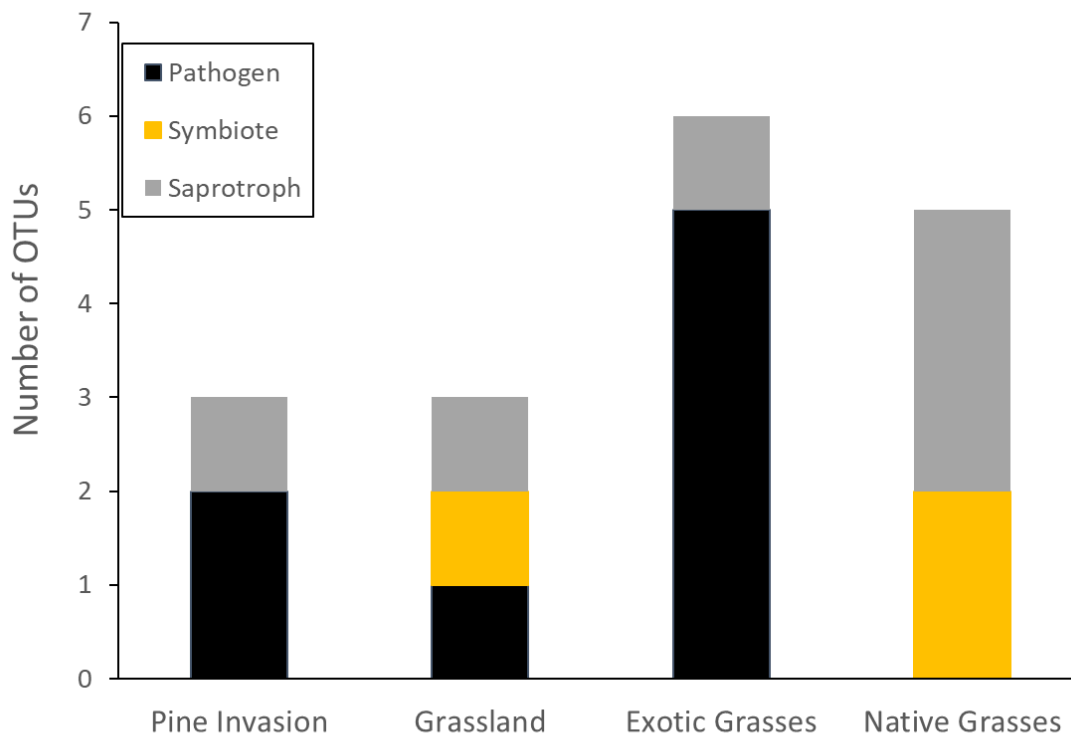
Figure 5: Top OTUs categorized by trophic mode from Table 1 which were significant in the displayed category (Provenance or Subplot), underline colours correspond to component colouring used in Figures 2-4

To further investigate the potential or most likely guild of the endophyte species (symbiotrophs, pathotrophs, and saprotrophs) were determined with FUNGuild (Nguyen et al. 2016) and analysed with mixed effects models. Some OTUs were classified in more than one guild, as could be expected of generalist fungi, in all cases results for top OTUs FUNGuild analysis was affirmed with literature review, Chapter 4 Appendix Table 3. All guilds were significantly

65

influenced by pine: symbiotrophs ($F = 8.02$, $p = 0.004$) and pathotrophs ($F = 6.60$, $p = 0.009$), while saprotrophs were significantly influenced by both pine ($F = 8.99$, $p = 0.002$) and provenance ($F = 4.63$, p = 0.032). This combined with data from Table 1 is shown in Figure 5.

Discussion

All exotic and native grasses included in this study had different fungal endophytes and differing levels of richness in grasslands as compared to pine-invaded areas. In addition, exotic grasses had different communities than native grasses across both habitats, though some endophyte species were shared. However, exotic grasses did not have a unique response to a pine presence, as the interaction term was not significant except for *Penicillium wellingtonense*. Therefore landscapes dominated by exotic grasses after a pine invasion are likely the result of a combination of a plant being exotic, thus more likely to host certain fungi, and pine effects, but not necessarily about an exotic grass and pine interaction, as I had hypothesized.

In Depth Analysis on Abundant OTUs

The community can be better understood by closer examination of the most abundant OTUs as they often drove community dynamics as seen in ordination (Figure 3) and are likely the source of many changes. To check the validity of sequence results I did a literature search of the most abundant OTUs (Chapter 4 Appendix, Table 3). 12 of the 26 identified in this study were previously documented as endophytes in grasses while 11 have been found within other plants and three in soil (Chapter 4 Appendix, Table 3). Given the generalist nature of some endophytes it is possible that endophytes usually associated with trees, forbes, or shrubs could also occur in grasses, or for soil fungi to also infect grass roots.

Presence in Aotearoa \ New Zealand

A recent search on Manaaki Whenua's Biota NZ site (biotanz.landcareresearch.co.nz) along with an additional literature review, found that 17 of my 26 most abundant endophyte species found in my study (65%) have previously been recorded in New Zealand (Chapter 4 Appendix, Table 3). The presence of four species was listed as uncertain or no status was listed, but closely related species in the same genus are listed as present (*Microdochium lycopodinum, Linnemannia aff. gamsii, Lachnum asiaticum, Cadophora orchidicola*). Three of the 26 species are listed as uncertain without any closely related species present (*Mycochaetophora, Tetrapyrgos subdendrophora, Paracamarosporium*), and only two species are currently listed as "absent" (*Penicillium sajarovii* and *Penicillium maclennaniae*) (Chapter 4 Appendix Table

3). Both *Penicillium* species came back at 100% identity, so are potentially the first documented in Aotearoa | New Zealand.

Trophic Mode Shifts across Provenance and Invasion

Most of the OTUs that showed a significant effect of provenance and were fungi mostly found in exotic plant species, are generally considered pathogens (*Pyrenophora* spp., *Mycochaetophora, Fusarium acuminatum*), whereas those that showed a significant effect of provenance and that were mostly found on native species tended towards symbiotrophy and saprotrophy (*Trichoderma ghanense, Linnemannia aff. gamsii*) (Chapter 4 Appendix Table 3). The OTUs that were significantly more abundant in pine plots were also identified as likely pathogens (*Diaporthe columnaris, Cladosporium delicatulum*) (Chapter 4 Appendix Table 3). Even though not significant in individual OTU models, it does appear that *Pezicula ericae* is a main driver in the invasion plot type effect as seen in the ordination as a strong vector (Figure 3) and heat maps (Figure 4) as dominating in many plants, though likely a trend reinforced by *Diaporthe columnaris* as the vector on the ordination also pulls towards the general grouping of invasion type shown by the ellipses (Figure 3). *Pyrenophora* seems to be a main factor behind the exotic clustering as it is more abundant in exotic species, significantly associated as shown in Table 1. Most OTUs occurred across both exotic and native grasses similar to what was seen by Visscher et al. (2021) on foliar fungal endophytes.

The higher abundance of pathogenic OTUs on exotic grasses and in pine invasion plots reinforces the potential for exotic grasses to act as reservoirs for pathogens and that the pine presence could lead to greater community pathogen load. While exotic plant species have been found to accumulate pathogens, it often does not negatively impact their growth (Flory and Clay 2013), and could lead to spillback into the native plant species. It appears provenance might be a less significant driver of fungal community compared to overall genetic similarity; as plants phylogenetically related are more likely to share fungi than those that are from the same region but from vastly different species (Koyama et al. 2019). Invasion from plants similar to natives might be more likely to directly interfere with the fungal community, so while pine invasion may indirectly change grass root fungal associates, the exotic grasses might cause more direct impacts on native grasses.

Limitations

My results are based on culturing and therefore have the expected culturing bias (Hyde and Soytong 2008). This means that they might not capture rare and slow-growing or otherwise less competitive fungi as noted in recent comparative (culture vs. direct sequencing) work by

Høyer and Hodkinson (2021).The benefit of culturing is that the fungi found are definitively alive and living within the roots. Also, I did collect comparable root samples for direct sequencing, which have been stored at -80˚C for future work. The results presented here may provide strong evidence for funding such research.

Overall Conclusion & Implications

While my work does not directly measure mechanisms, it does indicate a framework for potential pathways behind the exotic grass dominance post-pine. Further, my work addresses the need for more endophyte research as identified by Saikkonen et al. (2010a), particularly as it is from an under-studied group (wild grasses in Aotearoa | New Zealand) and investigates multi-species interactions (pines and various species of grasses). Removing and controlling pine invasions is a costly venture, especially if restoration is the goal. Managers should be aware that interactions with grasses and pines can create a soil legacy that remains and will affect future plant communities, leading to an environment that favours exotic grasses.

Chapter 5

General Discussion

Overview

My PhD thesis investigates the soil-based interactions between invasive plants and plant community, using invasive pines in Aotearoa | New Zealand as a model system. Pines are of great local concern in New Zealand as they are rapidly spreading, with roughly 30% of New Zealand, being susceptible to invasion and with current rates of spread of 5–6% per annum (Howell 2016) and costing $5.3 billion if no action is taken (Wyatt 2018). Unfortunately, many of the current restoration efforts can be ineffective in the long term as invasive plants, such as pines, can leave lasting soil legacies that can persist long after their removal (Dickie et al. 2014b). My work addresses a need for better understanding how the soil legacy can differentially affect specific members of the plant community, leading to plant communities different than those before or during the invasion. These legacy effects can help direct management practices depending on the intended end goal for an invaded, or previously invaded site.

Key Findings per Research Chapter

Chapter 2

Chapter two proved that a manipulation in plant community legacy factors resulted in a significant difference in plant response, measured as seedling biomass. I have shown that exotic-dominated and pine legacies led to larger exotic plants, specifically in pine and broom seedlings. This suggests a potential for re-invasion or invasional melt-down in areas where invasive plant species have dominated in the past. While there was not a change in mycorrhization of pine roots, there was a difference in fungal community identity due to legacy factors, resulting in essentially three distinct community types, one dominated by *Wilcoxina*, one dominated by *Inocybe* and one variable community not dominated by either of these species. The only significant predictor of biomass involving the fungal community was low diversity, though neither dominant fungal species was significant on its own, but low Shannon

diversity was driven by a pine presence, thus showing a potential mechanism for the pine legacy effect seen as a boost in pine seedling growth.

Chapter 3

Chapter three further reinforced the importance of plant legacies in soil, but potentially more importantly showed a community/competition context can change results, a caveat to potentially apply to results from my chapter two, which only used single species pots as indicators. The grasses grown in pine soil had high biomass as did the *Cytisus scoparius* plants (Chapter 2). This suggested shrub and grass species be used in chapter three. In chapter three, all plants seemed to benefit from a pine presence, but the effect was almost more so for native plants but not a sufficient boost in community/competition assays to overcome the inherent competitive advantage of exotics, in particular grasses.

Chapter 4

Based on the findings from previous chapters, in addition to field observations, I decided to focus on the pine-grass connection for chapter four. Endophytes were chosen as a potential biological source of changes due to their close connection to host plant health and interactions with changes in soil. Chapter four showed a difference in fungal endophytic community in exotic grasses versus native and a difference in response from pine invasion versus uninvaded grassland. Exotic grasses and those growing near pine had a greater abundance of potential pathogens, though many generalists are shared amongst all grasses.

Integration

Together these experiments investigate the topic of soil legacy effects between invasive pines and plant communities near these plant invasions. Chapter two showed that various legacy factors within the resident community could potentially promote invasion, the strongest being a large presence of exotic plants and a pine presence. These factors increased growth in both *Pinus contorta* and *Cytisus scoparius*, which are both exotic invasive plant species as well, so could lead to a feedback of enhancing other exotics or invasive plant species and possibly an invasive meltdown. While chapter two showed any pine presence could increase growth, chapter three showed that greater pine presence (via dominance of pines) can boost growth of most plants, but that when grown in a community the exotic plants, in particular grasses, dominated, overriding any potential benefit of the legacy to native plants. To follow up on the pine to grass connection and to look for potential biological drivers associated with a pine legacy, chapter four examined grass root endophytes from within pine invasion sites and in

nearby uninvaded grasslands. The endophytes showed a greater proportion of potentially pathogenic species within exotic grasses and within all grasses from pine invasion sites. This could be part of the reason why exotics dominate in competition, a tolerance to pathogens, or pathogen spill over into native plant species from pathogens living in exotics plant species. Regardless, my work shows a connection between pines and community, primarily in self-promoting and promoting other exotics and grasses.

Limitations

Knowing what I know now, there are several suggestions I would make if anyone chose to do similar work. My chapter three demonstrated the importance of community context, with implications for any future work on invasive plant legacies as well as other plant-soil-feedback studies. I only used single species pots for my chapter two, which might limit the applications of the result when considering the results from my chapter three. In my research chapter three I did not consider the previous plant community (though much of that data is available via Manaaki Whenua | Landcare Research vegetation surveys) which given results from chapter two could provide impactful legacy effects as well. I could use recent vegetation surveys from Manaaki Whenua | Landcare Research of the sample plots I used and apply the inherent legacy factors to my results. In some cases I did not process potentially pertinent soil chemistry measurements, which could explain some of the variance seen; for chapter two phosphorus was not measured, and no chemical measurements were made for the soil associated with the grasses sampled for endophytes in my chapter four.

There were also some technical limitations that could be improved. I would not directly seed grasses into live soil with other resident grass seeds, it is difficult to correctly identify sprouting grass seedlings, though I could have asked advice from a botanist to improve my identification and to potentially avoid some of the weeding errors that occurred.

I would have liked to have more replicates in most experiments, but time and space were limiting. Also I had a large amount of difficulty finding non-invaded grassland sites near any pine invasions, to the point where many of my grassland sites were actually control sites which had at least some limited pine presence.

In an ideal world many of these concepts would be tested over longer time scales than the growing periods I used, but my time was tightly constrained. However, the fact that there were measurable responses most likely indicates that the ecologically short time scale of my

experiments was sufficient for an impact; and that the short term differences in seedling growth could influence long-term trajectories.

Implications for Future Research and Applications

Community Context

Results from chapter three could have wide-reaching impacts as much plant-soil feedback and soil legacy research only uses one or a few indicator species (Kulmatiski et al. 2008, Van der Putten et al. 2013, Nuske et al. 2021) and rarely in a community context that allows for competition (Waller et al. 2020). Considering the different results from my community competition assays, findings from single species or limited competitive inclusion research, like pairwise only, could end with potentially misleading results, which might never occur in natural systems outside of a controlled greenhouse manipulative experiment.

Fungal Networks and Host Preferences

Findings from chapters two and four indicate that fungal associations with plant species are complicated and that there are more generalists and less species-specific associations than expected. On a larger scale though, differences in OTU abundances and overall richness were found to be significant amongst exotic and native host plants and affected by legacy factors such as exotic plant dominance and pine presence. Given my results with generally under-studied wild endophytic fungi in grasses (unlike the well-studied endophytes within agriculture), it seems worth investigating endophytes from other plants near invasions, particularly other invasives, perhaps like the shrub *Cytisus.* This could be greater strengthened by applying these findings in a controlled manipulated environment or creating a truly reciprocal system using both pines and grasses in bi-directional assays.

Implications for Management and Restoration

Given the nature of invasive pines and the extensive efforts in New Zealand and worldwide invested in control, being aware of legacy effects is quite relevant. As my work has shown *Cytisus* and exotic grasses tend to highly benefit from pine presence and that they also can dominate the plant community with a pine legacy. If restoration is the end goal for control efforts it would be prudent to manage other exotic plant species, in presence and seed sources, and or plant native species or nursery species to assist the re-establishment of a native grassland

Closing Remarks

My research has shown that plant community and invasive plant species legacy effects can be short-term though impactful on the plant community. Also my experiment including a community competition assay showed the importance of a community when measuring a community legacy. While much of my work infers a potential difficulty in restoration of invaded grasslands, as invasive species tend to boost themselves and other invasive species, the results can inform better management practices and assist in potentially greater long-term success in controlling invasive pines.

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Chapter 2 Appendix

37-P	92-P	111-C	33-P	148-H
145-H	44-C	87-H	159-P	50-H
104-H	85-H	141-C	92-C	64-P
122-C	153-P	14-P	46-C	140-C
124-P	162-P	171-C	148-P	3-H
9-H	54-H	68-C	40-P	173-C
158-H	128-H	42-P	116-P	78-H
126-H	66-H	88-C	43-P	10-H
31-C	174-H	172-H	46-P	130-P
76-H	120-C	57-P	158-P	150-C
176-P	151-H	155-C	93-C	61-P
139-P	80-H	91-H	30-C	11-C
140-P	20-P	67-P	23-P	103-C
84-C	50-C	51-C	45-C	180-P
23-C	26-C	75-P	70-C	173-P
102-C	18-P	168-P	75-H	153-C
58-H	74-H	123-C	180-H	48-H
74-P	72-C	109-C	143-H	96-P
158-C	12-H	13-H	85-P	164-C
169-H	112-C	140-H	72-P	104-C
156-H	38-C	105-C	65-H	7-H
34-P	10-C	152-H	77-P	142-C
82-P	135-H	114-P	141-P	39-C
154-H	119-P	32-H	160-P	17-P
117-H	176-H	46-H	122-P	104-P
63-P	64-H	132-P	59-C	112-H
129-C	107-P	111-H	81-C	45-H
139-C	43-H	41-C	166-P	139-H
179-H	133-P	127-C	148-C	50-P
55-P	28-P	122-H	87-P	22-H
52-P	29-C	12-C	79-P	65-C
58-C	124-C	63-H	128-C	106-C
171-H	13-P	163-C	149-H	101-H
62-C	71-H	97-P	2-H	155-P
59-H	99-P	121-H	152-P	24-H
30-P	102-P	8-P	156-C	130-H
115-H	107-C	165-H	76-C	70-P
159-H	141-H	123-P	51-P	159-C
16-H	94-H	36-H	5-P	103-H
150-H	54-C	53-H	171-P	67-H
60-P	83-H	68-P	174-C	121-C
96-C	7-P	113-C	87-C	70-H
51-H	14-H	88-P	114-H	91-P
12-P	118-C	27-P	144-C	68-H
120-P	53-P	55-C	124-H	33-H
86-C	19-P	49-H	142-P	9-C
151-P	138-H	62-P	131-C	55-H
32-P	103-P	101-C	116-C	154-C
64-C	66-P	57-C	60-H	44-H
14-C	154-P	142-H	168-H	81-P
1100C	112-P	155-H	127-H	114-C
131-H	36-C	97-C	22-P	162-H
10-P	126-P	136-C	20-H	144-P
39-P	29-H	60-C	35-H	63-C
21-C	15-P	5-C	82-C	164-P
170-H	177-P	91-C	7-C	108-H
95-H	22-C	150-P	102-H	125-P
6-P	137-H	30-H	9-P	16-P
3-P	119-H	56-C	24-P	72-H
167-C	78-P	106-P	77-H	40-H

175-H	35-C	146-P	78-C	157-H	95-C	80-C	32-C
35-P	163-H	41-P	100-H	48-P	169-P	11-H	177-C
126-C	127-P	130-C	25-C	74-C	27-C	84-H	89-P
52-H	137-P	145-P	83-C	164-H	166-H	65-P	168-C
167-H	176-C	134-H	89-H	108-P	11-P	38-P	81-H

172-C	21-H	80-P	23-H	138-P
61-C	165-P	8-H	34-H	110-P
42-H	6-C	29-P	4-P	44-P
105-H	62-H	3-C	157-P	79-H
1-C	16-C	97-H	177-H	143-C
135-P	1-H	156-P	41-H	133-C
172-P	100-C	105-P	71-C	173-H
101-H	20-C	134-C	24-C	13-C
98-H	108-C	109-P	67-C	147-P
129-P	136-P	69-C	169-C	144-H
132-C	85-C	178-H	180-C	167-P
93-P	128-P	31-H	49-C	52-C
149-P	83-P	143-P	42-C	99-C
120-H	37-H	113-P	146-H	175-C
151-C	61-H	26-H	86-P	131-P
39-H	136-H	82-H	109-H	147-H
84-P	49-P	6-H	28-C	25-H
5-H	147-C	175-P	115-C	160-H
90-H	69-H	149-C	117-P	89-C
157-C	33-C	94-C	79-C	27-H
73-P	56-H	94-P	118-P	121-P
17-C	47-P	107-H	18-H	66-C
110-H	161-H	146-C	135-C	2-C
4-C	179-C	178-P	53-C	8-C
163-P	152-H	71-P	88-H	98-P
25-P	36-P	86-H	54-P	17-H
69-P	160-C	99-H	21-P	165-C
134-P	111-P	106-H	15-C	162-C
2-P	170-C	166-C	153-H	123-H
48-C	31-P	73-C	18-C	116-H
37-C	90-P	45-P	178-C	93-H
47-C	145-C	137-C	77-C	179-P
133-H	113-H	28-H	138-C	34-C
26-P	59-P	73-H	161-P	118-H
132-H	95-P	100-P	58-P	40-C
1-P	117-C	129-H	19-C	98-P
38-H	115-P	170-P	4-H	57-H
15-H	43-C	119-C	161-C	125-H
96-H	75-C	92-H	90-C	125-C
56-P	174-P	47-H	19-H	76-P

Figure 1: Potting Diagram for Chapter 2. Numbers indicate code associated with community legacy soil, letters for seedling (P= *Pinus contorta*, C= *Cytisus scoparius*, H= *Holcus lanatus*)

Figure 2: *Holcus* seeding diagram

Tan circles represent seeds, black circles indicate planting locations within pot. This was an attempt to be able to identify emerging seedlings are those that were planted.

Figure 3: Experimental processing diagram

This flow diagram indicates soil treatment and pine legacy factors during each legacy phase.
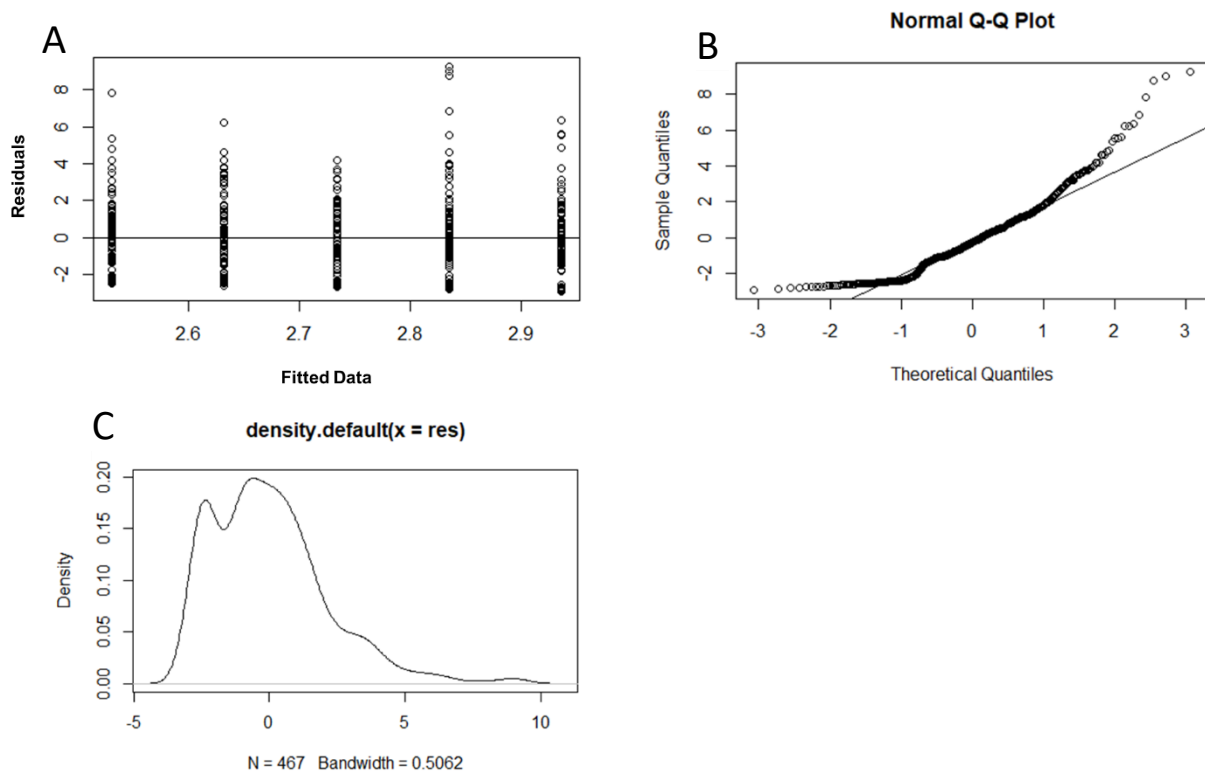
Figure 4: Normalcy testing of seedling biomass data

A: residual vs. fitted plot, B: Q-Q plot, C: density plot

Table 1: Model simplification process for pine seedling biomass modelling as shown in Chapter 2 Table 2, last row, most complicated model, same procedure used for all model simplifications

Model # : Equation	Compare (model numbers)	*AIC*	*BIC*	*P*
Model 1: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1: Pine Phase 2 + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass + Pine Phase 2:Grass + Exotic:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers + Pine Phase 1:Exotic:Grass + Pine Phase 2:Exotic:Grass + Pine Phase 2:Exotic:N-fixers + Exotic:Grass:N-fixers		352.41	432.036	
Model 2: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1: Pine Phase 2 +Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass +Pine Phase 2:Grass + Exotic:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers +Pine Phase 2:Exotic:Grass + Pine Phase 2:Exotic:N-fixers + Exotic:Grass:N-fixers	anova(1,2)	359.64	436.21	0.97
Dropping 3-way interaction (Pine Phase 1: Exotic : Grass) did not make a significant difference to the model				
Model 3: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 +Soil Nutrients Axis 2 + (1 |	anova(2,3)	361.19	434.69	0.65

Plant_community_number) + Pine Phase 1: Pine Phase 2 + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass + Pine Phase 2:Grass + Exotic:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers +Pine Phase 2:Exotic:Grass + Exotic:Grass:N-fixers				
Dropping 3-way interaction (Pine Phase 2: Exotic : N-fixers) did not make a significant difference to the model				
Model 4: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1: Pine Phase 2 + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass + Pine Phase 2:Grass + Exotic:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers + Exotic:Grass:N-fixers	anova(3,4)	364.82	435.26	0.55
Dropping 3-way interaction (Pine Phase 2: Exotic : Grass) did not make a significant difference to the model				
Model 5: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1: Pine Phase 2 + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass + Pine Phase 2:Grass + Exotic:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers	anova(4,5)	370.92	438.30	0.04
Dropping 3-way interaction (Exotic : Grass: N-fixers) did not make a significant difference to the model (using restricted $p< 0.01$ for 3-way interactions)				

Model 6: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 +Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1: Pine Phase 2 + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass + Pine Phase 2:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers	anova(5,6)	373.86	435.11	0.97
Dropping 2-way interaction (Exotic : Grass) did not make a significant difference to the model				
Model 7: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 +Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1: Pine Phase 2 + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers	anova(6,7)	373.83	432.02	0.54
Dropping 2-way interaction (Pine Phase 2: Grass) did not make a significant difference to the model				
Model 8: Seedling Biomass ~ Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:Grass + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers	anova(7,8)	373.83	432.02	0.62
Dropping 2-way interaction (Pine Phase 1: Pine Phase 2) did not make a significant difference to the model				

Model 9: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic + Pine Phase 2:Exotic + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers	anova(8,9)	374.85	429.97	0.83
Dropping 2-way interaction (Pine Phase 1: Grass) did not make a significant difference to the model				
Model 10: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic + Pine Phase 1:N-fixers + Pine Phase 2:N-fixers + Exotic:N-fixers + Grass:N-fixers	anova(9,10)	374.07	426.14	0.16
Dropping 2-way interaction (Pine Phase 2: Exotic) did not make a significant difference to the model				
Model 11: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic +Pine Phase 1:N-fixers + Exotic:N-fixers + Grass:N-fixers	anova(10,11)	373.01	422.01	0.15
Dropping 2-way interaction (Pine Phase 2: N-fixers) did not make a significant difference to the model				
Model 12: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 1 + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase	anova(11,12)	372.46	418.39	0.32

1:Exotic + Pine Phase 1:N-fixers + Grass:N-fixers				

Model 13: Seedling Biomass ~ Pine Phase 1 + Pine Phase 2 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic + Grass:N-fixers	anova(12,13)	371.41	414.28	0.32

Dropping 2-way interaction (Pine Phase 2: N-fixers) did not make a significant difference to the model

Model 14: Seedling Biomass ~ Pine Phase 1 + Exotic + Grass + N-fixers +*Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic + Grass:N-fixers	anova(13,14)	366.00	405.75	0.66

Dropping Soil Nutrients Axis 1 did not make a significant difference to the model

Model 15: Seedling Biomass ~ Pine Phase 1 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + *Inocybe* Dominated + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic + Grass:N-fixers	anova(14,15)	364.72	397.69	0.32

Dropping Pine Phase 2 did not make a significant difference to the model

Model 16: Seedling Biomass ~ Pine Phase 1 + Exotic + Grass + N-fixers + *Wilcoxina* Dominated + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic + Grass:N-fixers	anova(15,16)	360.94	397.69	0.14

Dropping Inocybe Dominated did not make a significant difference to the model

Model 17: Seedling Biomass ~ Pine Phase 1 + Exotic + Grass + N-fixers + Shannon	anova(16,17)	358.82	392.51	0.15

Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic +Grass:N-fixers				
Dropping Wilcoxina Dominated did not make a significant difference to the model.				
Model 18: Seedling Biomass ~ Pine Phase 1 + Exotic + Grass + N-fixers + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number) + Pine Phase 1:Exotic	anova(17,18)	363.17	393.80	0.06
Dropping 2-way interaction (Grass: N-fixers) barely did not make a significant difference to the model, though it was close to threshold of keeping.				
Model 19: Seedling Biomass ~ Pine Phase 1 + Exotic + Grass + N-fixers + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number)	anova(18,19)	364.42	391.98	0.07
Dropping 2-way interaction (Pine Phase 1: Exotic) did not make a significant difference to the model, though it was close to threshold of keeping.				
Model 20: Seedling Biomass ~ Pine Phase 1 + Exotic + Grass + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number)	anova(19,20)	361.25	385.75	0.50
Dropping N-fixers did not make a significant difference to the model				
Model 21: Seedling Biomass ~ Exotic + Grass + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number)	anova(20,21)	358.16	379.60	0.46
Dropping Pine Phase 1 not make a significant difference to the model				
Model 22: Seedling Biomass ~ Exotic + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number)	anova(21,22)	358.45	376.83	0.14
Dropping Grass not make a significant difference to the model				
Model 23: Seedling Biomass ~ Exotic + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number)	anova(22,23)	358.45	376.83	0.14
Dropping exotic made a significant difference to the model				

Model 24: Seedling Biomass ~ Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number)	anova(23,24)	359.99	375.30	**0.02**

Final Model: Seedling Biomass ~ Exotic + Shannon Diversity + Soil Nutrients Axis 2 + (1 | Plant_community_number)

A

B
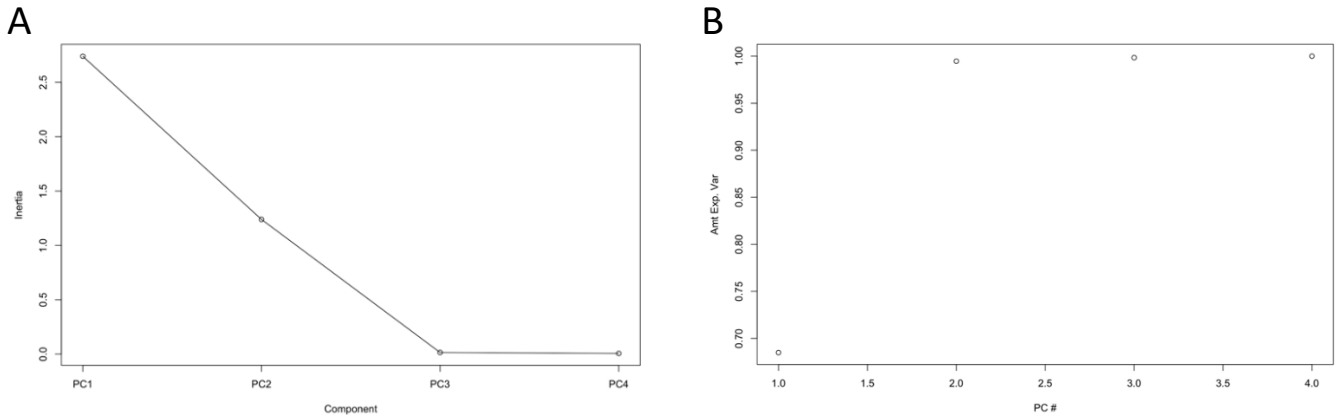
Figure 5: Nutrient PCA component exploration

A: scree plot indicating PC1 & PC2 most relevant, B: Amount of variance explained per component plot indicating PC1 most relevant and that PC3 & PC4 contribute less than 1% each

Table 2: Correlation for nutrient PCA to ordination components

	Dim.1	Dim.2	Dim.3	Dim.4
AN	0.604	0.389	0.007	0.000
tC	0.697	0.300	0.000	0.003
OM	0.696	0.301	0.000	0.003
AMN	0.743	0.249	0.008	0.000

A

B
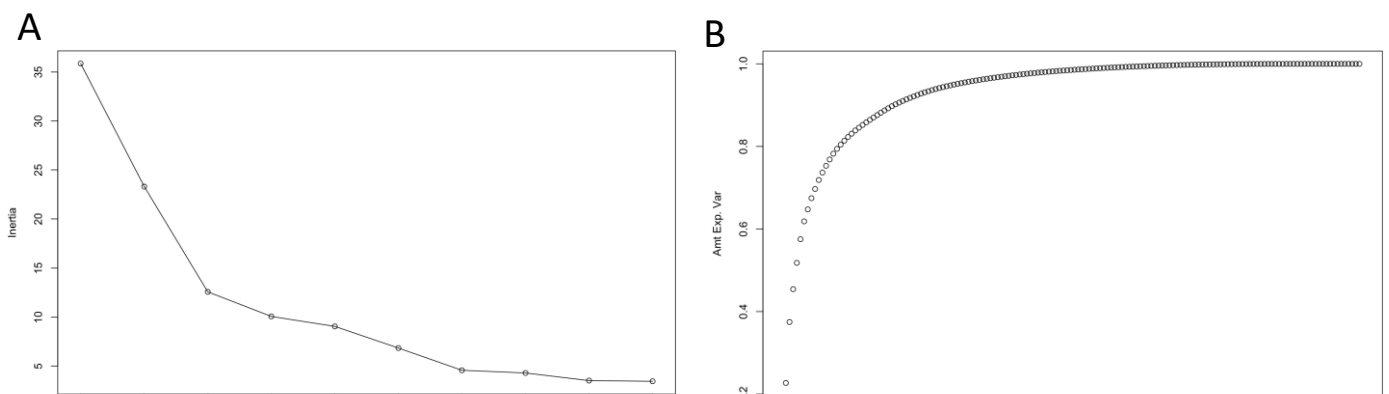
Figure 6: Fungal community PCoA component exploration

A: scree plot indicating PC1 – PC4 covering over 50% variance, B: Amount of variance explained per component plot shows PC5-6 begins the flattening of the curve indicating the less contributing components falling later

Table 3: Correlation for fungal community PCoA to ordination components, each row represents a community

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7
0.281	0.023	0.012	0.218	0.001	0.011	0.001
0.002	0.663	0.115	0.007	0.095	0.008	0.002
0.083	0.012	0.011	0.102	0.013	0.041	0.281
0.180	0.008	0.026	0.400	0.085	0.040	0.028
0.464	0.312	0.101	0.000	0.073	0.016	0.000
0.577	0.027	0.001	0.014	0.021	0.116	0.001
0.124	0.006	0.029	0.346	0.049	0.017	0.006
0.556	0.276	0.043	0.000	0.068	0.020	0.000
0.356	0.014	0.130	0.011	0.028	0.066	0.054
0.169	0.009	0.038	0.141	0.022	0.004	0.066
0.007	0.015	0.002	0.002	0.061	0.051	0.010
0.065	0.807	0.013	0.001	0.073	0.018	0.000
0.517	0.030	0.060	0.006	0.072	0.008	0.030
0.311	0.010	0.374	0.003	0.002	0.065	0.001
0.466	0.319	0.052	0.005	0.095	0.020	0.001
0.126	0.308	0.008	0.007	0.290	0.012	0.054
0.369	0.012	0.473	0.005	0.007	0.004	0.004
0.036	0.014	0.113	0.194	0.101	0.169	0.000
0.532	0.278	0.039	0.000	0.073	0.017	0.000
0.010	0.001	0.040	0.004	0.053	0.003	0.172
0.047	0.004	0.004	0.253	0.011	0.086	0.018
0.312	0.008	0.000	0.036	0.002	0.131	0.009
0.447	0.024	0.140	0.106	0.001	0.003	0.004
0.208	0.031	0.020	0.065	0.040	0.064	0.021
0.107	0.006	0.095	0.037	0.000	0.093	0.016
0.272	0.010	0.017	0.005	0.001	0.202	0.001
0.024	0.015	0.034	0.089	0.036	0.025	0.011
0.466	0.018	0.211	0.001	0.000	0.012	0.001
0.512	0.293	0.041	0.000	0.083	0.018	0.000
0.229	0.007	0.017	0.354	0.108	0.039	0.043
0.065	0.744	0.008	0.000	0.064	0.011	0.002
0.537	0.035	0.041	0.023	0.105	0.006	0.096
0.307	0.023	0.154	0.171	0.044	0.143	0.004
0.266	0.010	0.353	0.001	0.037	0.002	0.000
0.088	0.004	0.006	0.002	0.030	0.031	0.001
0.071	0.005	0.024	0.007	0.021	0.045	0.390
0.235	0.008	0.561	0.029	0.029	0.007	0.000
0.387	0.012	0.280	0.016	0.023	0.063	0.000
0.054	0.754	0.002	0.000	0.020	0.001	0.000
0.343	0.016	0.171	0.125	0.010	0.018	0.000
0.264	0.010	0.425	0.024	0.041	0.001	0.000

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7
0.037	0.698	0.013	0.002	0.085	0.032	0.007
0.245	0.016	0.048	0.001	0.000	0.207	0.010
0.081	0.779	0.006	0.002	0.051	0.011	0.000
0.005	0.675	0.001	0.033	0.194	0.014	0.006
0.174	0.003	0.007	0.001	0.081	0.015	0.035
0.109	0.011	0.065	0.151	0.006	0.022	0.000
0.085	0.003	0.048	0.000	0.045	0.022	0.000
0.444	0.251	0.025	0.000	0.069	0.017	0.004
0.272	0.009	0.005	0.407	0.075	0.006	0.003
0.014	0.001	0.011	0.000	0.034	0.022	0.006
0.235	0.014	0.010	0.103	0.000	0.102	0.060
0.104	0.031	0.131	0.023	0.143	0.136	0.157
0.079	0.010	0.102	0.069	0.011	0.131	0.227
0.541	0.283	0.044	0.001	0.071	0.021	0.000
0.288	0.028	0.013	0.268	0.063	0.002	0.000
0.164	0.004	0.012	0.434	0.026	0.014	0.002
0.053	0.746	0.007	0.000	0.076	0.010	0.002
0.441	0.298	0.020	0.007	0.117	0.008	0.001
0.230	0.231	0.038	0.002	0.074	0.031	0.005
0.363	0.011	0.422	0.010	0.018	0.005	0.001
0.145	0.006	0.020	0.473	0.063	0.042	0.035
0.630	0.138	0.058	0.000	0.108	0.030	0.000
0.400	0.022	0.273	0.009	0.022	0.088	0.005
0.048	0.001	0.062	0.001	0.010	0.009	0.001
0.011	0.034	0.012	0.004	0.165	0.052	0.014
0.052	0.805	0.026	0.002	0.069	0.019	0.000
0.521	0.273	0.057	0.000	0.062	0.016	0.000
0.393	0.019	0.004	0.312	0.038	0.000	0.001
0.552	0.272	0.043	0.001	0.063	0.019	0.000
0.207	0.007	0.601	0.032	0.013	0.044	0.001
0.062	0.007	0.097	0.027	0.010	0.048	0.395
0.167	0.007	0.018	0.449	0.068	0.046	0.038
0.362	0.020	0.047	0.108	0.019	0.101	0.045
0.182	0.112	0.004	0.039	0.153	0.083	0.031
0.082	0.772	0.010	0.001	0.059	0.012	0.000
0.222	0.006	0.035	0.023	0.140	0.001	0.019
0.506	0.310	0.029	0.001	0.115	0.009	0.000
0.000	0.631	0.011	0.000	0.174	0.029	0.000
0.003	0.630	0.044	0.000	0.104	0.050	0.003
0.084	0.762	0.007	0.001	0.041	0.008	0.000
0.107	0.013	0.062	0.187	0.009	0.107	0.105
0.282	0.032	0.117	0.125	0.133	0.000	0.000
0.436	0.014	0.263	0.035	0.005	0.006	0.001

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7
0.541	0.265	0.042	0.001	0.060	0.019	0.000
0.539	0.293	0.036	0.000	0.082	0.022	0.000
0.104	0.139	0.023	0.000	0.016	0.028	0.010
0.244	0.012	0.018	0.184	0.005	0.059	0.010
0.033	0.000	0.002	0.040	0.004	0.008	0.015
0.336	0.001	0.016	0.019	0.005	0.139	0.008
0.063	0.020	0.142	0.254	0.173	0.162	0.006
0.038	0.000	0.000	0.009	0.012	0.014	0.028
0.208	0.007	0.598	0.029	0.017	0.032	0.001
0.013	0.000	0.067	0.176	0.175	0.191	0.026
0.002	0.013	0.034	0.000	0.052	0.082	0.007
0.120	0.191	0.001	0.188	0.273	0.003	0.002
0.545	0.274	0.042	0.001	0.065	0.020	0.000
0.221	0.026	0.092	0.233	0.040	0.009	0.096
0.061	0.005	0.060	0.007	0.066	0.002	0.014
0.310	0.010	0.007	0.042	0.004	0.194	0.013
0.249	0.029	0.193	0.241	0.143	0.001	0.009
0.173	0.007	0.002	0.001	0.028	0.129	0.002
0.151	0.011	0.040	0.001	0.000	0.057	0.141
0.377	0.017	0.016	0.098	0.008	0.035	0.013
0.005	0.000	0.003	0.000	0.023	0.007	0.002
0.087	0.778	0.008	0.002	0.037	0.005	0.000
0.000	0.028	0.032	0.001	0.076	0.015	0.006
0.219	0.008	0.005	0.151	0.006	0.049	0.006
0.537	0.270	0.040	0.000	0.065	0.018	0.000
0.252	0.019	0.119	0.044	0.002	0.186	0.085
0.156	0.016	0.055	0.170	0.106	0.006	0.003
0.352	0.018	0.404	0.066	0.000	0.037	0.002
0.228	0.015	0.002	0.147	0.098	0.065	0.006
0.090	0.792	0.011	0.002	0.044	0.005	0.001
0.008	0.027	0.029	0.007	0.127	0.044	0.017
0.162	0.007	0.029	0.000	0.014	0.218	0.007
0.086	0.776	0.002	0.002	0.021	0.001	0.000
0.019	0.005	0.032	0.023	0.020	0.019	0.107
0.126	0.007	0.002	0.001	0.032	0.055	0.002
0.169	0.004	0.016	0.043	0.131	0.246	0.008
0.305	0.035	0.161	0.015	0.286	0.057	0.002
0.170	0.006	0.039	0.070	0.019	0.027	0.103
0.126	0.248	0.246	0.057	0.078	0.015	0.004
0.358	0.008	0.087	0.009	0.000	0.082	0.018
0.016	0.016	0.002	0.000	0.069	0.056	0.011
0.011	0.002	0.035	0.000	0.058	0.020	0.020
0.080	0.750	0.008	0.002	0.062	0.009	0.001

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7
0.341	0.014	0.011	0.263	0.044	0.013	0.003
0.171	0.012	0.050	0.093	0.001	0.021	0.278
0.171	0.030	0.181	0.290	0.099	0.001	0.071
0.567	0.030	0.001	0.038	0.140	0.000	0.004
0.086	0.759	0.009	0.002	0.054	0.011	0.001
0.245	0.020	0.101	0.006	0.111	0.010	0.013
0.258	0.014	0.074	0.047	0.083	0.011	0.028
0.212	0.012	0.004	0.002	0.028	0.174	0.001
0.030	0.010	0.015	0.000	0.027	0.034	0.002
0.556	0.284	0.043	0.000	0.073	0.019	0.000
0.539	0.268	0.045	0.001	0.060	0.019	0.000
0.090	0.020	0.080	0.166	0.148	0.199	0.004
0.113	0.016	0.039	0.143	0.034	0.001	0.000
0.533	0.029	0.055	0.078	0.025	0.002	0.000
0.365	0.012	0.193	0.001	0.004	0.014	0.003
0.164	0.008	0.023	0.097	0.023	0.072	0.000
0.468	0.015	0.093	0.135	0.050	0.006	0.002
0.070	0.655	0.001	0.000	0.000	0.005	0.000
0.084	0.400	0.189	0.011	0.111	0.030	0.005
0.065	0.000	0.010	0.017	0.001	0.005	0.001
0.057	0.003	0.075	0.001	0.000	0.024	0.336
0.552	0.271	0.043	0.001	0.063	0.019	0.000
0.396	0.033	0.168	0.095	0.107	0.025	0.004
0.509	0.012	0.295	0.005	0.019	0.012	0.000
0.462	0.012	0.056	0.031	0.001	0.105	0.007
0.290	0.012	0.333	0.000	0.001	0.080	0.004
0.001	0.711	0.140	0.012	0.081	0.008	0.000
0.001	0.027	0.012	0.005	0.116	0.035	0.006
0.100	0.008	0.121	0.038	0.004	0.023	0.342
0.497	0.294	0.084	0.000	0.067	0.015	0.000
0.082	0.003	0.016	0.000	0.001	0.050	0.090

Table 3: Top 50 Most abundant OTUs from pine roots.

OTU Name	Percent Identity	DNA Sequence	BLAST ID	FUNGuild	FunGUILD Trophic Mode
Otu1	100	CTCCTGGTATTCCGGGAGGCATGCCTGTTCGAGCGTCATTAAATACCTCTCAAGCACCATTGCTTGGTCATGGAAGATGAGTATGCTTGCATTCTCCCTTCTGAAATTCAAAGGCGGATCATCTCATATTCCCTGGCGTAGTAAGTGTTTATCTTTCGCTTGGAGTGTGTGACAGTCTTGCCATCGACCCCCATTATTTCAAGGTTGACCTCGGATCAGGTAGGGATACC	Wilcoxina_mikolae	Ectomycorrhizal	Symbiotroph
Otu2	100	CCCTTGGCTATTCCGAGGGGCATGCCTGTTTGAGTATCATGAACACCTCAACTCTCATGGTTTGCCATGATGAGTTGGACTCTGGGGGTTTTGCTGGCCTGTGGTCGGCTCCTCTCAAATTAATCAGCCTCCCAGTGTTTGGTGGCATCACGGGTGTGATAAATATCTACGCTCGCTGTTGTCTGCCAGGTAACCTTTGGTCACAAAGGTTTGCTGGAGCTCACAGATGTCTCTCCTCAGCGAGGACAGCTTTTTTAACGTTCGATCTCAAATCAGGTAGGACTACC	Thelephora_terrestris	Ectomycorrhizal-Undefined Saprotroph	Saprotroph-Symbiotroph
Otu3	95.045	CCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCAGTAATACTCTCCAGCCCTGCTGGGTGTTGGGTGTTTGTTCCGCTGCGCGCGTGAACTCGCCTCAAATGCATTGGCAGCCCGCCGTCCCGTGTGGGAGCGCAGCACATTTTGCGCTCTCTGCTGCGGACGGCAGCGTCCACAAGTCTATACTTTTATTTGACTCGGATCAGGTAGGGATACC	Pleosporaceae_sp	Endophyte-Lichen Parasite-Plant Pathogen-Undefined Saprotroph	Pathotroph-Saprotroph
Otu4	100	CCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCCAGCCCTGCTGGGTGTTGGGCGTTTTGTTCCGCTGCGCGCGTGAACTCGCCTCAAATGCATTGGCAGCCCGCCGTCCTGTGTGGGAGCGCAGCACATTTTGCGCTCTCCACTGCTGACGGTGGCATCCACAAGTCTACCATTTTACGCTTGACCTCGGATCAGGTAGGGATACC	Plenodomus_biglobosus	Undefined Saprotroph	Saprotroph
Otu5	100	TCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATCAATCTCTCAACTACATCAATCTTTCTGGTTTGGTGTAGCCTTTGGATGTGGGGGTTTTATTTTGCTGGCCTCTATTAAACGAGGTCAGCTCCCCTGAAATTTATTAGCGGTATCTGAGCAGAGACCTACTACAGGTGTGATAAATTATCTATGCCTTGGTAATGCTGCATCAACAGATTGTGCTGCTTCCAGTGAATCATTTGACAAATTTGACCTCAAATCAGGTAGGACTACC	Inocybe_sp	Ectomycorrhizal	Symbiotroph
Otu6	100	CTTCCGGTATTCCGGAAGGCATGCCTGTCCGAGCGTCATCAAAAACCACTCAAGTAGTTTTGCTTGGTTATGAAAGAAGAGTTTGCTTGCGAATTCCCTTTCGAAATTCAATGGCGGAGGGTCTCATGATTCCGGCGTAGTAATAACTTATTCGCTTGGTCATTGTGACAATCCTGCCTCAAACCCCCAATTCTAGTGTTTGACCTCGGATCAGGTAGGGATACC	Pustularia_sp	Dung Saprotroph-Ectomycorrhizal-Soil Saprotroph-Wood Saprotroph	Saprotroph-Symbiotroph
Otu7	100	CCATTGGTATTCCGATGGGCATGCCTGTTCGAGCGTCATTATCCTCCCTCAAACCTCGTGTTTGGTGTTGGACCGCGTTGGCCGAGCGACCAACTGGTCTCAAAGACAATGACGGCGTCCGTGGGACCCTCGGTGCAACGAGCTTCTAAGGAGCACGCGTCGAGTTTCAAGGACCCTCCGGGCCGGTCTTACCTCTATCTTCTCAGGTTGACCTCGGATCAGGTAGGAATACC	Herpotrichiellaceae_sp	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	Pathotroph-Saprotroph
Otu8	97.447	CCATTGGTATTCCGATGGGCATGCCTGTTCGAGCGTCATTATCCTCCCTCAAACCTCGTGTTTGGTGTTGGACCGCGTTGGTCGAGCGACCAACTGGTCTCAAAGACAATGACGGCGTCCGTGGGACCCTCGGTGCAACGAGCTTTTTAACGAGCACGCGTCGAGTTGCAAGGACCCTCCGGGCCGGCCTTGACCTCTATCTTCTTAGGTTGACCTCGGATCAGGTAGGAATACC	Chaetothyriaceae_sp	Epiphyte-Plant Pathogen	Pathotroph-Symbiotroph

OTU Name	Percent Identity	DNA Sequence	BLAST ID	FUNGuild	FunGUILD Trophic Mode
Otu9	94.977	CCTTCGGTATTCCGTTGGGCATGCCTGTTCGAGCGTCATT TAAACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTTCCGC CTCAGCGCGTGGACTCGCCTTAAATTCATTGGCAGCCGGT AGATTGGCTTCGTGCGCAGCACATTGTGTCACGATTTCAGT ATACTTCCTCCCATTAAGCCTCCTTTTTACTTTGACCTCGG ATCAGGTAGGGATACC	Pleomassariaceae_sp	Plant Pathogen-Plant Saprotroph	Pathotroph-Saprotroph
Otu10	94.785	CTTCTGGTATTCCGGAAGGTACATCTGTTTGAGTGTCATTC ATCTCTCAACTCTACCATTTTTTGAGTGTGTATGGGTTGGAT GTGAGGGTTGCAGGCTGCGAAGCCAGCTCCTTTGAAATGC ATTAGCTGGGAACTAGTACATCGTTTCAAGTGCTTTTTGATG CTTGAAGACCTCCTGGTGAGATAATTGTCTACGCCGTTGGT TTAGTCATTGCACCTATTTGGACTTTGGATTGTGCCTGCTT CTAACTGTCTCATTGCGAGACTAGTCCGCTACAGGGCTAC TATTGACTAGTGTGACCTCAAATCAGATGGGACTACC	Flagelloscypha_minutissima	Undefined Saprotroph	Saprotroph
Otu11	100	CCTCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATT ATCACCCCTCAAGCCCGGCTTGTTGTTGGATGCAGCGCTT ATCCCGCTCCTCCCAAAGATAATGACGGCGTCTGCGACGA CTCCTGTACACTGAGCTTTCGGGCACGTACACGGCTAGAA GTCCAGACCCGGTCGCCGTCCCCCCCGCGGGGGACACCC ATTACCACAAGGTTGACCTCGGATCAGGTAGGAATACC	Cyphellophora_sp	Animal Pathogen-Undefined Saprotroph	Pathotroph-Saprotroph
Otu12	100	CCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATC TACACCCTCAAGCTCTGCTTGGTGTTGGGCGTCTGTCCCG CCTTCGCGCGCGGACTCGCCCCAAATTCATTGGCAGCGG TCCTTGCCTCCTCTCGCGCAGCACAATTGCGTCTGCGGG GGGGCGTGGCCCGCGTCCACGAAGCAACATTACCGTCTTT GACCTCGGATCAGGTAGGGATACC	Paraphaeosphaeria_sporulosa	Undefined Saprotroph	Saprotroph
Otu13	100	CCTTTGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATT ATCACCCCTCAAGCCCTCGGCTTGGTGTTGGACGGTTTGG TGGAGGCCCCCTCGGGGGCTCCTGCCCCTCCCAAAGACA ATGACGGCGGCCTCGTTGGACCCCCGGTACACTGAGTTCT TCACGGGACACGTATCGGACACATGGGTTTACGGGACACG GTCTGCCTCCCCTCAGGGAGAATCTTTCTAAGGTTGACCT CGGATCAGGTAGGGATACC	Exophiala_equina	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	Pathotroph-Saprotroph
Otu14	100	CCTTCGGTATTCCGTTGGGCATGCCTGTTCGAGCGTCATT TAATCATTCAAGCTCTGCTTGGTGTTGGGTGTTTGTTCCGC CTCAGCGCGTGGACTCGCCTTAAATTCATTGGCAGCCGGT ATGTTGGCTTCGTGCGCAGCACATTGCGTCGCGATTCTGG CAGGCCTCCTCCCATTAAGCTTCTTTTTAAGTTTGACCTCGG ATCAGGTAGGGATACC	Pleotrichocladium_opacum	Wood Saprotroph	Saprotroph
Otu15	95.816	CTGCTAGCATTCTGGCAGGCATGCCTGTTCGAGCGTCATT TCAACCCCTCAAACTTTATCGTTTGGTGTTGAGGTTCTATAAT TATAGGCCTCTAAATCTATTGGCAGAACGTCATAAACTCTCA ATTGCAGTAAATTTTATTTCTATTGAAGAATTTTGATTGTACT AGCCGTAAAACACAGCGTGCTCTTTTTGAGCCCTATTTTTTA CAAGGTTGACCTCGGATCAGGTAGGAATACC	Sporidesmiella_sp	Undefined Saprotroph	Saprotroph
Otu16	100	CTGGCAGTATTCTGCCAGGCATGCCTGTCCGAGCGTCATT TCACCACTCAAGCTCTGCTTGGTGTTGGAGGACCCGCGTT TAGTCGCGGGCCGCCGAAATGCATCGGCTGTTGTATATAC AGCTTCCCTGTGTAGTAAATGCTTAGCTTTACACTTTGAAAC TTTTATATAACATGCCGAAAAACCCTCAACTTTTGAAAGGTT GACCTCGGATCAGGTAGGAATACC	Ceratocystidaceae_sp	Plant Pathogen	Pathotroph

OTU Name	Percent Identity	DNA Sequence	BLAST ID	FUNGuild	FunGUILD Trophic Mode
Otu17	100	TCCCTGGTATTCCGGGGAGCACGCCTGTTCGAGTGTCATG AAACTCTCAAACAAAGTAGATTTGTTTCTACCTCTGTTTGGA TTTGGACTCTGCTGCGTCAATGCGGCTGGTCTTAAATATAT TAGCTGATCCTAGCGAAGGTTTGGTTCTACTCAGCATGATA ATTATCTGATGTTGAGGACAGTCTTAGGACTGGCCAGGCTC TCTATGGATTGCTTCTAATCGTCTTTGGACAATCATTCAATA TCTGACCTCGAATCAGGTGGGACTACC	Corticiaceae_sp	Lichen Parasite-Plant Pathogen-Wood Saprotroph	Pathotroph-Saprotroph
Otu18	100	CTCCTGGTATTCCGGGAGGCATGCCTGTTCGAGCGTCATT AAATACCTCTCAAGCAGATTTGCTTGGTCTTGGAAGATGAG TTTGCTTGCATTCTCCCTTCTGAAATTCAAAGGCGGATGAT CTCATATCCCCAGGCGTAGTAAGATTATCTTTTCGCATGGT GTGTGTGATAGTCCAGCCGTCAACCCCCAATTTTTTCAAGG TTGACCTCGGATCAGGTAGGGATACC	Trichophaea_sp	Dung Saprotroph-Ectomycorrhizal	Saprotroph-Symbiotroph
Otu19	100	TCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTA AATTCTGTCAAAACATGCACTTGAGTGTGTTTTGGATTGTGG GAGTGTCTGCTGGCTTTATGAGCCAGCTCTCCTGAAATACA TTAGCTTTGGGGGGGAGGTGCCAAGTCACTTCTGCCTTTC CATTGGTGTGATAGATGAATTAACTTATCTACGCCAGGAAAG CAGGCTTCAGGTGATGCACTGTGATCTCTCTCTGCTCTCT AATTGACATTTGTCTGATAACTTGACCTCAAATCAGGTAGGA CTACC	Amanita_muscaria	Ectomycorrhizal-Undefined Saprotroph	Saprotroph-Symbiotroph
Otu20	100	TCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATTA AATCCTCAACCCCGTCCGTCGGTCGGGGCTTGGACTTTGG AGCGTGCTGGCGAAGGTCGGCTCCTCTTTAAATGCATCAG CGGAGAAAAACAAACCTTTTTTCCCCCTTCAGCGTGATAAC TGTGTTGCGCTGTGGTCGCGAAAGGGTCCGCTCATAATCG TCCTCGGACAAACACCGAATCTGTTTTGACCTCAGATCAGG TAGGACTACC	Atheliaceae_sp	Ectomycorrhizal-Lichen Parasite-Lichenized-Plant Pathogen	Pathotroph-Saprotroph-Symbiotroph
Otu22	97.727	CCTTTGGTATTCCATAGGGCATGCCTGTTCGAGCGTCGTC TCAAACCTTCAAGCCTAGCTTGGTGTTGGGTGACTGTCCC GCGTCACCGCGCGGACTCGCCTCAAAGTCATTGGCAGCA GACTCGGTAGCTAATTGCGCAGCACATCGCGCCAGAAGCT CCCCGTCCGCTATCCACGAGCACAGTCTCATCAGTTTGAC CTCGGATCAGGTAGGGATACC	Clohesyomyces_aquaticus	NA	NA
Otu23	100	CCCTTGGTTATTCCTTGGAGCATGCCTGTTTGAGTATCATG AACACCTCAACTCTCATGGTCTTCCATGATGAGCTTGGACT TTGGGGGTTTGCTGGCCCTGCGGCCGGCTCCTCTCAAAT GAATCAGCTTGCCAGTGTTTGGTGGCATCATGGGTGTGATA ACTATCTACGCTGCGGGTCGTCTGCCAGGTAACCCTCAGC GATGAGGGTTCATTGGAGCTCATAAACGTCTCTCCTCGGC GAGGACAACTTTTGAACGTTCGATCTCAAATCAGGTAGGAT TACC	Thelephoraceae_sp	Ectomycorrhizal-Undefined Saprotroph	Saprotroph-Symbiotroph
Otu24	96.751	CCTTTGGTATTCCGAAGGGTACGCCCGTTTGAGCGTCATT GTAATCTCACTTCTATAACTTTGTTGTTGTGGAATGTGGACT TGGACGTCTGCCGTGTCAACGGCTCGTCTTAAATGCCTGA GTGTACCCCGCTTTGCGGCGTATTCGGTGTGATAAACATTT CACCGGAGTTTGGTCTCTGACCGCGCGTTTAGCAATTGGTG GGCTCTATGCTTTCCAACCGTCTCTAATGGGACAATCTTTG ACAATTTGACCTCAAATCGGGCGGGACTACC	Serendipita_vermifera	Orchid Mycorrhizal	Symbiotroph
Otu25	100	CCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATT TGTACCCTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCCCG CCTTCGCGTGTGGACTCGCCTTAAAGTCATTGGCAGCCGG AATAATTCTGGGGAACGCAGCACAACTGCAGCCTCCATTTT ACGCCGAGCGTCCAGTAAGCCTTTTTTTTCACGTTTGACCT CGGATCAGGTAGGGATACC	Neopyrenochaeta_sp	NA	NA

OTU Name	Percent Identity	DNA Sequence	BLAST ID	FUNGuild	FunGUILD Trophic Mode
Otu26	100	CCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCTTCAAGCTATGCTTGGTGTTGGGTGTTTGTCCTCTCCCCTGCGTTTGGACTCGCCTTAAAGTCATTGGCAGCCTGTATATTGGTTTTGAGCGCAGCACATTTTGCGTCTTGCATCTAGTAATACTAGCATCCATCAAGCCCATTATCACTTTTGACCTCGGATCAGGTAGGGATACC	Ophiosphaerella_sp	Undefined Saprotroph	Saprotroph
Otu27	100	CCCTTGGTATTCCGAGGGGCATGCCTGTCCGAGCGTCATTATGACCACTCAAGCCTGGCTTGGTGTTGGGGCCCGCGGTTCCGCGGCCCTTAAAATCAGTGGCGGCGCCATCTGGCTCTGAGCGTAGTAATACTCCTCGCTATAGAGTCCGGGTGGATGCTTGCCGGCAACCCCCCATCTCACGGTTGACCTCGGATCAGGTAGGGATACC	Hyaloscypha_sp	Undefined Saprotroph	Saprotroph
Otu28	100	CCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATCTACACCCTCAAGCACTGCTTGGTGATGGGCGTCTGTCCCGCCTTCGCGCGCGGACTCGCCCCAAAGGCATTGGCAGCGGTCCCGATCGCCCTCTCGCGCAGCACATTTGCGCTTCTCGAGGCGGCGGGTTCGCGTCCACGAAGCCCTTTTTTACCACGTTTGACCTCGGATCAGGTAGGGATACC	Laburnicola_centaureae	Endophyte-Lichen Parasite-Plant Pathogen-Undefined Saprotroph	Pathotroph-Saprotroph-Symbiotroph
Otu29	100	CCCCTGGTATTCCGGGGGGGCATGCCTGTTCGAGCGTCATTTCACCACTCAAGCCTCGCTTGGTATTGGGCAACGCGGTCCGCCGCGTGCCTCAAATCGACCGGCTGGGTCTTCTGTCCCCTAAGCGTTGTGGAAACTATTCGCTAAAGGGTGTTCGGGAGGCTACGCCGTAAAACAACCCCATTTCTAAGGTTGACCTCGGATCAGGTAGGGATACC	Cladosporium_tenuissimum	NA	NA
Otu30	100	TCTTTGGTATTCCGAAGAGCATGCTTGTTTGAGTATCAGTAAACACCTCAAAGCTTTTGGATTTTTTTAATCGAAAAGCTTTGGACTTGAGCAATCCCAACACCAATCTTTTTGAGATCGGTGGCGGGTTGCTTGAAATGCAGGTGCAGCTGGACATTCTCCTGAGCTAAAAGCATATTCATTTAGTCCCGTCAAACGGATTATTACTTTTGCTGCAGCTAACATAAAGGGAGTTTGACCGTATTGGCTGACTGATGCAGGATTTCACAAGGGTCGGCAACGATTCTTGTTAAAACTCGATCTCAAATCAAGTAAGACTACC	Mortierella_elongata	Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph	Saprotroph-Symbiotroph
Otu31	94.737	CCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGTGTCATTAAATTCTCAACCTTGAAAGACTTTGTTGTCTTTTCAATGGCTTGGATGTGAGGGTTTTGCTGGCTTCCTTCAGTGGATGGTCTGCTCCCTTTAAATGCATTAGTGAGATCCTTTGTGGACGGTCACTTGGTGTGATAATCTATCTACGCCGCTTGTACTGTGAAGCAAAACTTGTGGGAACTCGCTTATAACCGTCTGTAATGGACAATTTTCTGATATTTGGCCTCAAATCAGGTAGGACTACC	Mycena_sp	Leaf Saprotroph-Plant Pathogen-Undefined Saprotroph-Wood Saprotroph	Pathotroph-Saprotroph
Otu32	99.679	TCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATCGAAACATCAGATCGAAGCTTTCGACCTCGTCGGAGCTCGGTTTGGACTTATGGGAGTCTGCGGGCGAACCTCCCGTCGGGGGGACGTCGGCTCTCCTCAAAAGCATCAGCGTTGGGTGCGAGCCTCGCGTGGCACGGCCTCTTCGACGTCATAATGACCGTCGTGGGCTGGAAGTGCGTGGATCGTAGATGCCCCGTCGCTCTCCAACTCTGCGAGCCCGGTCCGTCCGGCCGCGTGTTATCGAAGCTTGACCTCAAATCAGGTAGGACTACC	Scleroderma_bovista	Ectomycorrhizal	Symbiotroph
Otu33	100	TCCTTGGTATTCCTTGGAGCATGCCTGTTTGAGTATCATGAAATTCTCAAAGCAAACCTTTTGTTAATTCAACTGGCCTTGCTTTGGACTTGGAGGTCTTGCAGATGTCACAGTCTGCTCCTCTTAAATAAATTAGCTGGATCTCGGTATACACTTGGTTCCACTCGGCCGTGATAAGTATCACTCGCTGAGGACACTGTAAAAGGTGGCCAGGCAATACAGATGAACCGCTTCTAATAGTCCATTCACTTGGACAATTACACTTATGATCTGATCTCAAATCAGGTAGGACTACC	Ceratobasidium_sp	Endomycorrhizal-Plant Pathogen-Undefined Saprotroph	Pathotroph-Saprotroph-Symbiotroph

OTU Name	Percent Identity	DNA Sequence	BLAST ID	FUNGuild	FunGUILD Trophic Mode
Otu35	100	CCGCTGGTATTCCGGCGGGCATGCCCGTTCGAGCGTCAT TTTAATCCCTTCCCGGGAGACTCTTCTATTCTGTAGTGGAG TCGCACGGGGCGTTGGCACTGGGCGCCTCATTTTTTTCAG GCGGCCGGAGCCGAAATGAAGCGGCGGACTCGCGGCGG CCCCTAGCGCAGTAGAATAACCCTTCTCGCTTAGGAGAGC CGCAGCGGCGGACCACAGCCCCTACATACAAAACCTATAT ATATAAAACTTGACCTCGGATCGGGTAGGACTACC	Valsaceae_sp	Endophyte-Plant Pathogen-Undefined Saprotroph	Pathotroph-Saprotroph-Symbiotroph
Otu36	100	CCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCAT TGCTACCCCTCAAGCGCGGCTTGTGTGTTGGGCGCTGTC CCTCCCCTTCTTCGGGGGGACAGGCCTGAAAGGCAGTGG CGGCGTCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTC ACCCGCTCTGTGAGGATCCGGCCGGGGCCTGTCTCACCC CCAATCTTCTCTAAGGTTGACCTCGGATCAGGTAGGGATAC C	Sagenomella_verticillata	Undefined Saprotroph	Saprotroph
Otu37	93.607	CCCGTGGCATTCCGCGGGGCATGCCTGTTCGAGCGTCAT AATGACCCATCAAGCCTCGGCTTGGTCTTGGGGCCTGCGG TCTTCGCATCCCCTAAACCCAGTGGCAGTGCGATCGAGCT CTGAGCGTAGTAATTCTTCTCGCTATAGGGTCTCGGTCGT CGCTCGCCAGCAACCCCCTCAATTACTATCGGGTTGACCT CGGATCAGGTAGGGATACC	Pezoloma_sp	NA	NA
Otu38	100	CCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATT TGTACCTTCAAGCTTTGCTTGGTGTTGGGTGTTTGTCTCGC CTTTGCGTGTAGACTCGCCTTAAAAGAATTGGCAGCCGGC GTATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCACT CATAACGACGACGTCCAAAAGTACATTTTTACACTCTTGAC CTCGGATCAGGTAGGGATACC	Neomicrosphaeropsis_cytisicola	Animal Pathogen-Plant Pathogen-Undefined Saprotroph	Pathotroph-Saprotroph
Otu39	100	CCATTAGTATTCTAGTGGGCATGCCTGTTCGAGCGTCATTT CAACCCTTAAGCCTAGCTTAGTGTTGGGAGACTGCCTAATA CGCAGCTCCTCAAAACCAGTGGCGGAGTCTGTTCGTGCTC TGAGCGTAGTAATTTTTTATCTCGCTTCTGCAAGCCGGCCA GACGACAGCCATAAACCGCACCCTCTCGGGGGGCACTTTT TTAATGGTTGACCTCGGATCAGGTAGGAATACC	Microdochium_sp	NA	NA
Otu40	96.071	CCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGTGTCATTA AATTCTCAACCTTGAAAGACTTGTCTTTTCAATGGTTTGGAT GTGAGGGTTTTGCTGGCTTCCTTCAGTGGATGGTCTGCTC CCTTTAAATGTATTAGTGAGATCCTTTGTGGACGGTCACTT GGTGTGATAATTATCTACGCCGCTTGTACTGTGAAGCAAAA TTTGTGGGAACTTGCTTATAACCGTCTGTAATGGACAATTTT CTGAACTTTGGCCTCAAATCAGGTAGGACTACC	Mycenaceae_sp	Leaf Saprotroph-Wood Saprotroph	Saprotroph
Otu41	100	CCCTTGGCATTCCATGGGGCATGCCTGTTCGAGCGTCATC TAAACCCTCAAGCCCCCGGCTTGGTGTTGGGTGCCTGTCC CCGCTCCCCGCGCGGACTCACCCCAAATGCATTGGCAGC CGCCTCTCGGCTTCTTGCGCAGCACAGTGCGCAGCGAGG CGAGGTGAGGCGTGCGTCCAGCAAGCAACCACCCAAGTTT GACCTCGGATCAGGTAGGGATACC	Herpotrichiellaceae_sp	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	Pathotroph-Saprotroph
Otu43	100	CCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTT GTACCCTCAAGCTTTGCTTGGTGTTGGGCGTTTTTTGTCTCT GGTCCGCCAGCGACTCGCCTTAAAATCATTGGCAGCCGGC CTACTGGTTTCGGAGCGCAGCACAAATTTGCGCTTCTTCC AGCAGCGGTCCGCGTCCATGAAGCCACTTTTTTCAACGTTT GACCTCGGATCAGGTAGGGATACC	Pleosporaceae_sp	Endophyte-Lichen Parasite-Plant Pathogen-Undefined Saprotroph	Pathotroph-Saprotroph
Otu44	100	CCACTAGTATTCTGGTGGGCATGCCTGTTCGAGCGTCATT TCAACCCTCAAGCCTGGCTTGGTGTTGGGGCTCTGCGCCT GCAGTCCCTTAAATCCAGTGGCGGACACGCTAGGTCTCCG AGCGCAGTAGTTTCTTCTCGCTCAGGGCGTCCGGCGTGG GCTTGCCTCGCACCCATCTTATCAAGGTTGACCTCGGATC AGGTAGGAATACC	Ramichloridium_sp	NA	NA

OTU Name	Percent Identity	DNA Sequence	BLAST ID	FUNGuild	FunGUILD Trophic Mode
Otu45	94.714	CCTTTGGTATTCCGAAGGGCATGCCTGTTCGAGCGTCATT ATCAAAGCATCAAGCTTGGCTTGTCGTTGGGCCCTTTATCA CCTGGTGATAGGTCCAAAAGATAATGAGCGGTGCCGTAAG GACTCTATATGCAACAAGCTTCTAACAGCACGCATGTAGTG GTCATATGGCCCGGTTTACCCCTTTATTTCTCAAGGTTGAC CTCGGATCAGGTAGGAATACC	Herpotrichiellaceae_sp	Animal Pathogen-Fungal Parasite-Undefined Saprotroph	Pathotroph-Saprotroph
Otu46	100	CCTCTGGTATTCCGGAGGGCATGCCTGTTTGAGTGTCATG TAGACTCAATCCCTCGGGTTTCCGAGGAGATTGGACTTGG GTGTTGCCGCTCTGCCGGCTCGCCTTAAAAGACTTAGCGG GATAGCACCGTAGTCGGCGTAATAAGTTTCGTCGGTGAAG GTTGTGATGACTGCTTACAATCGCCCTCGGGCAATTTTTGA CTCTGACCTCAAATCAGGTAGGACTACC	Saitozyma_podzolica	NA	NA
Otu47	86.806	TCCTTGGAATTCCGAGGAGCATGCCTGTTTGAGTGTCATGA AACCCTTCAAACCTCATGGCTGATTAGTCTTTGAGGCTTGG ATTGTGGAGTGTGCCGAGTAAAAAGAGAGGACCTCTCTCAC CGGCTCCTCTGAAATGCATCAGCGAAGCTCCGACCACAGG AAAACTGCTTAGCTTTTGGTTTGATAATCTCTTTGTCTACCT CCATGGCTATTTGCATCCTTGCTGGTATAAGATTCGCTTAC AATCTTATCCTTTGACATTTTGACCTCAAATCAGGTAGGACT ACC	Tricholomataceae_sp	Ectomycorrhizal-Fungal Parasite	Pathotroph-Symbiotroph
Otu48	100	CCCCTGGCATTCCGGGGGGGCATGCCTGTCCGAGCGTCAT TTCTGCCCCTCAAGCACGGCTTGTGTGTTGGGTGCGGTCCC CCCGGGGGACCTGCCCGAAAGGCAGCGGCGACGTCCGTCT GGTCCTCGAGCGTATGGGGCTTTGTCACTCGCTCGGGAAG GGCTGGCGGGGGTTGGTCACCACCAAAATTTTACCACGGT TGACCTCGGATCAGGTAGGAGTTACC	Talaromyces_purpureogenu s	Undefined Saprotroph	Saprotroph
Otu49	100	CCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGTGTCATTA AATTATCAACCTTGTTCGCTTTTACGAGCTTGAGCGAGGCT TGGATGTGAGGGCTTGCTGGCTTCCTTCAGTGGATGGTCT GCTCCCTTTAAATGCATTAGTGGGATCTCTTGTGGACCGTC ACTTGGTGTGATAATTATCTATGCCATTTGACTGTGAAGCAA AATTATGGGAACCTGCTTATAACCGTCTCGCAAGGGACAAT TTAATTGACTATTTGACCTCAAATCAGGTAGGACTACC	Mycena_sp	Leaf Saprotroph-Plant Pathogen-Undefined Saprotroph-Wood Saprotroph	Pathotroph-Saprotroph
Otu51	96.203	CCATTGGTATTCCGATGGGCATGCCTGTTCGAGCGTCATT ATCCTCCCTCAAACCTCGTGTTTGGTGTTGGGCCGCGTTG GTCGAGCGATCAACTGGTCTCAAAGATAGTGACGGCGTCC GTGGGACCCTCGGTGCAACGAGCTTTTAACGAGCACGCGT CGAGTTTCAAGGACCCTCCGGGCCGGTCTAGACCTTTATA TCTTTCTCAGGTTGACCTCGGATCAGGTAGGAATACC	Herpotrichiellaceae_sp	Animal Pathogen- Fungal Parasite- Undefined Saprotroph	Pathotroph-Saprotroph
Otu52	100	CCTTCGGTATTCCGTTGGGCATGCCTGTTCGAGCGTCATT TAAACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTTCCGT CTTAGCGCGTGGACTCGCCTTAAAATTCATTGGCAGCCGGT ATGTTGGCTTCGTGCGCAGCACATTGCGTCATGATTTTAGC GTACCTCCTTCCATTAAGCTTTTTTTTTAGTTTGACCTCGGAT CAGGTAGGGATACC	Melanommataceae_sp	Wood Saprotroph	Saprotroph
Otu53	98.846	TCTTTGGTATTCCGAAGAGCATGCCTGTTTGAGTGTCATGA ATCTCTCAAATACAATAATTTTTTCTTTAATTGTTGTATTTGG ACTTGGAAGCTGTTGGCGCAAGTCGACTCTTCTCAAATTCA TTAGCTGGGGTTTATATAGTTGGATCCTTGGTGTGATAATTA TCTACGCCTTGAAGTCCCTGTAGACTCTGCTTCAAATCGTC TCTTCATGAGACAATATTTGAATCATCTGACCTCAAATCAGG TAGGACTACC	Waitea_circinata	Plant Pathogen	Pathotroph

OTU Name	Percent Identity	DNA Sequence	BLAST ID	FUNGuild	FunGUILD Trophic Mode
Otu54	81.867	CTTCTGGTATTCCGGAAGGCATGCTTGTTTGAGTATCAGTA AATACCCTCAACTCCTAACATATTTTGTCACTTCTTTCTCAA TTTGGGTTTGTGGTGCAAAAAGGAAGAGTTGGATTTGAGCA AAATTCCCTTGTTCCAAATTTGGAATTGCAGAGAATTTGCTT GAAATTCAGGTTCGGCCAAGACAATTTTTGTCCTACAAGCA ATTTTCACTTTATCCCGTCAAACGGATAATATCTTTTTGCTT TGGTGGTGGAATTTGTCAAAACGGCTCGACTGATGCAGATG GTTTTTTGGGTTGAAATATTTCGACCAAAAAACCGTAATCTC GATCTCAAATCAAGTAAGACTACC	Mortierellales_sp	NA	NA

Chapter 3 Appendix
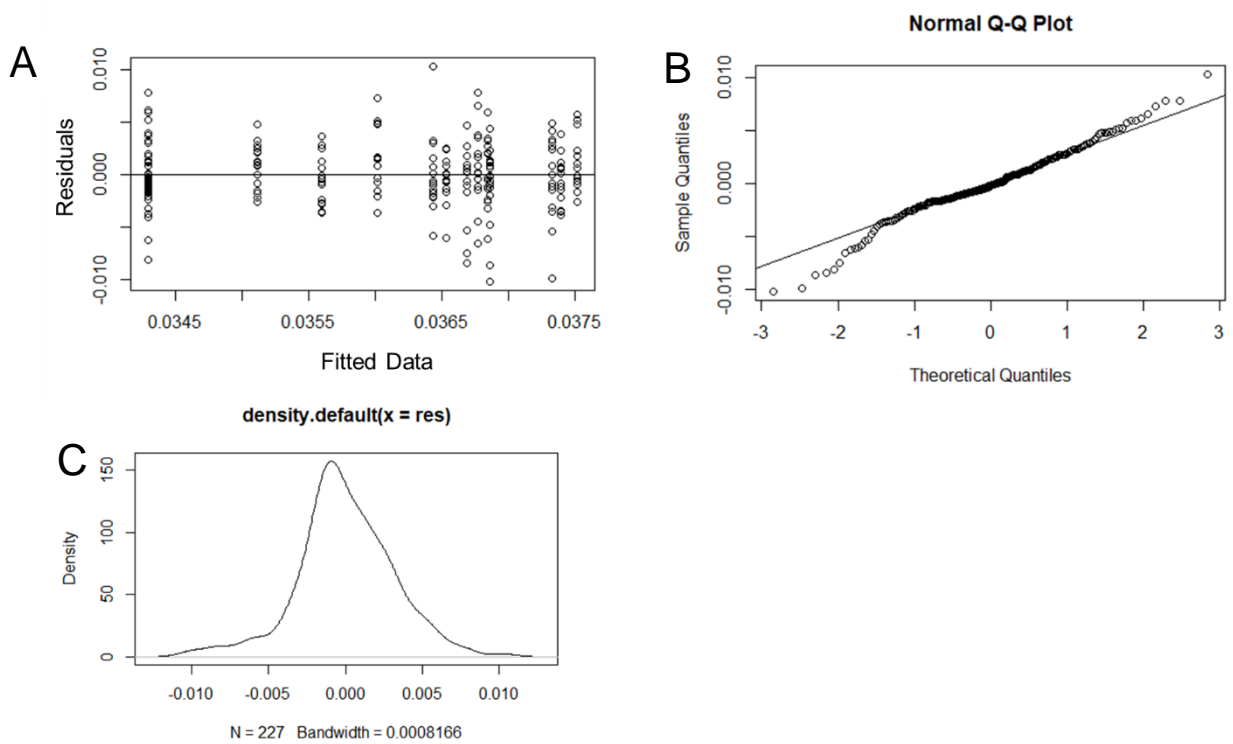
Figure 1: Normalcy testing of plant growth rate data

A: residual vs. fitted plot, B: Q-Q plot, C: density plot
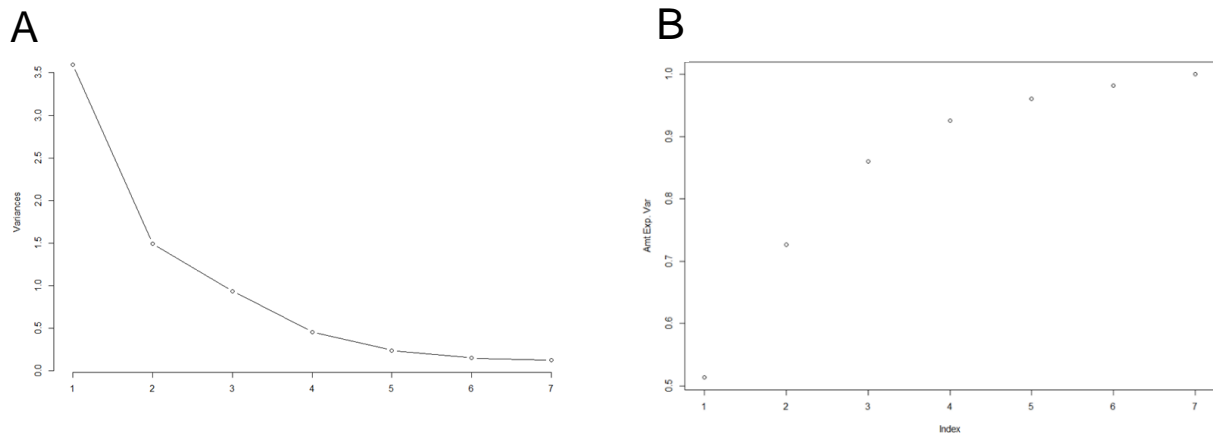
Figure 2: RDA component exploration

A: scree plot indicating Dim1 & Dim2 most relevant, B: Amount of variance explained per component plot indicating the curves starts to flatten with Dim3 to Dim4

Table 1: Correlation for plant species and growth rate to ordination components RDA

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7
CYTSCO	0.070	0.087	0.836	0.001	0.005	0.000	0.001
DACGLO	0.605	0.244	0.031	0.036	0.001	0.081	0.002
FESACT	0.428	0.413	0.002	0.026	0.130	0.000	0.001
FESARU	0.166	0.685	0.064	0.004	0.046	0.034	0.000
VERHUL	0.621	0.023	0.000	0.348	0.000	0.006	0.002
POACIT	0.848	0.014	0.003	0.041	0.021	0.006	0.067
SOPMIC	0.854	0.026	0.001	0.003	0.040	0.024	0.052

Table 2: Model simplification as shown in Chapter 3 Figure 3 and Table 2

Model : Equation	Compare (model numbers)	*AIC*	*BIC*	*P*
Model 1: Growth Rate ~ Pine Dominance * Functional Group * Provenance * Competition + (1|Species) + (1|Plot)		–1694.469	–1629.395	
Model 2: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Functional Group + Pine Dominance + Provenance + Functional Group:Provenance + Pine Dominance: Competition + Functional Group:Competition + Provenance: Competition + Pine Dominance: Functional Group:Provenance + Pine Dominance:Functional Group:Competition + Pine Dominance: Provenance:Competition + Functional Group:Provenance:Competition	anova(1,2)	–1708.342	–1646.693	0.375 4
Dropping 4-way interaction (Pine Dominance: Functional Group: Provenance: Competition) did not make a significant difference to the model				
Model 3: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Functional Group + Pine Dominance:Provenance + Functional Group:Provenance + Pine Dominance	anova(2,3)	–1723.638	–1665.414	0.000 3187

:Competition + Functional Group:Competition + Provenance:Competition + Pine Dominance:Functional Group:Competition + Pine Dominance: Provenance:Competition + Functional Group:Provenance:Competition				

Dropping 3-way interaction (Pine Dominance: Functional Group: Provenance) did not make a significant difference to the model

Model 4: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Functional Group + Pine Dominance:Provenance + Functional Group:Provenance + Pine Dominance: Competition + Functional Group:Competition + Provenance: Competition + Pine Dominance:Functional Group:Competition + Pine Dominance: Provenance:Competition	anova(3,4)	-1736.85	-1682.051	0.537

Dropping 3-way interaction (Functional Group: Provenance: Competition) did not make a significant difference to the model

Model 5: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Functional Group + Pine Dominance:Provenance + Functional Group:Provenance + Pine Dominance: Competition + Functional Group: Competition + Provenance:Competition + Pine Dominance:Provenance:Competition	anova(4,5)	-1749.43	-1698.056	0.058

Dropping 3-way interaction (Pine Dominance: Functional Group: Competition) did not make a significant difference to the model

Model 6: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Functional Group + Pine Dominance:Provenance + Pine Dominance:Competition + Functional Group:Competition + Provenance:	anova(5,6)	-1759.397	-1711.448	0.71

Model	anova			p
Competition + Pine Dominance: Provenance:Competition				
Dropping interaction (Functional Group: Provenance) did not make a significant difference to the model				
Model 7: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Provenance + Pine Dominance:Competition + Functional Group:Competition + Provenance: Competition + Pine Dominance: Provenance:Competition	anova(6,7)	−1775.356	−1730.832	0.38
Dropping interaction (Pine Dominance: Functional Group) did not make a significant difference to the model				
Model 8: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Provenance + Pine Dominance:Competition + Functional Group:Competition + Provenance: Competition	anova(7,8)	−1785.998	−1744.899	**0.02**
Dropping 3-way interaction (Pine Dominance: Provenance: Competition) did make a significant difference.				
Model 9: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance:Provenance + Pine Dominance:Competition + Provenance: Competition + Pine Dominance: Provenance:Competition	anova(7,9)	−1766.188	−1725.089	**<0.01**
Dropping 2-way interaction (Functional Group: Competition) did make a significant difference.				
Final Model: Growth Rate ~ Pine Dominance + Functional Group + Provenance + Competition + (1 | Species) + (1 | Plot) + Pine Dominance: Provenance + Pine Dominance: Competition + Functional Group:Competition + Provenance: Competition + Pine Dominance: Provenance:Competition				

Chapter 4 Appendix
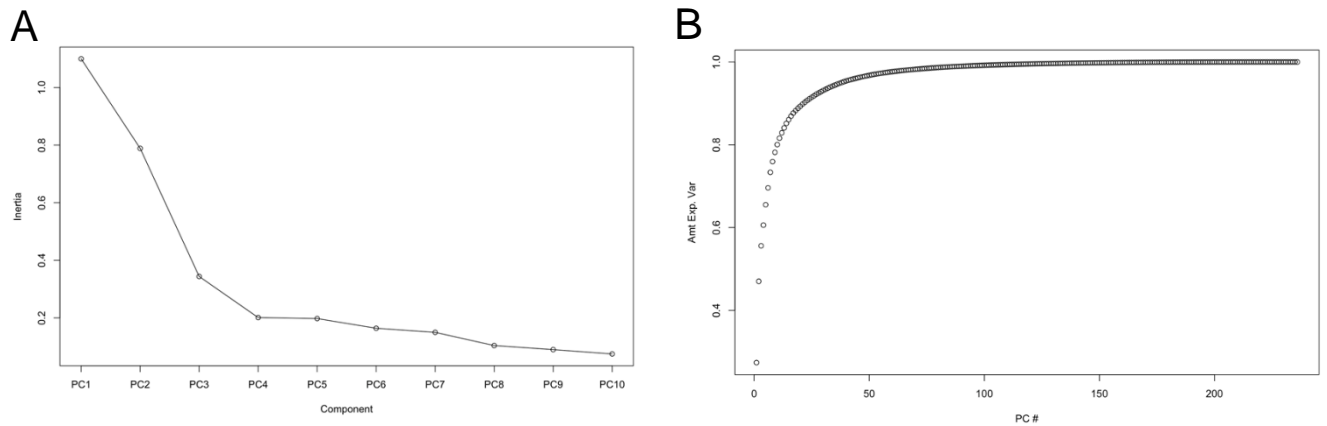
Figure 1: Fungal OTUs PCoA component exploration

A: scree plot indicating PC1 – PC10 covering 80% variance, B: Amount of variance explained per component plot shows around PC8 begins the flattening of the curve indicating the less contributing components falling later
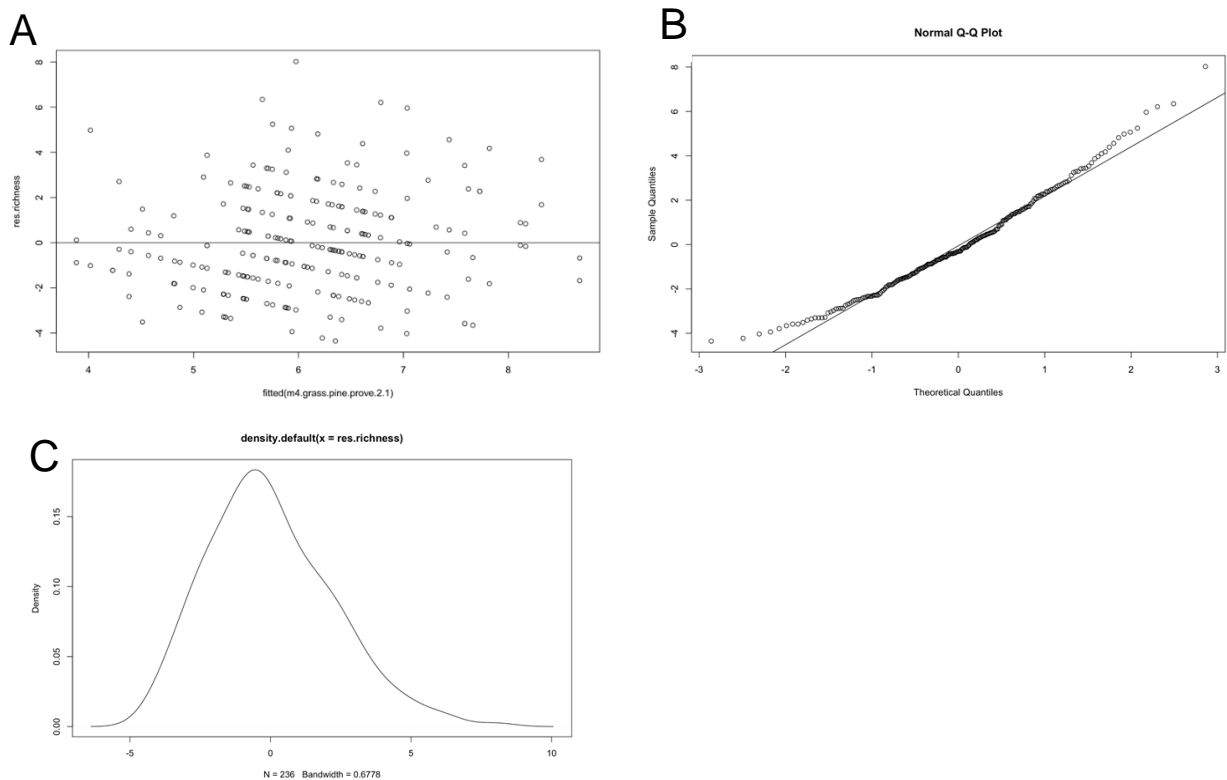
Figure 2: Normalcy testing of OTUs by treatment

A: residual vs. fitted plot, B: Q-Q plot, C: density plot

Table 1: Correlation for fungal OTUs PCoA to ordination components, each row represents a grass sample

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
0.008	0.009	0.000	0.000	0.003	0.001	0.000	0.000	0.000	0.000
0.008	0.005	0.002	0.000	0.004	0.000	0.001	0.000	0.000	0.000
0.006	0.005	0.002	0.000	0.002	0.000	0.002	0.000	0.000	0.000
0.004	0.002	0.000	0.001	0.000	0.000	0.002	0.000	0.001	0.000
0.000	0.000	0.002	0.000	0.000	0.000	0.002	0.000	0.000	0.000
0.001	0.001	0.001	0.000	0.000	0.000	0.009	0.001	0.000	0.000
0.003	0.007	0.000	0.001	0.000	0.000	0.002	0.000	0.001	0.000
0.000	0.000	0.001	0.000	0.000	0.000	0.004	0.000	0.000	0.000
0.000	0.000	0.002	0.000	0.004	0.000	0.002	0.000	0.000	0.000
0.000	0.002	0.002	0.001	0.000	0.000	0.003	0.000	0.000	0.000
0.000	0.000	0.001	0.000	0.002	0.001	0.000	0.000	0.001	0.000
0.002	0.002	0.000	0.000	0.000	0.001	0.002	0.000	0.000	0.000
0.006	0.000	0.001	0.001	0.000	0.001	0.003	0.001	0.000	0.000
0.003	0.003	0.002	0.000	0.004	0.000	0.006	0.000	0.000	0.000
0.003	0.000	0.002	0.002	0.000	0.000	0.001	0.000	0.001	0.000
0.006	0.008	0.000	0.000	0.000	0.001	0.003	0.000	0.001	0.000
0.001	0.002	0.006	0.001	0.000	0.004	0.003	0.000	0.000	0.000
0.002	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
0.009	0.001	0.000	0.002	0.001	0.000	0.000	0.000	0.000	0.000
0.004	0.002	0.003	0.000	0.006	0.000	0.003	0.000	0.001	0.000
0.003	0.000	0.000	0.003	0.000	0.000	0.002	0.000	0.000	0.000
0.008	0.004	0.004	0.000	0.001	0.002	0.000	0.001	0.000	0.001
0.014	0.001	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000
0.005	0.014	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.000
0.007	0.004	0.002	0.000	0.003	0.000	0.000	0.001	0.000	0.000
0.000	0.001	0.000	0.001	0.003	0.001	0.000	0.000	0.000	0.000
0.007	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001
0.006	0.007	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000
0.005	0.007	0.000	0.001	0.003	0.000	0.000	0.000	0.000	0.001
0.002	0.006	0.002	0.000	0.006	0.001	0.000	0.001	0.000	0.003
0.002	0.003	0.001	0.000	0.001	0.000	0.002	0.001	0.000	0.001
0.025	0.002	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000
0.022	0.000	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.000
0.005	0.014	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.017	0.001	0.000	0.000	0.001	0.001	0.001	0.000	0.000	0.000
0.001	0.007	0.000	0.001	0.001	0.002	0.000	0.001	0.001	0.000
0.007	0.001	0.001	0.002	0.001	0.001	0.000	0.000	0.000	0.000
0.012	0.000	0.001	0.001	0.000	0.000	0.001	0.000	0.000	0.000
0.000	0.000	0.003	0.001	0.000	0.003	0.001	0.005	0.001	0.000
0.002	0.007	0.000	0.000	0.000	0.000	0.002	0.000	0.001	0.001

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
0.012	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.000
0.014	0.004	0.002	0.001	0.000	0.001	0.001	0.000	0.000	0.000
0.003	0.011	0.001	0.001	0.000	0.000	0.000	0.001	0.001	0.000
0.007	0.000	0.001	0.000	0.002	0.000	0.000	0.001	0.000	0.000
0.004	0.000	0.003	0.000	0.004	0.002	0.000	0.000	0.002	0.000
0.008	0.001	0.008	0.000	0.000	0.000	0.000	0.001	0.001	0.002
0.005	0.021	0.003	0.002	0.000	0.000	0.000	0.001	0.001	0.000
0.003	0.007	0.003	0.000	0.002	0.004	0.001	0.004	0.000	0.000
0.015	0.004	0.000	0.001	0.001	0.001	0.000	0.002	0.000	0.000
0.005	0.011	0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.003
0.002	0.005	0.006	0.003	0.000	0.000	0.000	0.000	0.001	0.000
0.014	0.008	0.000	0.001	0.000	0.000	0.001	0.002	0.001	0.000
0.010	0.000	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.001	0.001	0.002	0.000	0.000	0.000	0.001	0.000	0.000
0.009	0.000	0.001	0.000	0.004	0.000	0.000	0.000	0.001	0.000
0.001	0.006	0.007	0.004	0.000	0.000	0.001	0.000	0.000	0.000
0.006	0.000	0.018	0.000	0.000	0.000	0.001	0.000	0.000	0.000
0.007	0.023	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.008	0.002	0.001	0.000	0.004	0.001	0.000	0.000	0.003	0.000
0.000	0.000	0.000	0.000	0.004	0.002	0.000	0.000	0.000	0.000
0.005	0.009	0.003	0.002	0.000	0.000	0.000	0.000	0.000	0.000
0.002	0.008	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.000
0.019	0.007	0.001	0.000	0.000	0.001	0.000	0.000	0.001	0.000
0.007	0.015	0.001	0.000	0.001	0.001	0.000	0.000	0.001	0.000
0.009	0.001	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000
0.004	0.017	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.026	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.004	0.016	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.002	0.008	0.001	0.003	0.000	0.001	0.000	0.000	0.000	0.000
0.008	0.003	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.001	0.013	0.000	0.000	0.001	0.000	0.000	0.000	0.000
0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.001	0.001	0.004
0.011	0.000	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.000
0.002	0.007	0.001	0.002	0.001	0.000	0.000	0.000	0.000	0.000
0.001	0.004	0.005	0.002	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.001	0.002	0.000	0.000	0.001	0.000	0.001	0.000
0.001	0.001	0.001	0.001	0.002	0.004	0.000	0.000	0.000	0.000
0.003	0.003	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.001	0.000	0.001	0.001	0.002	0.002	0.000	0.000
0.010	0.001	0.000	0.003	0.001	0.001	0.001	0.000	0.000	0.001
0.004	0.001	0.000	0.001	0.000	0.001	0.001	0.001	0.000	0.000
0.010	0.001	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
0.007	0.003	0.000	0.001	0.001	0.001	0.000	0.001	0.000	0.001
0.001	0.002	0.001	0.000	0.003	0.001	0.000	0.000	0.000	0.000
0.007	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.005	0.021	0.003	0.002	0.000	0.000	0.000	0.001	0.001	0.000
0.007	0.003	0.001	0.000	0.002	0.000	0.000	0.001	0.000	0.000
0.002	0.007	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000
0.004	0.010	0.002	0.002	0.001	0.000	0.000	0.000	0.000	0.000
0.001	0.000	0.003	0.004	0.000	0.001	0.000	0.000	0.000	0.000
0.001	0.003	0.000	0.000	0.002	0.000	0.001	0.000	0.001	0.000
0.002	0.012	0.002	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.001	0.000
0.007	0.001	0.006	0.002	0.000	0.000	0.000	0.000	0.000	0.000
0.002	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
0.002	0.005	0.001	0.001	0.001	0.000	0.001	0.000	0.000	0.003
0.000	0.002	0.002	0.003	0.000	0.000	0.001	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.007
0.002	0.005	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.001	0.001	0.000	0.002	0.000	0.005	0.000	0.000	0.000	0.000
0.000	0.001	0.000	0.006	0.000	0.000	0.001	0.000	0.000	0.000
0.011	0.000	0.001	0.000	0.003	0.000	0.000	0.000	0.000	0.000
0.010	0.001	0.000	0.001	0.001	0.001	0.000	0.000	0.001	0.000
0.003	0.007	0.001	0.002	0.001	0.000	0.000	0.000	0.000	0.000
0.002	0.002	0.005	0.000	0.000	0.002	0.000	0.000	0.001	0.000
0.002	0.002	0.003	0.000	0.001	0.002	0.000	0.000	0.000	0.000
0.000	0.002	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
0.007	0.001	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.001
0.010	0.000	0.000	0.003	0.000	0.000	0.001	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.001	0.000	0.001	0.005	0.000	0.000	0.001	0.003
0.015	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.005	0.009	0.005	0.001	0.001	0.001	0.000	0.000	0.001	0.000
0.008	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.001	0.000
0.010	0.001	0.002	0.000	0.001	0.000	0.000	0.000	0.001	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.001	0.000	0.003	0.003	0.000	0.001	0.000	0.000	0.000	0.000
0.003	0.006	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.002	0.003	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.012	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.001
0.012	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000
0.013	0.001	0.002	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.001	0.000	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000
0.004	0.013	0.002	0.001	0.000	0.000	0.000	0.001	0.001	0.001
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
0.026	0.000	0.002	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.001	0.000	0.002	0.001	0.000	0.000	0.001	0.000
0.003	0.005	0.004	0.002	0.000	0.000	0.000	0.000	0.001	0.000
0.012	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
0.003	0.003	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000
0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.000
0.013	0.008	0.000	0.001	0.000	0.000	0.001	0.001	0.001	0.000
0.004	0.003	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000
0.004	0.005	0.000	0.001	0.000	0.001	0.000	0.002	0.003	0.000
0.000	0.000	0.000	0.000	0.002	0.001	0.000	0.000	0.000	0.000
0.004	0.003	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
0.006	0.018	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.007	0.000	0.003	0.002	0.000	0.001	0.001	0.000	0.001	0.000
0.003	0.004	0.000	0.000	0.000	0.001	0.000	0.001	0.001	0.000
0.000	0.000	0.000	0.000	0.004	0.003	0.000	0.000	0.000	0.000
0.001	0.002	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.001	0.000	0.005	0.005	0.000	0.001	0.000	0.000	0.000	0.000
0.004	0.004	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000
0.001	0.000	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.001	0.003	0.002	0.000	0.000	0.000	0.000	0.005	0.000	0.000
0.000	0.000	0.002	0.001	0.000	0.005	0.000	0.002	0.000	0.000
0.000	0.000	0.001	0.000	0.002	0.002	0.000	0.001	0.000	0.000
0.001	0.004	0.002	0.002	0.000	0.000	0.003	0.000	0.001	0.000
0.000	0.003	0.002	0.002	0.000	0.000	0.005	0.000	0.001	0.000
0.001	0.000	0.001	0.000	0.001	0.001	0.006	0.000	0.000	0.000
0.000	0.000	0.002	0.001	0.000	0.005	0.000	0.000	0.001	0.000
0.000	0.000	0.001	0.000	0.000	0.000	0.006	0.000	0.001	0.000
0.000	0.001	0.001	0.000	0.003	0.000	0.000	0.000	0.000	0.000
0.002	0.002	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000
0.000	0.000	0.000	0.003	0.003	0.003	0.000	0.002	0.000	0.002
0.003	0.004	0.000	0.000	0.000	0.001	0.004	0.000	0.000	0.000
0.002	0.003	0.001	0.000	0.000	0.001	0.001	0.001	0.000	0.000
0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000
0.000	0.000	0.001	0.000	0.000	0.000	0.001	0.000	0.004	0.001
0.001	0.003	0.000	0.004	0.001	0.003	0.000	0.000	0.000	0.000
0.001	0.001	0.001	0.001	0.003	0.005	0.000	0.000	0.000	0.000
0.001	0.000	0.004	0.004	0.000	0.001	0.000	0.000	0.000	0.000
0.009	0.003	0.002	0.001	0.000	0.000	0.000	0.001	0.000	0.000
0.003	0.000	0.001	0.000	0.000	0.000	0.001	0.002	0.001	0.000
0.000	0.000	0.001	0.000	0.002	0.000	0.000	0.000	0.001	0.000
0.012	0.000	0.001	0.000	0.001	0.000	0.001	0.000	0.000	0.000

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
0.001	0.002	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.003	0.000	0.000	0.004	0.000	0.001	0.001	0.000
0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.006	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.001	0.005	0.001	0.000	0.002	0.000	0.005	0.000	0.000	0.000
0.000	0.000	0.001	0.000	0.001	0.000	0.007	0.000	0.001	0.000
0.001	0.000	0.003	0.004	0.000	0.000	0.000	0.000	0.000	0.000
0.005	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000
0.005	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.002
0.004	0.008	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000
0.005	0.008	0.000	0.001	0.003	0.000	0.000	0.000	0.000	0.000
0.001	0.001	0.000	0.003	0.001	0.004	0.004	0.000	0.000	0.000
0.008	0.001	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.002	0.000	0.003	0.000	0.003	0.000	0.000	0.000
0.001	0.004	0.001	0.000	0.000	0.000	0.000	0.001	0.001	0.000
0.001	0.003	0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.001	0.001	0.002	0.002	0.003	0.006	0.000	0.000	0.000	0.000
0.001	0.000	0.002	0.004	0.000	0.001	0.002	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.001	0.005	0.003	0.000	0.000	0.000	0.000	0.002	0.001	0.001
0.002	0.012	0.001	0.000	0.000	0.000	0.005	0.000	0.000	0.000
0.005	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.004	0.001	0.001	0.001	0.003	0.001	0.000	0.002	0.000	0.000
0.013	0.000	0.002	0.000	0.001	0.000	0.000	0.000	0.000	0.003
0.007	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.001	0.002
0.002	0.004	0.000	0.000	0.002	0.000	0.000	0.000	0.001	0.000
0.002	0.004	0.003	0.002	0.000	0.000	0.000	0.000	0.001	0.000
0.004	0.007	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000
0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000
0.000	0.001	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.000
0.006	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001
0.008	0.000	0.001	0.000	0.001	0.001	0.000	0.001	0.000	0.001
0.004	0.008	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.000	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000
0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.011	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.001	0.014	0.000	0.000	0.001	0.000	0.000	0.000	0.000
0.009	0.010	0.003	0.001	0.000	0.000	0.000	0.000	0.001	0.000
0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.009	0.006	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.001

Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10
0.009	0.007	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000
0.010	0.000	0.001	0.000	0.002	0.000	0.001	0.001	0.000	0.000
0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.000
0.003	0.005	0.001	0.000	0.000	0.000	0.001	0.003	0.001	0.000
0.020	0.005	0.003	0.000	0.000	0.001	0.001	0.000	0.001	0.000
0.002	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.010	0.008	0.001	0.000	0.000	0.003	0.000	0.001	0.000	0.000
0.005	0.006	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
0.003	0.001	0.001	0.000	0.002	0.001	0.000	0.000	0.000	0.003
0.005	0.008	0.002	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.015	0.005	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000
0.005	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.004	0.000	0.004	0.001	0.000	0.000	0.000	0.000	0.000	0.000
0.007	0.001	0.001	0.002	0.000	0.001	0.001	0.000	0.000	0.000
0.000	0.000	0.000	0.001	0.002	0.000	0.001	0.000	0.000	0.000
0.014	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.004	0.004	0.004	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000
0.011	0.000	0.001	0.001	0.004	0.000	0.000	0.000	0.001	0.000
0.002	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.014	0.000	0.001	0.003	0.002	0.001	0.000	0.002	0.000	0.001
0.006	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001

Table 2: Model simplification information: OTU2, *Pyrenophora spp.*

Note same procedure used for all other 25 OTUs

Model : Equation	Compare (model numbers)	AIC	BIC	P
Model 1: OTU2 ~ Treatment * Provenance +(1|Plot/Treatment) +(1|Species)		573.1	600.8	0.002
Model 2: OTU2 ~ Treatment + Provenance + (1|Plot/Treatment) + (1|Species)	anova(1,2)	571.7	595.9	0.02
Dropping interaction did not make a significant difference to the model				
Model 3: OTU2 ~ Provenance + (1|Plot/Treatment) + (1|Species)	anova(2,3)	572.9	593.8	0.12
Model 4: OTU2 ~ Treatment + (1|Plot/Treatment) + (1|Species)	anova(2,4)	573.9	594.7	**0.01**
Dropping treatment did not make a significant difference to the model, but dropping provenance did				
Final model: OTU2 ~ Provenance + (1|Plot/Treatment) + (1|Species)		572.9	593.8	0.12

Table 3: Top 26 Most Abundant OTUs from fungal isolates from grass root endophytes.

Known habitat and source provide the most contemporary example within following priorities: within grass roots > within grasses > within plant roots > within soil

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU13	CCTTGATAAGTTGGGGGGTTGCTGGCCAGCATCCACCGGACGTCTGTAGCGAGAGTATGTACTACGCTTAGAGCCAGATGGCGCCGCCACTGATTTTAAGGCACACCGGGACCGGTGACGCCCAAGACCAAGCAGAGCTTGAGGGTTGTAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTATATAGTACTCAGACGACACTAACATTCAGGTTTTGAGGTCCTCTGGCGGGCGCGCCCTAGCCGGAGCCAGGGGCGGGGCCGGGGCCCCGCGGCCCGCCAAAGCAACGAAGGTATAACGACACAGGGTGGGAGAGGTCTACCCAAAGGGCAGAGTCTCTGTAATGATCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTACTT	Pezicula ericae	99.62	Roots of Vaccinium membranaceum & Gaultheria shallon	Chen et al. 2016	present
OTU2	CGAGGTCAAACGTTGAAAAAGTGGCTTCATGGACGCGGACCGCTGCTGGAAGAAGCGCAAATTTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATGATTTTAAGGCGAGTCGCTGGCGGACCAGAGACAAAAACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAATTTATTACATTGTTTTGCTGACGCTGATTGCAATTACAAAAAGGTTTGAATGGTCCTGTTGGTGGGCGAACCCACCAAGGAAACAAGTAGTACGCAAAAGACATGGGTGAATATGGAGTCAGGCTGGCCGAAAAGACGCCCGCCCTGTCGCTTCAGCACCGCAGATGCGGCGGAAACGGCAGAATAGACGCCCTCCCGGGGATCCCAGCCCGCTTGCATATTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTACT	Pyrenophora spp.	99.67	Leaves of Dactylis glomerata	Márquez et al. 2007	present

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU6	TTGGGGGGTTCTGGCAGGTATCCAGGGGAACTCCATCGCGAGAAGAATTACTACGCGTAGAGCCACACCGGCACCGCCACTAGTTTTAGGGGCTGCGGAACCGCAAACCCCAATACCAAGCCAGGCTTGAGTGGTTGTAATGACGCTCGAACAGGCATGCCCTGCGGAATACCACAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTATATAGTACTCAGACATCACTAAAATTCAGAGTTTGATCCTCTGGCGGGCACATGCAGGCAGAGCCCACAGTGGAGGCCACGGCCCGCCAAAGCAACAAAAGTATGTAGACACGGGTGGGATTCACTCGGCCCCTAGCTCGAGGGCTAGGGCATCAGTATCCCGCGCGACTGGTAATGCTGTGAGTTTAGAACAGCCCCACAAGGTCGAGTAATCGACGTCGCTTGGGTACACCGGTGATCGTTTTTAATGATCCTTCCGCAGGTTCACCTACGGACGCAGAAACCCTAGAGGTCTCCCTCCAGGCTCGACTATATCTTAGATAGGGTTCTCCTACCCACAACCACTTAGTCTGT	*Phialocephala spp.*	100	Roots of *Cynodon dactylon & Pennisetum clandestinum*	Wong et al. 2015	present
OTU79	GATCCGAGGTCAATTTTCAGAAGTTGGGGGTTTAACGGCAGGGCACCGCCGGGCCTTCCAGAACGAAATATAATTACTACGCTCGGGGTCCTGGCGAGCTCGCCACTAGATTTCAGGGCCCGCCCTTTTACAGGCGGTGCCCCAACACCAAGCAATGCTTGAGGGTTAAAATGACGCTCGAACAGGCATGCCCTCCGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTTATGTTTTTTACTCAGAGATTCACTAAGAAACAAGAGTTTGGTTGGCCGCCGGCGGGCTGCTCCTCGTTTCCGAGGGGCCTCAGTGAGGCCGGCCTGCGCCGAGGCAACAGTAAGGTATAAGTTCACAAAGGGTTTCTGGGTGCGCCGAAGCGCGTTCCAGCAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTTTTACTT	*Diaporthe columnaris*	99.45	Leave, roots, & stems of *Pouteria reticulata*	Spear & Broders 2021	present
OTU3	CCGAGGTCACATTTCAGAAGTTGGGTGTTTTACGGACGTGGACGCGCCGCGCTCCCGGTGCGAGTTGTGCAAACTACTGCGCATGAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGGGCCGGGATCCCGTCTTAGGGGTTCCCGAAGTCCCCAACGCCGACCCCCCGGAGGGGTTCGAGGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATTTTGAATTTTTTGCTCAGAGCTGTAAAAATAACGTCCGCGAGGGGACTACAGAAAGAGTTTGGTTGGTCCCTCCGGCGGGCGCCTGGTTCCGGGGCTGCGACGCACCCGGGGCGTGACCCCGCCGAGGCAACAGTTTGGTATGGTTCACATTGGGTTTGGGAGTTGTAAAACTCGGTAATGATCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGACTTTTACT	*Trichoderma ghanense*	99.61	Leaves of *Dactylis glomerata*	Márquez et al. 2007	present

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU31	ATCCGAGGTCAACCATTAAAAAGTGCTGCCGAGGCAAGCGGTTTTTGGCTATCGTCTAGACGTGTTCAAAAGCGAGAATAGAATTACTGCGCTCAGAGTACGTAAAAACTCTGCCCACTGGTTTTGAGGAGCTGCGTATTAGGCAGTCTCCCAACACTAAGCTAGGCTTAAGGGGTTGAAATGACGCTCGAACAGGCATGCCCACTAGAATACTAATGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTTATTTCTTAGTTTGATTCAGAATAACAAAAATTAACAAGAGTTTAGATGTCCGCCGGCTTCCAGCACCCTTTCGGGTACCTTCCACCGAGGCAACAGTGGTAAGTTCACATGGTTTTAGGAGTTAAAAACTCTGTAATGATCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTACTTC	*Microdochium lycopodinum*	100	Leaves of *Indocalamus longiauritus*	Huang et al. 2020	present (genus Microdochium)
OTU49	TCCGAGGTCACCTTGATAGATGGGGGGTTGCTGGCCAGCATCCACCGGGCGTCTGTAGCGAGAGGATGTACTACGCTTAGAGCCAGGTGGCGCCGCCACTGATTTTAAGGCACACCGGGGACCGGTGACGCCCAAGACCAAGCAGAGCTTGAGGGTTGTAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTATATAGCACTCAGACGACACTAACGTTCAGGTTTTGAGGTCCTCTGGCGGGCGCGCCCCAGCCGGAGCCGGGGGCAGGGCCGGGGCCCCGCGGCCCGCCAAAGCAACAAAGGTACAACGACACAGGGTGGGAGGTCTACCCAGGGGGGCAGAGTCTCTGTAATGATCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTACTTC	*Pezicula rhizophila*	99.81	Leaves & roots of *Erica tetralix, Calluna vulgaris, Vaccinium vitis-idaea,* & *Vaccinium myrtillus*	Chen et al. 2016	present
OTU4	GTAAAAATTAGGGGTTGCTGGCAAGTAAACCTACCGGACTCAATCGCGAGGAGTATTACTACGCGTAGAGCCGACAGGCACCGCCACTGACTTTAGGGGCCGCGAGACCGCGAACCCCAATACCAAGCGAGAGCTTGAGTGGTTATAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTTATATAGTACTCAGACATCACTAAAAACAAGAGTTGTGGTCCTCTGGCGGGCACGCAACAGCCGAAGCCGCTGGCACGAGGCGGCCCGCCAAAGCAACAAAGGTAATTTATTCAAGGGTGGAGTTCAGGACCGAGCTTCTTCGAGAGGCCCGACGACTCTAAACCTTACCGAAGTAGGGTAGCCCCAGGGAGCAAGGCTCCGCGGGCGCTGTCTATCCTTTGCTCTAGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTT	*Mycochaetophora spp.*	99.83	Roots of *Zea mays*	Moll et al. 2016	unclear

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU14	GATCCGAGGTCAACCTTGTGTAAAAGATGGGGC TTTCACGGCCGGAACCCGCGACACCTCCCTAGC GAGATATTTTACTACTACGCTCGGAGTTATAGCG AGCCCGCCACTAGCTTTCAGGGCCTACGGCAGC CGTAGGACCCCAACACCAAGCGGGGCTTGAGG GTTGAAATGACGCTCGAACAGGCATGCCCGCCA GAGTACTGGCGGGCGCAATGTGCGTTCAAAGAT TCGATGATTCACTGAATTCTGCAATTCACATTACT TATCGCATTTCGCTGCGTTCTTCATCGATGCCAGA ACCAAGAGATCCGTTGTTGAAAGTTTTAACTTAT TTGCTTGTTTTCTCAGAGAGGCCACTAAAATACA AAAGAGTTTTGGTACGCCGGCGGGCACCCCCCG CGAGGGAGGCAGCGGCCCCAGGGAGGGGCCGC CCGCCGAGGCAACGTCTAACGGTACGTTCACAA TGGTTTTGGGAGTCTTTTTAGAACTCTGTAATGAT CCCTCCGCTGGTTCACCAACGGAGACCTTGTTAC GACTTTTACTT	*Lachnum controversum*	98.83	Stems of *Micanthus floridus* & *Micanthus sinensis*	Wu 2004	present
OTU22	AACCTTTCAGAAGTGGGGGGTTTAACGGCGTGG CCACGCTGTTTTCCAGTGCGAGGTGTGCTACTAC GCAGAGGAAGCTACAGCGAGACCGCCACTATA TTTGGGAGCCGGCGCGCCCCGGGGGGCACGCCG ATCTCCAACACCAAGCCCGGGGGGCTTGAGGGTT GAAATGACGCTCGAACAGGCATGCCCGCCAGA ATACTGGCGGGCGCAATGTGCGTTCAAAGATTC GATGATTCACTGAATTCTGCAATTCACATTACTT ATCGCATTTCGCTGCGTTCTTCATCGATGCCAGA ACCAAGAGATCCGTTGTTGAAAGTTTTGATTTAT TTAATCATTTACTCAGAAGATACTGTATAAAATC AAGGGTTTGGGTCCTCTGGCGGGCTGCCGAAGC AGGCACCGCCGAGGCAACAAATGGTATGTTCAC AGGGGTTTGGGAGTTGTAAACTCGGTAATGATC CCTCCGCTGGTTCACCAACGGAGACCTTGTTACG ACTTTTACTTC	*Ilyonectria mors-panacis*	100	Roots of *Panax quinquefolius*	Westerveld & Shi 2021	present
OTU75	GATCCGAGGTCAACATTCAGAAGTTGGGGTTTT ACGGCATGGCCGCGCCGCGTTCCAGTTGCGAGG TGTTAGCTACTACGCAATGGAGGCTGCAGCGAG ACCGCCAATGTATTTCGGGGACGGCGCCGCCCA GAAGGGCAGAGCCGATCCCCAACACCAAACCC GGGGGGCTTGAGGGTTGAAATGACGCTCGAACA GGCATGCCCGCCGGAATACCAGCGGGGCGCAAT GTGCGTTCAAAGATTCGATGATTCACTGAATTCT GCAATTCACATTACTTATCGCATTTTGCTGCGTTC TTCATCGATGCCAGAACCAAGAGATCCGTTGTTG AAAGTTTTGATTTATTTGTTTGTTTTACTCAGAAG TTACAATAAGAAACATTAGAGTTTGGGTCCTCTG GCGGGCCGTCCCGTTTTACGGGGCGCGGGCTGA TCCGCCGAGGCAACATTAAGGTATGTTCACAGG GGTTTGGGAGTTGTAAACTCGGTAATGATCCCTC CGCTGGTTCACCAACGGAGACCTTGTTACGACTT TTACTT	*Fusarium acuminatum*	100	Leaves & stems of *Triticum aestivum* , *Cucurbita pepo* , & *Solanum tuberosum*	Summerell et al. 2011	present

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU29	TAAAAGAAGCTTAATGGGAGGAGGCCTGCCAGAATCGCGACGCAATGTGCTGCGCACGAAGCCAACATACCGGCTGCCAATGAATTTAAGGCGAGTCCACGCGCTGAGGCGGAACAAACACCCAACACCAAGCAGAGCTTGAATGATTAAATGACGCTCGAACAGGCATGCCCAACGGAATACCGAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAATATTTGTTTTTACTGAAGATACTGCTACTACAAAGGTTTTTGTTGGTCCTCGTGGCAGGCAAGCCCACCGAGGAAACGAACGGTACTCATAAACAAAGGGTGCCATTTGGCGCGTTTTAAGGCCGCCGCCGTGCAAGCATTTCTGCAAGCACCATTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTT	*Pleotrichocladium opacum*	99.81	Wood of *Thuja occidentalis*	Hernandez-Restrepo et al. 2017	present
OTU46	TTAGAAATGGGGGTTGTTTTACGGCGTAGCCTCCCGAACACCCTTTAGCGAATAGTTTCCACAACGCTAGGGGACAGAAGACCCAGCCGGTCGATTTGAGGCACGCGGCGGACCGCGTTGCCCAATACCAAGCGAGGCTTGAGTGGTGAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAGGGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAGTTTTAATTTATTAATTAAGTTTACTCAGACTGCAAAGTTACGCAAGAGTTTGAAGTGTCCACCCGGAGCCCCCGCCCGAAGGCAGGGTCGCCCCGGAGGCAACAGAGTCGGACAACAAAGGGTTATGAACATCCCGGTGGTAAGACCGGGGTCACTTGTAATGATCCCTCCGCAGGTTCACCTACGGAGACCTTGTTACGACTTTTACT	*Cladosporium delicatulum*	99.81	Roots & leaves of *Arnebia euchroma*	Jain et al. 2021	present
OTU56	GATCCGAGGTCAACCTGGAAAAAATTTTGGTTGATCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGATCGGAGGACGGGGCCCAACACACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAAATAATTTATATTTTCACTCAGACTACAATCTTCAGACAGAGTTCGAGGGTGTCTTCGGCGGGCGCGGGCCCGGGAGCGTAAGCCCCCCGGCGGCCATAAAGGCGGGCCCGCCGAAGCAACAAGGTACAATAAACACGGGTGGGAGGTTGGACCCAGAGGGCCCTCACTCAGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTA	*Penicillium sajarovii*	100	Leaves & stems of *Pinus ponderosa* & *Pseudotsuga menziesii*	Ridout et al. 2017	absent

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU110	AGTTTTACAAGAATCGTTGCCGACCCTTGTGAAATCCTGCATCAGTCAGCCAAAACGGTCAAACTCCCTTTATATTAGCTGCAGCAAAAGTAATAATCCGTTTGACGGGACTAAATAAATATGCTTTTAGCTCAGGAGAATGTCCAGCTGCACCTGCATTTCAAGCAACCCGCCACTGATCTAAAAAAGATTGGTGTTGGGATTGCTCAAGTCCAAAACTGTTTTTCAAAAAATAAAAAAGAGTTTTGAGGTGTTTACTGATACTCAAACAAGCATGCTCTTCGGAATACCAAAGAGCGCAATATGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAGTTGTATTTTGAATTATTTTATTCATAATATTTTTCAGACAAAGAGTTAAAAAAATAGGTTGATGTTTGGCCGATCTCCATAAAGAAGACCGACTGACATTGCACACAAGGTGGATATGGATTTAAAAAGTGCCTAAAAACACTTATTATGAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTA	*Linnemannia aff. gamsii*	100	Soil associated with *Pinus cembra*	Telagathoti et al. 2021	present (genus Linnemannia)
OTU54	AAGTTTTGGTTGATCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGAAGGGGGACGAGACCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAAATAATTTATATTTAGACTCAGACTGCAATTTTCATACAGAGTTCAAGGTGTCTTCGGCGGGCGCGGGCCCGGGGGGCAGATGCCCCCCGGCGGGCCGTGAGGCGGGCCCGCCGAAGCAACAAGGTACAATAAACACGGGTGGGAGGTTGAATTCAGAGAATTCTCGCTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCCCAATTTTTTCCCCCAAAAAATTTCATAGGGGGAAGAAAAA	*Penicillium fellutanum*	100	Soil associated with *Rhizophora annamalayana*	Kathiresan & Manivannan 2010	present
OTU53	GATCGAGTTTACAAAGGCCAGCCGAAGCTGTCTCTGTGAATCCTGCATCAGTCAGCACAAGAACTAATCTCCTTTATGTTAGCTGCAGCAAAGGTAATAATCTGTTTTTTTAGGCAGACTAAATAGATATGCTTATAGCTCAGAGAAAAGTCCAGCTGCACCTGCATTTCAAGTAACCCGCCGCTTTTCGGTGAGAAAAGCGTTGGGATCACTCAAGTCCAGCTCCCATTTCAAAAAAGAAAGGGAAGTTGAGGTGTTTACTGATACTCAAACAAGCATGCTCTCCGGAATACCAGAGAGCGCAATATGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAGTTGTATTTTGAATTAAGTTATTCATAATATGTTTCAGACAAAATCACTAAAGTTCTGAGTAGATATAAATCCCAAAGGTGACCAACGGATTGTTACAGCCGTGACCTCCAGTGAGATGACATTGCACACAAGGTGGATATGGATTTTTGAAAGTGCCATAAAAACACTTGATTATGAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCTTATTTACCCCCAAAAAAAAAAATCAAAAAGGGGGGGGAAAAAAGCCCCCCCCGCCGCCAATTGCCTCATGGTGGAACCCCACCAAATTGTGGCCTCGAGGCGGGCCTCCCCCCCCCCCGAAAGAAGTGGTTGGAACCGGGAGGTCTTTAAACCCCCCCCCCCCAATTCAATTGTGTTTGCCCCCATTTCCGCCCTTCCCCTAAAAACTTAATTTATAGGCCCCCCCCCCCCCCCTGGGGTGCCAAGGGCCCCCCCCTCACGCCTCTGCTACCGAAAGGACCTGTTGCTTAAGCAAAAACTTTGCTACCGACTTTTACTTCC	*Mortierella alpina*	100	Roots/corms of *Crocus sativus*	Wani et al. 2016	present

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU21	CGCCGGCCGGGCCTACAAGAGCGGGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGGAAGGGGGACGGGGCCCAACACACCAGCCGGGCTGGAGGGCAGCAATGACGCTCGGACAGGCATGCCCTCCGGAATACCAGAGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAATTAATTTAATAATTGTCTCAGACTACATACTTCAGACGAGTTCACAGGTGGCTTCGGCGGGCGCGGGCCCGGGGGGCAGATGCCCCCCGGCGGCCGTGAGGCGGGCTCGCCGAAGCAACAAGGTTCGTTAAACACGGGTGGGAGGTTGGACCCCGAGGGGCCCTCACTCAGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTCC	*Penicillium wellingtonense*	99.82	Soil from Wellington, New Zealand	Houbraken et al. 2011	present
OTU57	CTGAAAAAGATTGATTGTTGTCGGCAAGCGCCGGCCGGGCCTACAAGAGCGGAAGACGAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGAAGGAGGACGGGGCCCAACACACAAGCCGTGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTTATTTAGCTAATTGCTCAGACTGCAATCTTCAGACAGCGTTCAATGGTGTCTTCGGCGGGCGCGGGCCAGAGGGCAAAAGCCCCCCGGCGGCCGTGAGGCGGGCCCGCCGAAGCAACAAGGTACGATAAACACGGGTGGGAGGTTGGACCCAGAGGGCCCTCACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTC	*Penicillium maclennaniae*	99.82	Leaves & stems of *Nicotiana tabacum*	Xing et al. 2022	absent
OTU82	AATTTGGGGTTGCTGGCAAGCATCTCCCCAGACCCTATAGCGAGAAAATTACTACGCGTAGAGCTGAAGAGCACCGCCACTAGTTTTAAGGCCCGCCAGACGGCGAAGCCCAACACCTAGCCAGGCTAGATTGGTATAAATGACGCTCGAACAGGCATGCCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAAATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTTAATAGTACTCAGACGACACTAACATTCAGAGTTTAGGGGTCCTCTGGCGGCCACGCTAGACGCGAATCTAGGTGCACGAGGCGCGGCCCGCCAAAGCAACATTCTATAAAGATATACAAGGGTGGGAGATCTACCCCGAAGGGCATGAACTCTGTAATGATCCTTCCGCAGGTTCACCTACGGAACGGCTAACCTCTGTAGTTTCCTAGAGAGCCCGACTATATCTTAAGCAGGACGTGCCTACCCACAACCACTTAGTCTGTGAACGTTCCCCGTAGGCCTAGGCCGTTAGGGGCTTCGCTGCGGATTATCCATTGTCGCATCCTAGCAGATTCTGACCTCCAGCGTGATTAACACTGGCCCACCCTGCATTTCTAGAGGGTGTTGGTACTGCTAGGCTTTAGGAGATCCCCGCAATTCGATTATGTTGCCGCCACAGCGA	*Lachnum virgineum*	97.87	Roots & stems of *Brachypodium rupestre*	Durán et al. 2021	present

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU17	TGATTTGAGATCGAGTTGAACAACACATAAGTGTCGTAAAATCCTGCATCAGTCAGCAAAGAGGACAATTATCCTTTATGTTAGCTGCAGCAAAAGTAATAATCCTGTTTGACGAGGACTAAATAGATATGCTTTTAGCTCAGATAAAAGTCCAGCTGCACCTGCATTTCAAGCCGCCCGCCTACCGGTGAAGGTCGTTGGGATAGCTCAAGTCCACCCTCTCTTTTTTTGTCAAAAAGAGAGGTTGAGGTGTTTACTGATACTCAAACAAGCATGCTCTTCGGAATACCAAAGAGCGCAATATGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACGTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTTGAAAGTTATATTTTGAATTAAGTATATTCATAATATGTTATCAGACGAATGTTGTTAAAGATATAGGTTGATATTTATGAAGGGAAAGAAAAGACTTTCCCAACAAACATTGCACACAAGGTGGATATGGATTTTTAAAGAGTGCCTAAAACACTCGTTTGTGAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTAC	*Podila minutissima*	99.83	Roots associated with *Pinus spp.*, *Picea spp.*, & *Quercus spp.*	Vandepol et al. 2020	present
OTU118	AGGTCACCTGAAAAAATTGGGGGGTTGCTGGCAAGCATCTCCACAGACCCTATAGCGAGAAGAATTACTACGCGTAGAGCTGAGGAGCACCGCCACTGATTTTAAGGCCCGCCAGACGGCGAAGCCCAACACCTAGCCAAGCTAGATTGGTATTAATGACGCTCGAACAGGCATGCCCCCCGGAATACCGAGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTTAATAGTACTCAGACGACACTAATATTCAGAGTTTGGGATCCTCTGGCGGGCACGCCGGACGCGAATCCGGGCAAGCCAGGCTTGCGGCCCGCCAAAGCAACATTTGATAATGATACACAAGGGTGGGAGATCTACCCCGAAGGGCATGAACTCTGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTTC	*Lachnum asiaticum*	97.54	Roots & soil associated with *Aucuba japonica*	Degawa et al. 2011	present (genus Lachnum)
OTU43	TTAGGGGTTGCTGGCAAGTAGACCTACCGGACTCAATCGCGAGGAGTATTACTACGCGTAGAGCCGACAGGCACCGCCACTGATTTTAGGGGCCGCGAAACCGCGAACCCCAATACCAAGCGAGAGCTTGAGTGGTTATAATGACGCTCGAACAGGCATGCCCCCCGGAATACCAGAGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTATTATATAGTACTCAGACATCACTAAAAACAAGAGTTGGGGTCCTCTGGCGGGCACTCAACAGCCGAAGCCGCTGGCACGAGGCGGCCCGCCAAAGCAACAAAGGTAGTTTATTCAAGGGTGGAGTTCAGGACCGAGCTTCTCCGAGAGGCCCGACGACTCTGAACCCTACGGGAGTAGGGTAGCCCCCGGGAGCGAGCTCCGCGGGCGCTGTCTATCCTTTGCTCTAGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTACTT	*Cadophora orchidicola*	99.67	Roots from *Picea abies*, *Fraxinus excelsior*, & *Acer pseudoplatanus*	Stroheker et al. 2021	present (genus Cadophora)

OTU Name	DNA Sequence	BLAST ID	Percent Identity	Known Habitat	Source	NZ Status
OTU5	TGTCTACAGTTGTAGACGGTTCGAAGCAGACAA TCCTGACTCAAAAGACAGAAAGAGCAAGTAAA TGCAACTCTCCACACCAAGACTGTAAACAGCTT GAAAGGGGTTCTAATGAGCATGCATTCAAAACCA TAGCGTAGATAATTATCACACTAAGGATGAACA CAAACGGGTCCACTAATGTATTTCAAGGGAGCT GAACATTTAAAACGCCAGCAAAGCCCTCACATC CAAGCTTCAAAAAGACAAAGCTTTTGAAGTTGA GAATTTAATGACACTCAAACAGGCATGCCTCTC GGAATACCAAGAGGCGCAAGGTGCGTTCAAAG ATTCGATGATTCACTGAATTCTGCAATTCACATT ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCG AGAGCCAAGAGATCCGTTGCTGAAAGTTGTATA AGGTTTAAAGGGTCAATCAAGCCCCAATAAAAG ACATTCATGACATTCATAGAGTAGGTAAGATAC ATAGAACCTAGAGTACTAAGGCCTTGGCGTTAA CCTAGACCCATTCCTCAGAGCTCTACAATAAGTG CACAGGTGGAGAAGGATGAATAGAAGCAGCAA GCACATGTCTAGTTAAAGACCAGCTCAGCCACTT CAGTACATTCAATAATGATCCTTCCGCAG	*Tetrapyrgos subdendrophora*	98.15	Roots from *Stenotaphrum secundatum*	Vinnere et al. 2005	unclear
OTU50	GATCCGAGGTCACCTGTAAAAATTGGGGGTTCT GGCGAGCCACCGGGGGAACTCTATAGCGGGAG TATGTACTACGCTTAGAGCCCACCGGCGCCGCCC TAATTTTGAGGGCCGCGGAGACCGCGTGCCCCA ATACCACGCTGGCGTGAGTGGTTAAATGACGCT CGAACAGGCATGCCCTGCGGAATACCACAGGG CGCAATGTGCGTTCAAAGATTCGATGATTCACTG AATTCTGCAATTCACATTACTTATCGCATTTCGCT GCGTTCTTCATCGATGCCAGAACCAAGAGATCC GTTGTTGAAAGTTTTAACTATTAGATAGTACTCA GACATCACTAACATTCAGAGTTTGGTTCTCTGGC GGGCACACACGAGCAGAGCCCGCAGTGGAGGC CACGGCCCGCCAAAGCAACAATAGTATGTAGAC ACGGGTGGGTGTAAGCTCCTAACCGCTGTTTCCA ACGTTCGGTAGCCTCACTTGTAATGATCCTTCCG CAGGTTCACCTACGGAAACCTTGTTACGACTTTT ACTC	*Phialocephala fortinii*	99.81	Roots from *Vaccinium vitis-idaea, Empetrum nigrum, Calluna vulgaris,* & *Fagus sylvatica*	Zijlstra et al. 2014	present
OTU18	CCGAGGTCAAGACGGTAATGTTGCTTCGTGGAC GCGGGCTGCCCACCTCGAGAAGCGCAATGTGCT GCGCGAGAGGAGGCAAGGACCGCTGCCAATGA ATTTGGGGCGAGTCCACGCGCGGAGGCGGGAC AGACGCCCAACACCAAGCAGAGCTTGAGGGTG TAGATGACGCTCGAACAGGCATGCCCCATGGAA TACCAAGGGGCGCAATGTGCGTTCAAAGATTCG ATGATTCACTGAATTCTGCAATTCACACTACTTAT CGCATTTCGCTGCGTTCTTCATCGATGCCAGAGC CAAGAGATCCATTGTTGAAAGTTGTAACGATTGT TTATATCAGAACAGGTAATGCTAGATGCAAAAA GAGTTTTAGCGTTCCAACGGCAGGTCGCCCCACC GAAGGAGAACGAAAGGTGCTCGTAAAAAAAG GATTCGGGTATGTGGAGCGTGAGAGTTTTACCTC TACCGCCCGGGGGGATGCCCCCGGGGGCCGCTG CCACACCGATGGGATAGATAATGATCCTTCCGC AGGTTCACCTACGGAAACCTTGTTACGACTTTTA CTTCC	*Paracamarosporium spp.*	97.36	Roots & stems of *Brachypodium rupestre*	Durán et al. 2021	unclear