Microbial community and functional gene profiling of kauri soil

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Abstract

Kauri (*Agathis australis*) is a significant native tree in Aotearoa New Zealand that holds cultural importance to Māori. The species plays a crucial role in maintaining ecological balance in kauri forests. Due to the intensified agriculture and timber industry many years ago, only about 1% of the original kauri forest remains today. This is now under threat from a soil-borne Oomycete pathogen known as *Phytophthora agathidicida*, that causes dieback disease in kauri. The immediate action to manage the spread of dieback disease and preserve the enduring health of kauri forests is paramount. Soil microbiota plays a crucial role in promoting plant health and preventing soil-borne diseases. The understanding of the microbial communities and diversity in kauri soils, such as in natural forests and anthropogenic settings, is limited. There is also sparse knowledge on possible microbial antagonists that can suppress plant diseases caused by pathogens like *P. agathidicida*. The main objective of this study was to characterise the microbial community structure in kauri soil, describe the functional potentials, and correlate these to the presence of the pathogen to the soil microbiome. Significant differences (p-values < 0.05) were found in soil microbial communities' diversity and species richness between soil from dieback symptomatic kauri trees and non-symptomatic kauri trees. Non-symptomatic kauri soils had a higher abundance of microbial genera such as *Penicillium, Trichoderma, Aspergillus, Streptomyces* and *Pseudomonas,* which have been reported to suppress growth of *Phytophthora* pathogens. These results may assist in the discovery of microbial taxa which enhance soil disease suppression.

The findings indicated notable variances in the signal intensities of carbon cycling genes and microbial defence genes in natural versus planted kauri soil (p values < 0.05). Specifically, the carbon degradation genes were significantly more abundant in the natural kauri soil, which can be attributed to the accumulation of litter in the forest. Furthermore, a range of genes involved in the nitrogen and phosphorus cycle processes, as well as genes that promote plant growth were identified. Notably, crucial genes, including PhID, which are linked to microbial defence and production of antimicrobial compounds capable of suppressing the growth of several *Phytophthora* species were also observed.

Through the in vitro screenings of fungal isolates, five different endophytes were identified to demonstrate varying levels of *P. agathidicida* growth inhibition in dual culture experiments. *Coprinellus micaceus* and *Ilyonectria mors-panacis* have shown to effectively inhibit the growth of *P. agathidicida* while also suppressing its zoospore formation. Further research needs to be conducted to understand the mechanism of inhibition and possible metabolite production of these isolates. It is worth considering these endophytes as potential biocontrol agents for plant pathogens, such as *P. agathidicida* in kauri.

Keywords: *Phytophthora agathidicida*; *Agathis australis*; kauri dieback; soil microbial community; high throughput sequencing; Geochip; taxonomic diversity analysis; functional gene analysis; in vitro screening; *Coprinellus micaceus*; *Ilyonectria mors-panacis*

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Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor used artificial intelligence tools or generative artificial intelligence tools (unless it is clearly stated, and referenced, along with the purpose of use), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Authors' contributions

The thesis is comprised of five peer-reviewed articles (Chapter 2, Chapter 3, Chapter 4, Chapter 5, and Chapter 6). Part of Chapter 5 has been published as a data submission and Chapter 6 has been published as a full article. Chapter 2, 3, and 4 are in preparation. Praveenth Lawrence as the first author had an overall contribution of 80% to each of the peer-reviewed manuscripts. He collected, analysed, and interpreted the data, and wrote the drafts of the manuscript. All other authors contributed to different capacities in conceiving and designing the study, in analysing and discussion of the results, and writing the final version of the manuscript.

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Chapter 1

Introduction

The spread of kauri dieback threatens Aotearoa New Zealand's ancient kauri forests, caused by the pathogen *Phytophthora agathidicida* (Beever et al., 2007). *Phytophthora agathidicida* is a highly infectious which spreads in kauri forests via soil, water, and root pieces (Beever et al., 2007; Bellgard, SE; Smith, C; Probst, 2019). Kauri trees are facing a grave threat from dieback disease, which underscores the pressing need for effective solutions to control its spread and safeguard the long-term health of kauri forests. Even prior to the identification of dieback as a significant danger, kauri forests were already among the most heavily disturbed indigenous forest ecosystems in Aotearoa New Zealand. In the early 1800s, following European settlement, the once-sprawling kauri forests were severely depleted due to unregulated logging for timber and land clearing for agricultural expansion. As a result of deforestation, the primary kauri forests that remain now only occupy less than 1% of their original area (Ecroyd, 1982; Steward, 2011). The role of soil microbiota in influencing the health of kauri trees is a necessary but understudied area of research. The soil microbiota is crucial in defence against soil pathogens and supporting plant health. They also aid the plant's immune response when facing pathogen attacks (Berendsen et al., 2012; Cook, 1985). To address this knowledge gap, the study aimed to analyse the diversity and potential functional genes of kauri soil microbiota and to screen kauri endophytes for their ability to inhibit the growth of the disease-causing Oomycetes *P. agathidicida*. The research involved studying soil microbial diversity and potential functional genes in planted kauri in anthropogenic (Auckland Botanic Gardens) and natural forest (Tairua and Whangapoa) settings. Additionally, the study correlated the phosphonate application on trees with the soil microbial community.

1.1 Thesis outline

The study aimed to explore the soil microbial communities associated with kauri trees. To achieve this, next-generation sequencing, GeoChip5 microarray, and culture-based techniques were employed in addressing the research questions. The research findings are presented in six chapters, starting with a detailed literature review in Chapter 2. There are four experimental chapters two of which have been published in Elsevier Data in Brief (part of Chapter 5 refer appendix C) Springer Journal of Plant Pathology (Chapter 6). Chapter 7 summarises the research findings and their implications, along with recommendations for future research.

1.2 Chapter 2

This chapter provides a comprehensive review of the existing literature that highlights the importance of kauri trees to the ecosystem and environment. It also discusses the current status of kauri dieback and the severity of the pathogen *Phytophthora agathidicida*. The chapter emphasizes the role of soil and root microbial communities in supporting kauri, especially in functions in nutrient cycles, microbial defence, plant growth promotion and disease suppression. Additionally, the chapter analyses the advancement of molecular techniques used to study microbial communities, such as high-throughput next-generation sequencing and Geochip microarray analysis. Finally, the chapter discusses the importance of identifying key beneficial microorganisms to suppress the growth of the pathogen and provide biological control.

1.3 Chapter 3

In this chapter the bacterial and fungal communities that are present in the soil and roots of kauri trees (*Agathis australis*) at the Auckland Botanic Gardens (ABG), Aotearoa New Zealand were described. The high-throughput next generation sequencing of the 16S rRNA and ITS gene regions was used to investigate the microbial diversity and taxonomic composition of kauri soils in three distinct gardens at ABG. It establishes the possible foundational microbial community structure in soil surround kauri trees.

1.4 Chapter 4

This chapter presents the variations in the diversity and composition of soil microbial communities associated with kauri dieback non-symptomatic and symptomatic trees. Non-symptomatic kauri refers to trees that do not express any symptoms of dieback disease, such as canopy thinning and bleeding. Symptomatic kauri, on the other hand, refers to trees that are expressing these symptoms. Additionally, the microorganisms were characterised on soil samples collected from phosphonate treated trees. The high throughput next generation sequencing of the16S rRNA and ITS gene region was used to characterise the soil microbial communities associated with dieback non-symptomatic and symptomatic kauri. Furthermore, soil DNA were tested for the presence of *P. agathidicida* using endpoint polymerase chain reaction and agarose gel visualization, amplifying the *P. agathidicida*-specific ITS region.

1.5 Chapter 5

This chapter characterised the soil microbial functional genes potentials that provide an understanding of different metabolic processes that contribute to the kauri ecosystem in the Auckland Botanic Gardens (ABG) and the Tairua and Whangapoa kauri forests in the Waikato region of New Zealand. The GeoChip 5.0M microarray-based metagenomics tool, developed by

Glomics Inc. USA. The GeoChip 5.0M tool containing 162,000 probes from 365,000 target genes (coding DNA sequence - CDS) and covers all taxonomic groups, including archaea, bacteria, fungi, protists, algae, and viruses was used. The investigation focused on the microbial functional gene profiles present in the kauri soil in both anthropogenic environments and natural forest environments.

1.6 Chapter 6

This chapter focused on the potential of fungal endophytes to affect the growth of PA. Seventeen endophytic fungal species previously isolated from kauri roots for their morphology and ability to counteract the plant pathogen were explored. Out of the seventeen, five fungal endophytes exhibited growth suppression of the pathogen in dual culture. *Pezicula sp*. partially inhibited the growth of *P. agathidicida*, whereas *Coprinellus micaceus* and *Ilyonectria mors-panacis* completely inhibited the growth of the pathogen. No oospores were observed in the dual culture, nor was the *P. agathidicida* viable when transferred into fresh culture media.

Chapter 2

Review: Understanding the microbial community and their significance on *Agathis australis* and its habitat

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2.1 Abstract

Kauri, a significant and iconic native tree of Aotearoa New Zealand. In the 19th century, virgin kauri forests covered 1.5 million hectares of Aotearoa New Zealand (Halkett et al., 1980). Currently, kauri is facing a *Phytophthora* dieback disease called kauri dieback. *Phytophthora agathidicida* is the causal agent of kauri dieback and is destroying kauri trees. Only 1% of the New Zealand virgin kauri forest remains.

Recent studies revealed that many soil-borne microorganisms had been found to systemically boost the defensive capacity of the trees by providing competition to pathogens for nutrient intake, thus preventing pathogen colonization and modulating plant immunity. In addition, the root microbiome consists of an entire complex of rhizosphere-associated microbes with their interactions influencing plant health. Endophytes are microorganisms that live inside plant tissues and are not harmful to the host plant (Hallmann et al., 1997). Specific endophytes have been shown to enhance plant growth, and increase plant resistance to pathogens, drought and even herbivores, such that their commercial potential has received much attention. To date, few studies have been conducted to study the microorganisms in the kauri soil and root and their possible association with tree health.

This review stresses the importance of studying the microbial community composition of kauri soil and its metabolic functions. It also highlights the need to screen endophytes that can inhibit the growth of *P. agathidicida* and investigate their potential as biological control agents for the kauri dieback pathogen. This may help understand the interaction between kauri and its associated microflora and determine their ability to inhibit the growth of *P. agathidicida* at different growth stages.

2.2 Introduction

Agathis australis (kauri), is a coniferous tree native to Aotearoa New Zealand. It belongs to the ancient family of Araucariaceae. The history of kauri is closely linked to the settlement of New Zealand. The indigenous Māori people had a significant relationship with kauri long before the arrival of Europeans to New Zealand. Kauri trees played a significant role in Māori culture through traditions, artwork, folktales, and legends. Over 1.5 million hectares of virgin kauri forests once covered New Zealand (Halkett, J., 1980). Subsequently the colonization of New Zealand by Europeans, the kauri timber and gum industries were established (Figure 1). Due to the appearance and working properties of kauri timber, it was highly sought after for many uses (Cheeseman et al., 2011; Steward, 2011). A massive amount of kauri forest was also transformed into farmland. This led to the damaging misuse of the species (Steward, 2011). In Aotearoa New Zealand, there are only 7,500 hectares of primary virgin kauri forests left. Additionally, there are 60,000 hectares of secondary kauri forests or savannahs that contain regenerating kauri. Most of these forests are located in remote areas on the Great Barrier Island, Coromandel Peninsula, and the North Auckland Peninsula. (Halkett, J., 1980).

Figure 2.1 Giant kauri woods that were chopped by Europeans in 1920 (Mahoney, 2007).

At present kauri dieback disease is destroying kauri. *Phytophthora agathidicida* is the primary causal agent of kauri dieback throughout lowland stands. Several symptoms arise, including the characteristic root and collar rot, resin-exuding lesions (Figure 2.2), severe chlorosis, canopy thinning and widespread tree mortality (Figure 2.3).

The extensive spread of *P. agathidicida* in major kauri stands, such as the Waitakere Ranges and Waipoua forest, in northern New Zealand, along with the current distribution of kauri forests, makes this pathogen a major concern for the long-term survival of this iconic tree species (Beever et al., 2007).

Figure 2.2: Globs of gum (left), develop into large bleeding lesions (right) (Stacey et al. Kauri care guide)

As a soil borne pathogen, the movement of infected water and soil plays a key role in the distribution of kauri dieback (Beever et al., 2007). It is expected that human activity has probably resulted in the increased distribution of this pathogen throughout kauri stands (Beever et al., 2007). To mitigate the impact of human activity, various measures have been implemented. These include the installation of boardwalks on frequently used forest tracks, public education on phytosanitary protocols, and closure of tracks in high-risk areas (Auckland Council, 2018). Where humans are concerned, lack of sanitation is a large contributor to dispersal of the pathogen. This is because the effectiveness of the signage accompanied by phytosanitary stations is largely determined by social compliance (Hill et al., 2017). It has been demonstrated that the spray application of TriGene, a disinfectant, can effectively eliminate *P. agathidicida* zoospores upon contact, as well as mycelium, within eight days of exposure and the ability to reduce oospore viability significantly (Bellgard & Williams, 2011).

Phosphonate can control plant diseases caused by *Phytophthora* spp. (Shearer et al., 2004). Studies showed that injecting phosphonate into the trunk can protect against *P. agathidicida* and lead to 100% survival rate. However, 20% of individuals showed signs of leaf yellowing. This raised phytotoxicity concerns about using phosphonate extensively (Horner & Hough, 2014).

Figure 2.3: Kauri tree infected by *Phytophthora agathidicida* leading to canopy thinning and widespread tree mortality (https://www.doc.govt.nz/nature/pests-and-threats/diseases/kauri-dieback/)

The soil in which plants grow is home to a diverse range of microbes that live in close association with plants. These microbial communities are known to be the largest reservoir of biological diversity discovered to date (Buée et al., 2009; Curtis et al., 2002; Gams, 2007; Torsvik et al., 2002). The rhizosphere is a narrow zone of soil that is affected by the substances secreted by roots. It has the potential to harbor as many as 10^{11} microbial cells per gram of root (Egamberdieva et al., 2008) and more than 30,000 prokaryotic species (R. Mendes et al., 2011). The root microbiome is a complex system of microbes and their genetic elements that interact with each other and have a significant impact on the overall health of the plant. In fact, the collective genome of this microbial community is much larger than that of the plant itself and is often referred to as the plant's second genome.

Endophytes are microorganisms that live inside plant tissues and are not harmful to the host plant (Hallmann et al., 1997). Endophytes inhabit most plants and have been isolated from various plant species (Ziadi et al., 2016). They live in intra- and intercellular spaces of the plant tissue interacting with the hosts, and a diverse array of species has been reported to be endophytic (Elbeltagy et al., 2001). Colonization may take place at the local tissue level or throughout the plant, with microbial colonies and biofilms residing latently in the intercellular spaces and inside the vascular tissues (Gage et al., 1996; Gopalaswamy et al., 2000; Hinton & Bacon, 1995; Sessitsch et al., 2002). Sturz & Nowak (2000), proposed that these endophytes originated from the rhizosphere or phylloplane micro-flora and observed that many rhizosphere bacteria could penetrate and colonize root tissue, providing a route into the xylem. In this vascular tissue, the microbes could transport themselves throughout the plant and colonize it systemically. Once inside the plant, endophytic populations have been observed to grow between 2.0 and 7.0 log10 cells per gram of fresh tissue (McInroy & Kloepper, 2007; Shishido et al., 1999).

Recent studies have revealed that endophytic microorganisms, such as rhizobacteria and mycorrhizae, play a crucial role in promoting plant growth and suppressing plant diseases. This is achieved through various means, including the production of growth-promoting substances and the enhancement of nutrient uptake by the plant (Feng et al., 2006). Therefore, a better understanding of endophytic microorganisms and microorganisms surrounding kauri may help to explain the biological defense of kauri. Further intensive studies may illustrate the changes occurring in the microbial flora due to diseases.

To our knowledge, only a few studies have been carried out to determine the diversity of microorganisms within and around kauri. There is a big gap in the information about the microbial community composition of kauri, overall soil health and abiotic factors and the diversity and the distribution of endophytes with the potential to be biological control agents living in *A. australis* roots are poorly understood. Understanding the microbial composition may reveal possibilities for natural disease management such as biocontrol or companion planting that will be able to provide kauri with enduring protection from pathogens without compromising ecosystem integrity.

This review aims to present a comprehensive understanding of the microbial community in kauri roots and the surrounding soil to date and highlight the importance endophytes that can act as biological control agents for the kauri dieback pathogen.

2.3 Kauri – Iconic tree of Aotearoa

The New Zealand endemic tree *Agathis australis* commonly known as kauri belongs to Araucariaceae family of the Pinophyta division (Henkel & Hochstetter, 2011). Araucariaceae are one of the most primitive families of living conifers, with a fossil record from Triassic times (“Two New Genera of Triassic Trigoniidae from New Zealand,”).

Development and diversification of Araucariaceae family occurred in both Jurassic and Cretaceous hemisphere and remained as important component of Gondwana vegetation

(Kershaw & Wagstaff, 2001). The genus *Agathis* consists of 21 species that originated during the Cretaceous period (Barton, 1978). *Agathis* is distributed in New Zealand, New Caledonia, Australia (Queensland), Solomon Islands, Vanuatu, Fiji, Sumatra and The Philippines (Hooker et al., 2017; Whitmore et al., 1980; Kirk et al., (1874).

Figure 2.4: Tane Mahuta (images by Donnabella Lacap-Bugler)

The species *Agathis australis* originated during the Oligocene era and is one of the most ancient living trees in the world (Fleming, 1975; Potter, 2004). They are dominant trees of low stands, often appearing in mixed forests (Ecroyd, 1982). Ecologically, kauri support many native species and biodiversity, earning them an essential keystone species (Ecroyd, 1982). Kauri has a great influence on the surrounding forest composition and structure. Their unique ecological place is their remarkable ability to modify the soil (Padamsee et al., 2016; (Macinnis-Ng et al., 2017). Kauri can grow on infertile low-nutrient soils and effectively intakes nitrogen and phosphorus.

They can overcome competitors in nutrition in a process known as leaching (Madgwick et al., 1982).

The kauri tree is the largest tree species in New Zealand (Cockayne et al., 1932); Reed et al., 1964). They have branchless, straight trunks, which can grow more than 12 metres in height (Roche, 2010), with an overall tree height between 30 – 60 metres (Allan, 1982; Hooker, 2017). The trunk diameter can be up to 5 metres wide (Steward, 2011). Mature trees form a crown and become spherical or flat-topped after emerging above the codominant forest canopy. These trees can live up to a maximum age of 1700 years (Cheeseman et al., 1914; Steward, 2011, & Beveridge et al., 2010).

Leaves can remain in trees for fifteen years and they are dull-green in colour (Silvester & Orchard, 1999a). Leaves are operational for 3 - 6 years, and budburst occurs between September and October (Ahmed, 1989; Bieleski, 1959). The oldest known kauri is Tane-Mahuta, Lord of the Forest (Figure 2.4).

Young kauri has a taproot and lateral roots (Ecroyd, 1982). Mature kauri has widespread root systems. These root systems consist of lateral roots that are attached to peg roots, allowing them to extend further into the ground approximately to 5 meters. The fine feeder roots penetrate throughout the litter and humus layer of the forest soil surface (Ecroyd, 1982). Kauri has a massive root system. Peg roots arrive from lateral roots and hold the tree firmly to the ground (Steward, 2011). The fine feeder roots persist right through the litter layer and the fine root hairs feed in the organic litter of the soil surface (Ecroyd, 1982). Kauri has well-developed tap root systems that utilize free-draining soil and are essential for nutrient uptake, optimum growth, and mycorrhizal symbiosis (Morrison et al., 1967; Padamsee et al., 2016). Family Araucariaceae are heterorhizic with indefinite long roots and spherical short roots (nodules); these nodules contain arbuscular mycorrhizal (AM) fungi (Khan & Valder 1972; Cairney 2000; Russell et al. 2002; Dickie & Holdaway 2010; Schwendemann et al. 2011).

2.4 *Phytophthora* diseases

Phytophthora is commonly known as water moulds requiring water for their reproduction cycle (Judelson et al., 2005). They are microscopic eukaryotic ‘fungus-like’ soil-borne pathogens that are able to produce both asexual and sexual spores (Krull et al., 2013). They belong to the family Peronosporaceae under the phylum Oomycota in the Stramenopila kingdom (Beakes et al., 2014; Dick et al., 2001). Species from this genus are considered as primary plant pathogens that can either be hemibiotrophic or necrotrophic (Jung et al., 2018; Thines et al., 2016; Jung et al., 2011; Beakes et al., 2012; Brasier et al., 2003). They can infect different species of tree, crop, and shrub across the world at different stages of the plant development (Jung et al., 2018; Erwin et al., 1996; Hansen et al., 2012). *Phytophthora* can cause damping-off of plant seedlings, aerial bleeding cankers on stems, root rot, and blight of foliage, buds, or fruits in different plant families (Erwin et al., 1996; Hardham et al., 2005; Cahill et al., 2008). *Phythophthroa cinnamomi*, *P. cryptogea*, *P. ramorum*, *P. palmivora*, and *P. nicotianae* can infect many woody plant hosts (Jung et al., 2018; Brasier et al., 2003). *Phytophthora* also includes the aquatic species, saprophytes, and opportunistic necrotrophic pathogens (Jung et al., 2018; Thines et al., 2016; Jung et al., 2011; Beakes et al., 2012; Brasier et al., 2003).

Phytophthora agathidicida was the most frequently observed kauri pathogen (Winkworth et al., 2021). Other *Phytophthora* species, including *P. cinnamomi* and *P. multivora*, ("Phytophthora: A Global Perspective," 2013) were also isolated from kauri dieback symptomatic trees, suggesting that these species also may contribute to kauri's overall poor health (Waipara et al., 2013).

2.4.1 *Phytophthora agathidicida*

In 2008, *P. agathidicida* was confirmed as an unwanted species in New Zealand (Bradshaw et al., 2020). Since the early 2000s, there has been an increase in the number of kauri stands with canopy thinning associated with kauri dieback. The disease has now been confirmed throughout the range of kauri forests. *Phytophthora agathidicida* is a severe threat to both individual kauri trees and the entire population. It has a significant impact on kauri ecosystems and their natural processes. Kauri forests are at risk due to this disease, and they are vital in carbon cycling and nutrient cycling (Madgwick et al., 1982). Losing forests due to invasive *Phytophthora* species will have a great impact on many ecosystems and natural processes. Given the current fragmented distribution of kauri forests today and the widespread dispersal of *P. agathidicida* throughout major kauri stands (e.g. Waitakare Ranges and Waipoua forest) in northern New Zealand, this pathogen poses a significant threat to the long-term survival of this iconic tree species (Beever et al., 2007; Waipara et al., 2013).

The life cycle of *Phytophthora agathidicida* involves both the sexual and asexual reproduction, producing oospores and motile zoospores produced within sporangia, respectively and the vegetative hyphal growth in habitats (Figure 2.5).

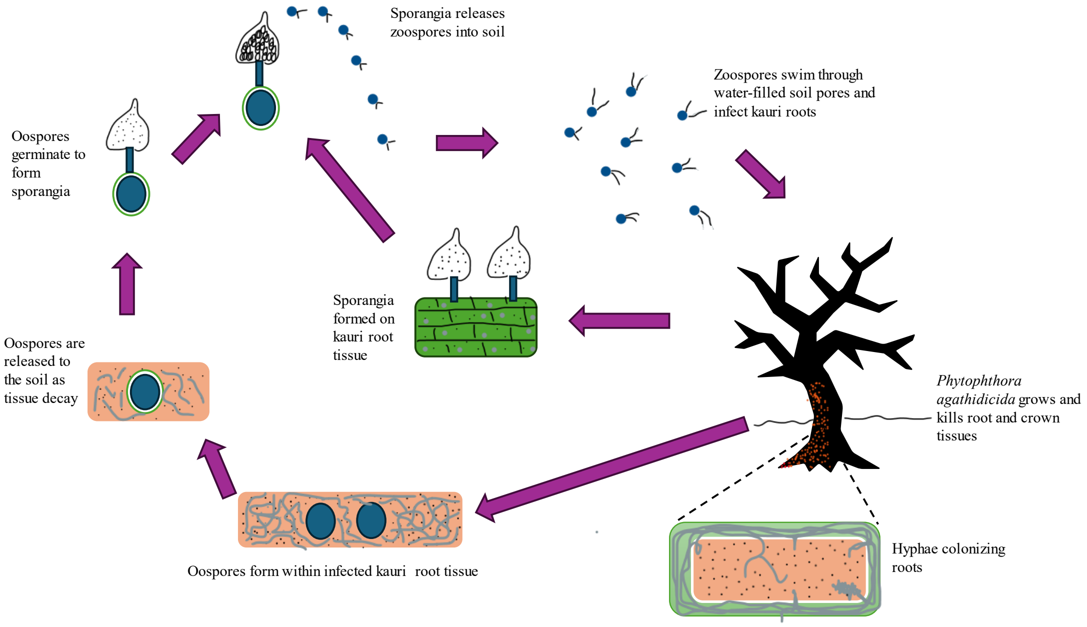

Figure 2.5: The life cycle of *Phytophthora agathidicida*.

Oospores have thick cell wall allowing them to survive during stress periods. They are produced through sexual reproduction and germinate to produce sporangia. Oospores can colonise kauri roots which then can produce sporangia. The sporangia then release zoospores that can colonise plant tissues which complete the life cycle. Zoospores are unicellular short-lived motile cells that can move through wet soil along chemotactic gradients towards kauri roots, where they encyst and form a penetration structure, allowing infection of the fine root epidermis and colonization of the cortex (Weir *et al.*,2015; Bellgard *et al.*, 2016).

2.4.2 Use of Phosphonate against *Phytophthora*

Previous studies have demonstrated that phosphonate is an effective means of reducing the impact of *Phytophthora* on susceptible species from various native plant communities in Western Australia (Shearer et al., 2004a; Tynan et al., 2001) and Victoria (Ali and Guest, 1998). Although phosphonate does not eliminate the pathogen, it slows its growth, which allows plants to isolate infections. Phosphonate-treated plants that are infected with *Phytophthora* may produce zoospores (Wilkinson et al., 2001). However, phosphonate can reduce the disease spread through a plant community for periods ranging from six months to more than five years (Shearer and Fairman, 1997a, b). While the fungicide effectively protects susceptible plant species against *Phytophthora*, the protection induced by phosphonate and its persistence in the plant vary between species (Barrett, 2001; Barrett et al., 2003, 2004), which affects the management of the phosphonate regimen for a given vegetation community.

Glasshouse studies on two-year old kauri seedlings, phosphonate was shown to be effective in providing protection against *P. agathidicida*; phosphonate injection into the trunk resulted in 100% survival rate following soil inoculation. Despite the evidence for phosphonate protection against *P. agathidicida*, there are ongoing concerns with phytotoxicity and interrogations about the commercial practicability of widespread use of phosphonate as a control measure. Phytotoxic symptoms (e.g. leaf yellowing) were observed in 20% phosphonate (200 ml/L Agrifos600®)-injected individuals (Horner and Hough et al., 2014).

Furthermore, phosphonate injections to the entire kauri forests were recognized as unsustainable given the huge costs and resources that would be involved (Horner and Hough et al., 2014). Therefore, chemical control usage (i.e. phosphonate) will likely only supplement other disease management strategies currently in place to mitigate kauri dieback, with a specific focus on shielding the largest and most notable kauri individuals.

2.5 Role of Soil Microbial Community in Plants

In the forest ecosystems that cover a substantial part of the Earth's surface, trees play an important role in soil microbe interactions. Trees are responsible for the bulk of the total primary production, and they often control the characteristics of the rest of the vegetation in a given ecosystem. The functional characteristics of dominant trees may affect microbial communities in several ways, including the production of litter, rhizodeposition, direct interactions with root-symbiotic microorganisms, indirect biotic effects on soil microorganisms mediated by herbivores or soil fauna or through the alteration of the microclimate ((Prescott & Grayston, 2013); Augusto et al., in press).

Microbes that inhabit the soil in which plants grow have a close association with the plants. Soil microbial communities characterize the highest of biological diversity known so far (Buée et al., 2009; Curtis et al., 2002; Gams, 2007; Torsvik et al., 2002). The rhizosphere is the narrow zone of soil that is influenced by root secretions. Largely, there are three discrete components of rhizosphere: soil, rhizoplane and root itself. Rhizosphere is thus the region of soil influenced by roots through releasing substrates to affect the microbial activity of 10^{11} microbial cells per gram root (Egamberdieva et al., 2008) and more than 30,000 prokaryotic species (Mendes et al., 2011). The rhizosphere microflora includes bacteria, fungi, nematodes, protozoa algae and microarthropods (R. Mendes et al., 2011). The diversity of microorganisms in the rhizosphere is the outcome of microbial interactions within the region (Somers et al., 2004). Rhizosphere inhabiting microorganisms compete for water nutrients and space. They improve their competitiveness by developing intimate association with plants (Berg et al., 2014).

The soil microbiome can be an indicator of the soil quality because its sensitivity to minor changes in the environment resulting from environmental stresses or natural alarms (Sharma & Gobi, 2016). High in species richness and diversity produce high functional redundancy within the soil microbiome, allowing rapid recovery during stress (Nannipieri et al., 2003). The high functional redundancy in soil microbial diversity also provides protection against soil-borne diseases (Brussaard et al., 2007; Garbeva et al., 2004; R. Mendes et al., 2011; Nannipieri et al., 2003; Ziadi et al., 2016). The growth in microbial diversity produces a microbiome that does not allow for pathogens to flourish since the high microbial diversity present in the soil keeps the pathogen “in check” (Garbeva et al., 2004; R. Mendes et al., 2011).

Root microbiome consists of the entire complex of rhizosphere-associated microbes, their genetic elements and their interactions influence plant health. In the rhizosphere, plant and microbes’ interactions play an important role on ecosystem processes. Plant microbe interactions in the soil can be mutualism or competition or parasitism or predation or pathogenesis. These interactions can either be beneficial impacts to the plant such as disease suppression, increased nutrient availability and increased immunity to abiotic and/or biotic stress or can be harmful to the plant. Pathogenic interactions can severely impact plant health. The success of a pathogen is influenced by the microbial community of the soil.

Rhizosphere bacteria that play a positive role on plant growth are referred to as plant growth promoting rhizobacteria (Kloepper, 1981). Currently plant growth promoting rhizobacteria include representatives from very diverse bacterial taxonomy (Lucy et al., 2004). Diverse bacterial strains have been used successfully for inoculations to promote plant growth. These microorganisms include *Bacillus* (Jacobsen et al., 2004), *Stenotrophomonas* (Ryan et al., 2009), *Azospirillum* (Cassán & Diaz-Zorita, 2016), *Rhizobium* (Long, 1996), *Pseudomonas* (Loper & Gross, 2007), *Serratia* (De Vleeschauwer & Lal, 1981) and *Streptomyces* (Schrey et al., 2007). Some fungi belonging to the genera *Ampelomyces*, *Coniothyrium*, and *Trichoderma* have also been described to be beneficial for the host plant (Harman et al., 2004).

The plant growth-promoting rhizobacteria have complex mechanisms to promote growth, protection and development of plants. Such mechanisms include biofertilization (increasing the availability of nutrients to plant), biocontrol (controlling diseases, mainly by the production of antibiotics and antifungal metabolites, lytic enzymes, and induction of plant defence responses) and phyto-stimulation (promoting plant growth, usually by the production of phytohormones). *Pseudomonas* and *Bacillus* are the most studied plant growth promoting bacterial genera, and often the significant bacterial groups in the rhizosphere (Morgan et al., 2005). In many cases of individual beneficial plant-microbe interactions, several mechanisms are involved, and this include but not limited to antagonism (Vacheron et al., 2013).

2.5.1 Antagonism

Root colonization functions as the transport system of antagonistic metabolites that are elaborated as direct inhibition of plant pathogens (Raaijmakers et al., 2009; Shoda, 1999). The direct inhibition includes antibiosis that inhibits microbial growth by diffusible antibiotics and toxins, volatile organic compounds and biosurfactants. Parasitism may involve production of extracellular cell wall-degrading enzymes such as chitinases and β-1,3- glucanase (Harvey et al., 2002; Ziadi et al., 2016). The degradation of pathogenicity factors of the pathogen such as toxins by the beneficial organism has also been reported as protective mechanism (Barea et al., 2005).

2.5.2 Antibiotics in biocontrol

To demonstrate the role of antibiotics in biocontrol, as example, *Bacillus subtilis* strains produce a diverse antifungal metabolite, e.g., kanosamine and lipopeptides from the surfactin, zwittermicin-A, Iturin and fengycin families (Romero et al., 2007). Dunne and collaborators (2000) demonstrated that overproduction of extracellular protease in the mutant strains of *Stenotrophomonas maltophilia* W81 resulted in improved biocontrol of *Pythium ultimum*. Excretion of chitinases and glucanases by species of *Trichoderma* and *Streptomyces* has also been shown to play an important role in mycoparasitism of phytopathogenic fungi (Josic et al., 2012).

Fluorescent *Pseudomonads* produce the antibiotic 2, 4- DAPG which is a protectant against soil borne protectant directly linked with disease suppression (Raaijmakers et al., 2009). *B. subtilis* also releases the antibiotics, surfactin and iturin into the rhizosphere that play an important role in suppressing the cucumber fusarium wilt caused by *Fusarium oxysporum* (Kinsella et al., 2009). The biocontrol agent *Bacillus subtilis* produces several classes of broad spectrum lipopeptide antibiotics which are effective suppressors of many plant pathogens, including species of *Fusarium, Pythium, Phytophthora, Rhizoctonia, Sclerotinia, Septoria,* and *Verticillium* (Nagórska et al., 2007).

2.5.3 Competition

Competition for resources such as oxygen and nutrients occur generally in soil between soil-inhabiting organisms. For biocontrol purpose, it occurs when the antagonist directly competes. Study by Ezziyyani and others revealed two compatible microorganisms *Trichoderma harzianum* and *Streptomyces rochei* both antagonistic to the pathogen *Phytophthora capsici,* which causes root rot in pepper and when used as control in the soil, the pathogen population was reduced by 75%. Vegetative growth of the mycelium *P. capsici* was inhibited in vitro on the second day after *P. capsici* and *T. harzianum* were placed in the opposite sides of the plate. *Trichoderma harzianum* was not only able to arrest the spread of pathogen but also to invade all the surface of pathogen colony (Figure 2.6).

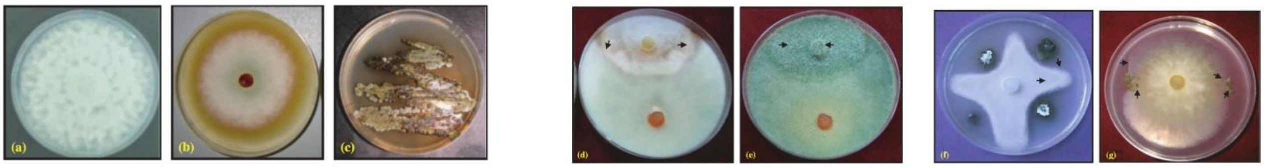

Figure 2.6. a-c *In vitro* growth of pathogen *Phytophthora capsici*(a), *T. harzianum* (b) *and Streptomyces rochei* (c). d and e *T. harzianum* inhibited *P. capsica* by hyper parasitism. f & g Synergic compatibility in the confrontation between antagonists (Ezziyyani et al., 2007).

2.5.4 Mycorrhizae

Mycorrhizae form mutualistic partnerships involving between fungus and a plant root. These are symbiotic associations, entirely mutualistic and both fungus and plant benefit (Bellgard & Williams, 2011). The benefits are mainly connected to improve nutrition of the host, extend decrease of hormonal balance, physical protection, chemical protection, and modification of other rhizosphere microorganisms.

Studies on *A. australis* root systems have identified and confirmed the presence of Arbuscular Mycorrhizae Fungi. These fungi enhanced the phosphorus absorption (Morrisson & English, 1967), heavy metal tolerance (Leyval et al., 1997) and protects the plants from pathogens (e.g. *Phytophthora)* (Bellgard & William 2011) (Vigo et al., 2000). Furthermore Padamsee et al., (2016) has described the presence of AMF in kauri roots observed under light microscope.

2.5.5 Endophytes

Endophytes are microorganisms that live inside plants tissues and are not harmful to the host plant (Hallmann et al., 1997). Microbial colonization of the tissues of plants has been described in almost plant species. Although many of these microbes are phytopathogenic, and a considerable number have also been found to colonize the plant without causing disease (Sessitsch et al., 2002). Such microbes are referred to as endophytes. Endophytes ubiquitously inhabit most plant species and have been isolated from a variety of plants (Ziadi et al., 2016). Colonization may take place at the local tissue level or throughout the plant, with microbial colonies and biofilms residing latently in the intercellular spaces and inside the vascular tissues (Gage et al., 1996; Gopalaswamy et al., 2000; Hinton & Bacon, 1995; Sessitsch et al., 2002). A diverse array of species has been reported to be endophytic (Elbeltagy et al., 2001). (Sturz &

Nowak, 2000)), proposed that these endophytes originate from the rhizosphere or phylloplane micro-flora, and observed that many rhizosphere bacteria could penetrate and colonise root tissue, providing a route into the xylem. In this vascular tissue, the microbes could transport themselves throughout the plant and hence colonise it systemically. Once inside the plant, endophytic populations have been observed to grow between 2.0 and 7.0 log10 cells per gram of fresh tissue (McInroy & Kloepper, 2007; Shishido et al., 1999). Certain endophytes have been shown to enhance plant growth, increase plant resistance to pathogens, drought and even herbivores, such that their commercial potential has received much study (Bacilio-Jiménez et al., 2001; Sturz & Nowak, 2000; Azevedo et al., 2000; Iwai et al., 2010; Shishido et al., 1999; James, 2000).

Specific endophytes have demonstrated to enhance plant health. The fungal endophyte from Yew tree *Paraconiothrium* SSM001 increase plant resistance to pathogens (Soliman et al. 2015). Norway spruce root endophyte *Phialocephala sphareoides* was able to inhibit phytopathogens *Heterobasidion parviporum*, *Phytophthora pini*, and *Botrytis cinereal* under in vitro conditions and promote root shoot ratio (Terhonen et al. 2016). Figure 2.6 endophyte *Nigrospora sphaerica* inhibiting *Phytophthora* pathogens in invitro conditions. *Colletotrichum tofieldiae*, a fungal endophyte of *Arabidopsis thaliana*, transfers macronutrient-phosphorus to shoots and promotes plant growth (Azevedo et al., 2000; Bacilio-Jiménez et al., 2001; Hiruma et al., 2016).

Dark septate endophytes (DSE), belonging to the class 4 endophytes, colonize their hosts' roots (Rodriguez et al. 2009). They are conidial or sterile septate fungal endophytes that form melanized structures, including inter- and intracellular hyphae and microsclerotia in the roots (Jumpponen & Trappe, 1998; Tellenbach et al. 2013).

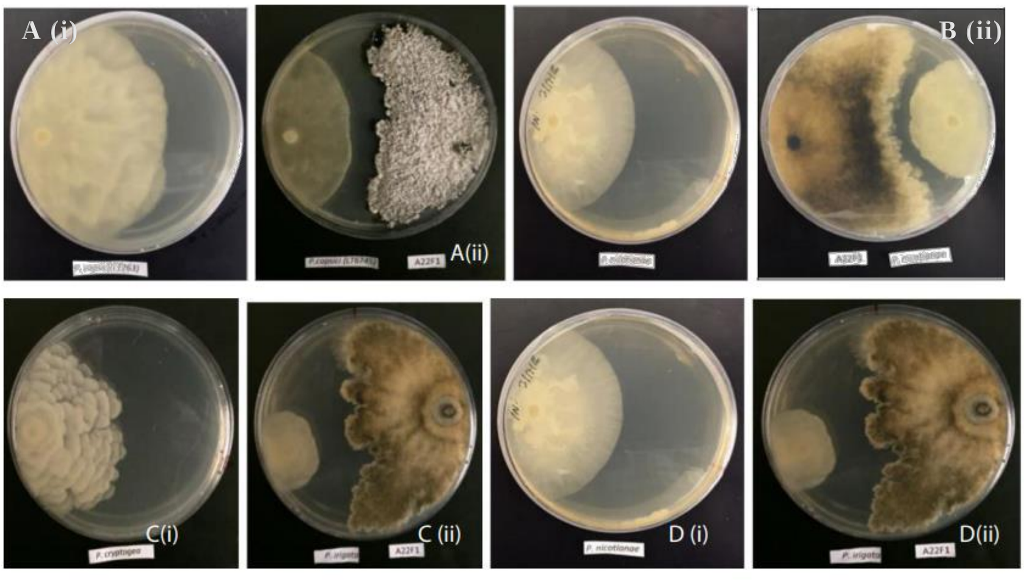

Figure 2.7: Inhibition of pathogen growth by endophyte *Nigrospora sphaerica* in dual cultures.

(Ai)-*Phytophthora capsici* alone, (Bi)-*Phytophthora nicotiana*, alone (Ci)-*Phytophthora cryptogea* alone and (Di)-*Phytophthora irritigata* alone. A(ii), B(ii), C(ii), D(ii) pathogen with endophyte (MT et al., 2018).

Biological control agents (BCAs), whose primary mode of action is through competition, sustaining high environmental population levels is essential to suppress target pathogens (Alabouvette et al. 2006). Moreover, the ecotoxicological risk and associated risk assessments required to facilitate their application are much lower as they inhibit pathogens through general ecological processes rather than the production of antimicrobial compounds (Köhl et al. 2019).

2.6 Aotearoa New Zealand Forest soil microbiome

New Zealand has a rich history of research on soil microbes, which includes exploring the interactions between carbon and nitrogen in above- and below-ground communities. This research also examined the impact of land management practices on soil microbes and the cycling of potentially pathogenic bacteria through the soil (Stott & Taylor, 2016)

Soil bacterial communities along a 6,500-year-old dune chronosequence under a lowland rainforest in Haast, New Zealand, revealed that bacterial communities showed patterns of change during pedogenesis. Alphaproteobacteria, Actinobacteria, and Acidobacteria were the most abundant bacterial taxa. These bacterial groups are closely related to nitrogen-fixing bacteria and suggest that heterotrophic nitrogen input may be important throughout the chronosequence. The changes in bacterial community structure were related to changes in several soil properties, including total phosphorus, Carbon:Nitrogen ratio, and pH. The abundance of Bacteroidetes, Actinobacteria, Cyanobacteria, Firmicutes, and Betaproteobacteria generally declined as soil aged, while Acidobacteria, Alphaproteobacteria, and Planctomycetes tended to increase (Turner et al., 2012).

In 2013 and 2014, a study was conducted across 110 sites in northern New Zealand, covering a land area of approximately 29,500 km^2. The study area consisted of diverse soil types, including indigenous forest, exotic forest, dairy pasture, dry stock pasture, and horticulture. The study found that bacterial communities, as well as specific taxa, could reflect changes occurring in the soil environment due to human activity. This was confirmed by the spatial pattern observed in Northland, where communities of bacteria with similar compositions formed clusters in different areas of the study, rather than a simple distance-based gradient of increasing dissimilarity being observed (Hermans et al., 2017).

(Byers, Condron, Donavan, et al., 2020) found significant differences in microbial diversity and composition of soil microbial communities associated with old growth kauri forests and adjacent pine plantations in Waipoua Forest, and the taxonomic groups contributing to significant differences were identified. The bacterial communities present in the soils of both kauri and pine trees display a high degree of diversity and low dominance values. The dominant bacterial phyla found in these soils are Proteobacteria, Actinobacteria, and Acidobacteria, which are commonly found in acidic coniferous forests (Lladó et al., 2017). Differences in the composition and taxonomy of fungal communities were observed between kauri and pine soils. These differences can be explained ecologically, as the role of fungi in the soil environment is both saprophytic and symbiotic. Therefore, the composition of the fungal community is often influenced by the inputs of tree litter and root exudates (Urbanová et al., 2015).

Furthermore, (Byers, Condron, O'Callaghan, et al., 2020a) also found that there were significant differences in the fungal diversity of microbial communities in asymptomatic and symptomatic soils. Interestingly, fungal diversity was found to be higher in symptomatic soils. Moreover, the composition of fungal and bacterial communities was also different in asymptomatic and symptomatic soils. Taxonomic analyses have revealed that several microbial taxa, such as *Penicillium, Trichoderma, Enterobacteriaceae, Actinobacter,* and *Pseudomonas,* were more abundant in asymptomatic kauri soils. Considering the current threat posed by kauri dieback disease, further understanding of how these differing microbial communities' impact on health of kauri forest fragments and their ability to respond to pathogen invasion is essential.

As far as we know, only a handful of research studies have been conducted to investigate the variety of microorganisms that exist within and around kauri trees. There is a big gap in the information about microbial community composition of kauri, overall soil health and abiotic factors. To gain a better understanding of the microorganisms that are present in a kauri environment, there is a need for further research. Research should aim to identify the specific microorganisms that are frequently present in kauri trees in Auckland, Northland, and Waikato, as well as compare the microorganisms found in different seasons. It is also important to investigate the development of roots and soil microbes in relation to the age of the kauri and compare the root and soil microorganism of planted kauri with naturally occurring kauri.

In addition, it is crucial to identify key microbes that can suppress the growth of pathogens that threaten the health of kauri trees. These microbes should be screened against *P. agathidicida* to assess their potential as biocontrol agents. By identifying and understanding these important microbial species, we can work towards protecting kauri trees and preserving their natural habitat. Furthermore, it is crucial to explore the plant-beneficial roles that these microbial taxa may possess. This can help in identifying microbial taxa that can support kauri health in the encounter of pathogen attacks.

2.7 Methods to study microbial community in environment

2.7.1 Amplicon sequencing

The recent development of metagenomic technologies such as high throughput sequencing (Shendure & Ji, 2008) and microarrays (He, Xu, et al., 2010) provide powerful tools for characterizing microbial communities. Information on microbial community and diversity can be obtained by using molecular approaches based on 16S rRNA, and internal transcribed spacer (ITS) gene analysis. This has been successfully used for microbial community analysis from great variety of environments, including soil ecosystems (Dunbar et al., 1999) and rhizosphere (Smalla et al., 2001).

The 16S rRNA gene have been used in majority of the studies targeting the Bacteria and Archaea (e.g. (Barberán et al., 2012; Mao et al., 2012; D. L. Sun et al., 2013). 18S rRNA gene and internal transcribed spacer (ITS) region gene have been used in several fungal and other eukaryotic analyses (e.g. (Lazarus & James, 2015; Nagahama et al., 1999). The ITS 1 region, located between 18S and 5.8S rRNA genes was more commonly targeted (e.g. Bazzicalupo et al., 2019; Bellemain et al., 2010), with more recent studies switching to the ITS2 region (between 5.8S and 28S rRNA genes; Bazzicalupo et al. 2013) (Figure 2.7)

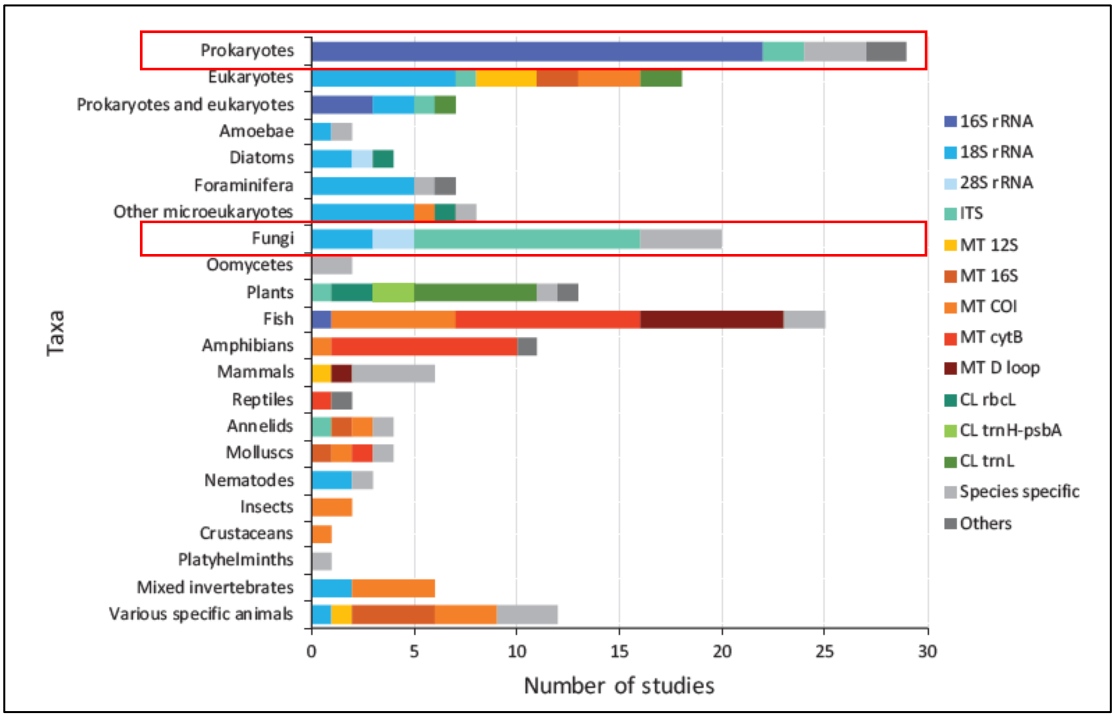

Figure 2.8**:** Gene regions targeted in studies focused on different taxa. rRNA indicates ribosomal RNA genes, MT indicates mitochondrial genes, and CL indicates chloroplast genes.

DNA-based methods have been used to characterise the community composition of microbes across a wide range of environments including soils (Griffiths & Philippot, 2013). The resulting datasets have generated valuable insights into variabilities in microbial communities across land-uses and landscapes, as well as key environmental drivers or correlates of prokaryote community composition such as variation in climate (Lear et al., 2003), pH (Lauber et al., 2009) and pollution gradients (Yergeau et al., 2012). High throughput amplicon sequencing has recently been used to investigate continental-scale distributions of bacteria (Barberán et al., 2015) and community responses to stresses such as disinfection (Lee et al., 2014).

2.7.2 GeoChip

Functional Gene Arrays (FGA) is another strong, influential, high-throughput tool to specifically, sensitively and quantitatively profile microbial communities and link their composition and structure with environmental factors and ecosystem functioning (HE et al., 2008). They are comprised of probes targeting key genes involved in microbial functional processes, such as biogeochemical cycling of carbon (C), nitrogen (N), sulfur (S), phosphorus (P), and metals, stress responses, and virulence and antibiotic resistance (He et al., 2012). Recently a variety of FGAs have been developed and applied for studying the functional diversity, composition, structure, function, activities, and dynamics of microbial communities from different habitats (Bodrossy et al., 2006; Hazen et al., 2010; He et al., 2007, 2012; Lu et al., 2012; McGrath et al., 2010; Taroncher-Oldenburg et al., 2003).

Soil has the most complex microbial communities among known environments, and FGAs have been used to address fundamental ecology theories (Zhou et al., 2008). GeoChip data were used to address ecological theories related to the gene–area relationship for better understanding of spatial scaling in forest soil microbial communities (Zhou et al., 2008). GeoChip was used to examine how elevated CO 2 affected soil microbial communities (He, Xu, et al., 2010). The results showed that the functional composition, structure, and metabolic potential of soil microbial communities shifted, which is significantly correlated with soil C and N contents and plant productivity (He, Xu, et al., 2010). GeoChip was also applied to profile rhizosphere microbial communities of *Candidatus Liberibacter asiaticus* infected citrus trees. The results showed that the communities shifted away from using more easily degraded sources of carbon to more recalcitrant forms, suggesting that the change in plant physiology mediated by '*Ca. L. asiaticus*' infection could elicit shifts in the composition and functional potential of rhizosphere microbial communities (Trivedi et al., 2012).

The latest version of FGA, known as GeoChip 5.0, utilizes the Agilent platform. Two formats have been created - a smaller one (GeoChip 5.0S) that primarily covers genes related to carbon, nitrogen, sulfur, and phosphorus cycling, as well as others that provide ecological services. The larger format (GeoChip 5.0M) contains functional categories involved in biogeochemical cycling of C, N, S, P, and various metals, stress response, microbial defense, electron transport, plant growth promotion, virulence, gyrB, and fungus-, protozoan-, and virus-specific genes. GeoChip 5.0M includes 161,961 oligonucleotide probes that cover 365,000 genes of 1,447 gene families from broad, functionally divergent taxonomic groups, including bacteria (2,721 genera), archaea (101 genera), fungi (297 genera), protists (219 genera), and viruses (167 genera), mainly phages (Z. Shi et al., 2019).

Computational and experimental evaluation have shown that the designed probes are highly specific and can detect as little as 0.05 ng of pure culture DNAs within a background of 1 µg of community DNA (equivalent to 0.005% of the population). Furthermore, there were strong quantitative linear relationships between signal intensity and the amount of pure genomic (99% of probes detected; r 0.9) or soil (97%; r 0.9) DNAs (Shi et al., 2019).

GeoChip 5.0 can be used to profile potential functional genes in kauri soil, understand the shift of potential functional genes during pathogen invasion, and learn about genes involved in various ecological functions.

2.8 Conclusion

Kauri is a majestic and iconic native tree that is endemic to New Zealand. Unfortunately, it is under threat from a deadly disease called kauri dieback, which is caused by the pathogen *Phytophthora agathidicida*. Recent studies have shown that certain soil-borne microorganisms can help to enhance the trees' natural defenses against the disease by competing with pathogens for nutrients, preventing pathogen colonization, and boosting plant immunity. The root and soil microbiome are a complex community of rhizosphere-associated microbes that interact with each other, influencing the health of the plant. Endophytes are microorganisms that live inside plant tissues and can enhance plant growth, increase resistance to pathogens, drought, and herbivores. However, there is still a significant lack of knowledge about the microbial community composition of kauri soil and root, overall soil health, and the abiotic factors that impact kauri growth. And the diversity and the distribution of endophytes with the potential to be BCAs living in *A. australis* roots are also poorly understood. More research is required to better understand the microorganisms present in kauri trees. This research should identify commonly found microorganisms in different regions and seasons and investigate the development of root and soil microbes in relation to kauri age. Comparing planted kauri with natural kauri using amplicon sequencing and Geochip can provide valuable insights. This review highlights the critical importance of studying the microbial community composition of kauri soil and their metabolic functions. It also emphasizes the need to screen endophytes that can inhibit the growth of *P. agathidicida* and investigate their potential as biological control agents for the kauri dieback pathogen. This research may help understand the interaction between kauri and its associated microflora and determine their ability to inhibit the growth of *P. agathidicida* at different growth stages.

Chapter 3

Kauri (*Agathis australis*) soil bacterial and fungal communities in an anthropogenic setting

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3.1 Abstract

Kauri (*Agathis australis*), a tree species iconic to Aotearoa, New Zealand, faces a grave threat from kauri-dieback disease caused by a *Phytophthora* infection. Recent studies have shown that soil microorganisms can boost the plant immune system by reducing pathogen colonization through competition for nutrients. The root microbiome, a complex system of diverse microbes with their genetic elements and interactions, also plays a critical role in maintaining plant health. The Auckland Botanic Gardens (ABG)in Aotearoa, New Zealand, features kauri trees in three distinct garden areas: Native Forest, Kauri Grove, and Rose Garden. These kauri trees are approximately twenty years old. To date, no studies have been reported to investigate the microorganisms in the planted kauri soil in an anthropogenic setting.

Soil samples were collected from four cardinal points of each stand. The environmental DNA (eDNA) obtained from all four cardinal points was analysed using a 16S rRNA and ITS1 amplicon sequencing approach. The bacterial and fungal communities associated with the rhizosphere of twenty-year-old kauri trees at ABG were characterised. The analysis revealed that Acidobacteriales is the most abundant bacterial order, and Mortierellales is the most abundant fungal order. Xanthomonadales, Rhizobiales, Rhodospirillales, Sphingomonadales, Hypocreales, Tremellales and Trichosporonales represented the bacterial and fungal core rhizosphere microbiome of *A. australis,* at Auckland botanic gardens. *Penicillium, Mortierella, Streptomyces, Nitrospira* and *Flavobacterium* genera were detected in the ABG soil. Bacterial endophytes such as *Pseudomonas*, *Rhizobium*, Enterobacteriaceae, *Staphylococcus* and *Burkholderia* were also identified in kauri roots at ABG. There were no significant differences between cardinal points, and distance samples from the 'bleeding tree' KG3 showed that Rozellomycota and Verrucomicrobia were higher towards the grass while Proteobacteria, Basidiomycota and Ascomycota were higher towards the KG4 tree.

3.2 Introduction

Kauri, (*Agathis australis*), stands out as an ancient coniferous tree only found in Aotearoa New Zealand. Its significance is deeply intertwined with the region's settlement history, as its timber was highly coveted for its aesthetic appeal and ease of use (Cheeseman et al., 2011; Steward, 2011). Due to the demand for kauri timber and the conversion of kauri forests into farmland, the species was extensively exploited, leading to the extensive degradation of the upper North Island’s Forest landscape (Steward, 2011). Currently, only 7,500 hectares of primary virgin kauri forests remain in Aotearoa New Zealand, while there are also 60,000 hectares of secondary kauri forests or savannah containing regenerating kauri, as noted by (Halkett, J., 1980).

Kauri dieback is a severe *Phytophthora* infection that is currently affecting kauri trees. The primary cause of this disease is *Phytophthora agathidicida*, which damages the nutrient-distributing tissues in the tree roots, leading to collar rot, and ultimately causing tree mortality (Bassett et al., 2017; Beever et al., 2007; Waipara et al., 2013; Weir et al., 2015). This disease is prevalent in kauri trees of all age groups in lowland stands of northern Aotearoa, New Zealand. The symptoms of kauri dieback include root and collar rot, resin-exuding lesions, severe chlorosis, canopy thinning, and widespread tree death. *Phytophthora agathidicida* has spread throughout major kauri stands such as the Waitakere Ranges and Waipoua forest in northern Aotearoa, New Zealand, putting the survival of this iconic tree species in danger (Beever et al., 2007; Waipara et al., 2013).

Kauri forests are a vital part of local and global carbon cycles, and soil and nutrient cycles (Madgwick et al., 1982). Losing forests due to invasive *Phytophthora* species will have a great impact on many ecosystems and natural processes. Given the current fragmented distribution of kauri forests today and the widespread dispersal of *P. agathidicida* throughout major kauri stands in northern Aotearoa New Zealand, this pathogen poses a significant threat to the long-term survival of this iconic tree species (Beever et al., 2007; Waipara et al., 2013).

Soil microorganisms play important roles in various biogeochemical cycles (Trevors, 1998; Wall & Virginia, 1999). They contribute significantly to plant nutrition (Timonen et al., 1996), plant and soil health (Filion et al., 1999; Srivastava et al., 1996), soil structure (Dodd et al., 2000; Wright & Upadhyaya et al., 1998), and fertility. Their role in the cycling of organic compounds impacts above-ground ecosystems (Timonen et al., 1996). For instance, soil microorganisms aid in plant nutrition by providing essential nutrients, improve plant health by suppressing harmful pathogens, contribute to soil structure by enhancing soil aggregation and stability, and boost soil fertility by decomposing organic matter. These processes highlight the crucial role of soil microorganisms in sustaining healthy soil ecosystems (Saeed et al., 2021).

Soil conditions play a vital role in enhancing plant productivity and nutrient availability. The rhizosphere, which is the soil surrounding plant roots, is home to a diverse range of microorganisms that interact with the roots and soil to influence plant growth (Dlamini et al., 2022). These microorganisms, including endophytes, symbionts, pathogens, and plant growth promoting rhizobacteria, work together to form the soil microbiome (Backer et al., 2018). Root exudates produced by the plants are used as substrates and signaling molecules for the microbes, and this complex relationship between the microbiome and plants is critical for plant health (Hayat et al., 2010; Raaijmakers et al., 2009).

Plants grow in close association with soil microbes, which represent the largest known biological diversity (Buée et al., 2009; Curtis et al., 2002; Gams, 2007; Torsvik et al., 2002). The rhizosphere, the narrow soil zone influenced by root secretions, may contain up to 10^{11} microbial cells per gram of root (Egamberdieva et al., 2008) and more than 30,000 prokaryotic species (R. Mendes et al., 2011).

Soil plays a crucial role in urban gardens, but the practices followed in urban allotment gardens can make their soil distinct from other types of soil (Tresch et al., 2018). These gardens are located within cities and comprise of materials and manipulated due to activities such as mixing, filling, transportation, and construction-related perturbations (Probst et al., 2023). Anthropogenic influences disrupt ecological gradients and connect unrelated habitats, causing urban soils to lack spatial logic (Probst et al., 2023; Schmidt et al., 2019). It is possible that human activities, such as urbanization, farming, use of pesticides, and pollution, may have an impact on the diversity of soil microbes (Dror et al., 2022). However, we do not yet know the soil microbial communities of planted kauri in an urban environment and how changes in microbial diversity might affect both above-ground ecosystems. Therefore, we must first study soil microorganisms before we can understand how changes in their community structure can influence ecosystem functions.

As far as we know, only a handful of research studies have been conducted to investigate the variety of microorganisms that exist within and around kauri trees. Furthermore, there has been no research conducted on the soil microbial community of kauri trees that are grown in a man-made or urban environment. There is a big gap in the information about microbial community composition of kauri, overall soil health and abiotic factors.

The aim of this study is to investigate the microbial community present within kauri trees and the surrounding soil, using molecular-based techniques such as sequencing of 16S rRNA and ITS1 loci. The diversity of bacteria and fungi can be crucial in enhancing soil quality by promoting soil agglomeration, increasing soil fertility, and aiding nutrient cycling. They may also play a significant role in improving plant health through various direct and indirect means. Furthermore, a healthy population of microorganisms in the rhizosphere can help plants cope with biotic and abiotic stresses, such as pathogens, drought, and soil contamination. This study aims to provide bacterial and fungal community structure in kauri soil in an anthropogenic environment.

3.3 Materials and Methods

3.3.1 Site description and soil and root sampling

Auckland Botanic Gardens is ~64 hectares and holds more than 10000 plants; located in the suburb of Manurewa, Auckland, New Zealand. The garden has kauri trees in three different garden types, namely Native Forest (NF), Kauri Grove (KG) and Rose Garden (RG). All kauri trees are ~20 years old. The Native Forest (NF) is a man-made forest that imitates the natural forest ecosystem of New Zealand. It is made up of indigenous plant species (https://www.aucklandbotanicgardens.co.nz/our-gardens/native-forest/). In the NF, there are only two kauri trees present, both of which were sampled for research purposes. The Kauri Grove (KG) contains around 100 kauri trees and surrounded with kauri grass (*Astelia trinervia)*. The Rose Garden (RG) has kauri trees with flowering plants surrounding them, giving it a look similar to that of a garden.

Ten trees were selected for sampling, two trees from the Native Forest, five from the Kauri Grove, and three from the Rose Garden. Kauri Grove tree number three (KG3) showed basal bleeding symptoms. Root samples were collected from Kauri Grove trees one and three (KG1 and KG3), both Native Forest trees (NF1 and NF2) and one and three tree of Rose Garden (RG1 and RG3). Soil samples were collected at every 100cm from the bleeding tree towards the grassland (TG) and towards the healthy tree (TKG4) (Table 3.1).

Soil and root samples were collected from the top 10 cm depth at four cardinal points of the tree 100 cm away from the tree trunk using a spade. Tree NF2 was located on a slope. North (up slop) and south (down slope) cardinal point samples were on the slope. The soil and root samples were stored in sterile bags, transported at 4 °C, and stored at –20 °C until further processing. Care was taken to prevent cross-contamination of soil samples by cleaning all sampling equipment with ethanol and using fresh gloves and boot covers between each tree sampled (Lawrence et al., 2023). Approximately 3g of roots were separated, washed, surface-sterilized, and homogenized using a motor and pestle with liquid nitrogen. Soil samples were taken from the bleeding tree (KG3) at 100cm, 200cm, 300cm, and 400cm towards a non-symptomatic tree (TKG4) and grass (TG).

Table 3.1: List of soil and root sample collected from different garden types within the Auckland Botanic Gardens.

Site	Trees Sampled	Soil Samples	Number of Soil Samples	Root	Number of Root Samples
Native Forest	Native Forest Tree 1 (NF 1)	Yes	4 (CP)	Yes	4 (CP)
	Native Forest Tree 2 (NF 2)	Yes	4 (CP)	Yes	4 (CP)
Kauri Grove	Kauri Grove Tree 1 (KG 1)	Yes	4 (CP)	Yes	4 (CP)
	Kauri Grove Tree 2 (KG 2)	Yes	4 (CP)	No	
	Kauri Grove Tree 3 (KG 3) *	Yes	4 (CP)	Yes	4 (CP)
	Kauri Grove Tree 4 (KG 4)	Yes	4 (CP)	No	
	Kauri Grove Tree 5 (KG 5)	Yes	4 (CP)	Yes	4 (CP)
Rose Garden	Rose Garden Tree 1 (RG 1)	Yes	4 (CP)	Yes	4 (CP)
	Rose Garden Tree 2 (RG 2)	Yes	4 (CP)	No	
	Rose Garden Tree 3 (RG 3)	Yes	4 (CP)	Yes	4 (CP)
Distance Samples	Towards KG 3 (100m, 200m, 300m and 400m)	Yes	4	No	
	Towards Grass (100m, 200m, 300m and 400m)	Yes	4	No	

*Indicates the bleeding tree and (CP) indicates samples collected from cardinal points.

3.3.2 Soil and root DNA extraction and amplicon sequencing

Soil and root DNA was extracted following the hot phenol-chloroform DNA extraction method (Lawrence et al., 2023). In brief, samples were incubated in phosphate buffer, SDS, CTAB, lysozyme and proteinase K at 60°C for one hour and purified using Phenol-Chloroform. Three extractions, using 0.5 g of soil per extraction, were taken per sample and DNA from the three replicate extractions were combined to provide a 100 ng/µl DNA for each soil sample. DNA samples were quantified using a Quant-iT dsDNA Assay kit (Invitrogen, California USA) on a

Qubit 4 Fluorometer (Invitrogen, California USA). Sample purity was measured using a Nanodrop Spectrophotometer to check for A260/A280 ratio of >1.8.

For bacterial microbial community identification, the 16S rRNA gene region was amplified using primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) to target the V3–V4 gene region (Fadrosh et al., 2014). The fungal internal transcribed spacer one (ITS1) region was amplified using the forward CTTGGTCATTTAGAGGAAGTAA and reverse GCTGCGTTCTTCATCGATGC primer set (Op De Beeck et al., 2014). AMPure XP beads (Beckman Coulter Life Sciences, USA) were used to purify the amplicons following the manufacturer's instructions. One microlitre of a 1:50 dilution of the final library was run on a Bioanalyzer DNA 1000 chip to verify the size. Successful libraries were sequenced on the Illumina MiSeq platform using MiSeq reagent kit v2 (300 cycles).

Preprocessing of the amplicon reads was performed using QIIME2 V 2022.2 following the prescribed pipeline (reference here). Amplicon sequence variants (ASV) were produced by the q2-deblur denoising method.

The taxonomic assignments of the ASVs were determined using classify-sklearn naïve Bayes in q2-feature-classifier (Bokulich et al., 2018).

The taxonomic classifiers were trained against the prokaryotic reference sequences from databases 'Green Genes 13.8' for 16 S rRNA classification (McDonald et al., 2023) and the fungal reference sequences from UNITE v.9 with dynamic use of clustering thresholds (Abarenkov et al., 2022). Sample reads were rarefied and used in all downstream analyses. q2 phylogeny plugin was used to construct phylogenetic tree (Maximum Likelihood tree), ASVs were aligned with MAFFT (Katoh & Standley, 2013) and tree was constructed using Fast Tree 2 (Price et al., 2010). Phyloseq object was created using 'phyloseq' (McMurdie & Holmes, 2013) and 'qiime2R' (Maruyama et al., 2020)R packages for further downstream analysis.

3.3.3 Data Analysis

Statistical tests and graphical representations were carried out in QIIME 2 2022.2, and R v. 4.3. The microbial composition of the samples was visualized using complex heatmaps. To show the order of abundances as proportions of each sample, "CLR" (Centred Log Ratio) transformation - 'microViz' (Barnett et al., 2021), 'ComplexHeatmap' (Gu et al., 2022), 'dplyr' (C. Wickham eyal., 2014) and 'tidyverse' (H. Wickham et al., 2019) were used. Venn diagrams were used to visualize number of ASVs and genera per sites using R packages 'MicrobiotaProcess' (Xu et al., 2023), 'vennDiagram' (H. Chen & Boutros, 2011; Schwenk, 1984) (Appendix A).

To characterise the alpha diversity, the following indices using q2-diversity: ASVs richness (i.e., observed ASVs), Faith's phylogenetic diversity, Pielou's evenness and Shannon's diversity index, which accounts for both richness and evenness were determined. To evaluate changes in microbial community composition (beta diversity), Bray-Curtis distance was calculated and were visualized using NMDS plots through 'microViz' and 'shiny' R packages. The differences in community composition (beta diversity) in response to garden types were analysed using permutational multivariate ANOVA (PERMANOVA) using qiime diversity beta-group-significance (Anderson, 2001).

Taxonomic association trees were used to visualize differential abundance testing, arranged in a heatmap-style tree structure. Linear regression modelling (Total sum scaling and MaAsLin2) was used to identify differential abundance, with taxon models organized by rank (H. Zhou et al., 2022). 'microViz', 'concorb'and 'dplyr' in the R package was used to test and produce the trees. All analyses were run in the R version 4.3.1. q2 phylogeny plugin was used to construct a phylogenetic tree (Maximum Likelihood tree) ASVs were aligned with MAFFT (Katoh & Standley, 2013) and tree was constructed using Fast Tree 2 (Price et al., 2010). Phyloseq object was created using 'phyloseq' (McMurdie & Holmes, 2013) and 'qiime2R' 2020) R packages for further downstream analysis.

3.4 Results

3.4.1 Soil Microbial Communities

The eDNA recovered from the two trees from NF, three from RG, and five from KG generated a total of 2,909,472 16S rRNA demultiplexed reads. 88,273 total ASVs and 2960 unique ASVs obtained after denoising representing nineteen phyla and seventy-three orders (Figure 5.1 and Figure 5.2). Samples were rarefied and 500 amplicon reads were considered for each sample in determining the alpha and beta diversity (Appendix A). A total of 1,248,524 quality ITS paired-end reads was generated which were assigned to 363,818 ASVs of which 1010 unique ASVs representing eleven phyla and eighty-eight orders (Figure 3.1). Acidobacteriales (15.6%), Xanthomonadales (10.55%), Rhizobiales (6.03%), Rhodospirillales (6.36%), and Sphingomonadales (7.06%) were the most abundant taxa at the order level (Figure. 3.2 and 3.3).

3.4.2 Alpha and Beta Diversity

The Kruskal-Wallis test was used to identify any differences in alpha diversity among the soil samples collected from the three garden types in Auckland Botanic Gardens. The results showed that there was no significant difference in alpha diversity among the four cardinal points regardless of the garden type (Appendix A). However, a significant difference was observed in bacterial alpha diversity Pielou Evenness between the Rose Garden and Kauri Grove (Table 3.1). The PERMANOVA analysis of the Bray Curtis distances reveal that there were significant differences in the bacteria and fungi beta diversity between the garden types. (Figure 3.4).

3.4.3 Differential Abundance

The study revealed that certain phyla displayed both positive and negative associations towards the different types of garden soil microbial community. It was also observed that five bacterial phyla were absent in KG soil compared to RG soil (Figure1). The Phylum Elusimicrobiota was found to be positively associated with KG soil, while Phyla Desulfobacterota, RCP2-54, and Chloroflexi were negatively associated with KG soil. As for RG soil, Actinobacteria and Acidobacteria were negatively associated, while Nitrospirae and RCP2-54 were positively associated. And NF soil was positively associated with Chloroflexi. NF, KG and RG were positively associated to fungal phyla Zoopagomycota, Basidiomycota and Aphelidiomycota (SI). According to the differential abundance at the genus level, there were significant positive coefficients observed for the following groups in KG soil: Planctomycete, *Bdellovibrio, Gynumella, Skermanella, Actinomadura, Telmatobacter, Granulicella, Acidopila,* and *Terriglobus*. And the following fungal genera had significant positive coefficients at the kauri grove site: *Penicillium, Pestalotiopsis, Leucosporidium, Cystofilobasidium, Saitozyma,* and *Solicoccozyma* (Figure 3.5).

Brevundimonas, Cystobacter, Candida, Streptomyces, Rugosimonospora, Lacibacter and Burkholderia were with positive coefficients and significant genera to NF soil (Figure 3.6).

Flavobacterium, Nitrospira, Norcardioides, Arthrobacter, Sporichthya, Luteibacter, Lysobacter, Thermomonas, Enterobacteriaceae family, *Nitrobacteria, Sphingobium, Porphyrobacter,* and *Naganishia* had positive coefficients and were significant in RG soil (Figure 3.7).

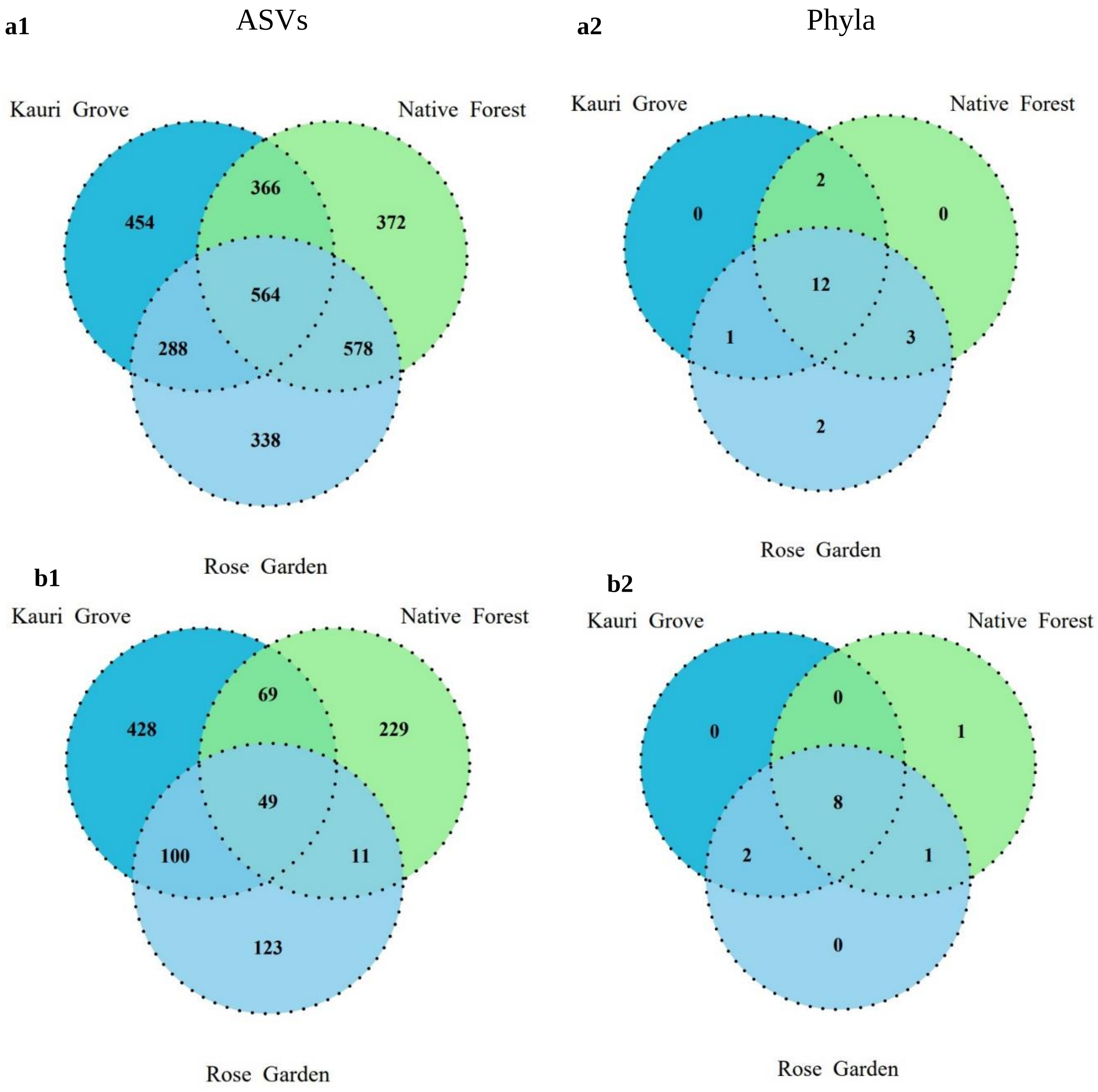


Figure 3.1: Distribution of bacterial and fungal ASVs and phyla detected in Kauri Grove, Native Forest and Rose Garden soil samples. ASV (a1 and b1) Phyla (a2 and b2).

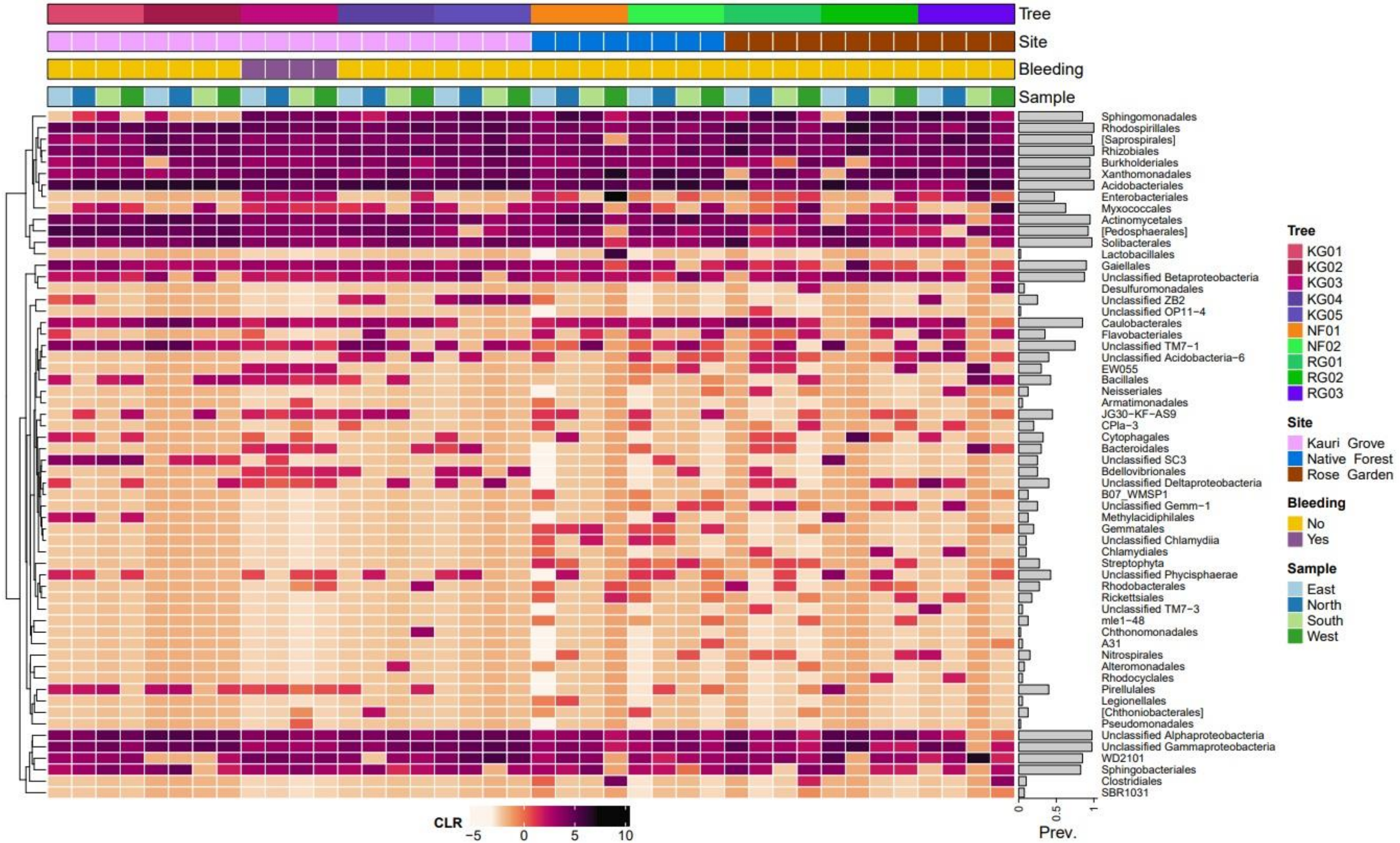


Figure 3.2: The relative abundance of bacterial taxa at the order level present in the ABG soil in centred log ratio (CLR).

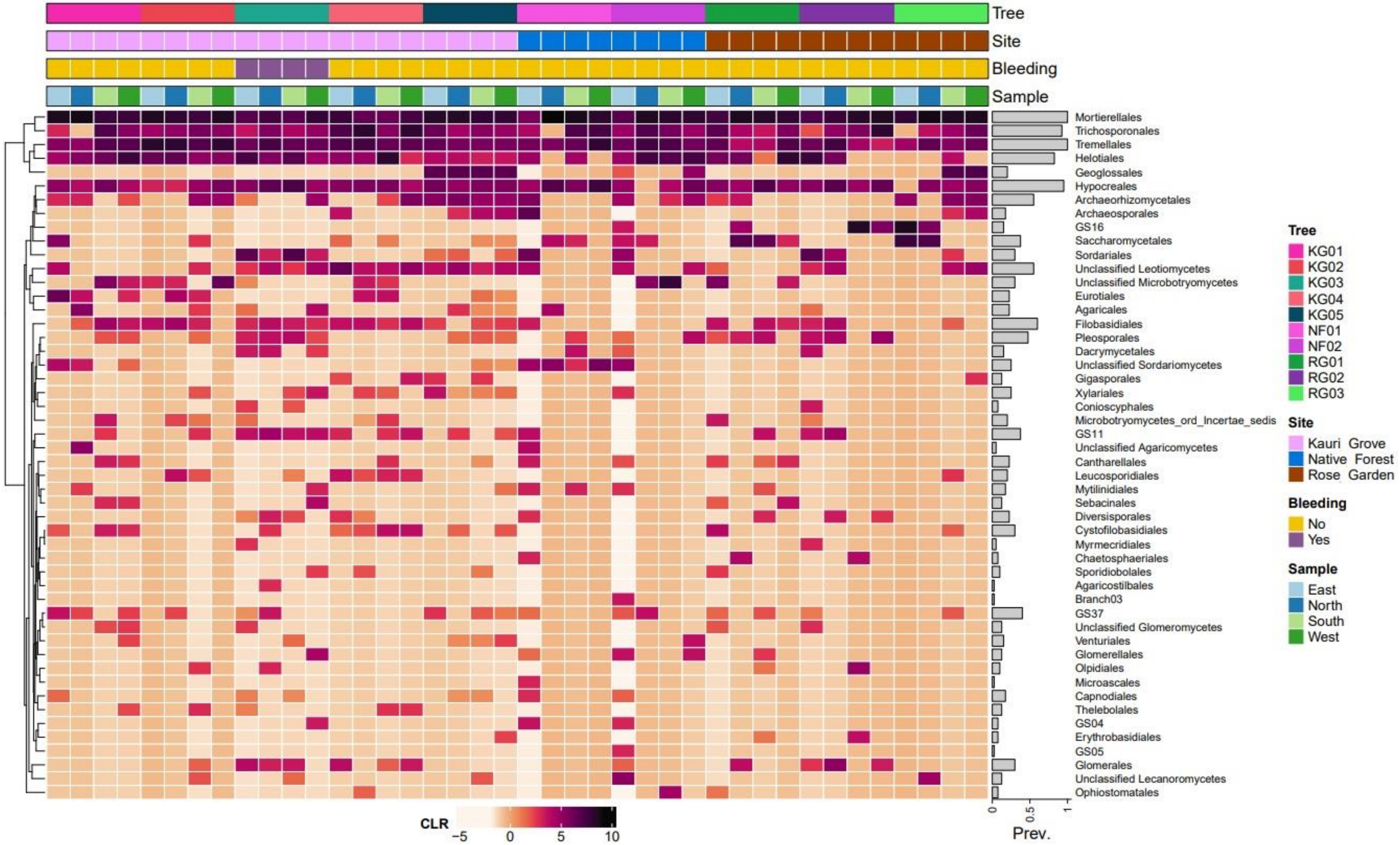
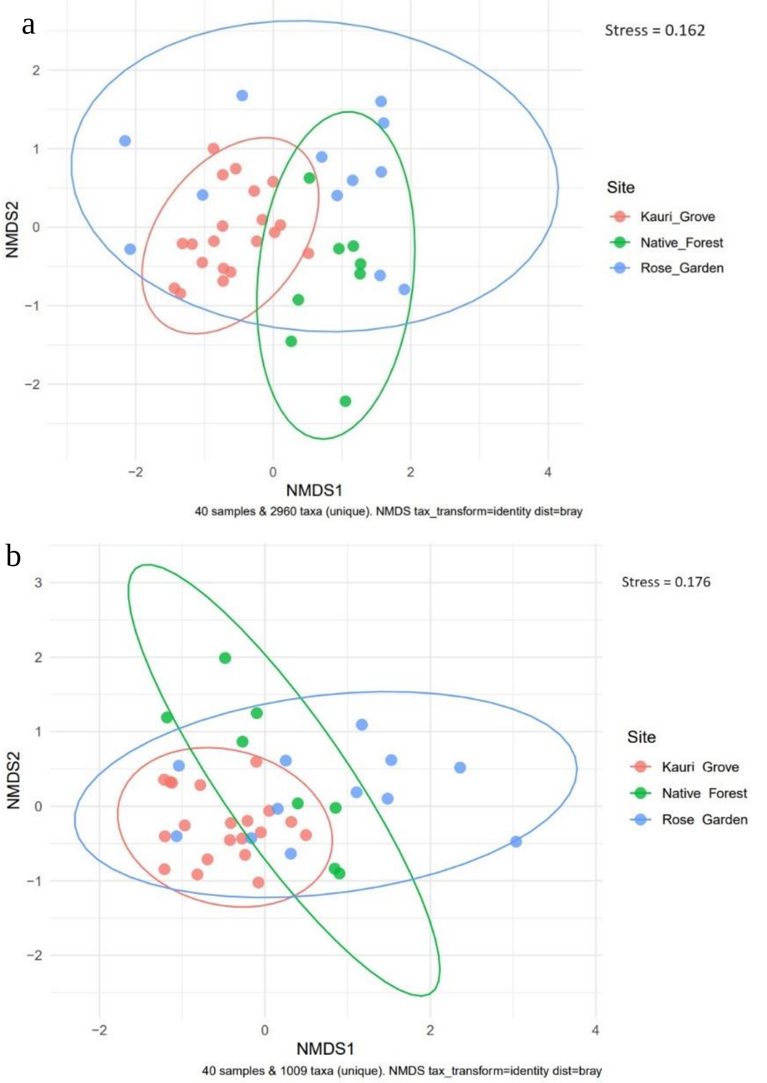


Figure 3.3: Relative abundance of the top 60 fungal taxa at the order level in the ABG soil in centered log ratio (CLR).

Table 3.2: Alpha diversity indices of bacterial and fungal communities

	Kauri Grove	Native Forest	Rose Garden	Kruskal-Walis test (p-value)
Bacteria				
Observed features	783.5+/-42.81	772.5+/-89.77	660+/-36.43	0.83
Faith PD	12.90+/-2.31	14.00+/-2.98	13.38+/-1.17	0.79
Shannon	7.26+/-0.37	7.01+/-1.19	7.029+/-0.294	0.38
Pielou Evenness	0.95+/-0.02	0.92+/-0.07	0.93+/-0.01	0.03*
Fungi				
Observed features	75.2 +/-9.84	92.5 +/-14.22	68 +/-10.65	0.58
Faith PD	6.011+/-2.76	7.33+/-4.45	5.91+/-1.15	0.683
Shannon	2.95+/-0.93	2.968+/-0.95	2.95+/-1.02	0.80
Pielou Evenness	0.71+/-0.15	0.74+/-0.15	0.71+/-0.08	0.614



Bray Curtis

PERMANOVA
Pseudo-F = 2.1568
p-value = 0.001**

Bray Curtis

PERMANOVA
Pseudo-F = 2.3865
p-value = 0.01*

Figure 3.4: Non-metric Multi-Dimensional Scaling plot showing the distance similarities among samples from the different garden types in ABG, a) bacteria, b) fungi. The ellipses represent 95% confidence level.

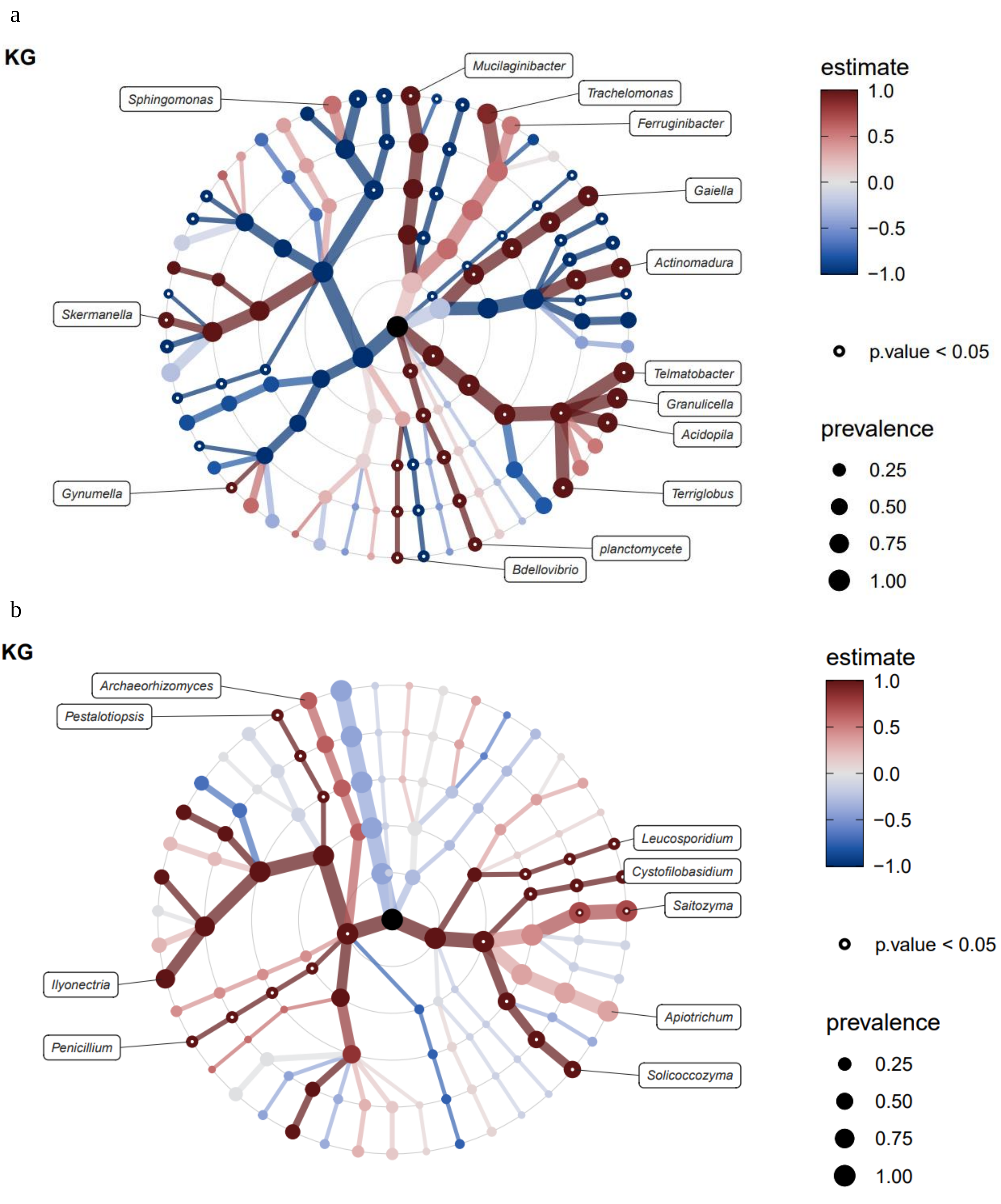


Figure 3.5: Differential abundance of genera presents in Kauri Grove soil. a) bacteria b) fungi. Red and blue are positive and negative coefficient estimates, respectively. The thickness of the branch is the prevalence. Significant genera are marked with an open circle.

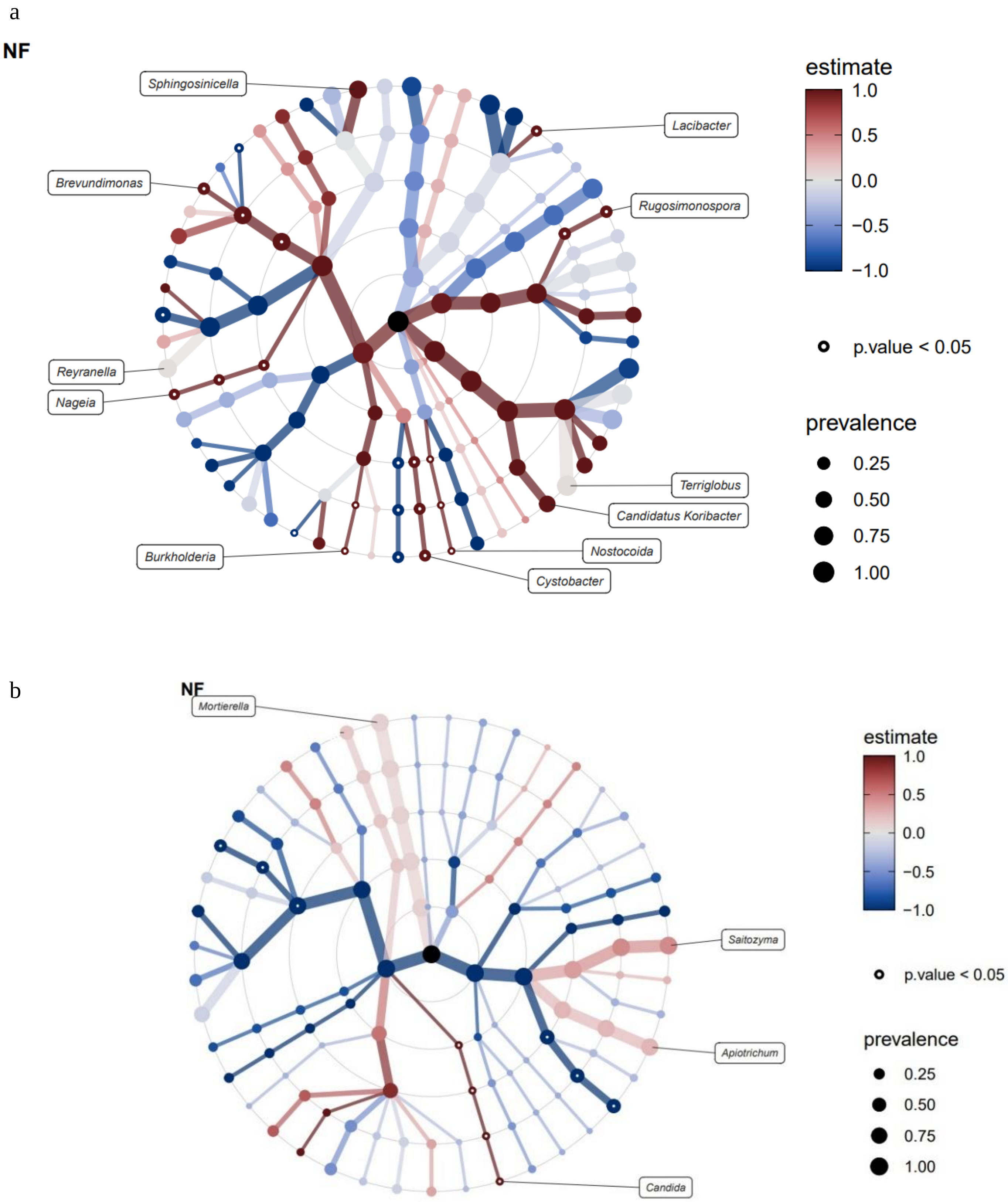


Figure 3.6: Differential abundance of genera presents in Native Forest soil. a) bacteria b) fungi. Red and blue are positive and negative coefficient estimates, respectively. The thickness of the branch is the prevalence. Significant genera are marked with an open circle.

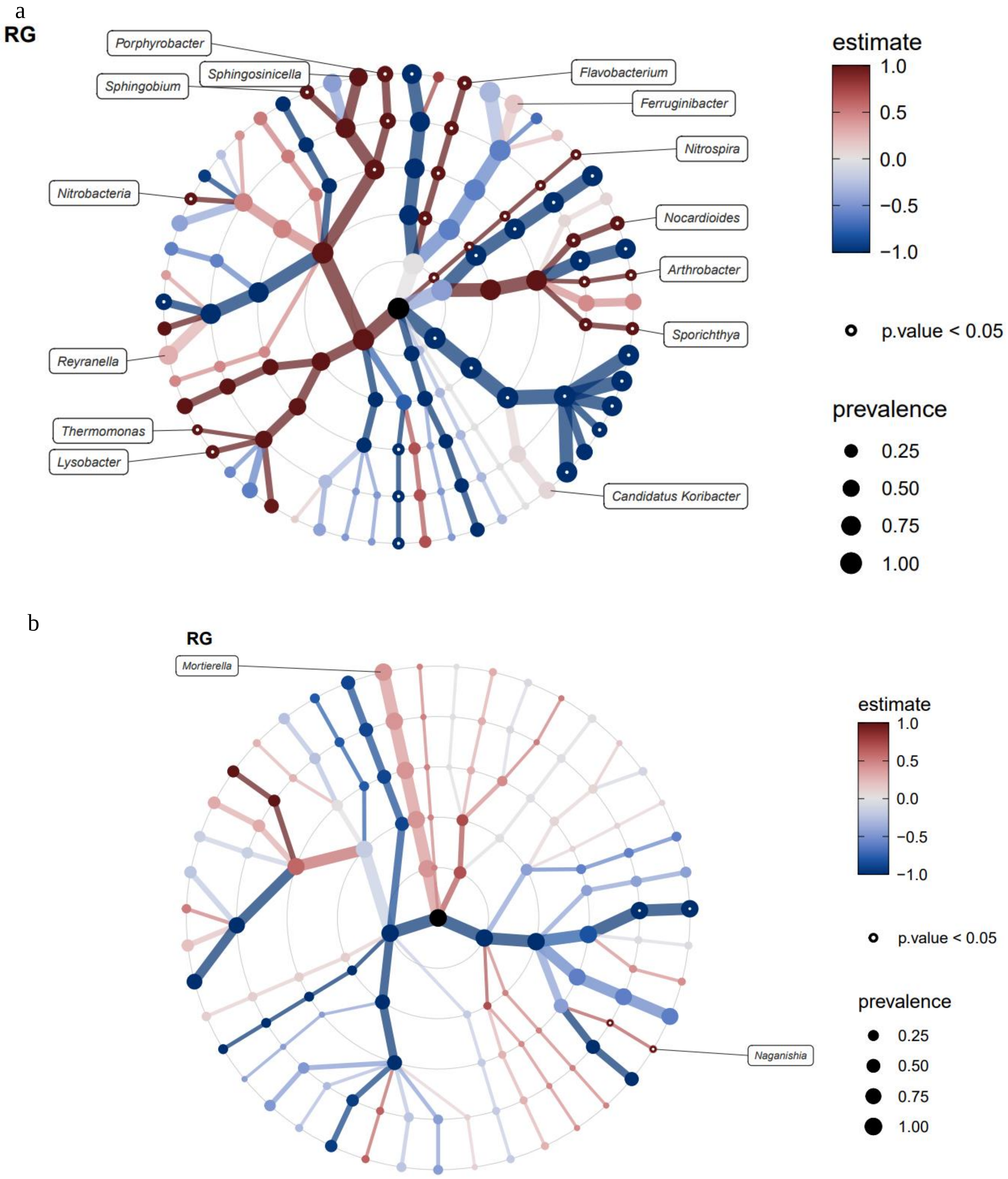


Figure 3.7: Differential abundance of genera presents in Rose Garden soil. a) bacteria b) fungi. Red and blue are positive and negative coefficient estimates, respectively. The thickness of the branch is the prevalence. Significant genera are marked with an open circle.

3.4.4 Cardinal point analysis

The study found that there was no significant difference in alpha and beta diversity between the cardinal points for all the trees. However, there were some interesting observations made regarding the microbial community of samples taken from the North and South cardinal points of the Native Forest tree 2, which was located on a slope. These observations were found to be different compared to the ones taken from the west and east cardinal points located at a plane. The relative abundance of phylum Actinobacteria was higher at the slope cardinal points compared to the cardinal points on the plane with 12% and 6.2% on the slope and plane, respectively. On the other hand, phyla Myxococcota, Bacteroidota, and Planctomycetota had lower relative abundance at the slope cardinal points compared to the plane, with 0.1%, 2.5%, and 2.2% on the slope, and 8.2%, 8.7%, and 8.2% on the plane, respectively (Figure 3.8).

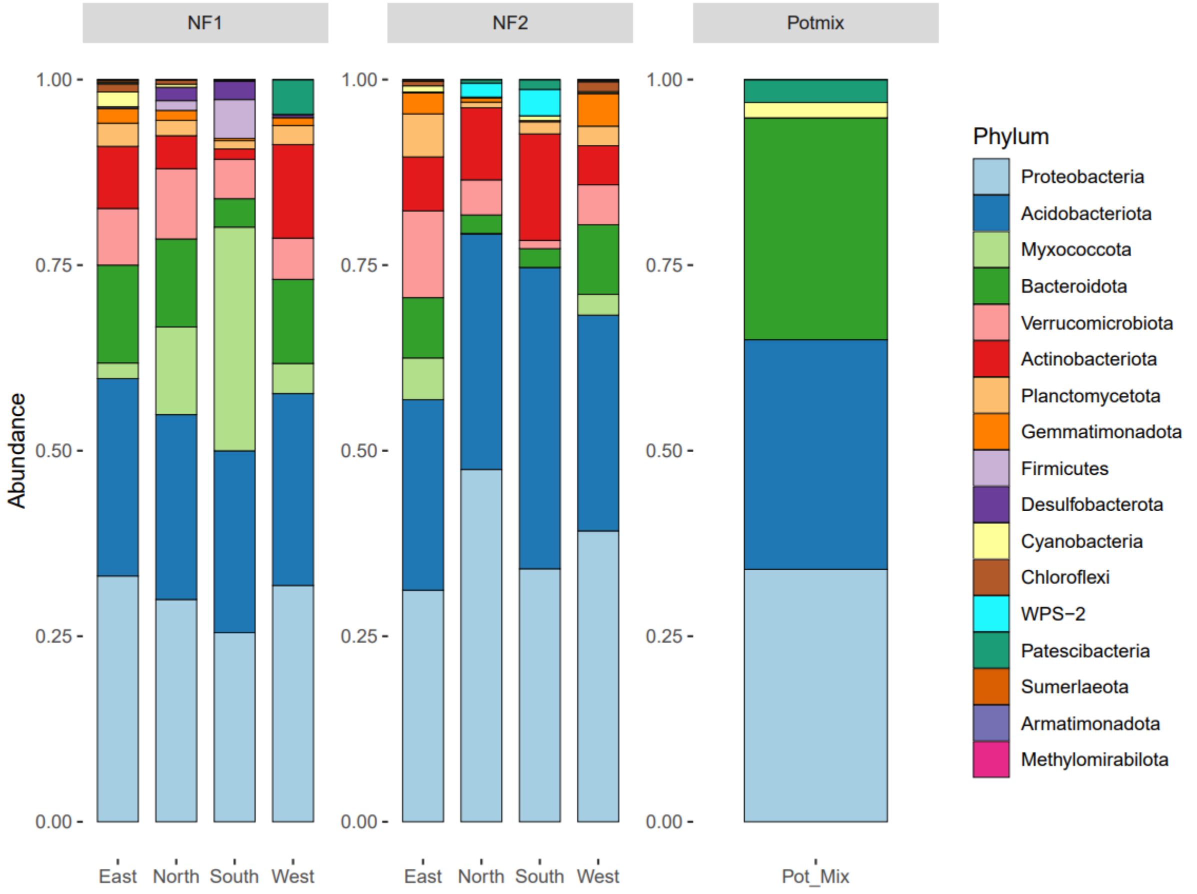


Figure 3.8: Relative abundance of bacterial community at the phylum level based on cardinal point from soil samples collected from the Native Forest trees.

3.4.5 Root Microbial Communities

A total of 1,582,259 16S rRNA demultiplexed reads were obtained from root samples at ABG, generating a total of 1,026,246 paired end reads. 101,922 total ASVs and 1200 unique ASVs were obtained after denoising, representing 14 phyla with a total of 48 different taxa at order level (Figure 3.9 and Figure 3.10). Samples were rarefied for 500 reads before alpha and beta diversity analysis (SI). Proteobacteria was the abundant phylum in the roots. Healthy tree KG1 and NF trees had high Proteobacteria of 77.96% and 88.0% respectively. Bleeding tree (KG3) and RG had 11.9% and 18.5% of Actinobacteria as their most abundant phyla. Phylum Chlamydiae, Chloroflexi, ODI, and WPS-2 were not detected in NF root and RG root. Planctomycetes, Verrucomicrobia and Tenericutes were not detected in NF samples only (Figure 9). Rickettsiales (18.38%), Actinomycetales (9.05%), Enterobacteriales (6.30%), and Sphingomonadales (7.06%) were the most abundant bacterial orders (Figure. 3.10).

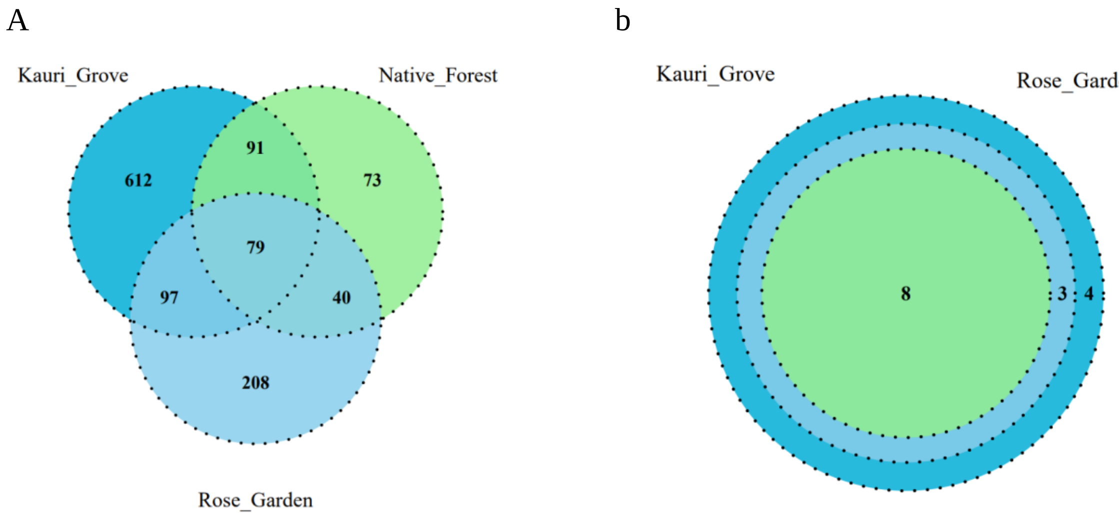


Figure 3.9: Venn diagram showing the number of ASV unique and Phyla shared among the samples. a) ASVs and b) Phyla

3.4.6 Root bacterial Alpha and Beta Diversity

The Wilcoxon test and PERMANOVA test were performed to identify any differences in alpha and beta diversity among the kauri root samples collected from the different garden types at the Auckland Botanic Gardens. The results showed that there was no significant difference in alpha and beta diversity among the three garden types (Figure 3.13 and 3.14).

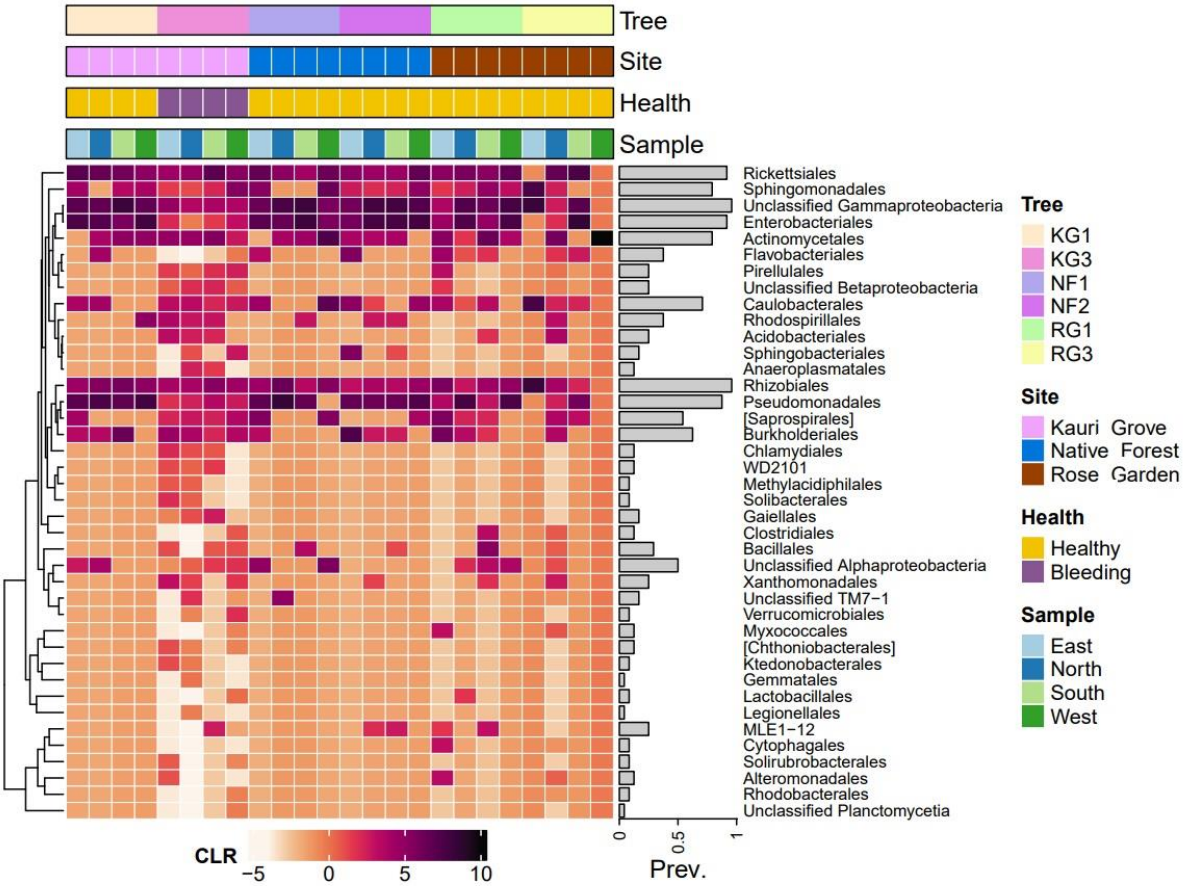


Figure 3.10: The relative abundance of the top 40 bacterial taxa at the order level in centered log ratio (CLR).

3.4.7 Root vs soil Microbial Communities

Root and soil bacteria were compared in KG1, KG3, NF1, NF2, RG1 and RG3 tree samples. Overall, 2482 ASVs were detected in the soil samples and 1200 ASVs were detected in root samples. 564 ASVs were common in both root and soil samples of these ASVs, 49 taxa at the orders level were common in root and soil samples (Figure 3.11). The soil samples did not detect Anaeroplasmatales, Ktenobacterales, Plantomycelia and Verrucomicrobiales (Figure 3.12).

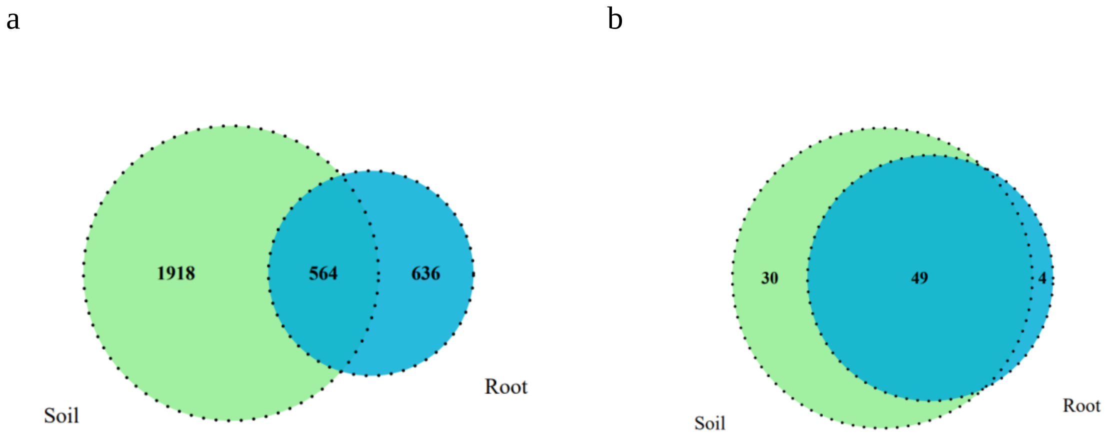


Figure 3.11: Venn Diagram showing the shared a) ASVs b) phyla.

3.4.8 Alpha and Beta Diversity

The Kruskal-Wallis test and PERMANOVA test was performed to identify any differences in alpha and beta diversity among root samples from the different garden types, respectively. The results showed that there was no significant difference in alpha (Figure 3.13) and beta diversity among the three garden types (Figure 3.14). Alpha diversity (Shannon diversity) of the soil was significantly higher compared to the roots in all garden types (Figure 3.13).

3.4.9 Genera has positive coefficient in the root samples

According to the differential abundance test, there were significant positive coefficients observed for the following bacterial genera in root samples: Planctomycete, *Conexibacter, Corynebacterium, Simkania, Nageia, Glucanacetobacter, Novosphingobium, Rhizobium, Ochrobactrum, Afipia,* Pseudomonadaceae - family, Enderobacteriaceae - family, *Arthrospira Staphylococcus* and *Peptoniphilus.* (Figure 3.15).

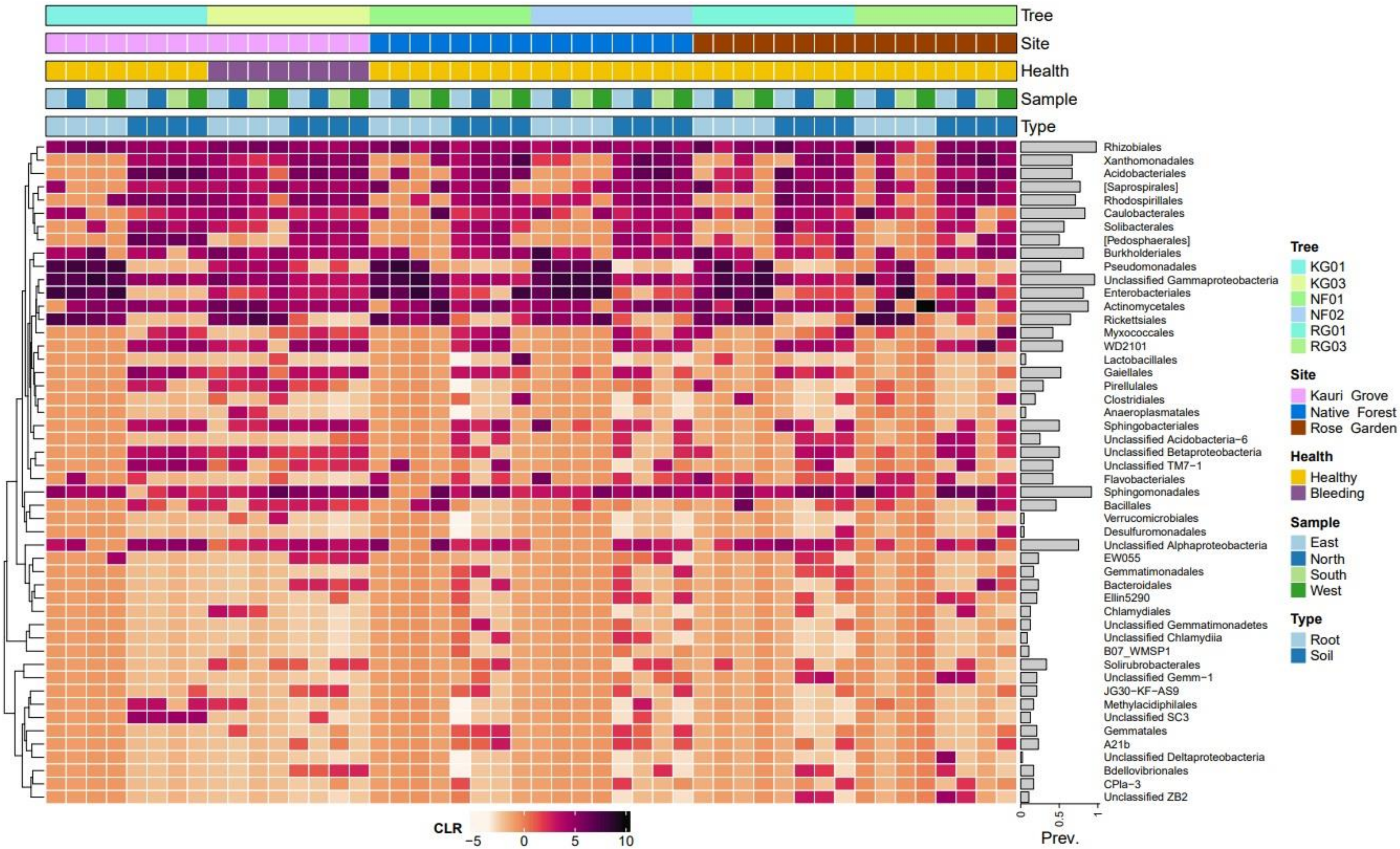


Figure 3.12: The relative abundance of the top 50 bacterial taxa at the order level present in the ABG roots and soil in centered log ratio (CLR).

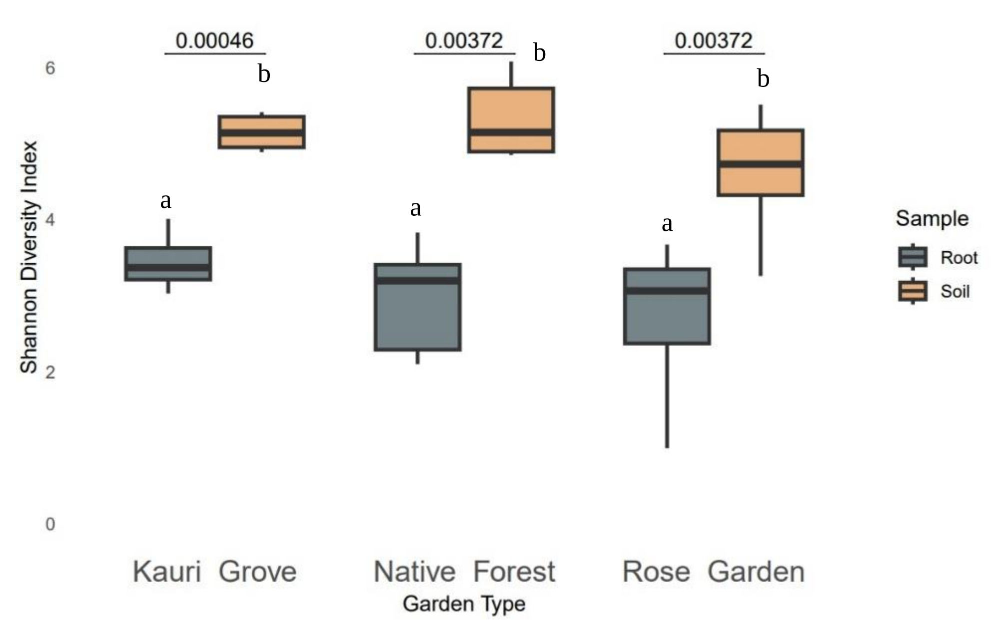


Figure 3.13: Boxplot of Shannon diversity index of soil and root samples. p-values of the Wilcoxon test.

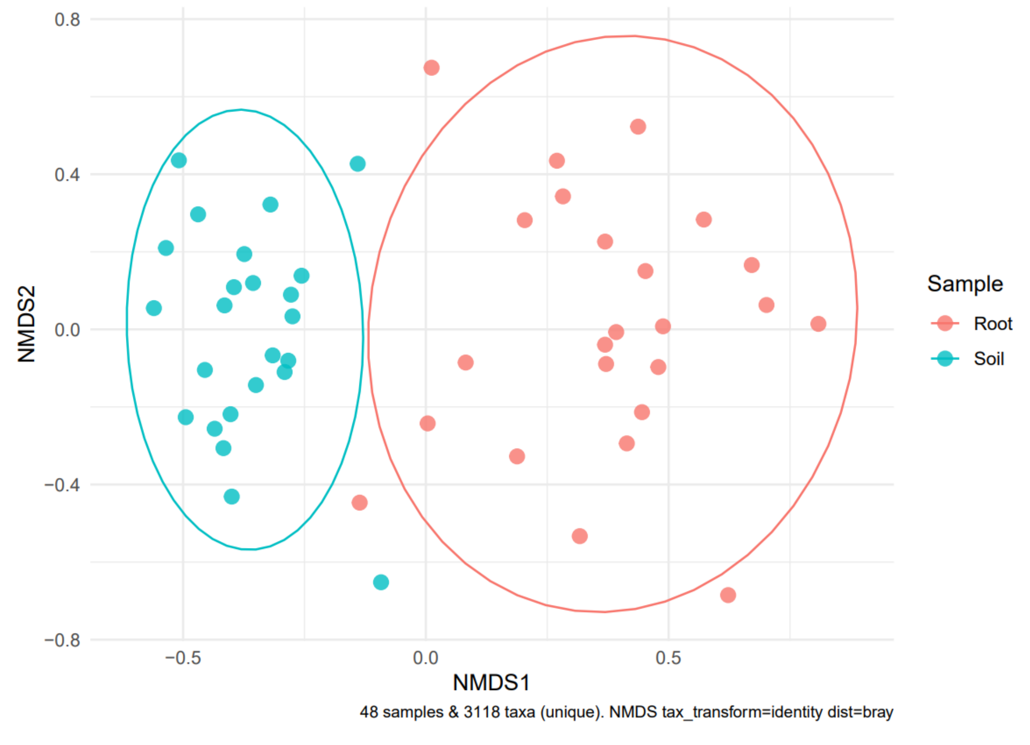


Figure 3.14: Non-metric Multi-Dimensional Scaling plot showing the distance similarities among soil and root samples in ABG. The ellipses represent 95% confidence level.

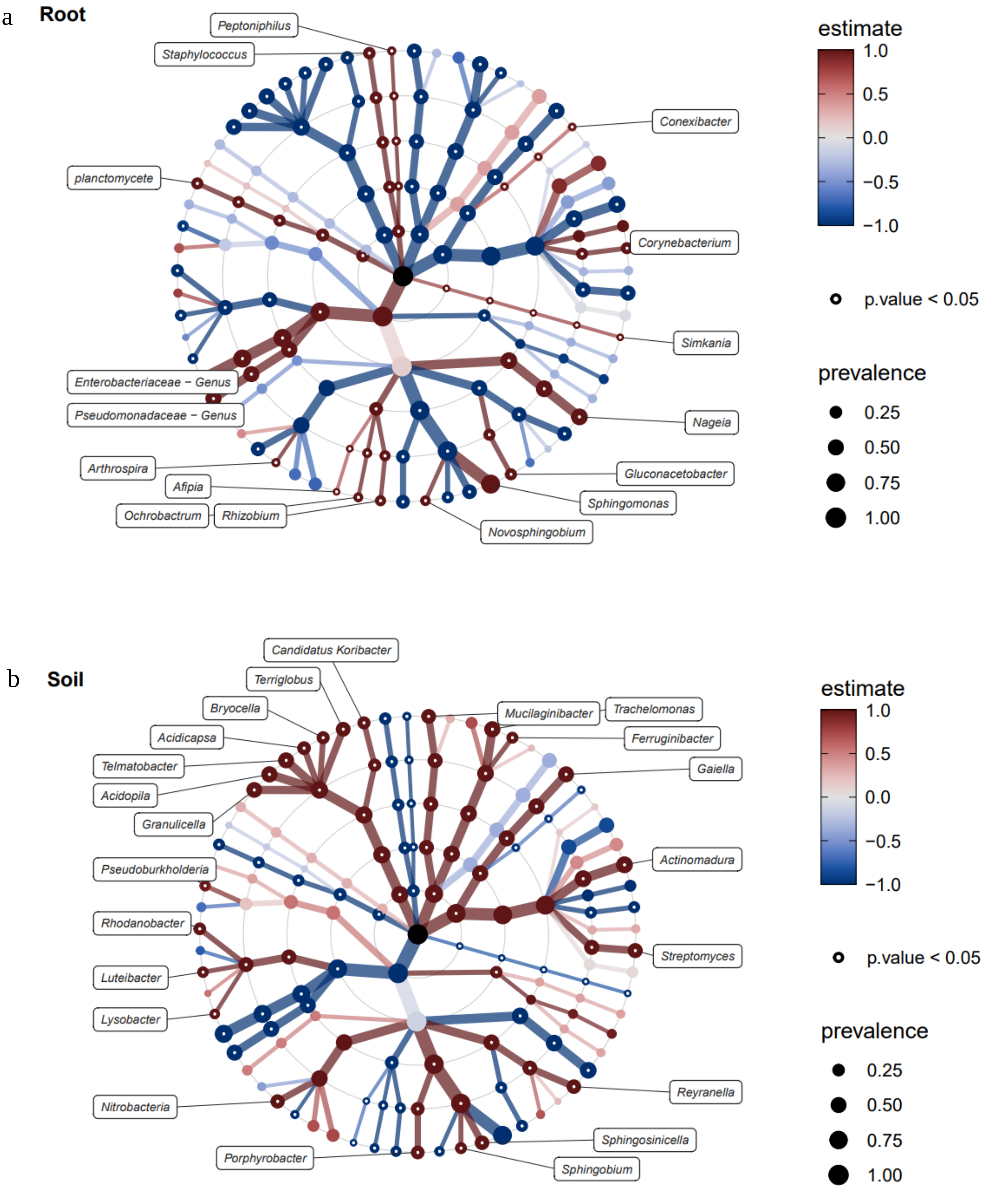


Figure 3.15: Differential abundance of genera presents soil and roots of ABG kauri. a and b represent root and soil, respectively. Red and blue are positive and negative coefficient estimates. The thickness of the branch is the prevalence. Significant genera are marked with a dot.

3.4.10 Microbial communities across a distance gradient from symptomatic tree.

The microbial communities were analyzed at different distances from KG3 (symptomatic tree = bleeding) towards KG4 and towards the garden lawn (TG). The results showed that the relative abundance of phylum Proteobacteria was 45.9%, 45.02%, 51.31%, and 35.11% at 100cm, 200cm, 300cm, and 400cm from KG3 towards KG4, respectively. At 100cm towards the grass (TG), the relative abundance of Proteobacteria was 45.74%, while at 200cm, 300cm, and 400cm, it was 21.72%, 25.23%, and 22.91%, respectively. These findings indicate that the relative abundance of Proteobacteria decreased as the distance from KG3 towards the grass increased (Figure 3.16).

The relative abundance of the Verrucomicrobia phylum was observed to be 4.07%, 0.95%, 4.91% and 7.75% at 100cm, 200cm, 300cm, and 400cm, respectively, towards KG4. Towards the grass (TG) at 100cm, the relative abundance of Verrucomicrobia was 4.33%, and at 200cm, 300cm, and 400cm, it was 33.80%, 16.73%, and 18.15%, respectively. These findings indicate that the relative abundance of Verrucomicrobia increased as the distance from KG3 towards the grass increased. And the relative abundance of phylum Acidobacteria was increasing towards KG4 and towards grass (Figure 3.16).

The fungal relative abundance of Ascomycota was found to be 32.2%, 10.4%, 71.3% and 9.2% at distances of 100cm, 200cm, 300cm and 400cm, respectively, towards KG4. For grass, the percentages were 64.8%, 15.4%, 22.1%, and 12.6% at distances of 100cm, 200cm, 300cm and 400cm, respectively. Basidiomycota had an abundance of 18.9%, 33.7%, 17.6% and 65.7% towards KG4 at distances of 100cm, 200cm, 300cm and 400cm, respectively. For grass, the percentages were 14.5%, 26.9%, 18.6%, and 14.1% at distances of 1m, 2m, 3m and 4m, respectively. Both Basidiomycota and Ascomycota were found to be more abundant between KG3 and KG4 and less so towards the grass. Rozellomycota had a higher abundance towards the grass, with percentages of 1.5%, 47.1%, 41.2%, and 44.8% at distances of 100cm, 200cm, 300cm, and 400cm, respectively (Figure 3.17).

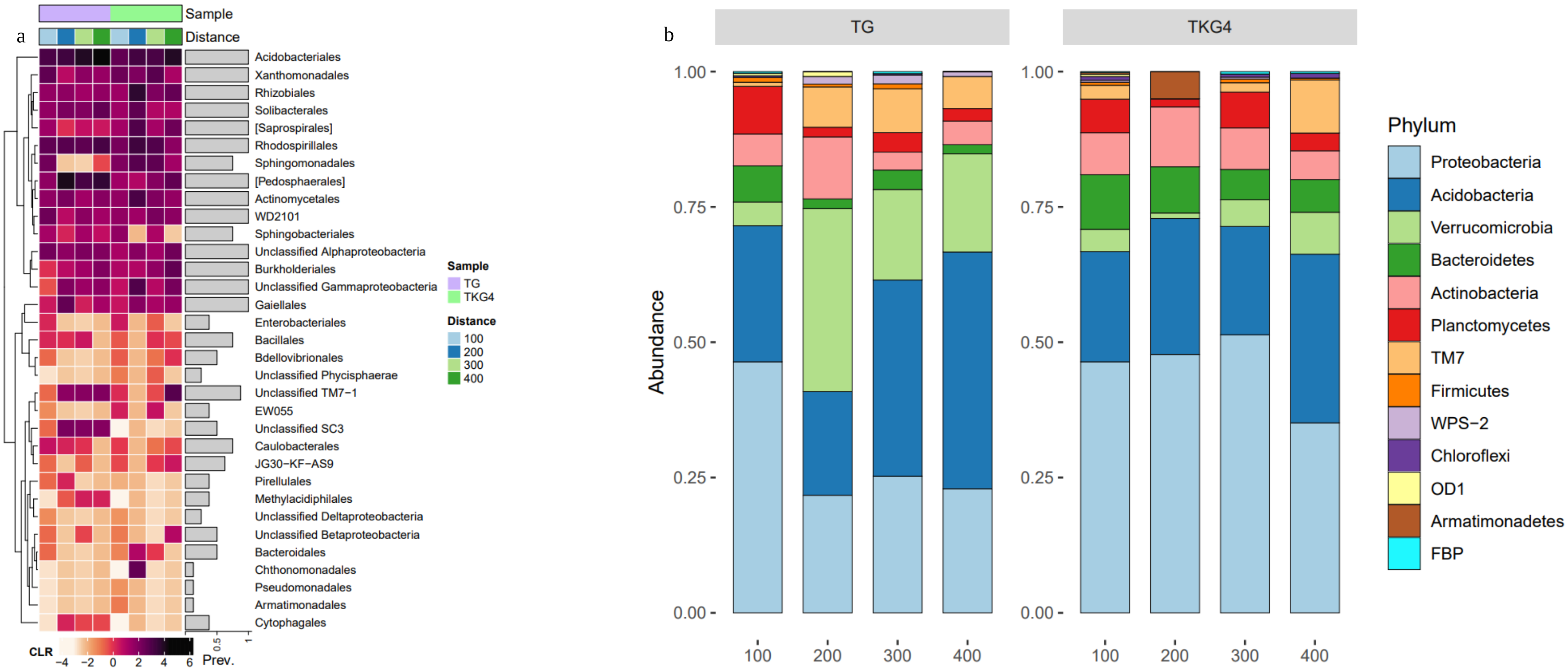


Figure 3.16: a) Relative abundance (CLR) of bacteria taxa at the order detected in the distance samples. b) Relative abundance of bacterial phyla detected in the distance samples. Distances are mentioned in centimeters (100cm, 200cm, 300cm and 400cm).

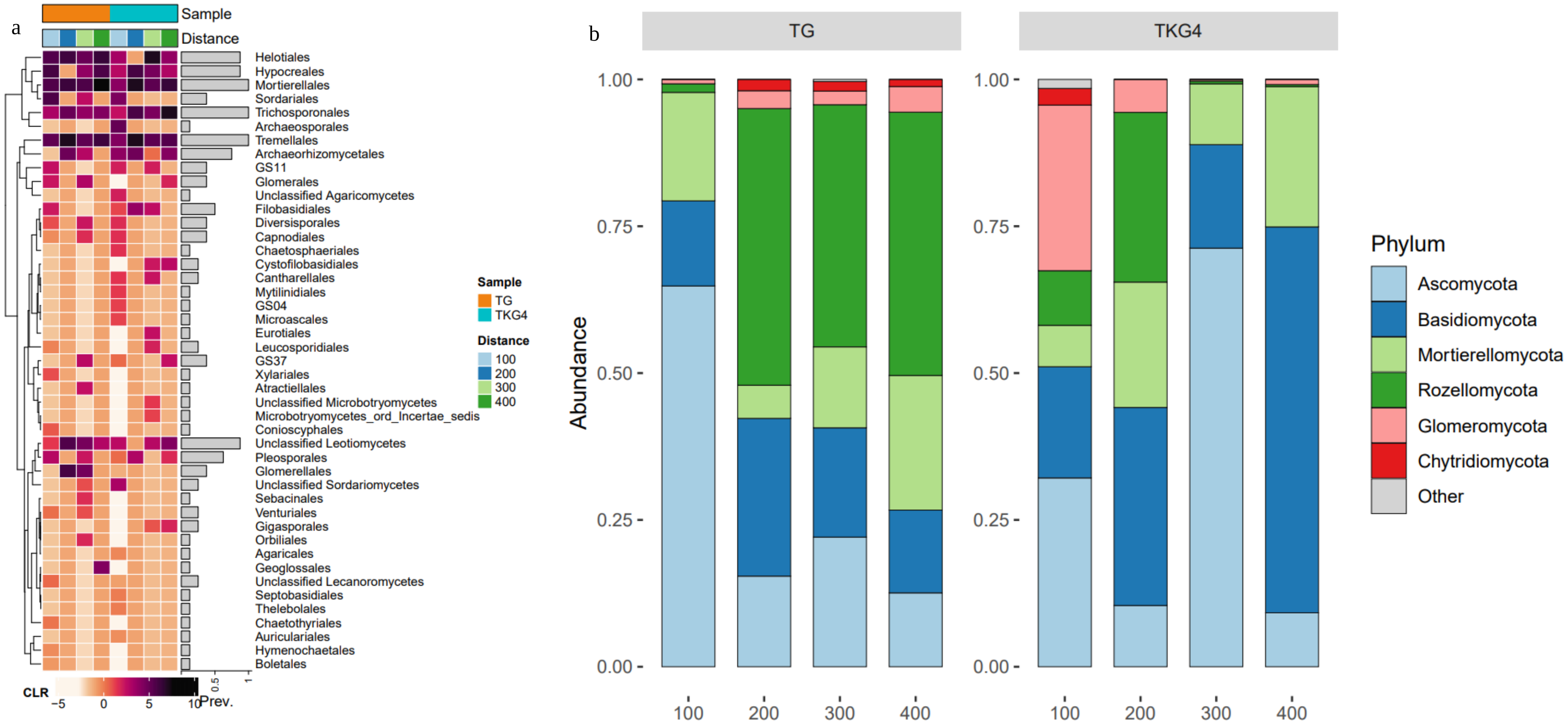


Figure 3.17: a) Relative abundance (CLR) of fungal taxa at the orders detected in the distance samples. b) Relative abundance of fungal phyla detected in the distance samples. Distances are mentioned in centimeters (100cm, 200cm, 300cm and 400cm).

3.5 Discussion

Amplicon sequencing technique was used to identify bacterial and fungal communities in planted native kauri located at Auckland Botanic Gardens, Auckland, New Zealand. The current study is documenting bacterial and fungal structure in the rhizosphere of planted *Agathis australis* in a garden environment.

3.5.1 Soil Microbial Communities

The kauri tree rhizosphere at ABG have bacterial communities that mainly consist of Acidobacteria, Actinobacteria, Bacteroidetes, and Proteobacteria (Figure 3.1). Previous studies have reported these phyla of rhizosphere bacteria to be dominant in various environments, including Norway spruce plantation soil (Uroz et al., 2013), German grassland and forest soils (Nacke et al., 2011), and planted conifers such as *Pinus tabulaeformis* (Chinese pine) (H. X. Yu et al., 2013), *Pinus massoniana* (Chinese red pine) (C. Shi et al., 2015), *Pinus sibirica* (Siberian pine) and *Pinus koraiensis* (Korean pine) in Russia (Naumova NB et al., 2015).

Mortierellomycota, Basidiomycota, Ascomycota, Rozellomycota and Glomeromycota were the most common fungal communities in the ABG kauri rhizosphere (Figure 3.1). This composition is similar to results observed in other studies on forest soil fungal communities in rhizosphere (D. Liu et al., 2018; Siles & Margesin, 2016; Zhu et al., 2022). Previous work has suggested that Ascomycota (Geml et al., 2014; T. Yang et al., 2017) or Basidiomycota (D. Liu et al., 2018) were the dominant phyla in most soil types. The results presented here align with previous studies that have shown Ascomycota to be associated with forest soil and conifers forest soil (H. X. Yu et al., 2013). While Poland forest soil study showed a significant presence of the Mortierellomycota phylum surrounding conifer trees compared to European larch (*Larix deciduas Mill.*), English oak (*Quercus robur L.*), English ash (*Fraxinus excelsior L.*), European beech (*Fagus sylvatica L.*) and European hornbeam (*Carpinus betulus L.*) (Szlachta et al., 2024). Previous investigations revealed that early stages of conifer trees rhizosphere are colonized by Ascomycota and Mortierellomycota primarily involved the degradation of simple polysaccharides and hemicellulose (Zhu et al., 2022). This might explain the abundance of Ascomycota and Mortierellomycota among the soil samples as the kauri trees at ABG are only about 20 years old.

The soil samples from the Native Forest contained most abundant phyla of Proteobacteria, Acidobacteria, Bacteroidetes, Verrucomicrobia and Actinobacteria. And phyla Chloroflexi, Planctomycetes and Firmicutes were also found. The NF is designed to emulate a natural forest and is composed of New Zealand's indigenous plant species. The forest soil consists diverse and plentiful microbial community, with bacteria being the most abundant microorganisms present (Hardoim et al., 2015; Nacke et al., 2012). The most abundant bacterial phyla of NF were found to be similar to that of temperature forests (López-Mondéjar et al., 2015) and conifer forests with highly abundant Proteobacteria, Acidobacteria, Bacteroidetes and Actinobacteria (Baldrian et al., 2012; Uroz et al., 2013). It also resembled the most dominant bacterial phyla found in urban forest sites across New Zealand (*The Effects of Urban Forest Restoration and Environmental Heterogeneity on Microbial Diversity and Ecosystem Functioning*, 1994). The bacterial community of Waipoua Kauri Forest in New Zealand primarily consisted of Proteobacteria, Actinobacteria and Acidobacteria (Byers, Condron, Donavan, et al., 2020).

Most abundant fungal phyla were Mortierellomycota, Basidiomycota, Ascomycota Rozellomycota and Glomeromycota. They were frequently reported as dominant phyla in natural forest soils (Z. Wang et al., 2022). Basidiomycota, Ascomycota, Chytriodiomycota and Mucoromycota fungal phyla were detected in urban forest sites across New Zealand (*The Effects of Urban Forest Restoration and Environmental Heterogeneity on Microbial Diversity and Ecosystem Functioning*, 1994). These phyla were well represented in NF soil samples except Mucoromycota which was detected only in two samples of NF. Waipoua Kauri Forest in New Zealand is primarily composed of abundant Basidiomycota and Ascomycota, followed by Mortierellomycota and Mucoromycota (Byers, Condron, Donavan, et al., 2020). The difference in the abundance may be due to difference in the age of the trees. Study by (Dang et al., 2017) revealed that rhizosphere fungal community significantly differ with the age of the stand. Phyla Kazan-3B-28, FBP and OP11 were not present in NF soil samples.

Same as NF, KG soil samples were also abundant with Proteobacteria, Acidobacteria, Bacteroidetes, Verrucomicrobia and Actinobacteria. However, Chlamideae, Gemmatimonadetes, Kazan-3B-28, OP11 and Nitrospirae were not detected in KG soil. And Aphelidiomycota and Blastocladiomycota were not detected in KG soil. This may be influenced either by kauri roots or kauri grass. RG had highest detection of bacterial and fungal phyla compared to NF and KG. Armatimonadetes and WSP2 were not detected in RG soil and only Blastocladiomycota was not detected compared to NF and KG. These bacterial and fungal phyla were detected in the Waipoua kauri forests (Byers, Condron, Donavan, et al., 2020).

3.5.2 Diversity Analysis

According to the results, certain phyla showed both positive and negative associations with the microbial community in garden soil. It observed that five bacterial phyla were absent in KG soil compared to RG soil, which could be a possible explanation for the significant difference between them (Figure 3.1). The phylum Elusimicrobiota was found to be positively associated with KG soil, while the phyla Desulfobacterota, RCP2-54, and Chloroflexi were negatively associated. On the other hand, Actinobacteria and Acidobacteria were negatively associated with RG soil, while Nitrospirae and RCP2-54 were positively associated. Moreover, NF soil was positively associated with Chloroflexi, and NF, KG, and RG were positively associated with the phyla Zoopagomycota, Basidiomycota, and Aphelidiomycota, Basidiomycota contains many ectomycorrhizal and saprophytic fungi, which are essential for decomposing macromolecular compounds. The kauri tree's basal bleeding could increase carbon and nitrogen in KG soil by increasing litter, leading to a rise in saprotrophic Basidiomycota (Zhu et al., 2022). Overall, the alpha diversity was low compared to other previous studies conducted in New Zealand kauri forests and Urban forests (Byers, Condron, Donavan, et al., 2020). This might be due to loss of DNA during extraction and limitations (Roopnarain et al., 2017; Trubl et al., 2019; Willner et al., 2012). The above-ground species may influence the diversity of the soil microbial community, and the trees in ABG are young kauri where roots may have not influence heavily on the microbial community (H. Li et al., 2018). However, the results aligned with a study conducted in the city of Coimbra, Portugal, regarding alpha diversity. The study compared the soil bacterial and fungal communities of public gardens and remnant forests (Barrico et al., 2018).

3.5.3 Key taxa at the order level detected in ABG kauri soil

In ABG, the bacterial order Acidobacteriales was the most prevalent, making up 15% of the seventy-three detected orders. The second and third most dominant orders were Sphingomonadales (Alpha proteobacteria) and Xanthomonadales (Gamma proteobacteria), with abundances of 10.55% and 7.06%, respectively. The top twelve orders, including Rhizobiales (Alphaproteobacteria), Saprospirales (Bacteroidetes), Rhodospirillales – purple sulfur bacteria (Alphaproteobacteria), Burkholderiales (Betaproteobacteria), and Myxococcales (Myxococcota), accounted for 75% of the bacterial community.

Acidobacteriales is a bacterial order commonly found in various ecosystems (Eichorst et al., 2020; Janssen, 2006). The pan-genomic profiles of Acidobacteriales suggest that they possess diverse metabolic pathways, hence are believed to play crucial roles in various ecological processes, such as regulating biogeochemical cycles, breaking down biopolymers, producing exopolysaccharides, and enhancing the growth of plants. Their widespread distribution and abundance in soil make them significant contributors to the overall ecosystem (Crits-Christoph et al., 2018; Kielak et al., 2017; Ward et al., 2009).

Sphingomonadales are often found in the vicinity of plants, and several strains have been isolated from the rhizosphere. While some of these strains have been categorized as pathogens, they may have different roles in the rhizosphere. For example, *Sphingomonas suberifaciens* is known to cause corky root disease in lettuce (H. Kim et al., 1998; Van Bruggen et al., 1990).

Furthermore, the bacterial core microbiome in the rhizosphere of *A. australis* consisted of the orders Burkholderiales, Rhizobiales, and Actinomycetales. Many conifer species have ectomycorrhizal (EM) root tips that are predominantly colonized by non-nodulating *Rhizobium* spp., as well as certain members of Burkholderiales, Xanthomonadales, and Actinomycetales (Burke et al., 2008; Izumi et al., 2006; Khetmalas et al., 2002; Lladó et al., 2017; N. H. Nguyen & Bruns, 2015). However, as *A. australis* only associates with arbuscular mycorrhizae (Padamsee et al. 2016), the bacterial orders detected could be associated with EM-associated plants in the vicinity (N. H. Nguyen & Bruns, 2015). For example, Burkholderiales and Rhizobiales are abundant root-associated bacterial orders containing nitrogen-fixing and mineral-weathering bacteria, and they are part of the core microbiomes for a wide range of plant hosts (Garrido-Oter et al., 2018; H. Sun et al., 2014; Yeoh et al., 2017). The *A. australis* fungal core microbiome is comprised of taxa within the orders of Mortierellales, Tremallales, Agaricales, Hypocreales, Helotiales and Trichosporonales. Numerous studies have reported the occurrence of Mortierellales in diverse environments such as in rocks, caves, mines, rivers, lakes, plant tissues, soils, and rhizospheres, including agricultural areas, at all latitudes (Held et al., 2020; Martino et al., 2003; Matei et al., 2020; T. T. T. Nguyen et al., 2019; Ozimek & Hanaka, 2021; Wani et al., 2017). Recent research using molecular methods revealed that *Mortierella* strains are the most common filamentous fungi found in soils around the world (Daghino et al., 2012; Grządziel & Gałązka, 2019; F. Li et al., 2018; Qiao et al., 2019; Smit et al., 1999; Wu et al., 2013; Yadav et al., 2015). The positive contribution of Mortierellales in the soils and plant tissues may lead to complex interactions between plants and microorganisms. (Mehrotra & Baijal, 1963). Mortierellales are found in the bulk soil, rhizosphere and plant tissues (Ozimek & Hanaka, 2021). These microorganisms are also responsible for improving access to the bioavailable forms of P and Fe in the soils, the synthesis of phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and the protection of plants from pathogens (Ozimek & Hanaka, 2021). *Mortierella* spp. were classified as saprotrophic microorganisms found in forest litter, and recent research has confirmed their value as decomposers in soils. These fungi have several key features, such as the ability to survive in unfavourable environmental conditions and using carbon sources found in polymers like cellulose, hemicellulose, and chitin (Wolińska et al., 2022).

Tremellales play a key role in decomposition (Banerjee et al., 2019; Ottesen et al., 2016) and produce a diverse range of bioactive metabolites (Sibanda et al., 2018). Tremellales taxa are highly host-specific and often limited to a single plant genus or species. (A.-H. Li et al., 2020; X.-Z. Liu et al., 2015).

3.5.4 Key genera identified based on the differential abundance analysis.

The ABG kauri soil key genera identified that are known to be with plant growth enhancing potential including *Flavobacterium, Brevundimonas, Streptomyces, Candidatus, Luteibacter, Sphingobium, Penicillium, and Arthrobacter.*

Nitrospira and *Nitrobacter* genera identified having positive significant association with RG have the potential to play a key role in nitrogen cycles (Daims et al., 2015). *Nocardiodies* genera have excellent and long-lasting adaptability. They can be microbial agents for phytoremediation (M. Song et al., 2022). *Lysobacter* is a new type of biocontrol bacteria with antimicrobial and PGP properties (Z. Dai et al., 2023). *Sporichthya* one of the genera was abundantly present in a rhizosphere which suppressed sweet peppers diseases (L.-N. Zhang et al., 2019). *Flavobacterium* exhibits growth-promoting qualities such as auxin, phosphate-solubilization, siderophore, salicylic acid, and chitinase production. It also has the ability to use 1-Amino Cyclopropan-1-Carboxylate as a nitrogen source. (Soltani et al., 2010). The genus *Sphingobium* is also a plant growth promoting rhizobacteria (PGPR), although it is mostly comprised of organisms that play an important role in biodegradation and bioremediation in sediments and soils (Boss et al., 2022). *Arthrobacter* has been found promoting growth in various plants, potential beneficial effects of this bacterial genus on plant growth and yield have been suggested and *Arthrobacter* species are known to produce different carotenoids (Y. chen Sun et al., 2022). *Luteibacter rhizovicinus* MIMR1 can colonize the rhizosphere of barley *in vitro*, promoting root development and plant growth (Guglielmetti et al., 2013).

Genera positively associated to NF such as *Brevundimonas* (Kumar & Gera, 2014), *Streptomyces* and *Candidatus* and have been well studied about their ability to enhancing growth of in plants (J. Peng et al., 2021; Z. Peng et al., 2022). *Streptomyces* is a significant producer of bioactive compounds, and therefore, it is extensively studied. These bioactive compounds include secondary metabolites such as antibiotics and extracellular enzymes, as well as antitumor and agro-active compounds. Furthermore, *Streptomyces* is crucial in the decomposition of cellulose and chitin (Olanrewaju & Babalola, 2019).

Penicillium spp. interact with the roots of crop plants, leading to improved plant growth. These fungi are known to secrete hormones, such as indole-3-acetic acid (IAA) and GA, which promote plant growth, and also play a role in phosphate solubilization, which could be a factor in their ability to enhance plant growth. *Bdellovibrio* rhizosphere soil controls bacteria, supports nutrient cycling and fosters microbial communities (Senthil Kumar et al., 2023). Certain species of *Penicillium* have been found to produce antibiotics that can Figureht against pathogens and activate multiple defense signals in plants, thus increasing their resistance (Radhakrishnan et al., 2014). Studies revealed that *Solicoccozyma* was able to promote shoot growth in maize and promote phosphorus fertilisation in combination with other microorganisms (Sarabia et al., 2018).

3.5.5 Root microbial Community

In the ABG, the roots of kauri trees were found to have high levels of the Proteobacteria. Similarly, in the Brazilian *Araucaria* Forest, bacterial phenotypic groups were analysed, which identified high sequences of Proteobacteria (Lammel et al., 2013). Proteobacteria were also detected in the roots of *Araucaria angustifolia* (Neroni & Cardoso, 2007) and conifers *Pinus thunbergii* and *Pinus flexilis* (Carper et al., 2018; Ma et al., 2020). In another separate study, the *Araucaria araucana* root microbiome also confirmed Proteobacteria as the dominant phylum. These results suggest that Proteobacteria may play a significant role in the root microbiome of Araucariaceae tree species and conifer species (Jaime Alarcón et al., 2020). KG samples were detected with fourteen phyla and had high alpha diversity compared to RG and NF roots. However, there were no significant differences between garden type or between trees in alpha and beta diversity.

Pseudomonas, Rhizobium, Enterobacteriaceae, *Staphylococcus* and *Burkholderia* were highly studied as bacterial endophytes were detected in kauri root at ABG (Figure 3.15). *Pseudomonas, Rhizobium,* and *Burkholderia* were the majority of the isolates of Brazilian *Araucauria* forest. It was noted that *Pseudomonas* did not nodulate and probably are endophytic bacteria in the Brazilian *Araucaria* forest study (Lammel et al., 2013). Root-associated bacteria were isolated from *Araucaria angustifolia* and revealed that key genera included Enterobacteriaceae and Pseudomonadaceae, which is also the case with *Agathis australis* at ABG (Figure 3.15) (Ribeiro & Cardoso, 2012). *Staphylococcus* genus was found as endophytes in rice plants (Chaudhry & Patil, 2016). *Rhizobium,* and *Burkholderia* are known as endophytic bacteria and have also been found inside nodules (Hoque et al., 2011; Li et al., 2008; Muresu et al., 2008). It has not yet been determined if they may play some role in the nodules. Pseudomonadaceae are well known as plant growth promoting bacteria, while some of them are known as plant pathogens (McSpadden Gardener, 2007).

3.6 Conclusion

The study has revealed that several important organisms responsible for crucial functions in the ecosystem have been found in the kauri soil of the Auckland Botanic Gardens. Acidobacteriales and Mortierellales are the most dominant bacterial and fungal orders respectively. There were significant genera such as *Penicillium*, *Mortierella*, *Streptomyces*, *Nitrospira* and *Flavobacterium* detected from ABG soil. *Pseudomonas*, *Rhizobium*, Enterobacteriaceae, *Staphylococcus* and *Burkholderia* were highly studied as bacterial endophytes were detected in kauri root at ABG. There was no significant difference between cardinal points. And distance samples from the bleeding tree KG3 showed that Rozellomycota and Verrcomicrobia were higher towards the grass and Proteobacteria, Basidiomycota and Ascomycota were higher towards KG4 tree. Further research is required to understand how microbial communities in kauri soils change due climate change, age, and physiochemical characteristics.

Chapter 4

Soil microbial communities in Kauri (*Agathis australis*) forests

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4.1 Abstract

New Zealand's ancient kauri forests are culturally iconic and ecologically significant. These forests are under threat from a highly virulent soil-borne pathogen called *Phytophthora agathidicida* that causes dieback disease in kauri trees. Kauri trees play a crucial role in sustaining the forest ecosystem by supporting a unique plant and soil environment. However, the impact of the disease outbreak and the following tree deaths in the kauri forests, particularly on the soil microbial communities, remains unknown. This lack of understanding is a cause for concern, as it is crucial to understand the full extent of the damage caused by this disease and develop effective strategies to protect these forests from extinction. In this study, soil microbial communities associated with various symptoms of kauri dieback and phosphonate treatment were analysed using high throughput amplicon sequencing. The study revealed significant variations in the alpha diversity of bacterial and fungal communities between soil of non-symptomatic and symptomatic kauri. Moreover, significant differences in the beta diversities of non-symptomatic and symptomatic trees, and phosphonate-treated and non-phosphonate-treated trees were observed. Previously studied disease suppressor microbial genera such as *Penicillium, Trichoderma, Aspergillus, Streptomyces, Clostridium, and Pseudomonas* were found to be significantly abundant in non-symptomatic kauri soil. These findings suggest a potential solution for discovering microbial taxa to suppress kauri dieback and emphasize the long-term impact this disease can have on kauri forests.

4.2 Introduction

Agathis australis, commonly known as the kauri tree, is a coniferous species that is endemic to Aotearoa New Zealand (Ecroyd, 1982). This majestic tree species has a rich history that is closely tied to the country's settlement and development, and it is treasured as a culturally significant (taonga) by the Māori people (Waipara et al., 2013). Kauri trees are considered one of the largest and longest-living conifer species in the world, with the ability to grow up to five meters wide and live for 600 to 1700 years (Ecroyd, 1982; Macinnis-Ng et al., 2017). In Aotearoa New Zealand there are only 7,500 hectares remaining primary virgin kauri forests and 60,000 hectares of secondary kauri forests or savannahs that contain regenerating kauri (Halkett, J., 1980). Kauri forests like other types of forests, play an essential role in the biogeochemical cycles (Madgwick et al., 1982).

Currently, there is plant dieback disease that is causing the destruction of kauri trees. The oomycete, *Phytophthora agathidicida* is the primary cause of kauri dieback disease in the lowland stands in Northern Island (Beever et al., 2007; Weir et al., 2015). The disease affects the roots and phloem tissues, resulting in collar rot that can affect trees of all ages (Beever et al., 2007). The pathogen famishes kauri by preventing the water and nutrients transportation throughout the tree, which leads to the canopy thinning over time. The scale of canopy thinning can differ. Figure 4.1 compares healthy kauri crowns to kauri suffering from severe dieback disease to a dead kauri described in Biosecurity New Zealand, Tiakina Kauri | Kauri Protection Agency (Identify the disease | Tiakina Kauri (kauriprotection.co.nz)). The disease symptoms include root and collar decay, lesions, significant yellowing of leaves, canopy thinning, of which lead to the death of many trees (Bassett et al., 2017; Waipara et al., 2013). The current distribution of kauri forests and the widespread dispersal of *P. agathidicida* throughout major kauri stands, such as the Waitakere Ranges and Waipoua Forest, means that this pathogen is a significant threat to the long-term survival of this iconic tree species (Beever et al., 2007; (Waipara et al., 2013).

Figure 4.1: The scale of canopy thinning. 1-Healthy crown, 2-Canopy thinning, 3- Thinning and some branch dieback, 4 – Severe dieback and 5 – Dead. (Identify the disease | Tiakina Kauri (kauriprotection.co.nz)).

Chemical agents such as phosphonate are known to be an effective control of many plant diseases caused by *Phytophthora* spp. (Horner & Hough, 2013a; Ziadi et al., 2016)(Ij & Eg, 2014). Recently, studies conducted in glasshouses on 2-year-old kauri seedlings showed that phosphonate provides effective protection against *P. agathidicida* (ref needed here). Injecting phosphonate into the trunk resulted in a 100% survival rate after soil inoculation with the pathogen. However, there are still concerns on the phytotoxicity and the practicality of using phosphonate as a control measure. In the Horner and Hough (2014) study showed 20% of individuals injected with phosphonate displayed phytotoxic symptoms such as leaf yellowing. The plant and soil microbial community interactions contribute to major biogeochemical cycles. The soil microbiome encompasses all rhizosphere-associated microbes, bacteria, fungi, and archaea, including their genetic elements and interactions that significantly influence the plant's overall health (Feng et al., 2006). Furthermore, they have a significant impact on the soil ecosystem as they help to maintain soil health, regulate nutrient cycling, and combat pollutants. Microbial communities are used as indicators to detect pollutant effects on biogeochemical processes and ecology (Yi et al., 2022). The diversity and structure of microbial communities are indicators of the nutrient status and self-healing capacity of the soil (P. Shi et al., 2017). Soil microbes live together in intricate ecological networks that involve various types of interactions between species, such as mutualism, competition, predation, and neutral relationships. Certain key species, known as keystone taxa, have a significant impact on the soil ecosystem's functional potential (Z. Yu et al., 2021).

Aotearoa New Zealand has a rich history of research on soil microbes. The studies include investigating the interactions between carbon and nitrogen in above- and below-ground communities, as well as examining the impact of land management practices on soil microbes. Additionally, these studies explore how potentially pathogenic bacteria cycle through the soil (Stott & Taylor, 2016). However, only a handful of research studies have been conducted to investigate the range of microorganisms that exist within and around kauri trees. Byers et al 2020 found a significant difference between the microbial diversity and composition of soil microbial communities in old-growth kauri forests and adjacent pine plantations in Waipoua Forest. The bacterial communities present in the soil of both kauri and pine trees showed high diversity and low dominance values. The dominant bacterial phyla found in these soils are Proteobacteria, Actinobacteria, and Acidobacteria, which are commonly found in acidic coniferous forests (Lladó et al., 2017). Furthermore, the differences in the composition of fungal communities were observed between kauri and pine soils. These differences were explained ecologically, as the role of fungi in the soil environment is both saprophytic and symbiotic. Therefore, the composition of the fungal community is often influenced by the inputs of tree litter and root exudates (Urbanová et al., 2015).

Another study also found significant differences in the fungal diversity in non-symptomatic and symptomatic kauri dieback soils (Byers, Condron, O'Callaghan, et al., 2020b). Interestingly, fungal diversity was found to be higher in symptomatic soils. Moreover, the composition of fungal and bacterial communities was also different in asymptomatic and symptomatic soils. Taxonomic analyses have revealed that several microbial taxa, such as *Penicillium*, *Trichoderma*, Enterobacteriaceae, *Actinobacter*, and *Pseudomonas*, were more abundant in asymptomatic kauri soils.

It is vital to conduct further research to determine how microbial communities impact the health of kauri forest fragments and their ability to respond to pathogen invasion. This is important given the current threat posed by kauri dieback disease. Moreover, it is essential to investigate the potential plant-beneficial roles that kauri soil microbial taxa may possess. Identifying microbial taxa that can support kauri health in the face of pathogen attacks can provide valuable insights for protecting these vital trees. Furthermore, a better understanding of soil microorganisms surrounding kauri may help to illustrate the changes occurring in the microbial flora due to diseases.

There is a significant lack of information about the microbial community structure of kauri soil, overall soil health, and the effects of abiotic factors. Examining the microbial composition could uncover potential options for natural disease management, such as biocontrol microorganisms that could offer long-lasting protection for kauri against pathogens without harming the ecosystem's integrity. The study aims to understand the microbial community structure in kauri soil using molecular-based methods by sequencing the 16S rRNA and ITS1 genes. This may provide new opportunities to illustrate the key microbial communities in kauri soil.

4.3 Materials and Methods

4.3.1 Site description and soil sampling

The study was conducted in the Waikato region of New Zealand. Soil samples were collected from 44 trees (15 from Tairua and 29 from Whangapoa) (Figure 4.2). The terrain of the site, tree health (canopy health score, yellowing leaves, basal bleeding) (Identify the disease | Tiakina Kauri (kauriprotection.co.nz)), tree circumferences at breast height (about 160 cm from the ground), and phosphonate treatment were recorded (Tables 4.1 and 4.2). Surface organic matter was removed to expose the soil, and soil samples were collected from four cardinal points at 100 cm from the trunk, at the surface and 10 cm depth. The roots of kauri trees usually extend three times the distance from the centre of the trunk to the edge of the canopy, and they are shallow. Soil samples from the Whangapoa kauri forests were pooled for each tree as surface samples and 10 cm depth samples and stored at -20°C until processed.

The tree health was evaluated on site by assessing the canopy and were classified according to the canopy scores. Trees with a canopy score below 1.5 were considered non-symptomatic, while those with a score between 1.5 to 2.5 were slightly thinning. Trees with a score of ≥2.5 were classified as symptomatic (Table 4.1).

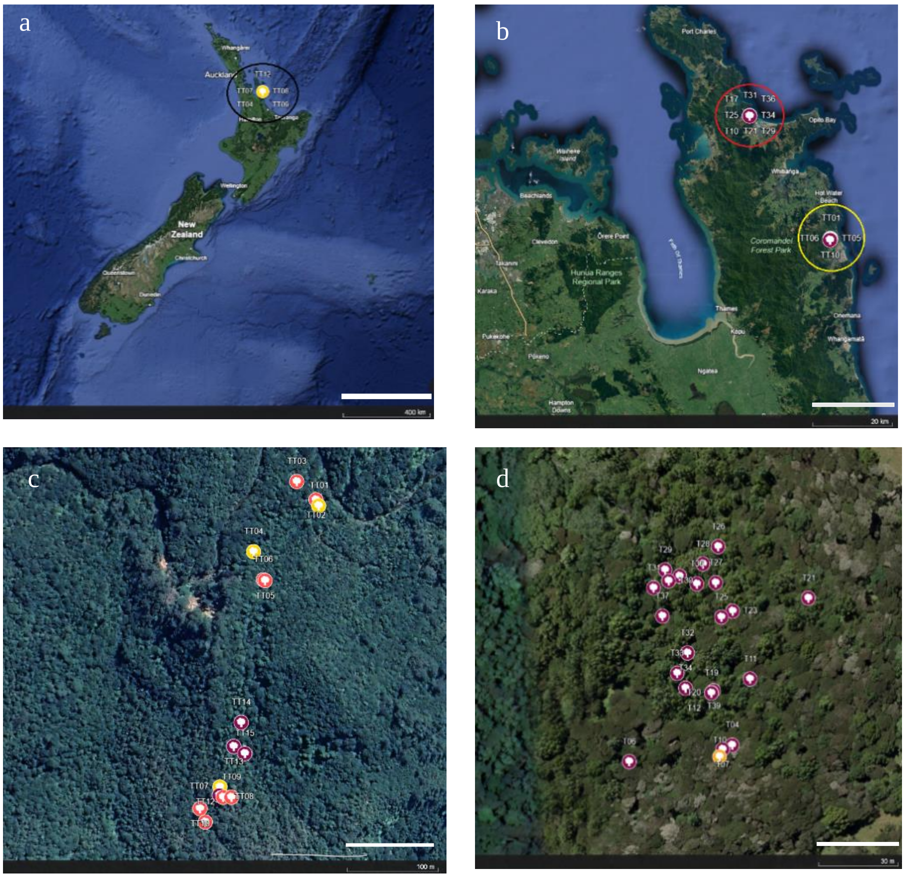


Figure 4.2: Location of two sampling sites in the New Zealand map (a), Location of Tairua site circled in yellow (b), and Whangapoa site circled in red (b), kauri tree locations at Whangapoa (d) and Tairua (c). Purple trees indicate kauri dieback symptomatic trees with a canopy score > 2.5, red trees with a canopy score between 1 to 2.5 and yellow trees with a canopy score below 1.5. Scale bars a - 400km, b - 20km, c - 100m, d - 30m.

4.3.2 Phytophthora agathidicida detection and identification

To identify *P. agathidicida* in soil DNA samples, the ITS1, 5.8S rRNA, and partial ITS2 region were amplified using endpoint polymerase chain reaction (PCR) with primers ITS PTA F2 (AACCAATAGTTGGGGGCGA) and ITS PTA R3 (CTCGCCATGATAGAGCTCGTC), as described by (Than et al., 2013). The *P. agathidicida* (ICMP 17027 strain) pure isolate was used as a positive control. PCR amplified product was visualised in 1% agarose gel electrophoresis (Bio-Rad, USA) gel image processed using SmartView pro 1100 Imager system, Taiwan. The bands were excised and purified using GFX PCR DNA and Gel Band Purification Kits Cytiva USA. Purified DNA samples were sequenced using Sanger sequencing platform. The sequence data were compared to reference sequences using the BLASTn tool in The National Centre for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the sequence identity.

4.3.3 Soil DNA extraction and amplicon sequencing

Soil DNA was extracted following the hot phenol-chloroform DNA extraction method (Lawrence et al., 2023). In brief, samples were incubated in phosphate buffer, SDS, CTAB, lysozyme and proteinase K at 60°C for one hour and purified using Phenol-Chloroform. Three extractions, using 0.5 g of soil per extraction, were taken per sample and DNA from the three replicate extractions were combined to provide a 100 ng/µl DNA for each soil sample. DNA samples were quantified using a Quant-iT dsDNA Assay kit (Invitrogen, California USA) on a Qubit 4 Fluorometer (Invitrogen, California USA). Sample purity was measured using a Nanodrop Spectrophotometer to check for A260/A280 ratio of >1.8.

For bacterial microbial community identification, the 16S rRNA gene region was amplified using primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) to target the V3–V4 gene region (Fadrosh et al., 2014). The fungal internal transcribed spacer one (ITS1) region was amplified using the forward CTTGGTCATTTAGAGGAAGTAA and reverse GCTGCGTTCTTCATCGATGC primer set (Op De Beeck et al., 2014). AMPure XP beads (Beckman Coulter Life Sciences, USA) was used to purify the amplicon libraries following the manufacturer's protocol. One microlitre of a 1:50 dilution of the final library was run on a Bioanalyzer DNA 1000 chip to verify the size. Successful libraries were sequenced on the Illumina MiSeq platform using MiSeq reagent kit v2 (300 cycles).

The amplicon sequence reads were processed in QIIME2 V 2022.2 following the prescribed pipeline (Liu et al., 2022). Amplicon sequence variants (ASV) were produced by the q2-deblur denoising method (Amir et al., 2017).

The taxonomic assignments of the ASVs were determined using classify-sklearn naïve Bayes in q2-feature-classifier (Bokulich et al., 2018).

The taxonomic classifiers were trained against the prokaryotic reference sequences from databases 'Green Genes 13.8' for 16 S rRNA classification (McDonald et al., 2023) and the fungal reference sequences from UNITE v.9 with dynamic use of clustering thresholds (Abarenkov et al., 2022). Sample reads were rarefied and used in all downstream analyses.

q2 phylogeny plugin was used to construct phylogenetic tree (Maximum Likelihood tree), ASV sequences were aligned using MAFFT (Katoh & Standley, 2013) and tree was constructed using Fast Tree 2 (Price et al., 2010). Phyloseq object was created using 'phyloseq' (McMurdie & Holmes, 2013)and 'qiime2R' (MARUYAMA et al., 2020) R packages for further downstream analysis.

4.3.4 Data Analysis

Statistical tests and graphical representations were carried out in QIIME 2 2022.2, and R v. 4.3. The microbial composition of the samples was visualized using complex heatmaps. To show the order abundances as proportions of each sample, "CLR" (Centred Log Ratio) transformation – 'microViz' (Barnett et al., 2021), 'ComplexHeatmap' (Gu, 2022), 'dplyr' (C. Wickham, 2014) and 'tidyverse' (H. Wickham et al., 2019) were used. To characterise the alpha diversity, the following indices using q2-diversity: ASVs richness (i.e., observed ASVs), Faith's phylogenetic diversity, Pielou's evenness and Shannon's diversity index, which accounts for both richness and evenness were determined. Phyloseq and ggplot R packages were used to visualize the Shannon's diversity indexes. To evaluate changes in microbial community composition (beta diversity), Bray-Curtis distance was calculated and were visualized using NMDS plots through 'microViz' and 'shiny' R packages. The differences in community composition in response to the variables were analysed using permutational multivariate ANOVA (PERMANOVA) using Qiime diversity beta-group-significance (Anderson, 2001). Taxonomic association trees were used to visualize differential abundance testing, arranged in a heatmap-style tree structure. Linear regression modelling (Total sum scaling and MaAsLin2) was used to identify differential abundance, with taxon models organized by rank (H. Zhou et al., 2022). 'microViz', 'concorb'and 'dplyr' in the R package weres used to test and produce the trees. All analyses were run in the R version 4.3.1.

4.4 Results

The eDNA collected from Tairua generated a total of 5,443,930 16S rRNA demultiplexed reads, with 133,543 total ASVs and 3,371 unique ASVs obtained after denoising, representing 13 phyla and 73 orders (Figure 4.2). To determine the alpha and beta diversity, samples were rarefied, and 100 amplicon reads were considered for each sample (Appendix B). In addition, a total of 5,457,455 quality ITS paired-end reads were generated and assigned to 1,521,494 ASVs, of which 1556 unique ASVs were identified, representing eight phyla and 88 orders (Figure 4.3).

Similarly, the eDNA collected from Whangapoa generated a total of 3,458,203 16S rRNA demultiplexed reads, with 81,365 total ASVs and 2,141 unique ASVs obtained after denoising, representing 13 phyla and 73 orders (Figure 4.2). Samples were rarefied, and 100 amplicon reads were considered for each sample to determine the alpha and beta diversity (Appendix B). Additionally, a total of 3,222,112 quality ITS paired-end reads were generated, and 1,420,131 ASVs were identified, of which 8,586 unique ASVs were detected, representing 11 phyla and 88 orders (Figure 4.3).

Tairua trees TT11, TT13, TT14 and TT15 were having canopy score above 2.5 (Table 4.1). Meanwhile, in the Whangapoa forest, we examined a total of 29 trees and found that 28 of them displayed symptoms with a canopy score above 2.5. Only one tree was found healthy, with a canopy score of 1. Among the 29 trees, 15 were treated with phosphonate, while 18 were observed with basal bleeding (Table 4.2).

4.4.1 P. agathidicida detection and identification

Soil samples were tested for the presence or absence of *Phytophthora agathidicida* (PA) using endpoint PCR. Five trees from the Tairua site and 12 trees from the Whangapoa site were PA-positive, as seen in the agarose gel, which indicated the expected PCR product of 82bps. The sequence data confirmed the presence of PA, with DNA sequences having >99% similarity with the *Phytophthora agathidicida* strain ICMP 17027 (KP29508) (Appendix B). In Tairua, trees TT02, TT03, TT10, TT12, and TT15 had positive results for PA. However, trees TT11, TT13, and TT14 had negative results, even though they had a canopy score of 2.5, which is an indication of kauri dieback disease symptomatic. None of the Tairua trees were found to have basal bleeding.

In Whangapoa, 12 trees tested positive for PA. Trees WT04, WT07, WT18, WT23, WT25, WT26, WT30, WT31, WT32, WT33, and WT34 were tested and found to be positive. Eleven surface samples and four samples taken at a depth of 10cm were positive for PA. Trees WT25, WT31, and WT34 were positive in both surface and 10cm depth. Conversely, WT32 had a positive test result only at a depth of 10cm, while WT26, WT23, WT07, WT04, WT33, WT18, WT29, and WT30 were PA positive in surface soil samples. Among the trees that tested positive for PA, WT23, WT07, WT31, WT32, WT18, WT29, and WT30 were also found to have basal bleeding. Other trees that tested positive for PA were non-bleeding. All trees in Whangapoa, except for WT10, which was not found to have PA, had a canopy score <2.5 and were therefore non-symptomatic. Out of two dead trees sampled, WT26 tested positive for PA, while WT27 had no PA detected in the soil samples (Table 4.2).

Table 4.1 Sample information of Tairua kauri soil

Tree Number	Terrain	Tree health (Canopy)	Phosphonate treatment	Symptom (Basal bleeding)	Canopy Health score	CBH (Inch)	PA presence
TT01	Gully	Non-Symptomatic	no	no	1	66	Negative
TT04	Ridge	Non-Symptomatic	no	no	1	9.6	Negative
TT07	Ridge	Non-Symptomatic	no	no	1	33	Negative
TT11	Ridge	Symptomatic	no	no	3	12.8	Negative
TT14	Gully	Symptomatic	no	no	3	19.5	Negative
TT02	Ridge	Slightly Thinning	no	no	2	79	Positive
TT03	Ridge	Slightly Thinning	no	no	2	16	Positive
TT05	Ridge	Slightly Thinning	no	no	2	73	Negative
TT06	Ridge	Slightly Thinning	no	no	2	18	Negative
TT08	Ridge	Slightly Thinning	no	no	2	26	Negative
TT09	Ridge	Slightly Thinning	no	no	2	41	Negative
TT10	Ridge	Slightly Thinning	no	no	2	77	Positive
TT12	Ridge	Slightly Thinning	no	no	2	12	Positive
TT13	Gully	Symptomatic	no	no	2.5	18	Negative
TT15	Gully	Symptomatic	no	no	2.5	8	Positive

Table 4.2 Sample information of Whangapoa kauri soil

Tree Number (ID)	Terrain	Tree health (Canopy)	Phosphonate treatment	Symptom (Basal bleeding)	Canopy Health score	CBH (Inch)	PA presence
WT04	Gully	Symptomatic	No	No	3	24.8	Positive*
WT06	Gully	Symptomatic	No	Yes	4	23.2	Negative
WT07	Gully	Symptomatic	No	Yes	3	56.1	Positive*
WT10	Gully	Non symptomatic	No	No	1	5.8	Negative
WT11	Ridge	Symptomatic	No	No	2.5	43.1	Negative
WT12	Ridge	Symptomatic	No	No	2.5	46.9	Negative
WT16	Ridge	Symptomatic	Yes	Yes	2.5	42	Negative
WT17	Ridge	Symptomatic	Yes	Yes	4.5	53	Negative
WT18	Ridge	Symptomatic	Yes	Yes	3	36	Positive*
WT19	Ridge	Symptomatic	Yes	Yes	3	91	Negative
WT20	Ridge	Symptomatic	Yes	Yes	3	50	Negative
WT21	Ridge	Symptomatic	Yes	Yes	2.5	35	Negative
WT22	Ridge	Symptomatic	Yes	Yes	3.5	48	Negative
WT23	Ridge	Symptomatic	Yes	Yes	3	55	Positive*
WT24	Ridge	Symptomatic	Yes	Yes	2.5	18	Negative
WT25	Ridge	Symptomatic	Yes	No	3	17	Positive***
WT26	Ridge	Symptomatic	Yes	No	5-dead	28	Positive*
WT27	Ridge	Symptomatic	Yes	Yes	5-dead	48.8	Negative
WT28	Ridge	Symptomatic	Yes	Yes	3	56.2	Negative
WT29	Ridge	Symptomatic	Yes	Yes	3	57	Positive*
WT30	Ridge	Symptomatic	Yes	Yes	2.5	18	Positive*
WT31	Ridge	Symptomatic	Yes	Yes		63	Positive***
WT32	Ridge	Symptomatic	No	Yes	3.5	45	Positive**
WT33	Ridge	Symptomatic	No	No	3.5	40	Positive*
WT34	Ridge	Symptomatic	No	No	4	20	Positive***

WT35	Ridge	Symptomatic	No	Yes	3.5	34	Negative
WT36	Ridge	Symptomatic	No	No	4	28	Negative
WT37	Ridge	Symptomatic	No	No	3	29.2	Negative
WT39	Ridge	Symptomatic	No	No	3	37.7	Negative

Note: * PA positive in the surface soil sample, **PA positive in the 10cm depth soil sample ***PA positive in both surface and 10cm depth soil samples, CBH – Circumference at breast height.

4.4.2 Bacterial and Fungal Community

The amplicon DNA sequencing data identified 13 and 11 different types of bacterial phyla from the Tairua and Whangapoa soil samples, respectively. Proteobacteria, Actinobacteria, Acidobacteria, Planctomycetes, and Chloroflexi make up 32.34%, 23.81%, 18.74%, 11.09% and 5.40% of the samples, respectively, making these phyla the most abundant group of microorganisms. At the order level, Actinomycetales, Acidobacteriales, Rhizobiales and Burkholderiales were most abundant with 23.13%, 15.4%, 12.6% and 9.54% respectively (Figure 3). Eight and 11 fungal phyla were identified in the Tairua and Whangapoa soil samples, respectively. Basidiomycota and Ascomycota account for 43.23% and 50.61%, respectively of the total fungal community. Hypocreales, Tremellales, Eurotiales, and Mortierellales were abundant fungal taxa at the order level detected in Tairua and Whangapoa soil samples 23.77%, 32.79%, 9.13% and 5.40% respectively (Figure 4). Based on the Wilcoxon rank sum test, bacterial and fungal phyla abundance in the surface and 10cm depth samples showed no significant difference ($p\text{-value}<0.05$). Additionally, Kruskal Wallis tests indicated no significant difference in bacterial and fungal phyla abundance among the cardinal point soil samples from the Tairua site (Appendix A).

4.4.3 Bacterial and fungal diversity

4.4.4 Tairua

4.4.4.1 Alpha and Beta Diversity

The Kruskal-Wallis test was used to detect any differences in alpha diversity (Shannon diversity) among the four cardinal points, tree health statuses, and sampling depths (surface and 10cm). The results indicated that there were no significant variations in the bacterial and fungal communities among the four cardinal points and sampling depths at the Tairua site. However, significant differences were observed in the bacterial and fungal alpha diversity among different tree health status in the Tairua site. Soil samples collected from symptomatic trees displayed a significantly higher Shannon bacterial diversity index ($p < 0.05$) compared to those obtained from non-symptomatic and slightly thinning trees (Figure 4.5). Soil samples collected from symptomatic trees displayed a significantly lower Shannon fungal diversity index (p value < 0.05) compared to those obtained from non-symptomatic and slightly thinning trees (Figure 4.6). There was no significant difference observed between non-symptomatic and slightly thinning trees (Figure 4.5 & 4.6).

The bacterial beta diversity showed no significant difference between the surface and 10 cm depth samples with a test statistic of 0.85 and a p-value of 0.622 (SI) of PERMANOVA results. However, there was a significant difference in bacterial beta diversity between different tree health states, with a test statistic of 6.48 and a p-value of 0.01 obtained from the PERMANOVA analysis of Bray Curtis distance (Figure 4.7a). The fungal community structure on the other hand also shows no significant differences in beta diversity considering the sampling depth. The PERMANOVA analysis further support this finding, with a test statistic of 1.56 and a p-value of 0.09 (SI). However, there was a significant variation in fungal beta diversity considering the tree health states. The PERMANOVA analysis of Bray Curtis distances gave a test statistic of 8.23 and a p-value of 0.01, indicating that symptomatic trees significantly differed from non-symptomatic and slightly thinning trees (Figure 4.7b). The bacterial NMDS plot revealed that the samples are grouped together compared to the fungal NMDS plot. This showed there was higher beta diversity among fungi samples compared to bacterial samples (Figure 4.7). Non symptomatic samples and slightly thinning samples were clustered together. Symptomatic samples were widely spread (Figure 4.7 b).

Figure 4.3: The heatmap shows the relative abundance of bacterial community structure based on the top 37 taxa at the order level, using centred logged ratio. TT denotes Tairua samples, WT denotes Whangapoa samples followed by the tree numbers. PA is detection of *P. agathidicida* test in the soil sample.

Figure 4.4: The heatmap shows the relative abundance of fungal community structure of the top 37 taxa at the order level using centred logged ratio. The trees named with "TT" are from Tairua while those starting with "WT" are from Whangapoa followed by the tree numbers. "PA" indicate the detection of PA in the sample.

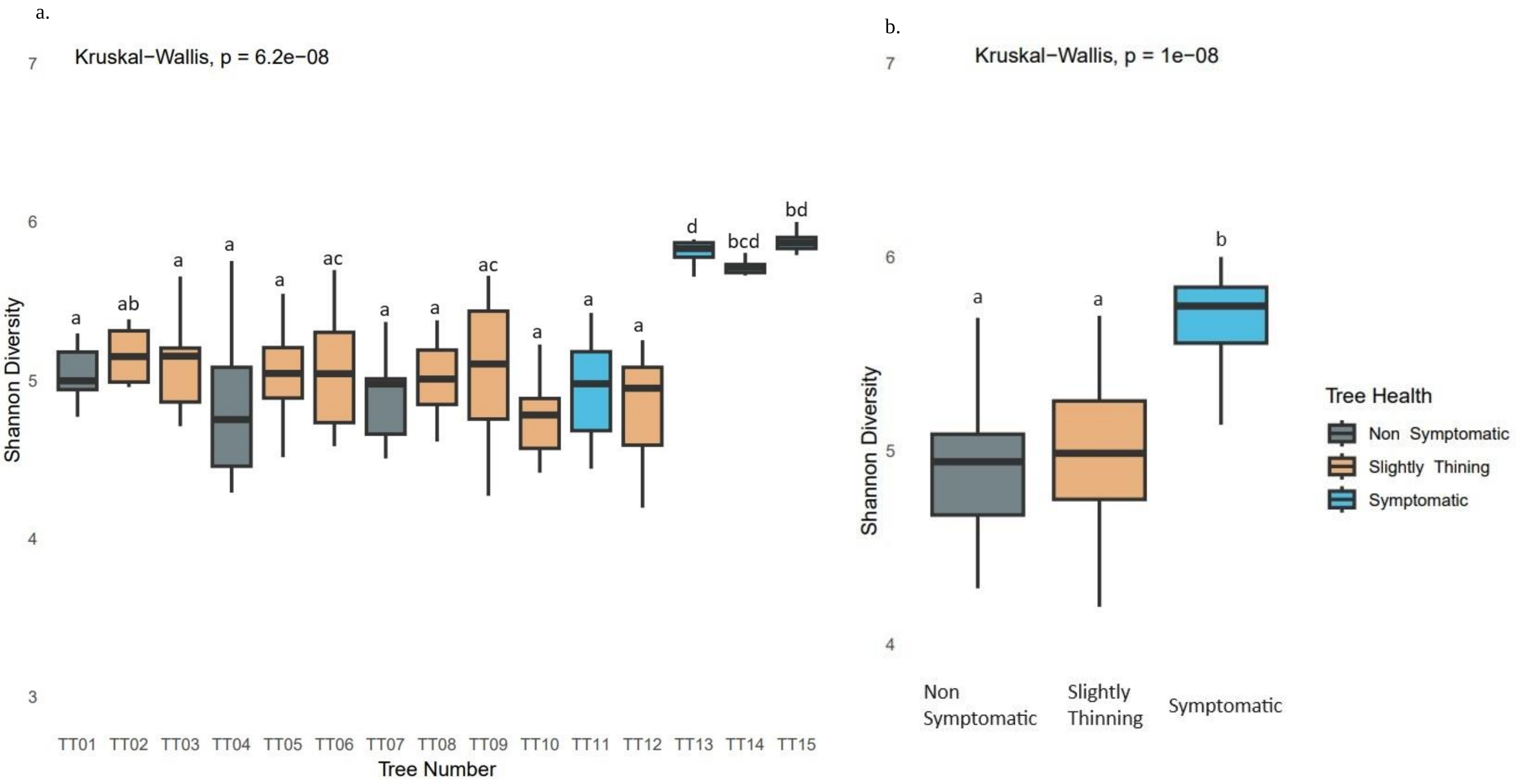


Figure 4.5: Boxplot of Shannon diversity indices based on tree samples (a) and tree health (b) of bacterial community in Tairua kauri forest.

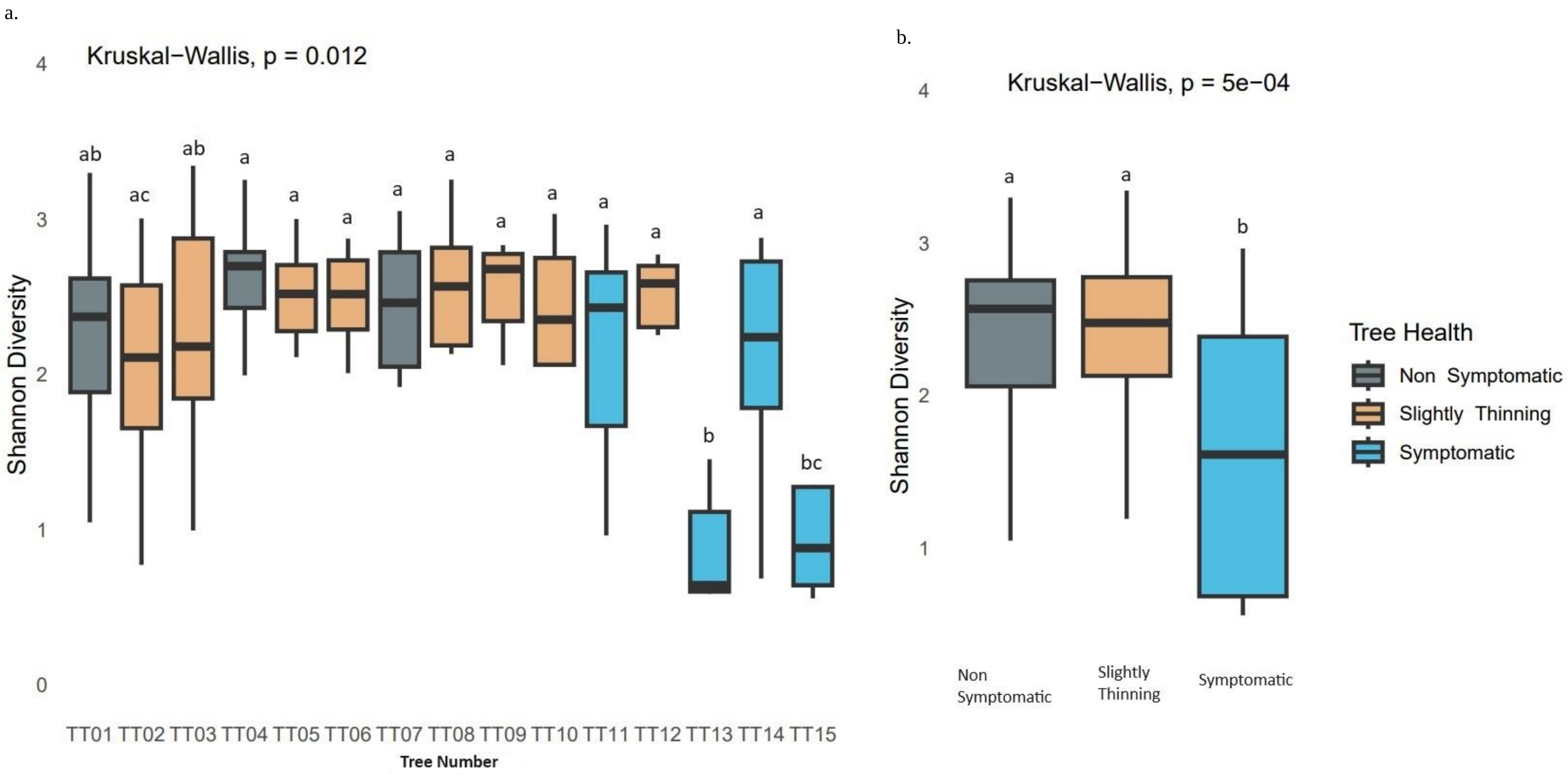


Figure 4.6: Boxplot of Shannon diversity indices based on tree samples (a) and tree health (b) of fungal community in Tairua kauri forest.

4.4.4.2 Differential Abundance

The study found that Phylum Verrucomicrobia had a significant positive association with symptomatic trees (estimate coefficient of 1.12, P-value = 0.0008). On the other hand, phyla Proteobacteria (estimate coefficient of -0.453, P-value = 0.001), Acidobacteria (estimate coefficient of -0.403, P-value = 0.002), and Actinobacteria (estimate coefficient of -0.623, P-value = 0.00005) had significant negative association with symptomatic soil. Furthermore, phylum Verrucomicrobia had a significant negative association with slightly thinning trees soil (estimate coefficient of -0.715, P-value = 0.005). Conversely, phyla Proteobacteria (estimate coefficient of 0.407, P-value = 0.001), Acidobacteria (estimate coefficient of 0.462, P-value = 0.009), and Actinobacteria (estimate coefficient of 0.277, P-value = 0.00483) were significantly positively associated with slightly thinning kauri soil. There was no significant association of taxa at the phylum level with non-symptomatic kauri soil in Tairua.

Phylum Mucoromycota had a significant positive association with non-symptomatic trees, with an estimated coefficient of 1.82 and a P-value of 0.01. On the other hand, Phylum Chytridiomycota had a significantly negative association with non-symptomatic kauri soil, with an estimated coefficient of 0.881 and a P-value of 0.03. Phyla Ascomycota and Motierellomycota were found to be positively associated with non-symptomatic, while Basidiomycota was negatively associated.

Phyla Rozellomycota and Glomeromycota were significantly positively associated with symptomatic kauri soil, while Ascomycota, Motierellomycota, and Mucoromycota were significantly negatively associated with symptomatic kauri soil with a *p*-value below 0.05. Basidiomycota was positively associated with symptomatic kauri soil. Slightly thinning kauri tree soil was also found to have Ascomycota and Mucoromycota significantly positively associated with a *p*-value below 0.05, while Rozellomycota, Glomeromycota, and Chytridiomycota were negatively associated with slightly thinning kauri soil with *p*-values below 0.05. Basidiomycota was also negatively associated with slightly thinning kauri soil.

The following bacterial and fungal genera have been found to be positively associated with different health statuses in Tairua kauri soil based. *Actinoallomurus, Streptomyces*, class Clostridiaceae, *Methylocapsa,* and *Rhodopila* were significantly positively associated with non-symptomatic kauri soil in Tairua (Figure 4.8). *Saccharomonospora, Rugosimonospora, Gaiella, Telmatobacter, Acidicapsa, Ktedonobacter, Brevundimonas,* and class Pseudomonadaceae were significantly positively associated with symptomatic kauri soil in Tairua (Figure 4.9). *Acidopila,* class Clostridiaceae, and *Nostocoida* genera were significantly positively associated with slightly thinning kauri soil in Tairua (Figure 4.10).

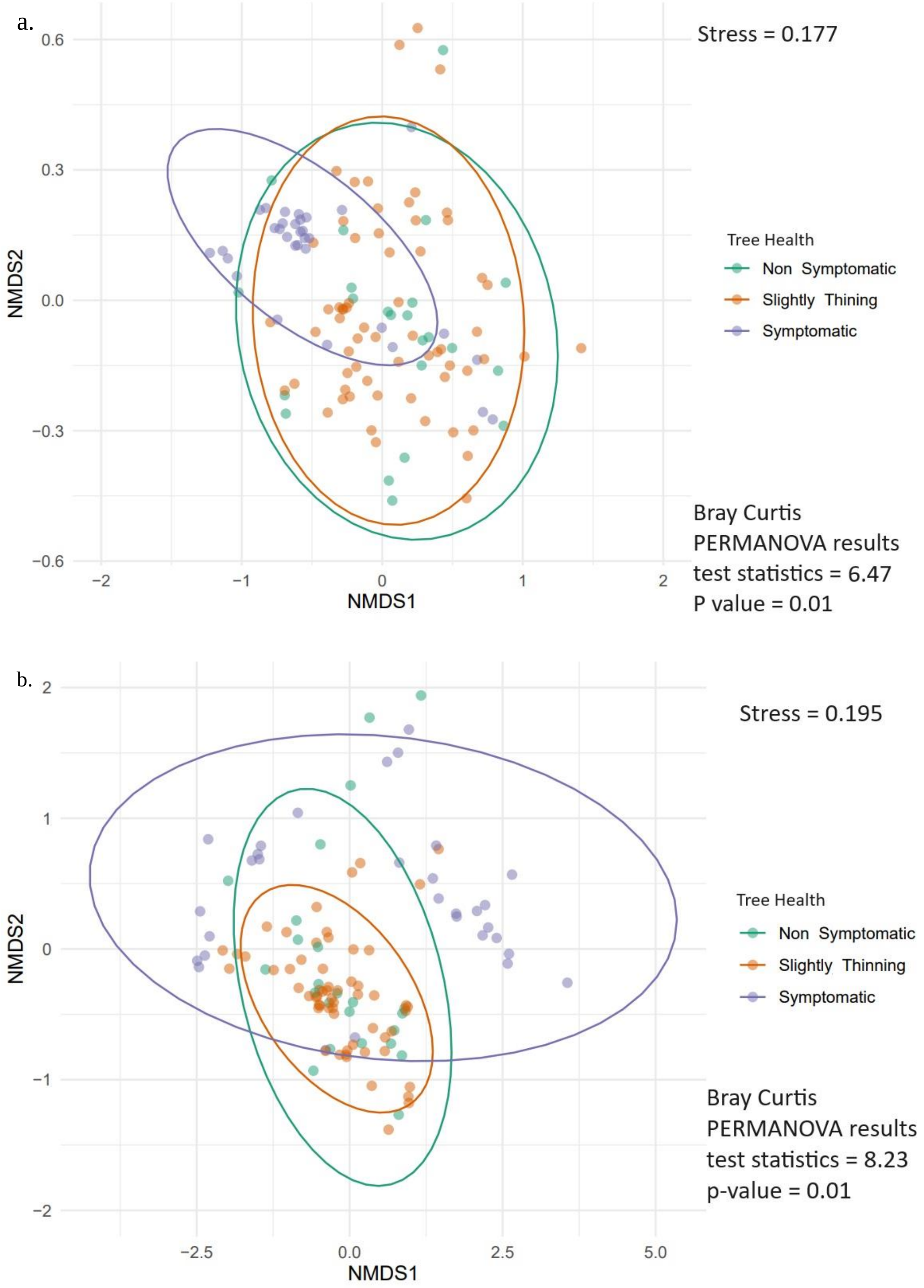


Figure 4.7: Non-metric Multi-dimensional Scaling plots of the Bray-Crutis dissimilarity indices. The eclipses denote the tree health statuses a) Bacteria and b) Fungi. The ellipses represent 95% confidence level.

Moreover, *Penicillium, Umbelopsis,* and *Leptobacillium* were significantly positively associated with non-symptomatic kauri soil in Tairua (Figure 4.11). *Saitozyma* and *Archeaorhizomyces* were significantly positively associated with symptomatic kauri soil in Tairua (Figure 4.12). Lastly, *Penicillium, Umbelopsis, Aspergillus, Trichorderma, Tolypocladium, Clonostachys, Mariannaea, Chloridium, Neopestalotiopsis, Scytalidium,* and *Sphaerographium* genera were significantly positively associated with slightly thinning kauri soil in Tairua (Figure 4.13).

4.4.5 Whangapoa

4.4.5.1 Alpha Diversity and Beta Diversity

In the Whangapoa forest, a total of 29 trees were included in the study. Of these, 28 trees showed dieback symptoms with a canopy score above 2.5. Only one tree had no symptoms, with a canopy score of 1. Of the 29 trees, 15 were previously treated with phosphonate in 2018, and 18 trees were observed to have basal bleeding (Table 4.1). The Kruskal Wallis test showed no significant difference in bacterial and fungal alpha diversity between surface and 10cm depth samples in the pooled soil samples collected from the cardinal points of the trees (SI). There were no significant differences observed between trees based on basal bleeding. The treatment of trees with phosphonate did not show significant differences either. Additionally, there were no significant differences observed comparing trees that tested positive for PA and those samples where PA was not detected (Figure 4.14, 4.15 and 4.16).

The PERMANOVA analysis conducted on the beta diversity using Bray Curtis distances for both 16S rRNA and ITS1 data showed that there was no significant difference in bacterial diversity based on the sampling depth (surface vs 10cm depth soil samples). Moreover, the analysis revealed that there is no significant difference between the samples that tested positive for PA and those that tested negative for PA. However, significant difference in the bacterial and fungal beta diversity was observed when comparing soil samples that were taken from trees that have been treated and not treated with phosphonate, as well as those trees that have evident basal bleeding and non-bleeding (Table 4.3 and Figure 4.17 and 4.18).

In Figure 17, the bacterial and fungal NMDS plots revealed that non-bleeding samples were clustered together compared to the basal bleeding kauri soil samples. In Figure 18, the non-phosphonate-treated bacterial and fungal samples were also grouped together in the NMDS plots, when compared to the untreated samples. Interestingly, the basal bleeding samples and phosphonate treated samples displayed greater diversity.

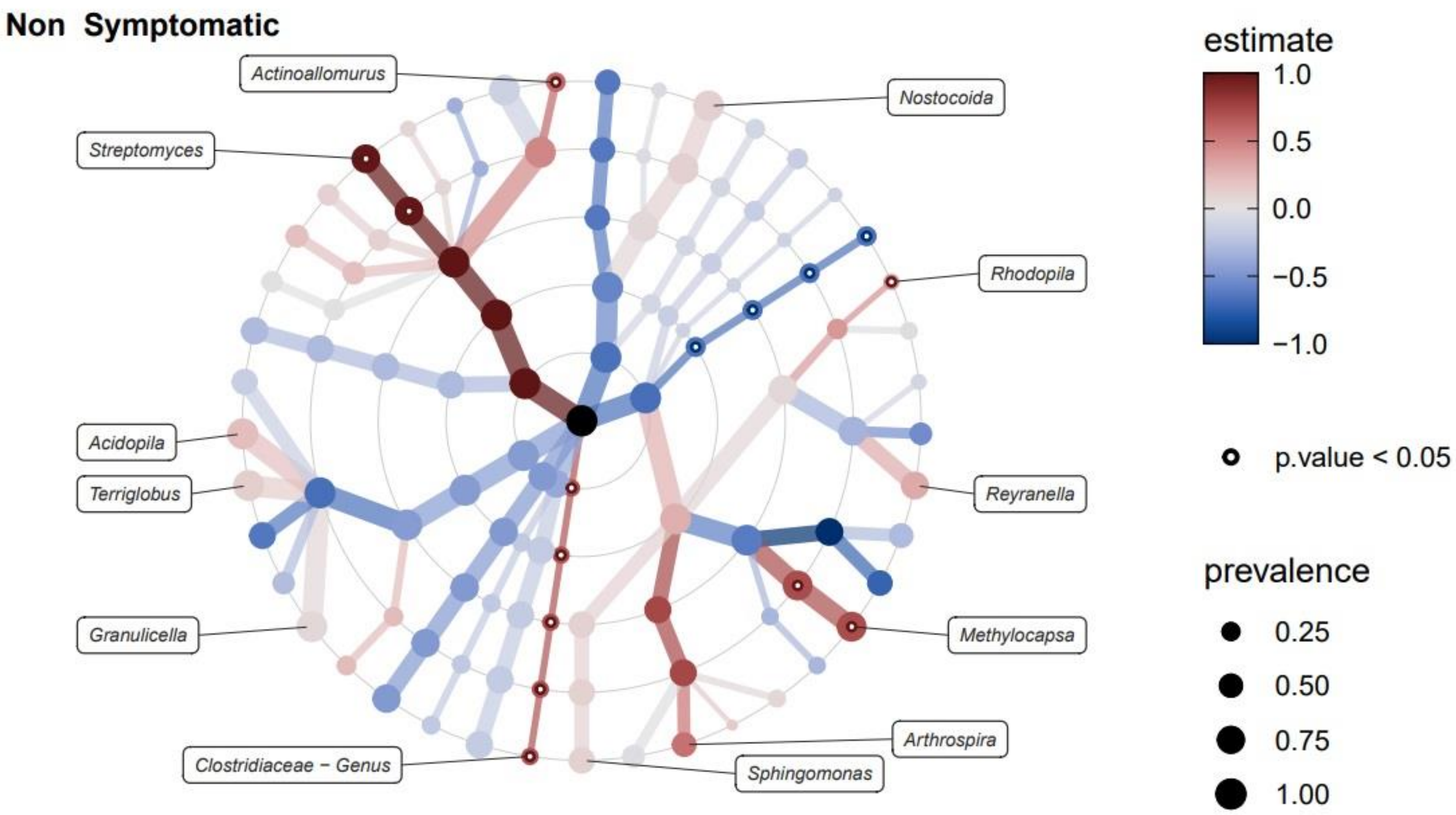


Figure 4.8: Taxonomic association tree plot showing the differential abundances of non-symptomatic (canopy score <1.5) soil bacterial taxa (genus level) in Tairua determined by linear regression correlation. Linear regression coefficient estimates are provided with the spectrum and significance marked with º. The taxonomy is organised by rank, expanding out from the central root node from e.g. Phyla around the centre to genera in the outermost.

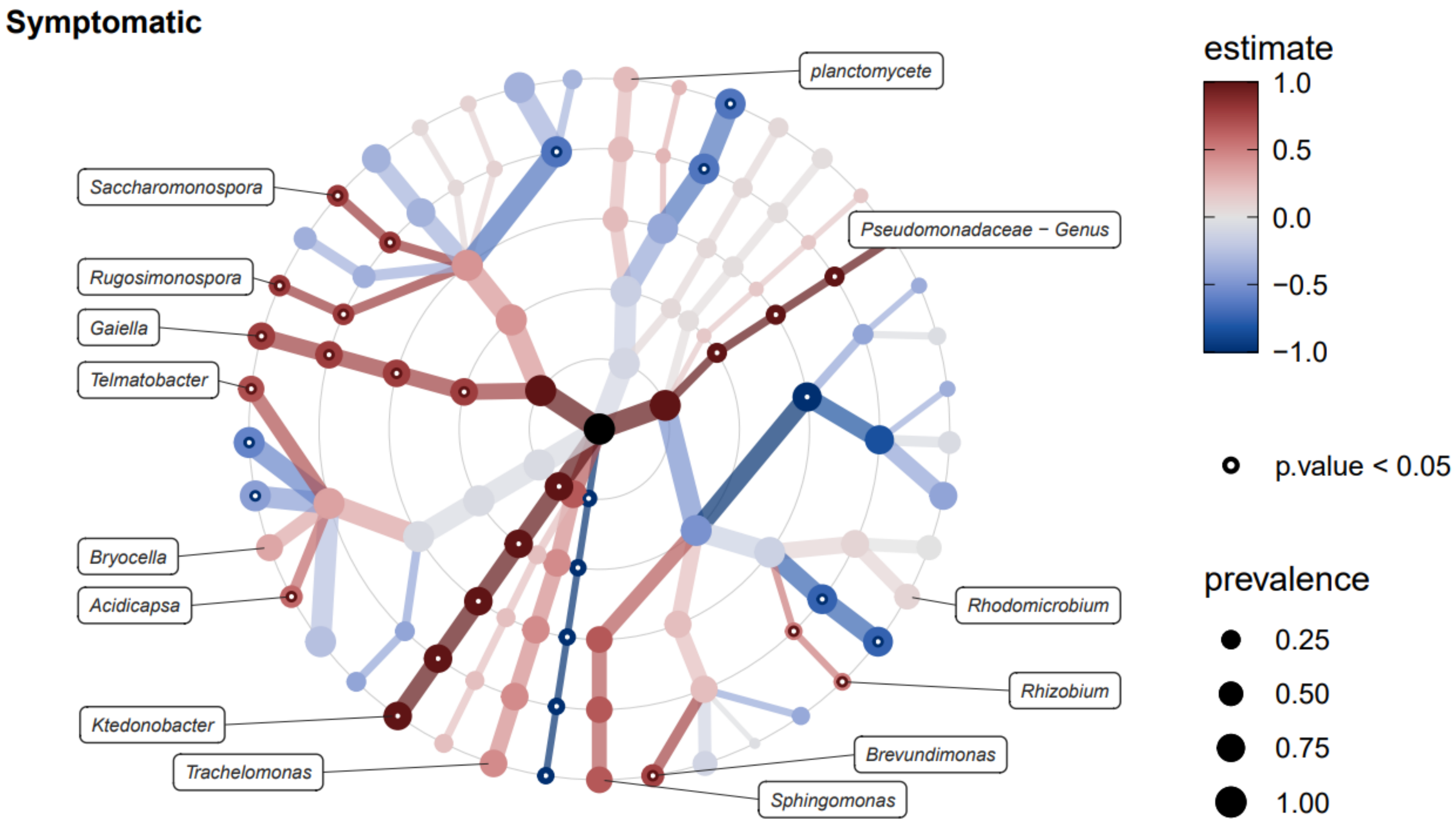


Figure 4.9: Taxonomic association tree plot shows the differential abundances of symptomatic (canopy score >2.5) soil bacterial taxa (genus level) in Tairua determined by linear regression correlation. Linear regression coefficient estimates are provided with the spectrum (blue and red) and significance marked with º. The taxonomy is organised by rank, expanding out from the central root node from e.g. Phyla around the centre to genera in the outermost.

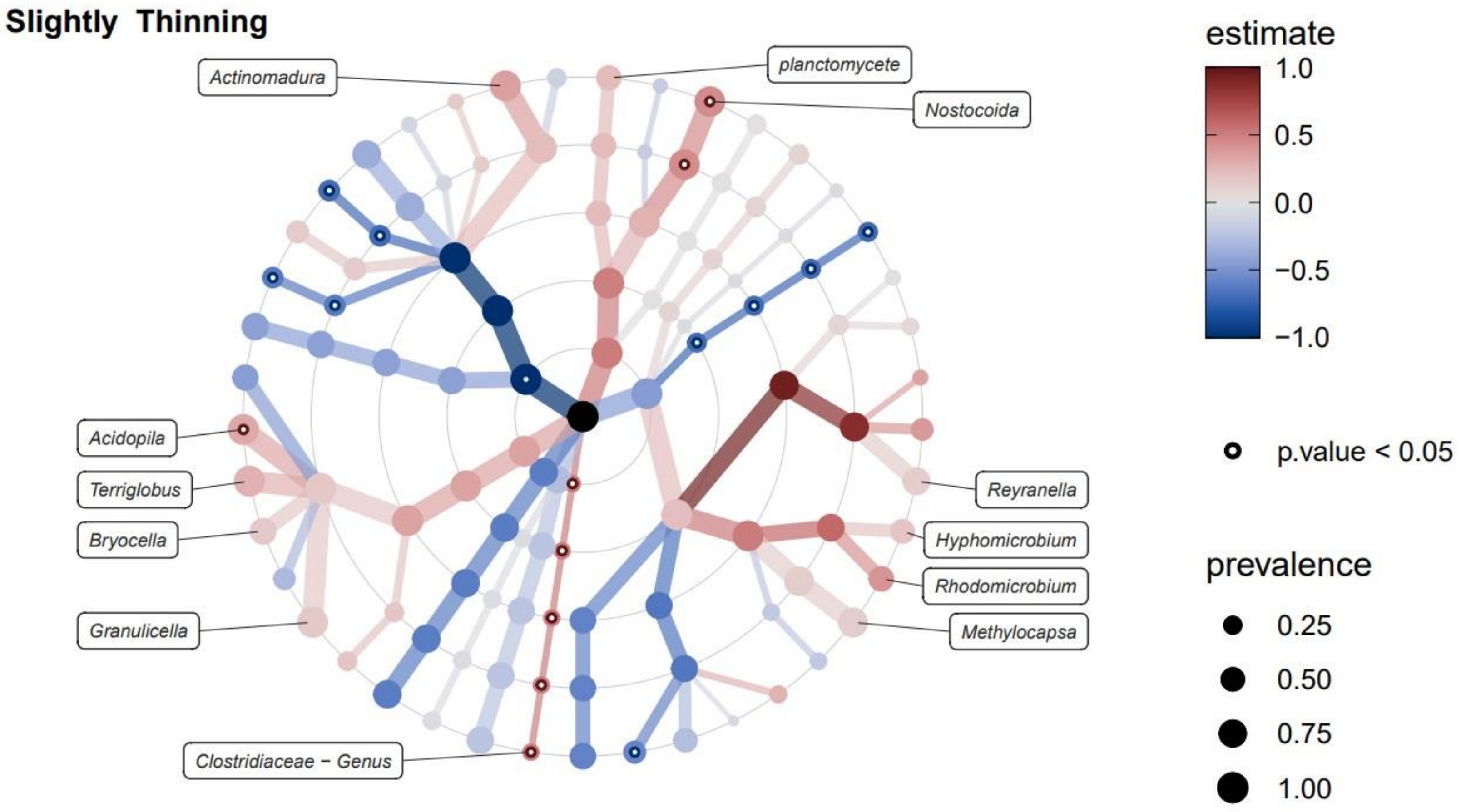


Figure 4.10: Taxonomic association tree plot shows the differential abundances of slightly thinning (canopy score 1.5 -2.5) soil bacterial taxa (genus level) in Tairua determined by linear regression correlation. Linear regression coefficient estimates are provided with the spectrum (blue and red) and significance marked with º. The taxonomy is organised by rank, expanding out from the central root node from e.g. Phyla around the centre to genera in the outermost.

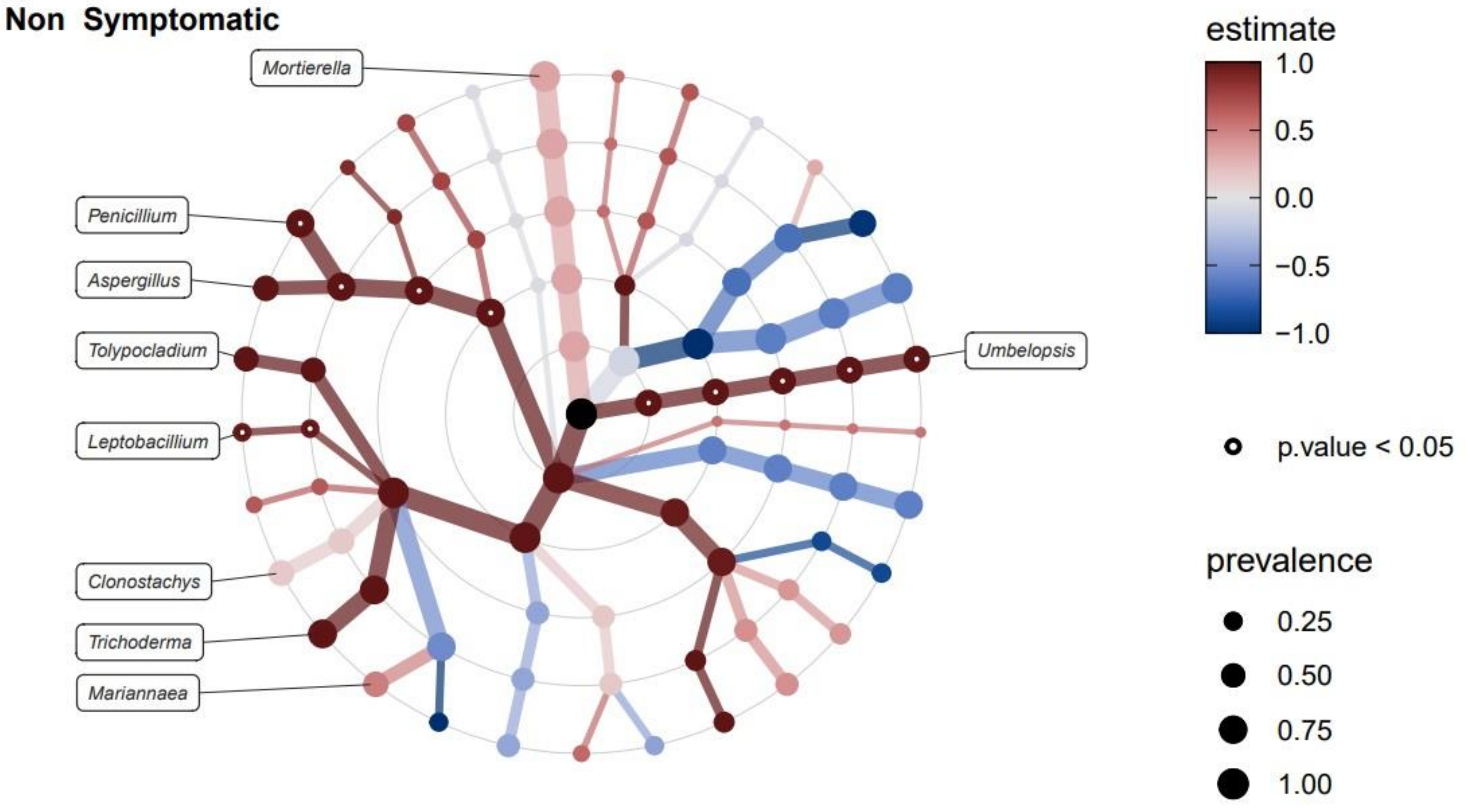


Figure 4.11: Taxonomic association tree plot shows the differential abundances of non-symptomatic (canopy score <1.5) soil fungal taxa (genus level) in Tairua determined by linear regression correlation. Linear regression coefficient estimates are provided with the spectrum and significance marked with º. The taxonomy is organised by rank, expanding out from the central root node from e.g. Phyla around the centre to genera in the outermost.

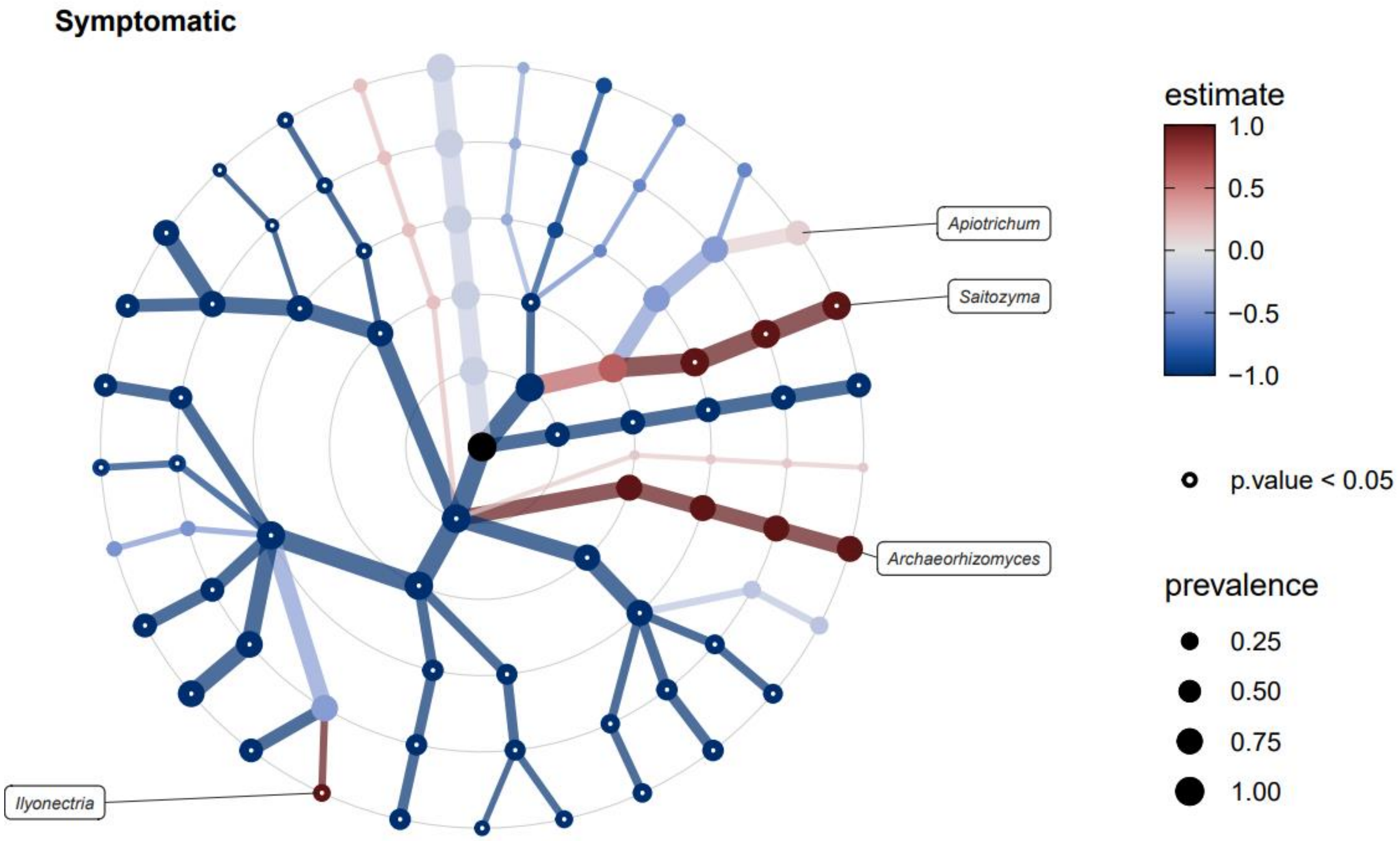


Figure 4.12: Taxonomic association tree plot shows the differential abundances of symptomatic (canopy score >2.5) soil fungal taxa (genus level) in Tairua determined by linear regression correlation. Linear regression coefficient estimates are provided with the spectrum (blue and red) and significance marked with º. The taxonomy is organised by rank, expanding out from the central root node from e.g. Phyla around the centre to genera in the outermost.

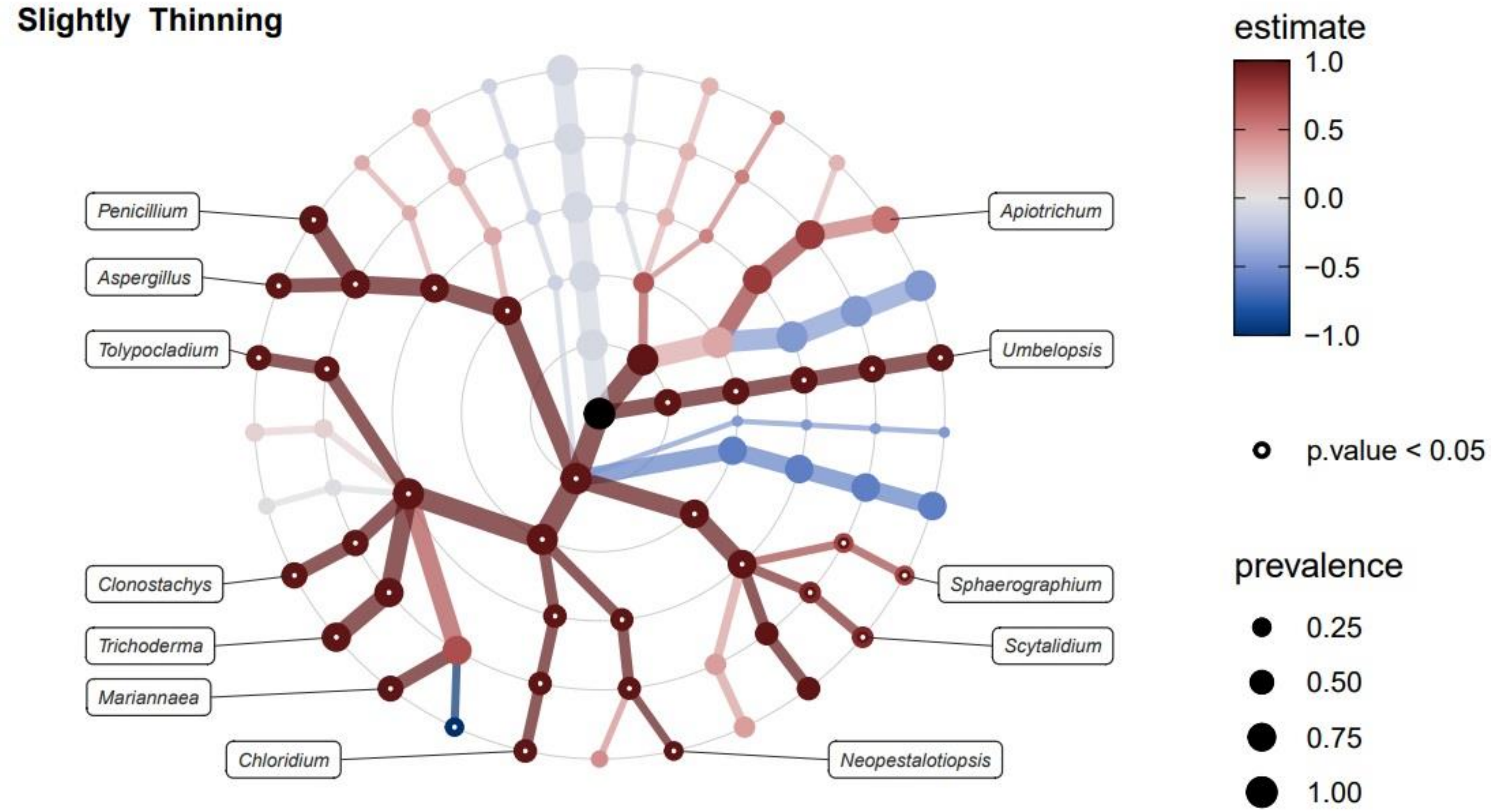


Figure 4.13: Taxonomic association tree plot shows the differential abundances of slightly thinning trees' (canopy score 1.5 -2.5) soil fungal taxa (genus level) in Tairua determined by linear regression correlation. Linear regression coefficient estimates are provided with the spectrum (blue and red) and significance marked with º. The taxonomy is organised by rank, expanding out from the central root node from e.g. Phyla around the centre to genera in the outermost.

4.4.5.2 Differential Abundance

Based on a differential abundance test conducted on the soil in Whangapoa, certain bacterial genera were found to have a significant positive association with basal bleeding kauri soil. These included Pseudomonadaceae, *Rhodomicrobium*, *Rhizobium*, and *Brevundimonas*. However, no fungal genera were found to be significantly positively associated with basal bleeding kauri soil (Figure 4.20 and 4.21).

Actinospica, *Methylocapsa*, and *Arthrospira* were discovered to have a significant positive association with non-bleeding kauri soil in Whangapoa (Figure 4.20). Moreover, several genera including *Umbelopsis*, *Talaromyces*, *Leohumicola*, *Oidiodendron*, *Unguicularia*, *Lachnum*, *Parafabraea*, *Gorgomyces*, *Chloridium*, *Leptobacillium*, *Metapochonia*, *Haglerozyma*, and *Exidia* were found to be significantly positively associated with basal bleeding kauri soil (Figure 4.21).

The differential abundance test results showed that certain bacterial genera, namely *Rhodomicrobium*, *Rhizobium*, and *Rugosimonospora*, *c*lass Pseudomonadaceae were significantly positively associated with phosphonate treated kauri soil. Similarly, fungal genera such as *Dendrochytridium, Phlalocephala, Infundichalara, Cryptosporiopsis, Cercophora, Malasuezia*, *Agaricas,* and *Mycena* were also found to be significantly positively associated with phosphonate treated kauri soil (Figure 4.22 and 4.22).

Actinospica, Methylocapsa, Actinoallomurus, and *Nostocoida* showed a significant positive association with phosphonate untreated kauri soil in Whangapoa. Several fungal genera including *Umbelopsis*, *Talaromyces, Leohumicola, Aspergillus*, *Penicillium, Trichoderma, Robillarda, Scytalidium, Trichocladium, Parafabraea, Geomyces, Chloridium, Stagonospora, Leptobacillium, Metapochonia, Haglerozyma, Piloderma*, *Formitiporula*, *Hypochnicium, Entoloma*, *Amanita, Russula,* and *Exidia* were found to be significantly positively associated with phosphonate untreated kauri soil (Figure 4.22 and 4.23).

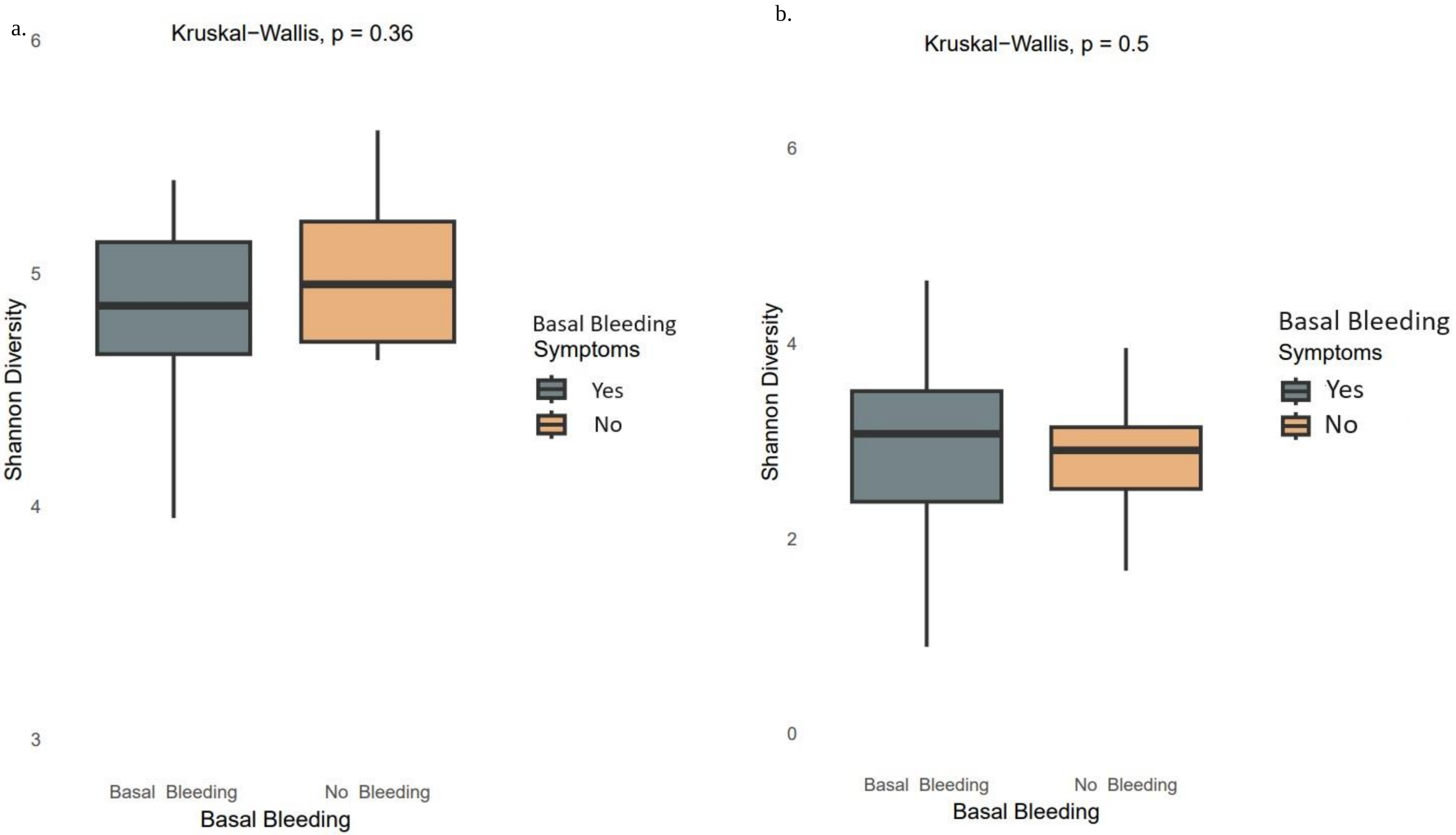


Figure 4.14: Boxplots showing the Shannon diversity indices of bacterial (a) and fungal (b) communities comparing the basal bleeding symptomatic trees and non-symptomatic trees.

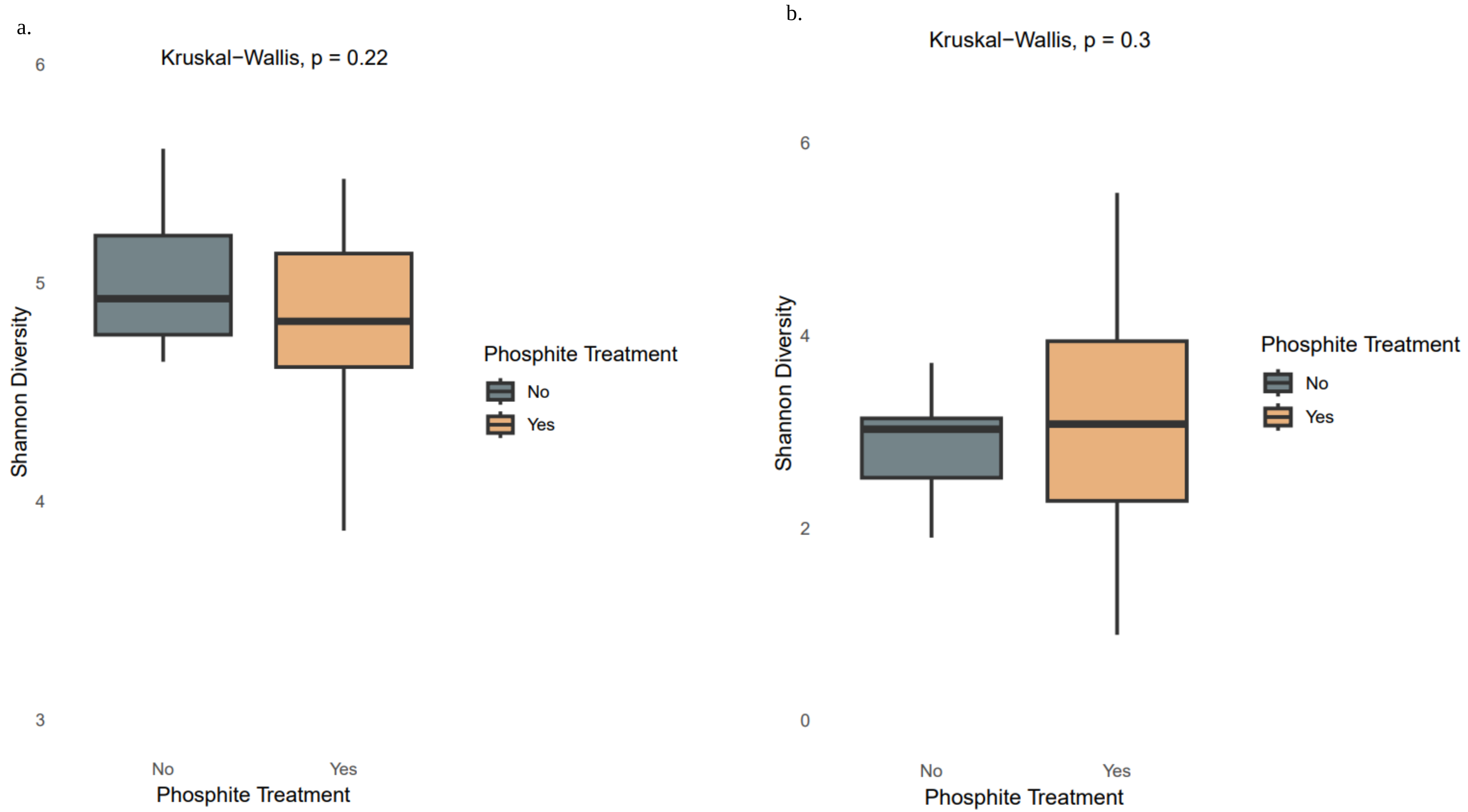


Figure 4.15: Boxplots showing the Shannon diversity indices of bacterial (a) and fungal (b) communities comparing the phosphonate treated and non-treated trees.

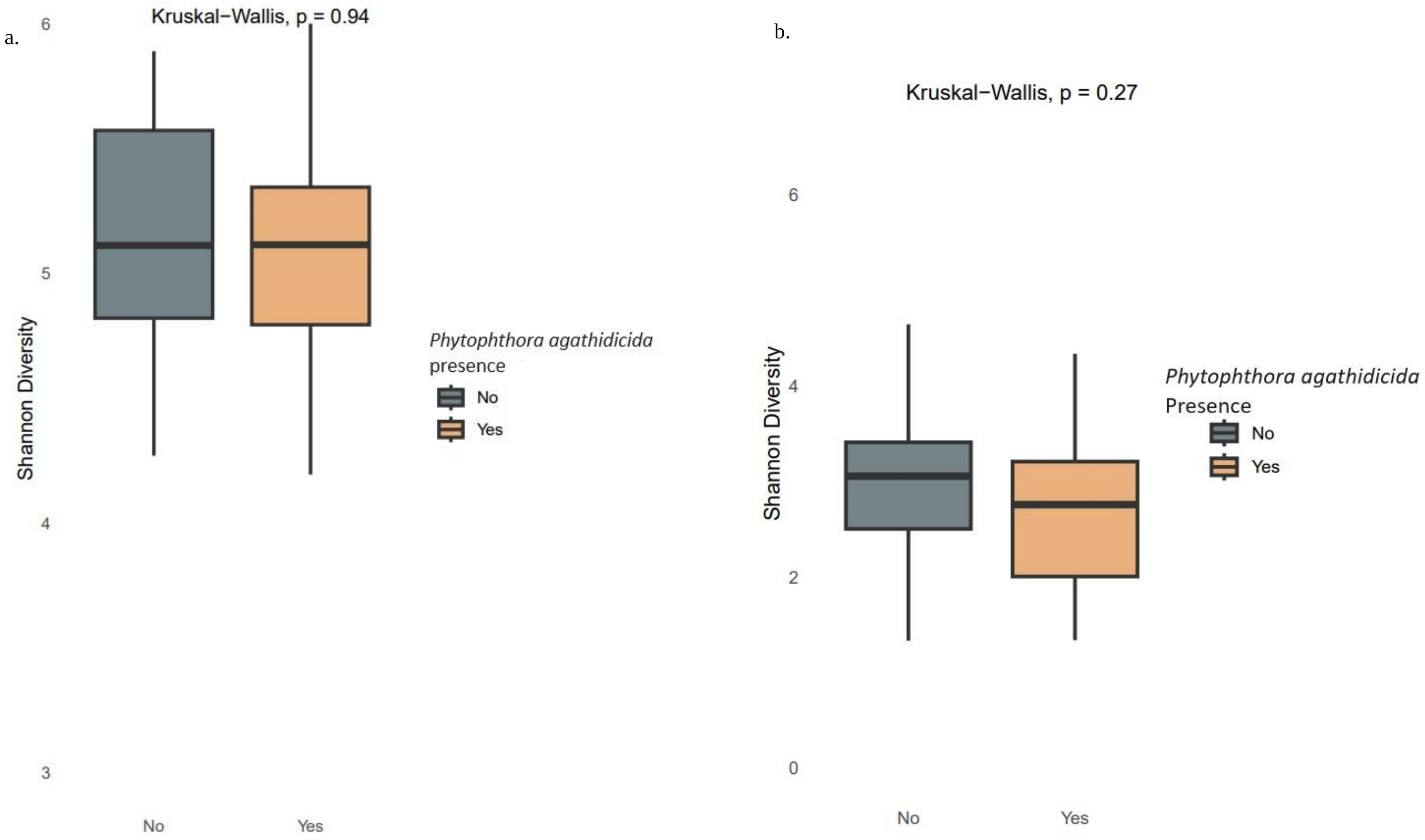


Figure 4.16: Boxplots showing the Shannon diversity indices of bacterial (a) and fungal (b) communities comparing the PA-detected and not-detected trees.

Table 4.3: PERMANOVA results of Whangapoa sample groups

Variable Group	**Number of Samples**	**Pseudo F**	***p*-value**
Bacteria (Bray Curtis)			
Sampling depth (Surface/ 10cm)	58	1.0754	0.263
PA detection (Presence / absence)	58	0.9769	0.507
Phosphonate treatment (Yes / No)	58	3.4715	0.001*
Dieback Symptoms (Bleeding/non-bleeding)	58	2.2694	0.001*
Fungi (Bray Curtis)			
Sampling depth (Surface/ 10cm)	58	1.0721	0.338
PA detection (Presence / absence)	58	0.8117	0.417
Phosphonate treatment (Yes / No)	58	3.7778	0.001*
Dieback Symptoms (Bleeding/non-bleeding)	58	1.621	0.035*

*Indicates the significant difference with *p*-value below 0.05.

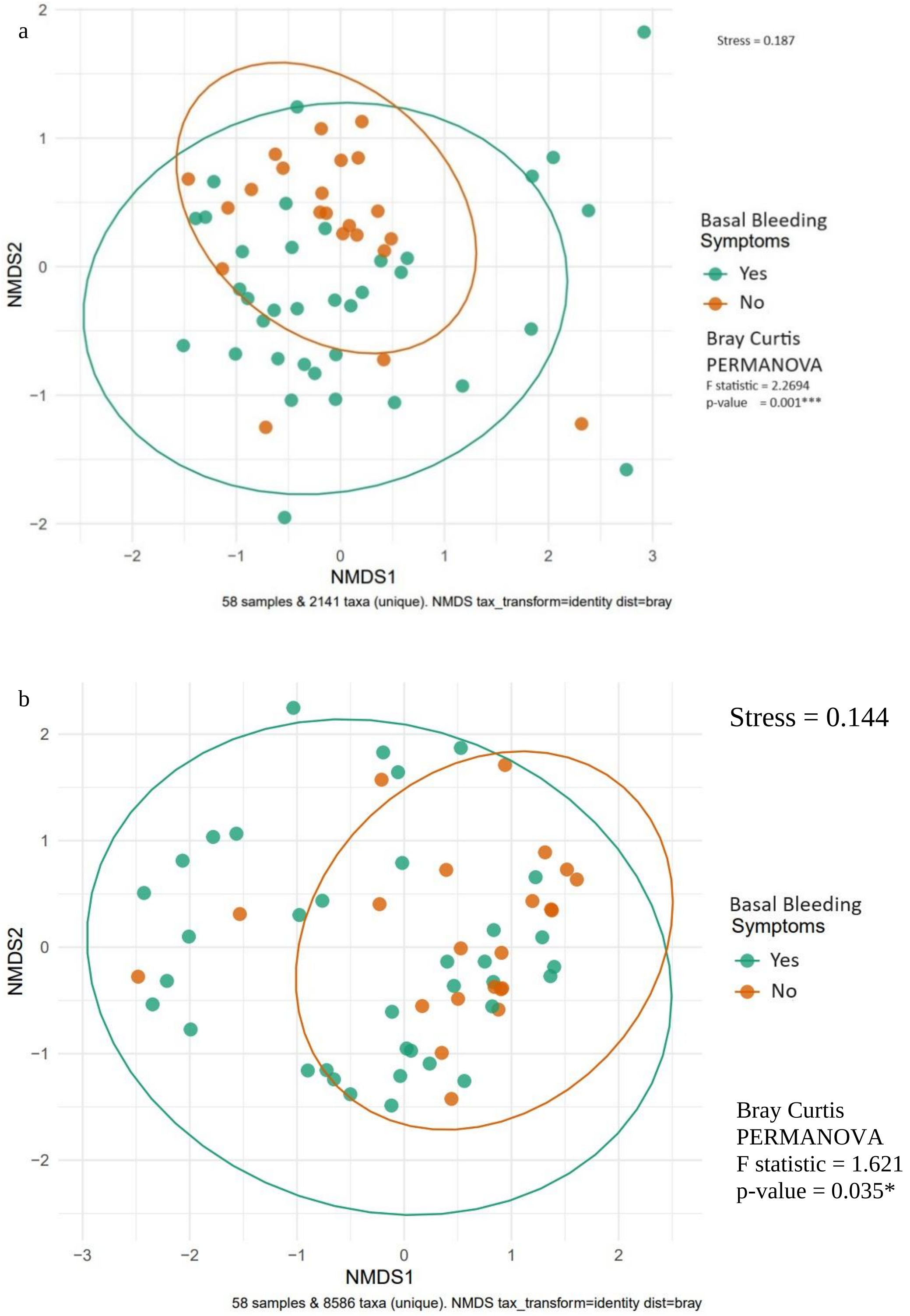
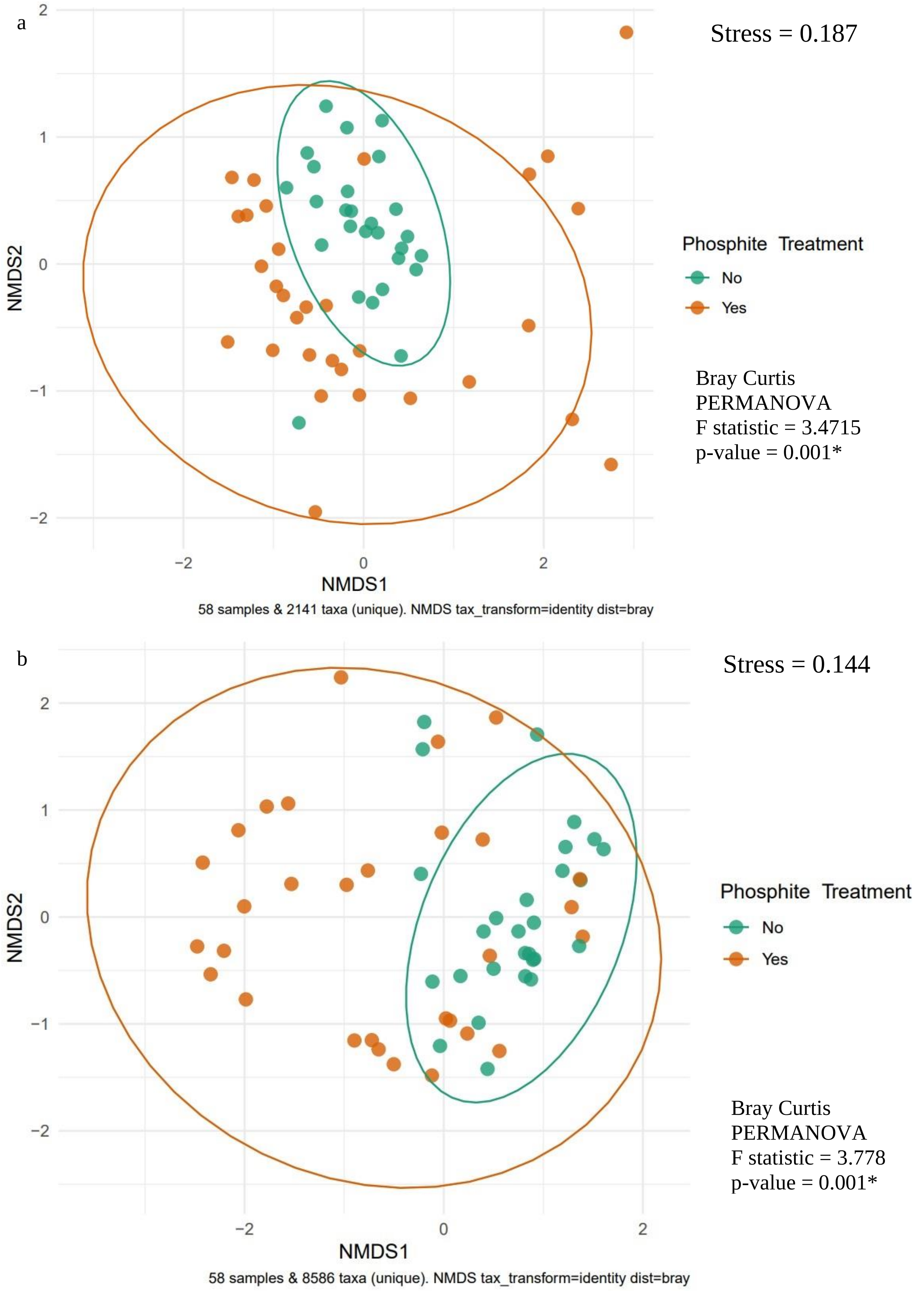


Figure 4.17: Non-metric Multi-dimensional Scaling plot showing soil microbial beta diversity Bray Curtis distances of basal bleeding (Yes) and non bleeding (No) trees. a) bacteria b) fungi.



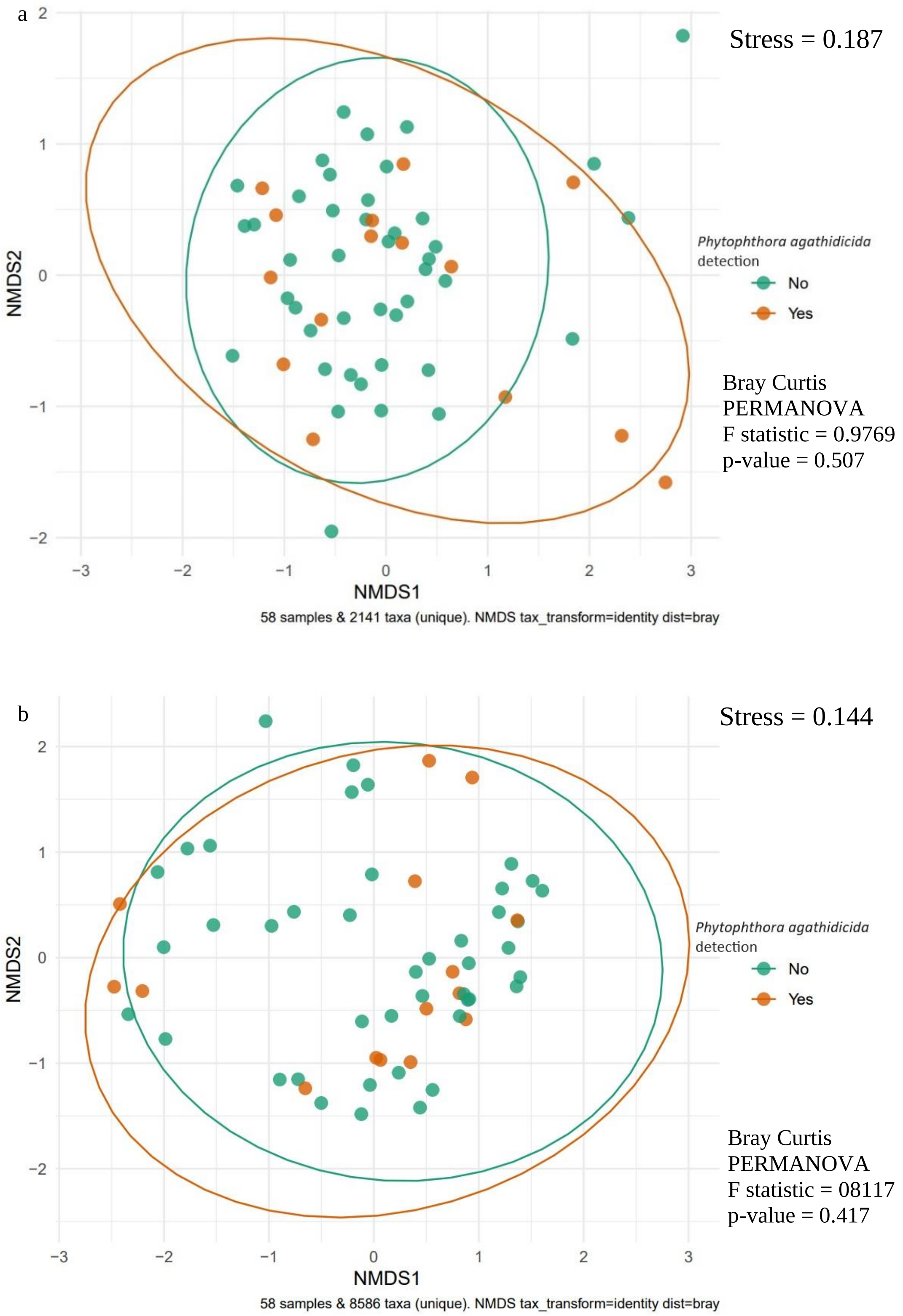
Stress = 0.187

Bray Curtis
PERMANOVA
F statistic = 3.4715
p-value = 0.001*

Stress = 0.144

Bray Curtis
PERMANOVA
F statistic = 3.778
p-value = 0.001*

Figure 4.18: Non-metric Multi-dimensional Scaling plot showing soil microbial beta diversity Bray Curtis distances of phophite treated (Yes) and non treated (No) trees. a) bacteria b) fungi.



Stress = 0.187

Bray Curtis
PERMANOVA
F statistic = 0.9769
p-value = 0.507

Stress = 0.144

Bray Curtis
PERMANOVA
F statistic = 08117
p-value = 0.417

Figure 4.19: Non-metric Multi-dimensional Scaling plot showing soil microbial beta diversity Bray Curtis distances of PA detected (Yes) and non detected (No) trees. a) Bacteria b) Fungi.

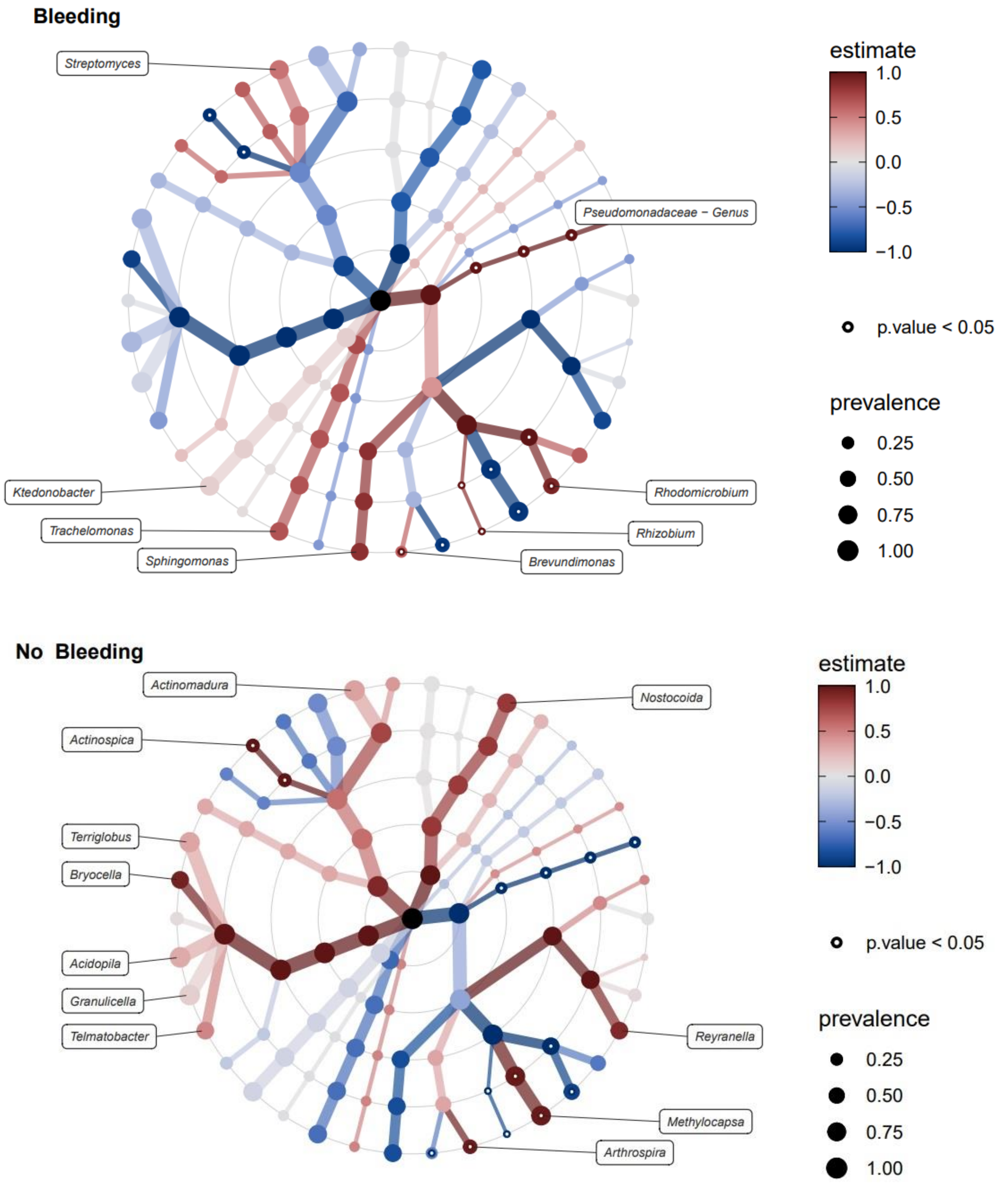


Figure 4.20: Taxonomic association tree plots display the varying levels of bacterial taxa (genus level) in the soil of kauri trees in Whangapoa, distinguishing between bleeding and non-bleeding trees. Linear regression coefficient estimates are provided along with the spectrum, and significance is marked with a degree symbol (°). The taxonomy is arranged by rank, with phyla located around the central root node, and genera located on the outermost portion of the plot.

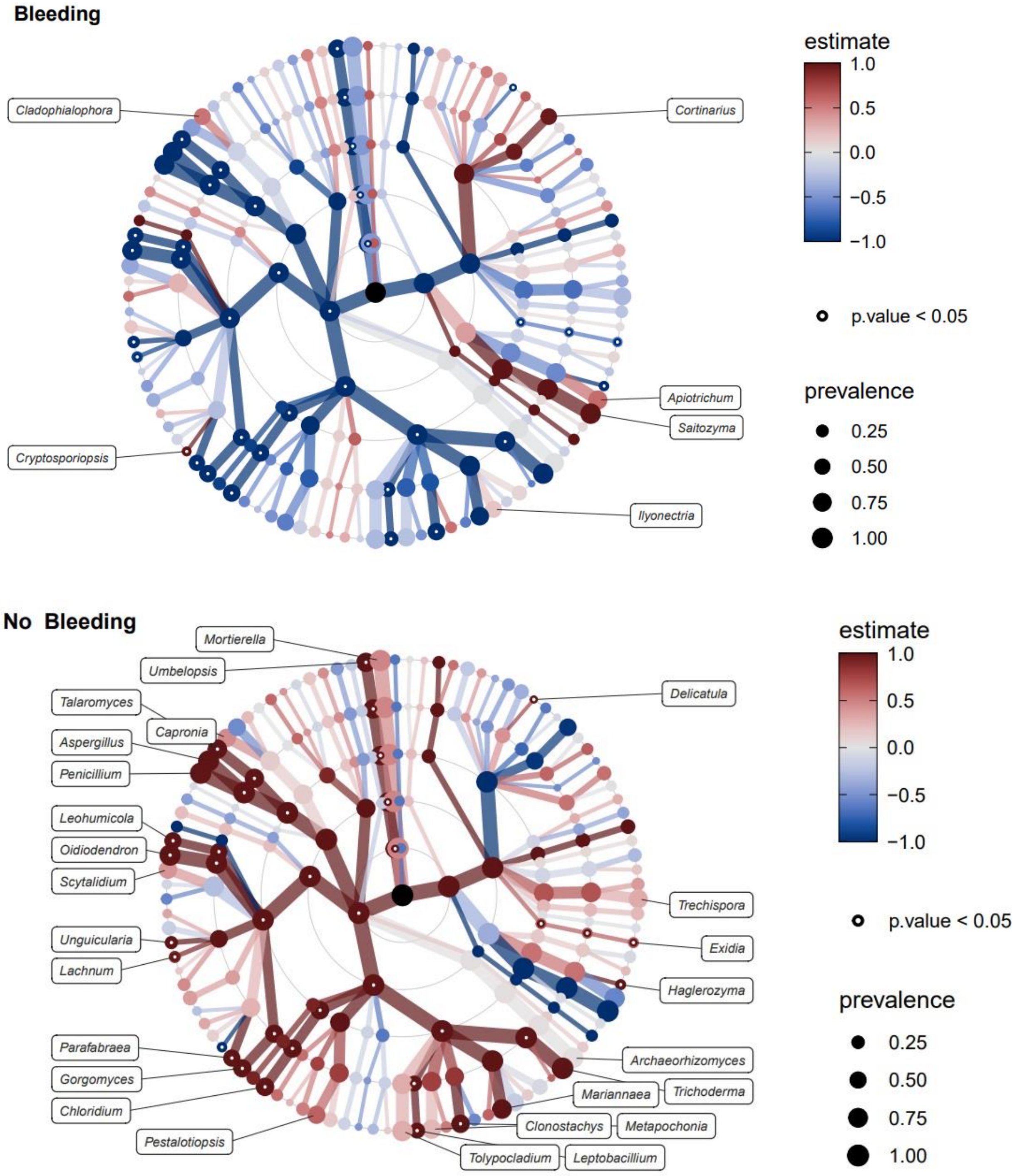


Figure 4.21: Taxonomic association tree plots display the varying levels of fungal taxa (genus level) in the soil of kauri trees in Whangapoa, distinguishing between bleeding and non-bleeding trees. Linear regression coefficient estimates are provided along with the spectrum, and significance is marked with a degree symbol (º). The taxonomy is arranged by rank, with phyla located around the central root node, and genera located on the outermost portion of the plot.

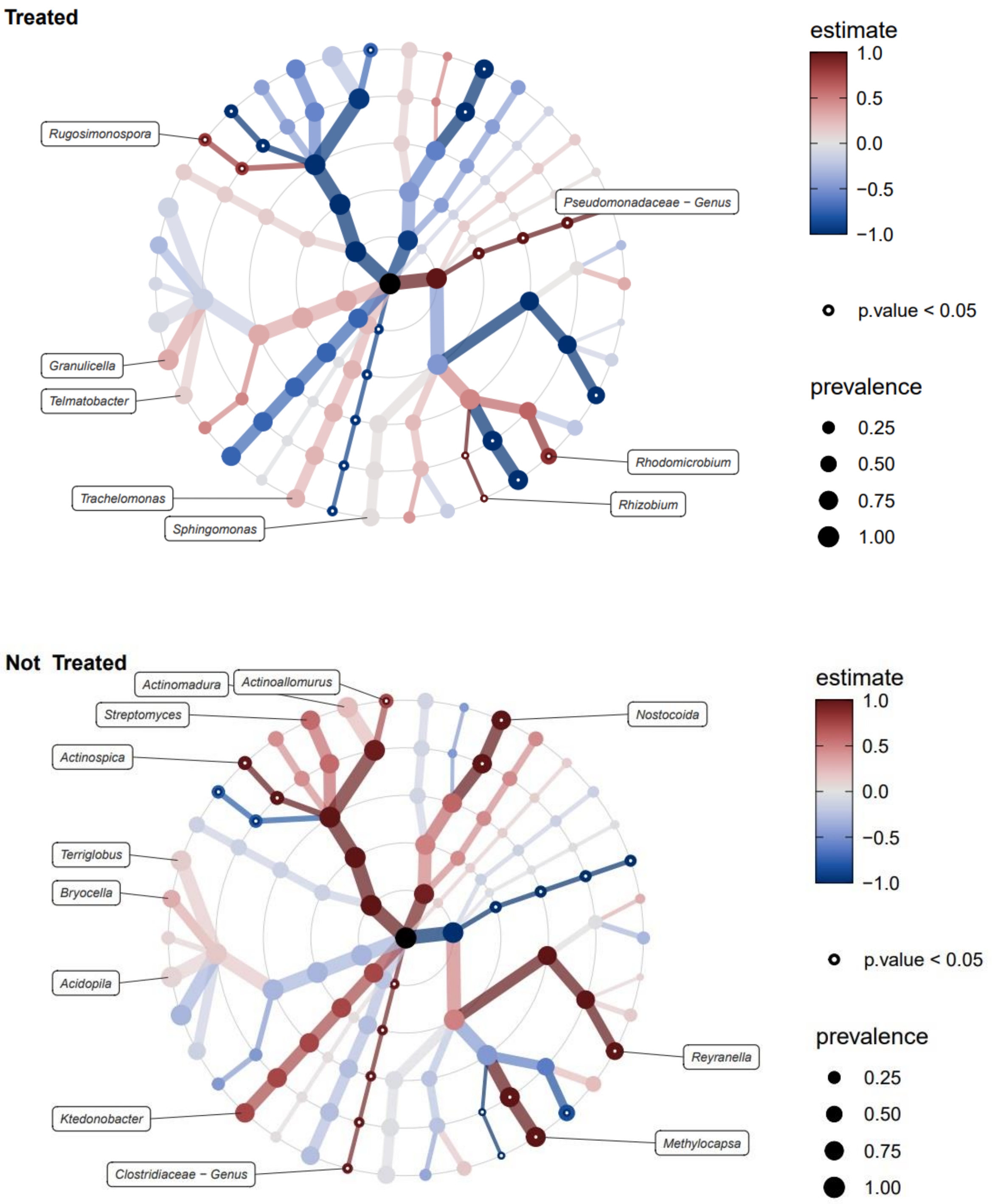


Figure 4.22: Taxonomic association tree plots display the varying levels of bacterial taxa (genus level) in the soil of kauri trees in Whangapoa, distinguishing between phosphonate treated and non-treated trees. Linear regression coefficient estimates are provided along with the spectrum, and significance is marked with a degree symbol (°). The taxonomy is arranged by rank, with phyla located around the central root node, and genera located on the outermost portion of the plot.

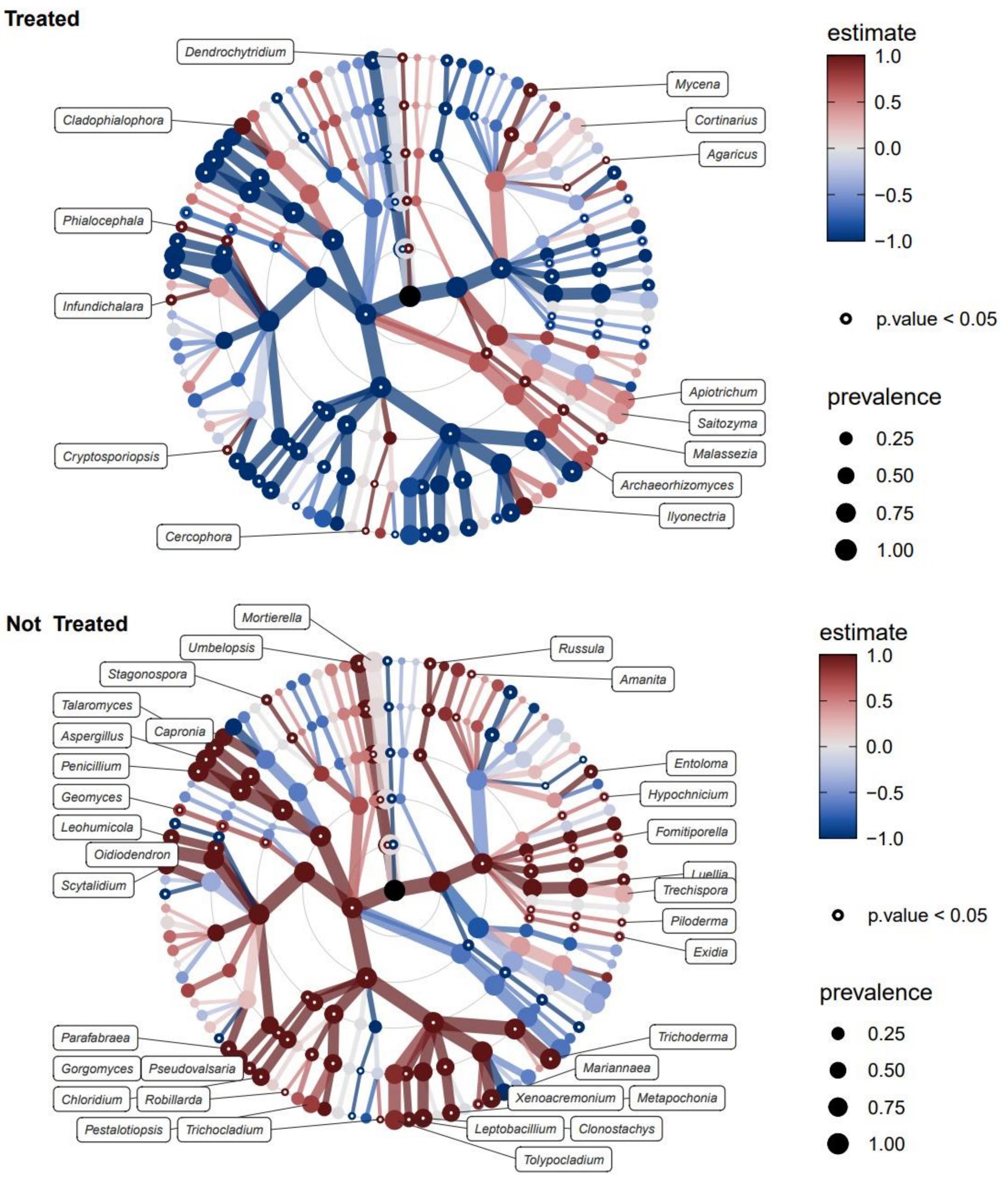


Figure 4.23: Taxonomic association tree plots display the varying levels of fungal taxa (genus level) in the soil of kauri trees in Whangapoa, distinguishing between phosphonate treated and non-treated trees. Linear regression coefficient estimates are provided along with the spectrum, and significance is marked with a degree symbol (º). The taxonomy is arranged by rank, with phyla located around the central root node, and genera located on the outermost portion of the plot.

Streptomyces, Actinoallomurus, Methylocapsa, class Clostridiaceae*, Penicillium, Aspergillus, Leptobacillium, Trichorderma* and *Umbelopsis* were positively associated to Non symptomatic, Nonbleeding and Not treated (phosphonate) kauri soil.

4.5 Discussion

In this study, the bacterial and fungal community structures of soil collected from Tairua and Whangapoa kauri forests were analysed using next generation sequencing (NGS) approach. Illumina amplicon sequencing (Mi*Seq*) has become the most popular NGS method for analysing microbial community structures and dynamics for various purposes, as documented in many studies (Nkongolo & Narendrula-Kotha, 2020).

4.5.1 PA Detection

The study found that only one symptomatic tree located in Tairua, Tree 15, tested positive for PA. This tree had a canopy score of 2.5. The proximity of this tree to a PA positive dead tree was only 1.5m. At Whangapoa, 12 trees were found to be PA-positive and symptomatic with basal bleed and high canopy score. The results were not surprising, as previous studies had indicated a high likelihood of PA-positive detection at symptomatic sites which could be associated with the spread of the pathogen within the site. The research on Chinese hickory (*Carya cathayensis* Sarg.) established that dieback disease caused by *Phytophthora cinnamomi* significantly impacts plant growth and nut production. The oomycete, *Phytophthora cinnamomi*, was also detected from symptomatic plantation soil (Tong et al., 2021). The detection of *Phytophthora nicotianae* and *Phytophthora citrophthora* was conducted using nested PCR in soils collected from five nurseries in citrus in Southern Italy (Grandgirard et al., 2002; Tong et al., 2021). As expected, in our study, all the trees that did not show any symptoms of the disease tested negative for PA as well, maybe the trees were not infected by the pathogen. However, trees with slightly thinning canopies (canopy score 2) tested positive for PA. Among the slightly thinning trees which tested positive, TT 3 showed signs of yellow leaves in Tairua. It was observed that out of the 28 symptomatic trees, only 12 were tested positive for PA in Whangapoa. However, this could be due to the small sample size of 0.5g of soil sample, which may result in a high degree of false negatives. It is possible that the DNA extraction and analysis by PCR may have missed the pathogen and during the pooling of the samples also chances of missing the pathogen (O’Brien et al., 2009).

4.5.2 Bacterial and Fungal communities

Acidobacteria, Actinobacteria, Bacteroidetes, and Proteobacteria are the most common bacteria phyla found in the rhizosphere of various environments, as reported by Nacke et al., 2011 and Uroz et al., 2013. Baldrian et al., 2012 found that these phyla also dominate in acidic coniferous forests, particularly in *Larix gmelinii* and *Pinus sylvestris* forest soil (Song et al., 2021). In New Zealand, (*The Effects of Urban Forest Restoration and Environmental Heterogeneity on Microbial Diversity and Ecosystem Functioning*, 1994)) detected similar bacterial phyla in urban forest sites, while (Byers, Condron, O'Callaghan, et al., 2020b) identified Proteobacteria, Actinobacteria and Acidobacteria as the primary bacterial community in Waipoua kauri and pine forest. Bacterial community of Tairua and Whangapoa resembles the above results with Proteobacteria, Actinobacteria and Acidobacteria as dominant phyla. However, the relative abundance of Proteobacteria was lower compared to the Waipoua pine and kauri forest, and *Acidobacteria* was higher. It was previously reported that soil acidity can shape the dominant bacterial groups in different types of soils (Yun et al., 2016). It was reported that relative abundance of Acidobacteria Group 1 and Group 3 were positively correlated with soil acidity (H. S. Kim et al., 2021) this might be a reason for the increase of Acidobacteria in kauri soil in Whangapoa and Tairua. Maybe the acidity of the soil was higher compared to Waipoa forest. Further studies on soil pH and chemical analysis required to understand the composition of Acidobacteria dominance.

In fungi, the current research builds upon previous studies of coniferous forests worldwide, including the Korean pine forest in China (Zhu et al., 2022), and forests of *Araucaria bidwillii, Araucaria cunninghamii,* and *Agathis robusta* in Australia (Curlevski et al., 2010a) having Basidiomycota and Ascomycota as most dominant phyla in the forest soil. Studies on Bunya pine forests soil in Australia (Curlevski et al., 2010b), Waipoua kauri and pine forest soil in New Zealand (Byers, Condron, Donavan, et al., 2020), and urban forests soil in New Zealand (*The Effects of Urban Forest Restoration and Environmental Heterogeneity on Microbial Diversity and Ecosystem Functioning*, 1994) also confirms that Ascomycota and Basidiomycota are the primary fungal phyla present in these forests. Furthermore, it was identified a higher abundance of Ascomycota in *Araucaria bidwillii, Araucaria cunninghamii*, and *Agathis robusta* forests, early stages of the Korean pine forest, and younger sites in urban forests in New Zealand. Kauri trees sampled in the Waikato region were still young, reflected by their CBH ranging 6 inches to 100 inches (Wunder et al., 2013). Similar to *Araucaria bidwillii, Araucaria cunninghamii,* and *Agathis robusta* forests, early stages of the Korean pine forest and younger sites in urban forests in New Zealand kauri soil in Whangapoa and Tairua are also abundant in Ascomycota. This may be due to the relatively young trees of kauri in the forest. Hypocreales, Tremellales, Eurotiales, Mortierellales and Archaeorhizomycetales were abundant fungal orders detected in Tairua (Figure 4.4). These results were mostly consistent with acidic coniferous forests and Waipoua Kauri forests (Byers, Condron, Donavan, et al., 2020; Byers, Condron, O'Callaghan, et al., 2020c).

4.5.3 Bacterial and Fungal Diversity

Several studies have been conducted to investigate the variation of soil microbial communities at different soil depths. Two of these studies were carried out in the Eurasian Steppe (Inner Mongolia, China) (Zhao et al., 2021) and in a forested montane watershed in Colorado, USA (Eilers et al., 2012). The results of these studies showed that soil samples taken at the surface and at a depth of 10cm did not differ significantly. However, significant differences were noted beyond a depth of 20cm in both cases. Similarly, our study on the kauri forest revealed that there were no significant differences between surface and 10cm depth soil samples. Moreover, the kauri lateral roots spread out more than five meters from the trunk, creating a similar ecosystem surrounding the tree (Padamsee et al., 2016; Yang Jaynie, 2022). As a result, no significant differences were observed among the soil samples taken from the four cardinal points. Therefore, samples collected at one meter from the trunk may be representative of the kauri root environment. However, this may vary depending on the specific environment, plants surrounding the kauri, and the slope of the cardinal point.

The study shows that trees with canopy thinning symptoms (canopy score > 2.5) had a higher bacterial Shannon diversity index compared to other tree health statuses. Trees TT13, TT14, and TT15, located within a 10-meter radius of a dead kauri tree that tested positive for PA (baiting method), had the highest bacterial Shannon entropy, as shown in Figure 5. This might be correlated with the presence of the dead kauri tree, as previous studies have indicated that bacteria play a crucial role in decomposing dead plant biomass and significantly contribute to decomposition processes in litter and soil (López-Mondéjar et al., 2016; Šturšová et al., 2012). In the current study, trees with symptoms (canopy score > 2.5) were observed to have lower fungal Shannon diversity index compared to those less symptoms (canopy score < 2.5). However, the NMDS plots showed that bacterial communities had lesser diversity between samples compared to fungal communities. This was evident in both Tairua and Whangapoa forest. This finding was similar to what was observed in a *Picea abies* forest, where a dieback disease significantly changes in the fungal community (Šturšová et al., 2014). The changes included a decrease in biomass, disappearance of fungi that were symbiotic with tree roots, and a relative increase in saprotrophic taxa. This observation was also evident in the current study where fungal diversity in the surface samples of symptomatic tree (TT11) at Tairua showing lower fungal diversity (Figure 4.4). Furthermore, the number of fungal OTUs shared by healthy

Pinus bungeana soil were significantly higher than those of unhealthy plants, indicating that the health conditions of the plants had a pronounced effect on the fungal community (J. Yang et al., 2022). The diversity patterns of soil microbial communities can be attributed to functional differentiation, with different microbial taxonomic groups demonstrating unique patterns and life history strategies (Schimel & Schaeffer, 2012). Fungi, in particular, vary in their ability to exploit resources and functions. Colonization of decomposed resources may alter community composition (Jung et al., 2018). In symptomatic soil, saprotrophic fungi such as *Mycena, Trichorderma, Umbelopsis, Cantharellales, Chaetothyriales, Mortierella, Leohumicola,* and *Apiotrichum* were detected. The accumulation of acidic podzols in the soil is caused by increased litter layer due to the dieback symptoms in kauri trees (Steward and Beveridge, 2010). The fungal Shannon diversity index is significantly low compared to slightly thinning and non-symptomatic trees. This might be due to the loss of symbiotic fungi of the dead tree and kauri dieback symptomatic status. Further research needs to be conducted to identify the cause of the difference in bacterial and alpha and beta diversities.

4.5.4 Differential Abundance

The dominant bacterial phylum, Proteobacteria, showed a positive association to healthy trees and slightly thinning trees, however, showed significantly negative association to symptomatic trees. Interestingly, previous studies have also identified a high abundance of Proteobacteria as a signature of disease-suppressive soils and healthy vegetation (Liu et al., 2016; Byers et al., 2020; Fernández-González et al., 2020), as this phylum contains a large number of biocontrol agents and growth-promoting bacteria (Mendes et al., 2011, 2013; Bruto et al., 2014). On the other hand, bacterial phyla such as Verrucomicrobia, and Bacteroidetes showed a clear positive association with symptomatic trees. More complex environments, featuring higher light availability and lower soil moisture, were found to favor these phyla over oligotrophic ones such as Proteobacteria and Acidobacteria in declining tree neighbourhoods. (D. Zhou et al., 2019).

4.5.5 Key genera with positive association to non-symptomatic kauri soil and their roles in biological control

Biocontrol has been proposed for pest and disease control for centuries. It involves the use of biological agents such as bacterial and fungal strains. The current study has identified several genera that are positively associated with non-symptomatic kauri soils, such as *Streptomyces, Actinoallomurus, Rodophila, Methylocapsa, Clostridium, Penicillium, Mortierella, Aspergillus, Trichorderma, Umbelopsis,* and *Leptobacillium* and these genera have been extensively studied for their ability to suppress diseases in plants (ref).

Streptomyces which is strongly positively associated to non-symptomatic kauri soil (Figure 4.8) are highly efficient in colonizing the rhizosphere, rhizoplane, and inner tissues of host plants as endophytes, due to their various features, such as multiplication rate, phytohormones, expression of genes controlled by quorum sensing, cellulases, amino acids, chitinase, lipase, and β-1,3-glucanase (Sousa & Olivares, 2016). The chemotactic movement of these microbes' aids in attracting *Streptomyces* to the rhizosphere through exudate attraction. Numerous studies have shown that the *Streptomyces* genera exhibit excellent biocontrol activities due to high production of bioactive compounds, which are utilized as defence mechanisms against plant pathogens. The mechanisms include synthesis of plant growth regulators (Goudjal et al., 2013), production of siderophores (Vijayabharathi et al., 2015) antibiotic production (Couillerot et al., 2013) secretion of volatile compounds, and competition for nutrients.

Plant growth-promoting *Streptomyces* species have been used as natural alternatives to synthetic fungicides (Sakineh et al., 2021). The dynamics of rhizosphere bacterial communities manipulated by a phosphate solubilizing *Streptomyces* strain have been studied using high throughput sequencing and microbiome profiling. The responses of rhizosphere bacterial communities of pepper plants treated with *Streptomyces* and inoculated with *Phytophthora capsici* have been explored. The study identified that these responses correlate with higher plant growth promotion and disease suppression by inhibiting the growth of the pathogen (Sakineh et al., 2021).

Secondary metabolites from *Streptomyces araujoniae* have been found to be effective in bending the hyphae and reducing the number of branches. They even break them, destroying the integrity of the hyphal cell membrane to inhibit the growth of *P. cinnamomi*. *Streptomyces araujoniae* also could inhibit the activity of *P. cinnamomi* cell wall-degrading enzymes. These findings suggest that *Streptomyces* is a naturally occurring biological control agent in the healthy kauri rhizosphere both at Tairua and Whangapoa, and it has the ability to suppress the growth of *Phytophthora*, making it an effective agent against kauri dieback pathogen *Phytophthora agathidicida*.

Actinoallomurus, also positively associated with non-symptomatic kauri soil (Figure 4.8), a genus of plant-associated endophytic Actinobacteria, has been suggested as a plant-growth-promoting agent due to its phytohormone-producing bacteria, plant-growth-promoting properties, and antimicrobial activity (Qin et al., 2011; Hamedi & Mohammadipanah, 2014). *Rhodopila* genus was positively associated to non-symptomatic kauri soil (figure 4.8) includes purple non-sulphur bacteria with vesicular intracytoplasmic membranes like *Rhodobacter* species, and *Rhodopila* can grow at low pH and influences nitrogen fixation by encoding nitrogenases (Novak et al., 2017). *Methylocapsa* (Figure 4.8) oxidises atmospheric methane aerobically and assimilates carbon from methane and carbon dioxide (Tveit et al., 2019). *Clostridium* also was positively associated with non-symptomatic kauri (Figure 4.8). The nitrogen-fixing bacterium *Clostridium* is a free-living microbe that exhibits antagonistic behaviour towards *Phytophthora capsici* in the rhizosphere of pepper plants (Li et al., 2022). This observation sheds light on microbial ecology and highlights the importance of inter-species interactions within this environment. *Clostridium*'s ability to regulate the population of *Phytophthora capsici* in the rhizosphere is a noteworthy aspect of its ecological (J. Dai et al., 2020; XU et al., 2023; Zeiller et al., 2015). Antimicrobial properties of *Actinoallomurus* and *Clostridium* against *Phytophthora* sp. may also provide protection against kauri dieback pathogen *Phytophthora agathidicida.*

Penicillium, Aspergillus, Trichorderma, and *Mortierella* were well-studied fungi that were positively associated with non-symptomatic kauri soil (Figure 4.11). *Penicillium* has been recognized as a rich source of bioactive metabolite (Bazioli et al., 2017). *Penicillium* spp., produces a range of medicinally important metabolites including antibacterial and antifungal (Korejo et al., 2014). Pepper (*Capsicum annuum*) wilting is caused by a complex of soil pathogens, among which *Phytophthora capsici* (Leonian) causes losses in fruit yield. There were suppressing soils containing *Penicillium* sp., antagonistic to the oomycete (Jiménez-Camargo et al., 2018). *Penicillium striatisporum* was isolated from the rhizosphere of chilli peppers. In dual culture agar plate assays, this isolate showed very high antagonistic effects on the mycelium growth of *Phytophthora* spp. (Ma et al., 2008). *Penicillium* is a naturally occurring biological control agent in the healthy kauri rhizosphere both at Tairua and Whangapoa. It has been detected in Waipoua asymptomatic kauri soil as well (Byers, Condron, O'Callaghan, et al., 2020b). It has proven its ability to suppress the growth of *Phytophthora*, making it an effective agent against the kauri dieback pathogen *Phytophthora agathidicida*.

Mortierella is a type of fungi that promotes plant growth. These microorganisms improve access to nutrients in the soil, protect plants from pathogens, and reduce the use of chemicals. Moreover, they significantly enhance plant health by supporting the performance of beneficial microorganisms (Ozimek et al., 2018).

Various species of the genus *Aspergillus* have been recognized as a rich source of biologically active secondary metabolite (Lubertozzi & Keasling, 2009). Aspergillus were found to have antifungal activity towards *Phytophthora,* and mycelial growth of *Phytophthora* species was inhibited. Germination of *P. capsici* zoospore was inhibited (Kang et al., 2005). *Aspergillus* is highly effective against *Phytophthora.* It has been discovered that compounds such as 3-Hydroxy-

2′,4,4′,6′-tetramethoxychalocone are responsible for this anti-*Phytophthora* activity. This compound is produced by *Aspergillus,* regardless of whether it is in contact with *Phytophthora parasitica.* The compounds significantly impact the growth and development of *P. parasitica,* inhibiting the germination of its zoospores, preventing the formation of sporangia, and causing abnormalities in hyphal growth. *A. flavipes* and its extracts are not toxic to common plant hosts like *N. benthamiana* and *S. lycopersicum* (El-Sayed & Ali, 2020).

Trichoderma species are commonly found in plant rhizosphere ecosystems. They are the most frequently isolated species in these environments (Harman et al., 2004). *Trichoderma* species have the potential to be biological control agents. They use various mechanisms including competition, antibiosis, mycoparasitism, and induction of plant defences (Shoresh & Harman, 2010). Recent studies have found that *Trichoderma* spp. can enhance plant growth and promote the synthesis of phenolic compounds, proteins, and amino acids (Shoresh & Harman, 2010). These compounds may directly inhibit pathogen such as *P. megakarya* development or be involved in metabolic pathways associated with disease resistance (Tchameni et al., 2017).

These results offer valuable guidance in the search for potential microbial antagonists against *P. agathidicida*. Further in vivo and in vitro studies are necessary to evaluate the suppressive capabilities of these potential biological control agents against kauri dieback disease.

4.5.6 Microbial community difference on basal bleeding symptoms

The structure and function of rhizosphere microbial communities are affected by the plant health status. There were no significant differences in the alpha diversity of bacterial and fungal communities in the soil of basal bleeding trees and non-bleeding trees. It is still too early to conclude whether these results can apply to other systems since very few studies have investigated the relationship between tree health and microbiome diversity. Recent research on acute oak decline in England found that the health of trees had only a minor impact on the soil microbiome of oaks, *Quercus* spp. The primary factor affecting the composition of the culturable microbiome was found to be geographical distance. However, there were fewer correlations between the microbiome of the oaks and the health status of the tissue (Denman et al., 2022). *Phytophthora cinnamomi* infected rhizosphere has increased the proportion of Pseudomonadales and Burkholderiales in the rhizosphere but reduced that of Actinobacteria, *Bacillus* spp. and Rhizobiales. This was partly evident in this study that class Pseudomonadaceae was found to have a positive association with bleeding symptomatic tree soil. However, Rhizobium also had a positive association with bleeding symptomatic tree soil (Figure 4.20). Many Actinobacteria, such as *Actinomadura* and *Actinospica,* were positively associated with non-bleeding kauri tree soil (Figure 4.20). Plant health potentially modifies the predicted functions by Rhizobacteria, reducing the proportion of categories linked with the lipid and amino-acid metabolisms whilst promoting those associated with quorum sensing, virulence, and antibiotic resistance. Many fungal genera were found positively associated with non-bleeding kauri soil. This indicates that maybe more fungi are symbiotically associated with healthy kauri (Figure 4.21).

4.5.7 Impact of phosphonate in kauri soil

Plants infested with *Phytophthora cinnamomi* and sprayed with phosphonate had a reduced number of Proteobacteria but a higher count of Actinobacteria and Chloroflexi (Farooq et al., 2022). Su et al. (2021a) conducted a study on the effects of phosphonate on the microbiome of tomatoes and found that it increased the prevalence of Proteobacteria and Actinobacteria, similar to the findings of our research. Actinobacteria, relative abundance increases effectively with phosphonate treatments, includes taxa such as *Streptomyces*, known to produce antibiotic compounds effective against *Phytophthora* (Farooq et al., 2022).

Phosphonate activates plant defence mechanisms (Ramezani et al., 2018; Mohammadi et al., 2020) and has a direct effect on the pathogen (Eshraghi et al., 2014; Achary et al., 2017; Gill et al., 2018). The phosphonate-treated kauri rhizosphere exhibited a more diverse bacterial community compared to non-treated rhizosphere (Figure 4.18). There are concerns about whether phosphonate is practical and safe for kauri protection. Further research can improve its effectiveness (Shearer et al., 2004).

4.6 Conclusion

The kauri forests of Aotearoa, New Zealand are not only culturally significant but also play a vital role in the ecosystem. However, a severe threat to their survival comes from the highly infectious soil-borne pathogen, *Phytophthora agathidicida*. The microbial communities present in the soil associated with the different symptoms and treatment of kauri dieback were presented here utilizing the high throughput amplicon sequencing of the 16S rRNA gene and ITS1 gene. The variations in diversity, taxonomic composition, and potential biological control options were identified. The results revealed significant variations in the bacterial and fungal communities' alpha diversity between non-symptomatic and symptomatic kauri soil. Moreover, significant differences in the beta diversities of non-symptomatic and symptomatic trees (presence of basal bleed), phosphonate-treated and non-treated trees were also observed. Microbial taxa that are known for their ability to suppress diseases, such as *Penicillium, Trichoderma, Aspergillus, Streptomyces, Clostridium,* and *Pseudomonas,* are significantly positively correlated with non-symptomatic kauri soil. Moreover, *Pseudomonas* and *Rhizobium* have been positively associated with phosphonate-treated kauri soil. The findings presented offer a promising avenue for identifying microbial taxa capable of having the potential of preventing kauri dieback, while underscoring the significance of this disease's lasting effects on kauri forests. This research affords valuable insight into the search of microbial antagonists to counteract *P. agathidicida*. Furthermore in vivo and in vitro investigations are necessary to evaluate the efficacy of these potential microbial control agents in identifying their potential on suppressing kauri dieback disease.

Chapter 5

Potential Functional Gene Profile of Kauri (*Agathis australis*) Soil in Natural Forest and Anthropogenic Environment

5.1 Abstract

Agathis australis (New Zealand kauri) is a significant and iconic native tree of Aotearoa New Zealand. Currently, *Phytophthora agathidicida* that causes kauri-dieback disease is killing kauri trees. Only 1% of the New Zealand virgin kauri forest remains. Recent studies revealed that many soil-borne microorganisms had been found to systemically boost the defensive capacity of the trees by providing competition to pathogens for nutrient intake, thus preventing pathogen colonization and modulating plant immunity. In addition, the root microbiome consists of an entire complex rhizosphere-associated microbes with their genetic elements and interactions that have influenced plant health. To date, very few studies have been conducted to investigate the microorganisms in the kauri soil and possible environmental driver.

To analyze the genes that contribute to soil microbial diversity of the kauri trees at Auckland Botanic Gardens (ABG) and the Tairua and Whangapoa kauri forests in the Waikato region of New Zealand, a microarray-based metagenomics tool called GeoChip 5.0M (developed by Glomics Inc. USA) was used. GeoChip 5.0M comprises 162,000 probes from 365,000 target genes (coding DNA sequence - CDS), which covers all taxonomic groups (archaea, bacteria, fungi, protists, algae, and viruses). The microbial functional gene profiles of kauri soil in man-made environments and natural forest environments were investigated.

A total of 946 gene families were detected from the ABG samples and 999 gene families belonging to 60 phyla were recovered from the Tairua and Whangapoa soil samples. The findings suggest significant differences in the signal intensities of carbon cycling genes and microbial defence genes between the ABG site and Tairua and Whangapoa. Moreover, the normalised signal intensity of carbon degradation genes in Tairua and Whangapoa kauri soil was significantly higher than in ABG kauri soil due to litter formation in the natural kauri forests. Several critical genes, such as PhID, have been detected related to microbial defence. These genes produce antimicrobial substances that can suppress the growth of several *Phytophthora* species. The kauri soil has been found to contain several plant growth-promoting genes, such as anti-pathogen, phytohormones, drought tolerance, and stress tolerance genes.

5.2 Introduction

The New Zealand Kauri, scientifically known as Agathis australis, is a coniferous tree that is native to Aotearoa, New Zealand. It belongs to the ancient Araucariaceae family. The kauri trees have an intricate and fascinating history associated with the settlement of New Zealand and the indigenous Māori people. Māori has a deep spiritual connection with the trees long before the arrival of Europeans. Kauri trees played a significant role in Māori traditions, art, stories, and legends. Before deforestation, the virgin kauri forests spanned over 1.5 million hectares of Aotearoa New Zealand (Halkett, J., 1980). After European colonization of Aotearoa New Zealand, the kauri timber and gum industries emerged. Kauri timber was highly sought after for its appearance and working properties (Cheeseman et al., 2011; Steward, 2011). A significant amount of the kauri forest was cleared for farming, leading to the misuse of the species and a complete transformation of the forest landscape in the upper North Island (Steward, 2011). Only 1% of the Aotearoa New Zealand virgin kauri forest remains (Halkett, J., 1980).

Currently, kauri trees are being devastated by a disease called kauri dieback. The leading cause of kauri dieback is *Phytophthora agathidicida*. The symptoms of this plant disease include root and collar rot, lesions that exude resin, severe chlorosis, thinning of the canopy, and widespread tree mortality (Beever et al., 2007). *Phytophthora agathidicida* poses a severe threat to kauri at both the individual and population levels. It has significantly impacted kauri ecosystems and their natural processes. Kauri forests are vital to local and global carbon cycles as well as soil and nutrient cycles (Madgwick et al., 1982). Losing forests due to invasive *Phytophthora* species will greatly impact many ecosystems and natural processes. Given the current fragmented distribution of kauri forests and the alarming widespread of *P. agathidicida* throughout major kauri stands in northern New Zealand, this pathogen poses a significant threat to the long-term survival of this iconic tree species (Beever et al., 2007; Waipara et al., 2013). Large-scale forest disease and dieback events are increasing globally, mainly due to biological invasions, climate change, and human activities (Ghelardini et al., 2016; Guégan et al., 2023; Pautasso et al., 2015). The destruction of these forests has disastrous consequences for biodiversity, carbon storage, and climate regulation (Narayan Pandey, 2002; Seidl et al., 2014). Human activities and soil-borne pathogens impact soil microbial communities and their function, including carbon and nitrogen cycling in managed forests (Byers, Condron, O’Callaghan, et al., 2020c; Kent & Triplett, 2002; Mendes et al., 2015). There is limited knowledge about the impact of human activities and the invasive kauri dieback pathogen on kauri soil microbial communities and their functional potential. However, this knowledge gap is significant because soil microbes play a crucial role in various biogeochemical processes, including carbon (C), nitrogen (N) and phosphorus (P) cycling. Consequently, they provide essential ecosystem services such as soil fertility and greenhouse gas mitigation (Luo et al., 2020; Y. P. Wang et al., 2010). Kauri dieback could greatly impact the long-term C and N cycling dynamics of kauri forests.

Microorganisms are a diverse group of organisms that form complex communities. These communities are critical to ecosystem functions, as they play integral roles in the biogeochemical cycling of carbon, nitrogen, sulphur, phosphorus, and metals. Soil microorganisms are closely associated with soil particles, particularly clay-organic matter complexes. These interactions are important for understanding the structure, functions, interactions, and dynamics of these communities (Nkongolo & Narendrula-Kotha, 2020; Sherameti & Varma, 2009). Soil biodiversity encompasses all flora and fauna that inhabit soil, classified by size as micro, meso, and macro (HWall et al., 2012; Sherameti & Varma, 2009). Soil is considered the most diverse ecosystem on earth from both a biological and physiochemical perspective (Brady & Weil, 1952; Wall et al., 2012; Kowalchuk et al., 2002). Soil is home to a vast array of organisms, a significant number of which have not yet been identified or studied.

Physical, biotic, and chemical factors significantly affect microbial population diversity and community structure. Such factors include moisture, temperature, predation, competition, pH, dissolved nutrients, salinity, organic matter, and natural and anthropogenic activities (Brady & Weil, 1952; Wall et al., 2012). Assessing the diversity and structure of microbial communities in soil remains one of the most challenging and fascinating aspects of microbial studies, given that over 99% of soil microorganisms have not been cultured (J. Zhou et al., 2010). In spite of the important role microorganisms play in ecosystem processes, the relationship between microbial diversity and ecosystem functions is still not well understood (Breure, 2020; Laureto et al., 2015; Zhou et al., 2010). GeoChip is a powerful metagenomics tool for analysing microbial communities, including their structure, metabolic potential, diversity, and their impact on ecosystem functions (Z. Shi et al., 2019).

With the development of advanced metagenomics technologies, it is now possible to study microbial communities in great detail. However, most of the research conducted has been limited to analysing microbial taxonomy, distribution, and diversity. It is crucial to investigate functional signatures of microorganisms, such as genes that are relevant to metabolic pathways, energetics, and regulatory circuits (Dick & Tebo, 2010; Pointing et al., 2009). The GeoChip microarray-based tool is widely used to study microbial gene diversity in different environments (He, Deng, et al., 2010; He et al., 2007; Lu et al., 2012; Van Nostrand et al., 2009a; Waldron et al., 2009; Wang et al., 2009) The latest version, Geochip 5.0 M, includes 162,000 probes from 365,000 target genes (coding DNA sequence - CDS). This covers taxonomic groups such as archaea, bacteria, fungi, protists, algae, and viruses (Shi et al., 2019). The current research aimed to investigate the abundance and functional profile of soil microbes that play a role in the cycling of carbon, nitrogen, and phosphorus, microbial defence against pathogens, and promotion of plant growth in kauri soils found in both native forest and man-made environments. The analysis was conducted using GeoChip 5 microarray technology. The study involved comparing the potential functional gene profile of natural kauri forests and planted kauri soils. Studying the microbial community and functional genes in the kauri soil environment is important in the context of kauri dieback.

5.3 Materials and Method

5.3.1 Site description and soil sampling

The study was conducted in Auckland (Auckland Botanic Gardens) and Waikato region (Tairua and Whangapoa), Aotearoa New Zealand. The site description and tree health characteristic were observed and recorded (Table 5.1). Auckland Botanic Gardens (ABG) in New Zealand is maintained by the local council and has sections called Native Forest (NF) , Kauri Grove (KG), and Rose Garden (RG). The NF section is designed to replicate a natural forest environment and features native trees and plants that are unique to New Zealand, such as kauri tree. The KG contains around 100 ~20-year-old kauri trees.

Approximately 100g of soil samples were collected from each of the four cardinal points at 100cm from the trunk (Figure 5.1), from the surface to 10cm depth. Samples were stored at -20^{o}C until processed.

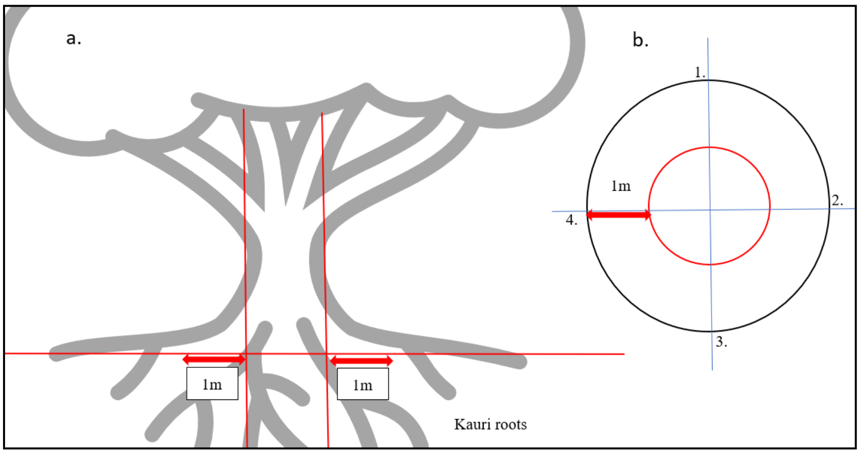


Figure 5.1: a.) The rootzone of mature kauri tree b.) Sampling points (four cardinal points) from the kauri trunk (Lawrence et al., 2023).

5.3.2 DNA extraction

Genomic DNA was extracted from 0.5g of soil from each cardinal point following the CTAB hot phenol-chloroform DNA extraction method. In brief, samples were incubated in phosphate buffer, SDS, CTAB, lysozyme and proteinase K in 60ºC water bath for one hour and purified using Phenol-Chloroform. Recovered DNA was quantified using a Quant-iT dsDNA Assay kit (Invitrogen, California USA) on a Qubit 2 Fluorometer (Invitrogen, California USA) according to manufacturer directions. Equimolar of genomic DNA from each cardinal point were pooled and sent to Glomics for GeoChip 5 analysis.

The DNA was purified, by adding 10 µL of NaCl (3M pH 5.2) (which is 1/10 of the DNA volume), followed by 200 µL of cold 100% ethanol (which is twice the DNA volume). The mixture was thoroughly mixed and then incubated in a negative twenty-degree overnight. The mixture was centrifuged at maximum speed (15000g) for 15 minutes. Supernatant was discarded. 500 µL of 70% cold ethanol was added, then vortexed slowly before being centrifuged at maximum speed (15000g) for 15 minutes. Supernatant was discarded and pellet was air dried for 5 minutes. The pellet was resuspended in 100 µL nuclease free water. DNA samples were quantified using a Quant-iT dsDNA Assay kit (Invitrogen, California USA) on a Qubit 2 Fluorometer (Invitrogen, California USA) according to manufacturer directions. Sample purity was assessed using a Nanodrop Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). DNA quality was evaluated by the absorbance ratios at A260/280 and A260/230. DNA with > 1.7 A260/280 and 1.8 A260/230 ratios recovered from each cardinal point were pooled per tree and used for further GeoChip analysis.

Table 5.1: Sample information of Geochip 5M analysis

Tree Number (ID)	Location	Site	Phosphonate Treatment	Basal Bleeding	Canopy Health Score	CBH (Inches)
TT06	Waikato	Tairua	No treatment	No	2	18
TT04	Waikato	Tairua	No treatment	No	1	9.6
TT14	Waikato	Tairua	No treatment	No	3	19.5
TT15	Waikato	Tairua	No treatment	No	2.5	8
WT10	Waikato	Whangapoa	No treatment	No	1	5.8
WT06	Waikato	Whangapoa	No treatment	Yes	4	23.2
WT16	Waikato	Whangapoa	Yes	Yes	2.5	42
WT04	Waikato	Whangapoa	No treatment	No	3	24.8
NF1	Auckland	ABG - Native Forest	No treatment	No	1	22.8
NF2	Auckland	ABG - Native Forest	No treatment	No	1	24.6
KG3	Auckland	ABG - Kauri Grove	No treatment	Yes	1	24.5
KG5	Auckland	ABG - Kauri Grove	No treatment	No	1	23.4

5.3.3 Geochip 5.0 experiment

Geochip 5.0M was manufactured by Agilent (Agilent Technologies Inc., Santa Clara, CA). The fluorescent Cy-3 labeling of DNA was accomplished using the random priming method with Klenow fragment. The DNA was subsequently purified with a QIAquick purification kit from Qiagen, CA, USA according to the manufacturer's instructions and dried. After resuspension of the labeled DNA in DNase/RNase-free distilled water, it was added to the vial containing the lyophilized 10× aCGH Blocking Agent and hybridization solution containing 10% formamide. The hybridization solution was transferred into the center of a gasket slide well from Agilent and then covered with an array slide. To ensure better results, SureHyb chamber was closed, and the hybridization process was allowed to proceed for 24 hours at a temperature of 67°C in an Agilent Hybridization Oven. Once hybridization was complete, slides were rinsed using Agilent wash buffer at room temperature. Imaging of the array was conducted with the NimbleGen microarray scanner at 633nm, and data extraction was performed using the Agilent Feature Extraction program, v11.5.

5.3.4 Microarray Data analysis

The data was extracted and uploaded onto the GeoChip data analysis pipeline (http://www.ou.edu/ieg/tools/data-analysispipeline.html). To ensure accuracy, all arrays in the experiment underwent a two-step normalization and quality filtering process (Liang et al., 2010; Tu et al., 2014). Initially, spots with low-quality were eliminated, which had a signal to noise ratio of less than 2.0. The average signal intensity of the five common oligonucleotide reference standard probes (CORS) was calculated for every subarray. The highest average value among all subarrays was utilized to normalize the signal intensity of samples in each array. For each array, the signal intensity was calculated, and the highest value was used to standardize the signal intensity of all spots in that array. This resulted in a normalized value for each spot in every array (Van Nostrand et al., 2009b).

The Shannon index and Simpson index were utilized to analyze the diversity of soil microbes and genes. Principal component analysis and correspondence analysis were employed to compare the samples. The statistical analyses were conducted in R (version 4.3.1) using various packages such as vegan, factoshiny, ggplot2, tidyverse, webr, dplyr, viridis, tidyr, circlize, chordiag, and heatmaply.

5.4 Results

5.4.1 Taxonomical results

GeoChip 5.0M comprises of 162,000 probes targeting 365,000 genes (coding DNA sequence - CDS), which span across all taxonomic groups (archaea, bacteria, fungi, protists, algae, and viruses).

Phylogenetically, it was found that in ABG, 674 gene families were derived from bacteria, 202 from archaea, 164 from fungi, 41 from Viridiplantae, 3 from Metazoa, and 17 from *Phytophthora*. Similarly, in Tairua, 698 gene families were derived from bacteria, 198 from archaea, 169 from fungi, 43 from Viridiplantae, 3 from Metazoa, and 18 from *Phytophthora*. In Whangapoa, 694 gene families were derived from bacteria, 199 from archaea, 170 from fungi, 43 from Viridiplantae, 3 from Metazoa, and 18 from *Phytophthora* (Figure 5.2).

Geochip 5.0M detected 49,005 probes, 946 gene families, 4,342 taxa, 102 phyla, and 995 genera in ABG kauri soil samples. The soil samples obtained from two different Native Forest trees, NF1 and NF2, displayed a total of 62,642,872 and 62,344,094 normalized signal intensities of genes, respectively. The soil samples from the Kauri Grove, KG3 and KG5 exhibited a sum of 66,489,421 and 63,035,864 normalised signal intensities, hybridised genes, respectively. The metal homeostasis gene category had the highest sum of normalised signal intensity out of all gene categories, with 62,885,341. On the other hand, the category of genes associated with protozoa had the lowest number of detected genes and sum of normalised signal intensity of only 574,856.01 (Appendix C). The GeoChip 5M detected 999 gene families, 55138 probes, 60 phyla, and 1301 genera from archaea, bacteria, Eukaryota, and viruses at Tairua and Whangapoa. The soil samples taken from four trees in the Tairua forest and four trees in the Whangapoa forest had a total of 731,604,797.30 and 755,643,441.70 normalized signal intensities of genes, respectively. The metal homeostasis gene category had the highest sum of normalised signal intensity out of all gene categories, with a total of 370,447,422.97. On the other hand, the category of genes associated with the virus had the lowest number of detected genes and the sum of normalized signal intensity of only 7,629,331.71 (Appendix C).

5.4.2 Microbial diversity

The species richness and alpha diversity were analyzed based on species identified in the GeoChip analysis. The richness (Chao) and diversity (Shannon indices) were all higher in Tairua and Whangapoa kauri soils than in ABG kauri soils (Table 5.2).

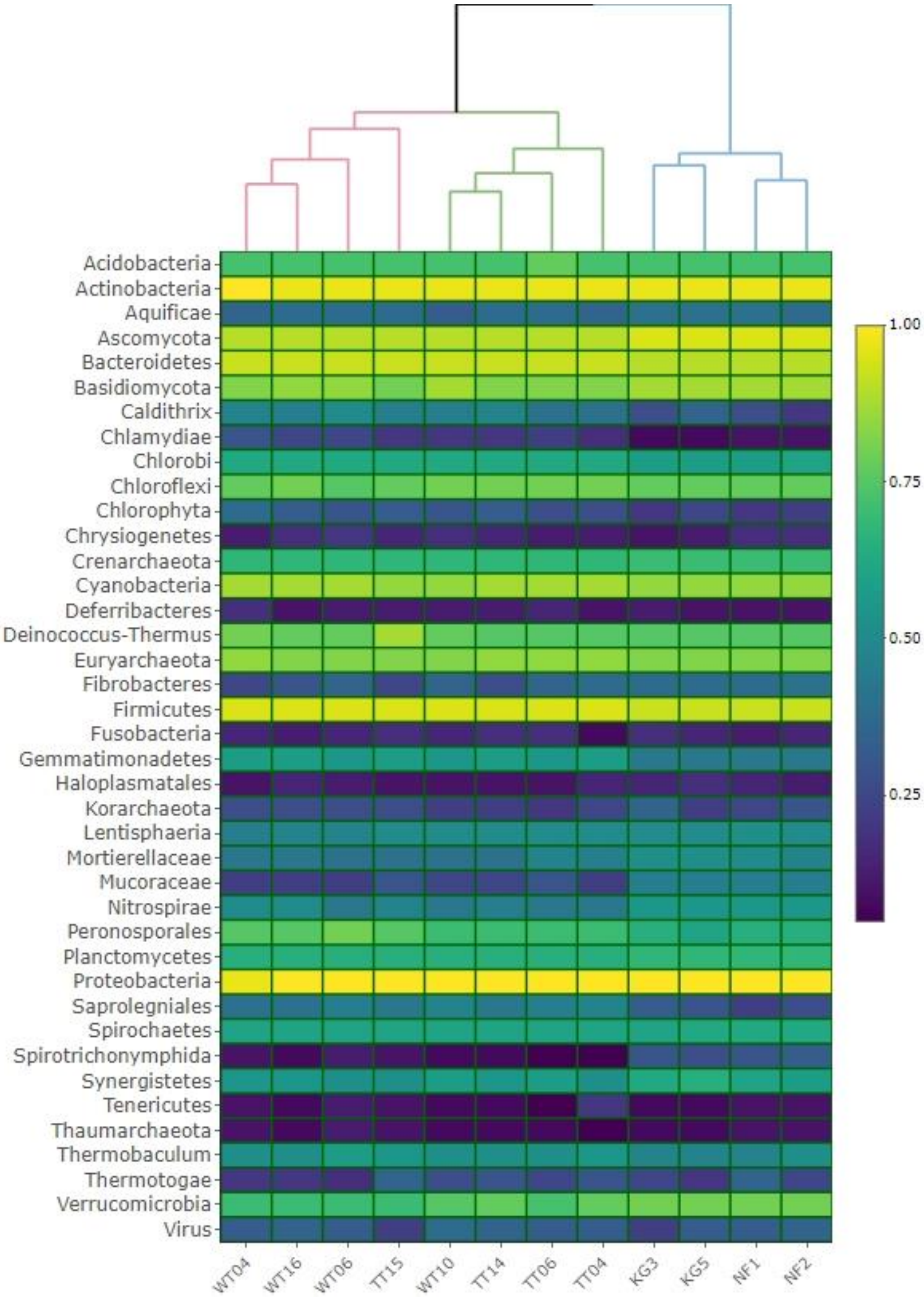


Figure 5.2: Taxonomy of genes detected at ABG, Tairua and Whangapoa kauri forest. NF1, NF2, KG3, and KG5 represent kauri tree samples from ABG, TT04, TT04, TT14 and TT15 from Tairua and WT04, WT06, WT10 and WT16 and Whangapoa.

Table 5.2: Alpha diversity indices for samples taken from the different locations

ABG

Samples	NF1	NF2	KG3	KG5	Mean	SD
Shannon	5.37	5.36	5.38	5.39	5.375	0.01236
Simpson	0.986	0.986	0.987	0.987	0.987	0.00062
Species Richness	675.87	711.30	730.70	745	675.75	16.859
Observed Species	745					
Chao	783.23					

Tairua

Samples	TT04	TT06	TT14	TT15	Mean	SD
Shannon	7.048	7.068	7.061	7.013	7.048	0.0245
Simpson	0.99	0.99	0.99	0.99	0.99	0.00011
Species Richness	3920	4309	4091	3909	4057.25	187.38
Observed Species	4515					
Chao	4807.72					

Whangapoa

Samples	WT10	WT06	WT16	WT04	Mean	SD
Shannon	7.04	7.045	7.029	7.018	7.033	0.012
Simpson	0.99	0.99	0.99	0.99	0.99	0.00005
Species Richness	4026	3946	4165	3879	4004	123.0095
Observed Species	4377					
Chao	4541.536					

5.4.3 GeoChip analysis of key functional genes.

PCA was performed based on all detected functional genes to determine the difference in the overall microbial community and functional structure of the samples. As shown in Figure 3, the four ABG soil samples (NF1, NF2, KG3, and KG5) were clustered together and well separated from the Whangapoa and Tairua forest kauri soil samples. The Whangapoa and Tairua samples were clustered together.

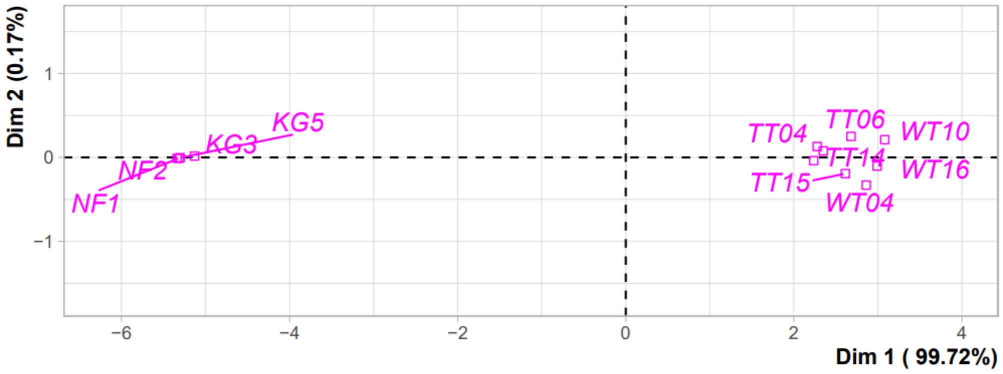


Figure 5.3: PCA analysis of kauri soil Geochip data of ABG, Tairua and Whangapoa samples.

Wilcoxon test confirmed significant differences were found in log10 means of normalised signal intensity of carbon cycling and microbial defence genes between ABG and Tairua and ABG and Whangapoa (Figure 5.4).

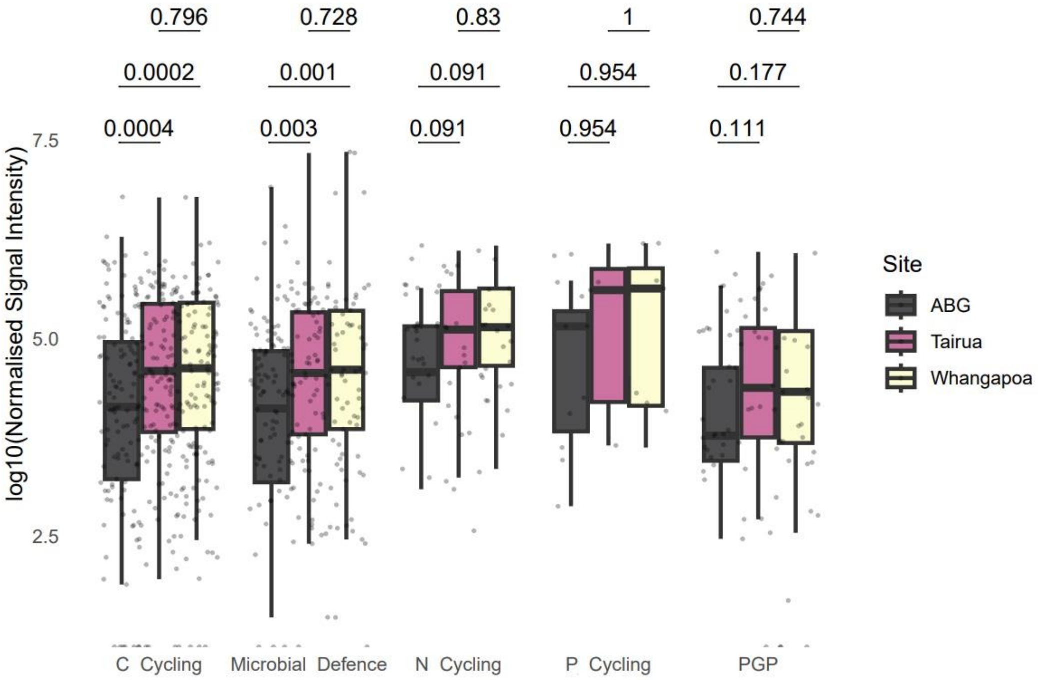


Figure 5.4: The log10 normalised mean signal intensity of gene categories detected GeoChip 5.0 in ABG, Tairua and Whangapoa. The significant differences between the sites were tested using the Wilcoxon test. Significant difference $p < 0.05$.

5.4.4 Carbon Cycling genes

Genes that are responsible for carbon degradation, carbon fixation, and methane metabolism were detected in the soils of ABG, Tairua, and Whangapoa. The log means of normalized signal intensities of genes related to methane metabolism were not significantly different between ABG, Tairua, and Whangapoa. However, there were significant differences in the log mean normalized signal intensities of genes related to carbon degradation and carbon fixation between ABG and Whangapoa. Moreover, there was a significant difference in the log mean signal intensity of genes related to carbon degradation between ABG and Tairua soil samples. Ace A and Ace B genes were not detected in ABG samples. Methane and cellulose fixation genes were only detected in ABG. The Mttb gene of methane metabolism was only detected in Whangapoa kauri soil.

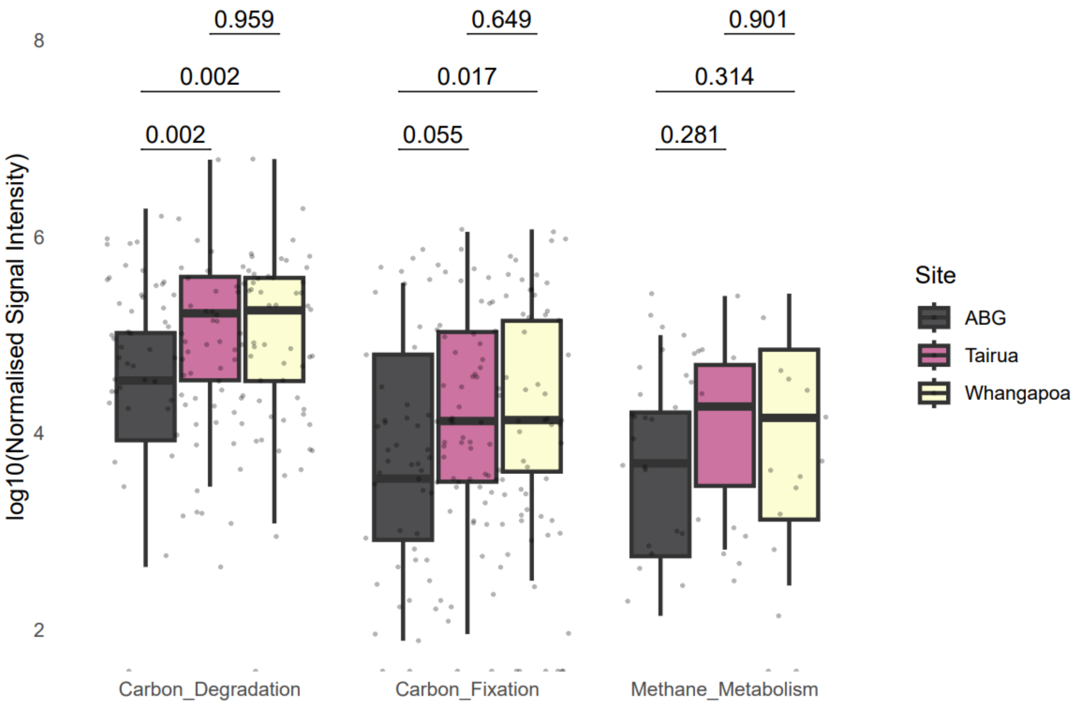


Figure 5.5: Normalised signal intensity (log10) of Carbon cycling gene categories detected in ABG, Tairua and Whangapoa kauri forests.

Carbon cycling genes were associated with three archaea phyla, twenty-five bacterial phyla, three fungal phyla, and one class from the kingdom Viridiplantae at ABG. The detected fungal classes in the samples include Basidiomycota, Ascomycota, and Mucoromycota. Phylum Chlorophyta was detected from the kingdom Viridiplantae. The samples collected from Tairua and Whangapoa were found to be associated with four archaeal phyla, two fungal phyla, and one phylum from the kingdom Viridiplantae. The genes present in Tairua were associated with nineteen bacterial phyla, while Whangapoa genes were associated with eighteen phyla. The phylum Tenericutes was found only in Tairua, and the phylum Chlorophyta was detected from the kingdom Viridiplantae. Mortierellales, Mucorales, and Spirotrichonymphida were detected only in the ABG samples. Chlamydiae was found in both Whangapoa and Tairua, but not in the ABG samples. Moreover, Tenericutes and Thaumarchaeota were exclusively detected in Tairua samples.

5.4.4.1 Carbon degradation genes

The Carbon degradation genes showed several carbohydrate-degrading genes, such as those specific to degrade starch, chitin, lignin, hemicellulose, cellulose, inulin, and pectin. Among carbon cycling functional gene families, the *amyA* gene, which degrades starch, had the highest signal intensity. The next highest signal intensity was observed for chitinase, which degrades chitin, followed by cutinase for cutin degradation, ara and xylanase for hemicellulose degradation, cellobiase, axe, and exoglucanase for cellulose degradation, and phenol oxidase for lignin degradation. Furthermore, pectin degradation (RgaE), chitin degradation (acetylglucosaminidase), tannin degradation (tannase_Cdeg) and hemicellulose degradation (xyla) were also detected (figure 5.7).

5.4.4.2 Carbon fixation genes

The C fixation genes in kauri soil includes several pathways such as the 3-hydroxypropionate/4-hydroxybutyrate cycle, Calvin cycle, dicarboxylate/4-hydroxybutyrate cycle, reductive acetyl-CoA pathway, and reductive tricarboxylic acid. Fifty six gene families were detected in all three sampling sites. During the carbon fixation process, certain gene families such as TIM, tktA, FBPase, RubisCO, GAPDH_Calvin, pgk, and PRI contribute to higher signal intensity in the Calvin cycle. Similarly, genes such as *CsoS1 CcmK*, *pcc*, *CODH*, and *FTHFS* are involved in the bacterial microcompartment cycle, multiple systems cycle, and reductive acetyl *Co*A pathway, respectively (figure 5.8).

5.4.4.3 Methane metabolism genes

The 17 methane metabolism gene families were detected in kauri soil in ABG, Whangapoa and Tairua. Including FmdB_fwdB, Ftr, HdrB, Hmd, Mch_methane, mcrA, Mer_methane, mrtH, MT2, mtaB methane formation genes and methane oxidation gene, mmoX (Figure 5.9).

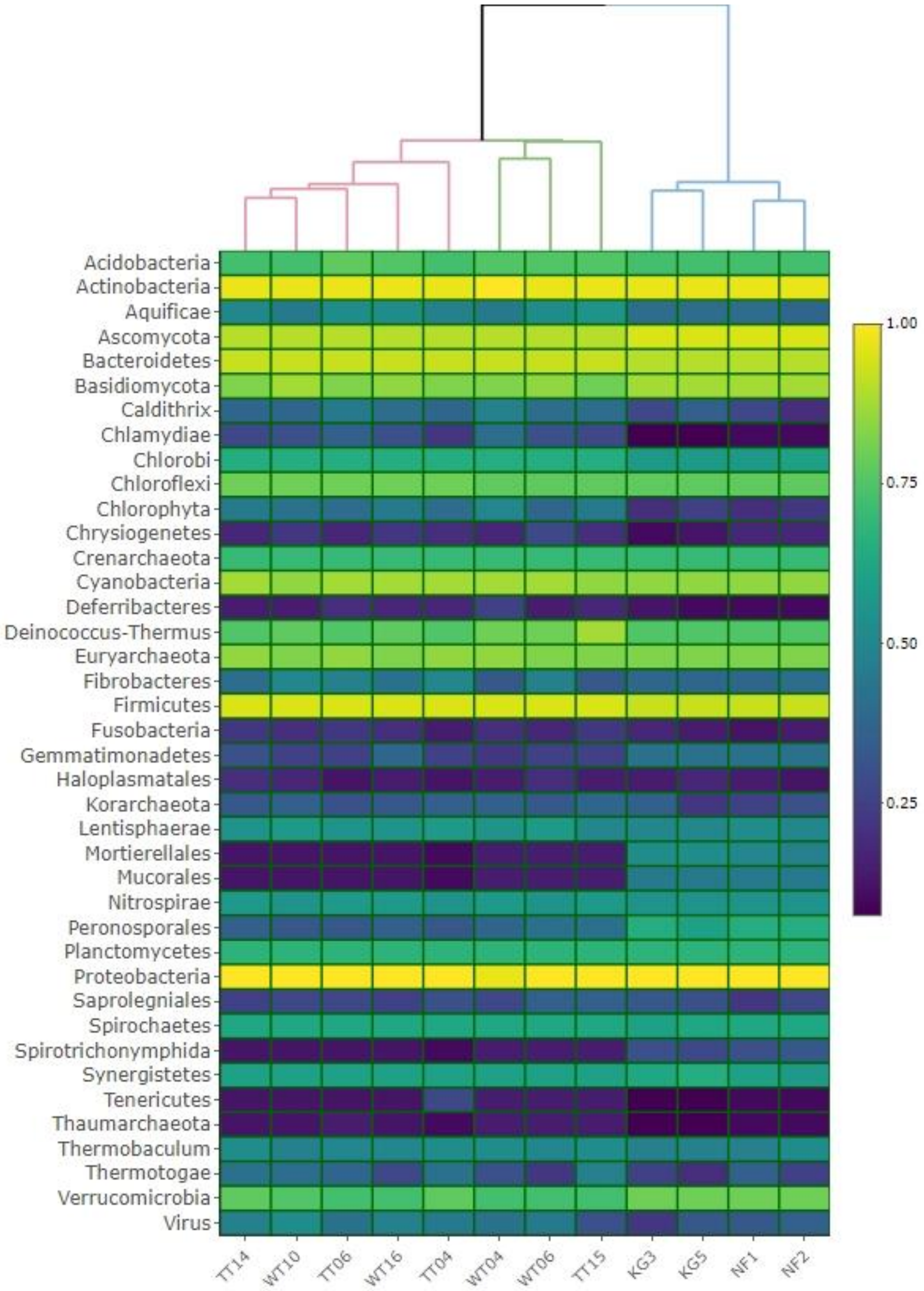


Figure 5.6: Taxonomy of genes detected in Carbon cycling genes at ABG, Tairua and Whangaopoa kauri forest. NF1, NF2, KG3, and KG5 represent kauri tree samples from ABG, TT04, TT04, TT14 and TT15 from Tairua and WT04, WT06, WT10 and WT16 and Whangaopa.

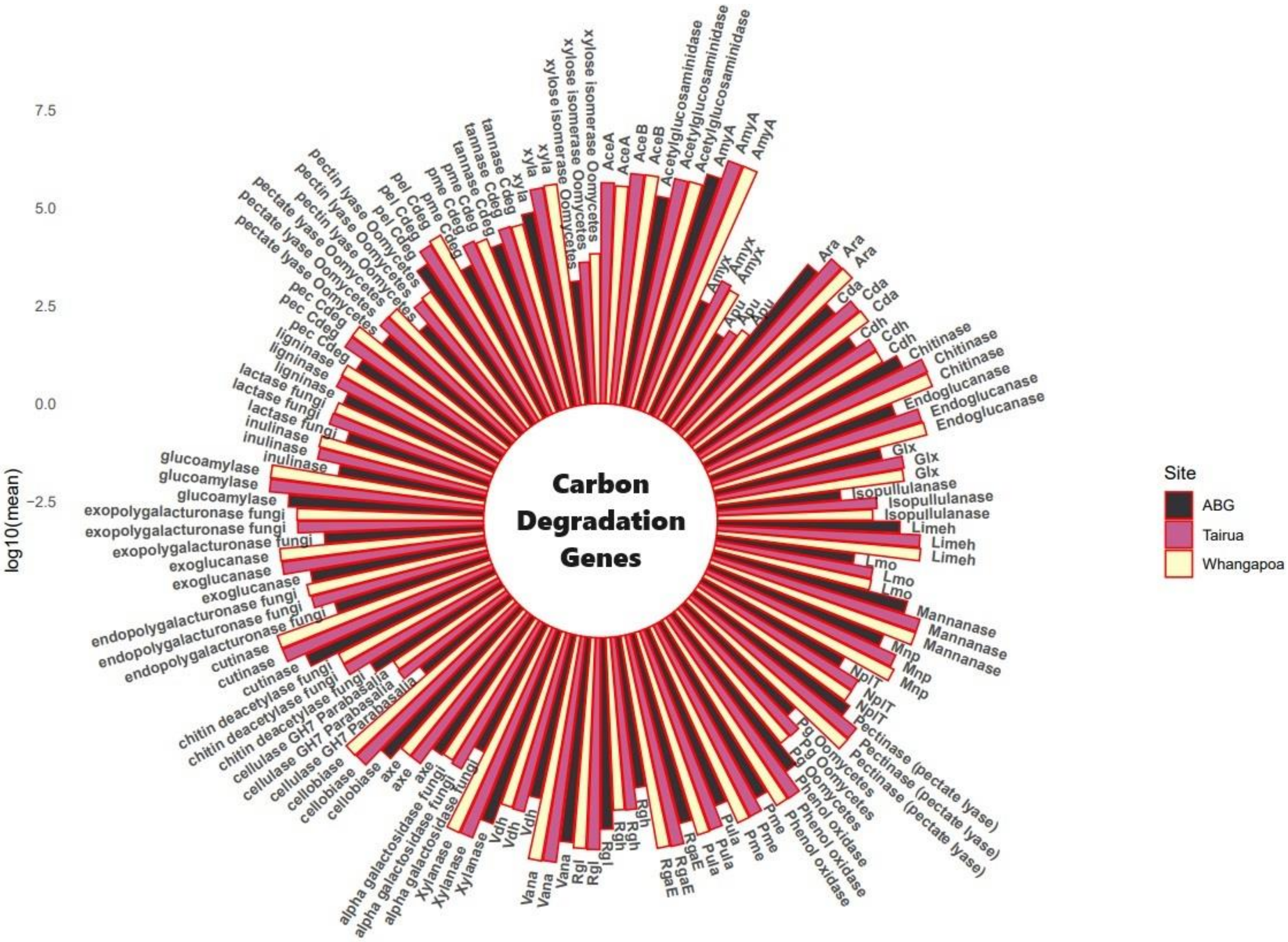


Figure 5.7: The normalized log means signal intensity of carbon degradation genes detected GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

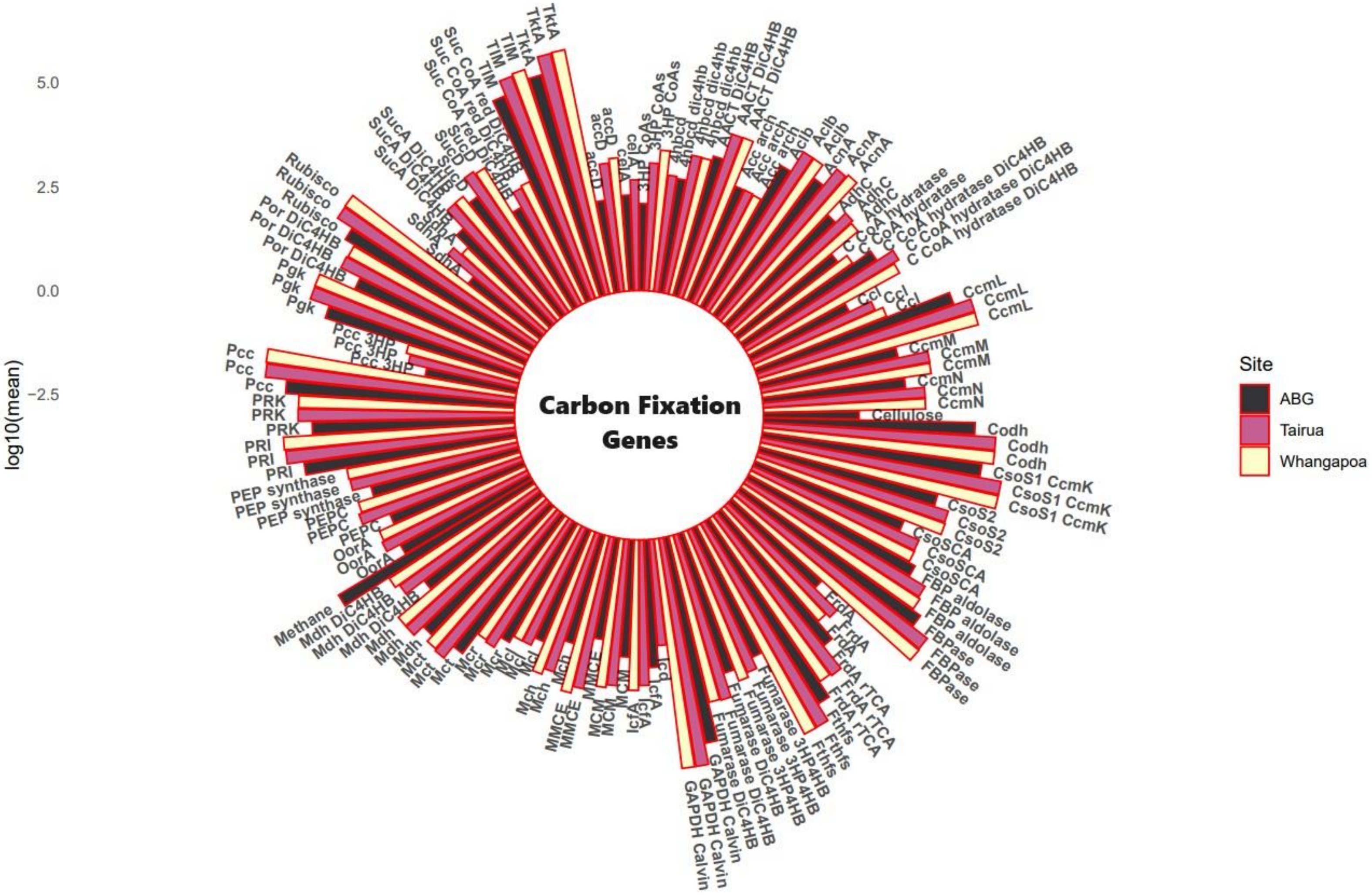


Figure 5.8: The normalized log means signal intensity of carbon fixation genes detected GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

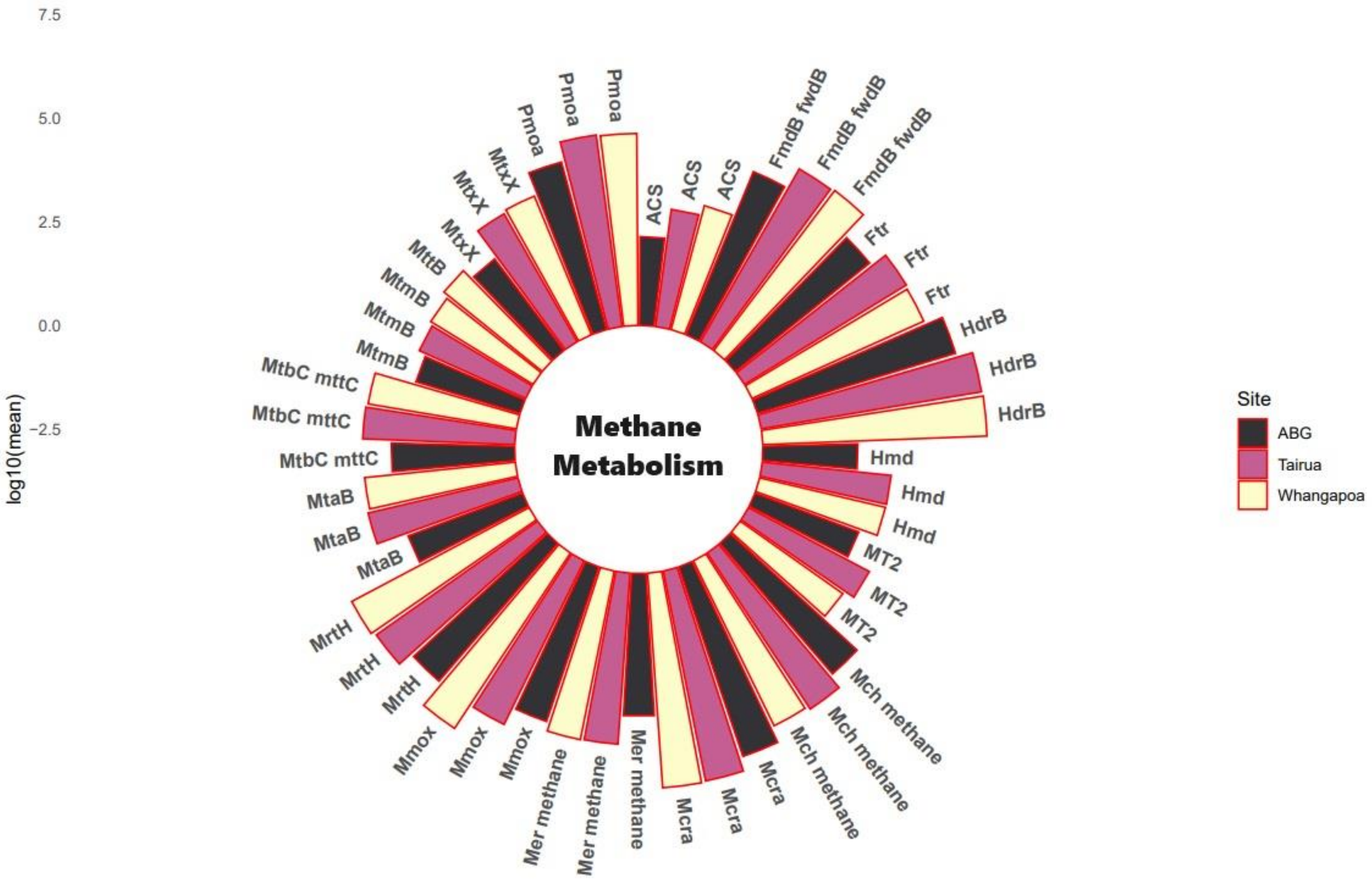


Figure 5.9: The normalized log means signal intensity of methane metabolism genes detected GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

5.4.5 Nitrogen cycle genes

The GeoChip analysis conducted on ABG, Tairua, and Whangapoa samples revealed the presence of genes associated with various nitrogen cycling processes. These processes included ammonification, anammox, assimilation, assimilatory N reduction, denitrification, dissimilatory N reduction, N assimilation, nitrification, and nitrogen fixation (figure 5.10). The analysis detected *gdh*, *glna* fungi, and *urec* genes associated with ammonification, Hzo and Hzsa gene families associated with anammox, narb, nasa, NiR, Nira, and Nirb associated with assimilatory N reduction, CnorB, Narg, Nirk, Nirs, Norb, and Nosz associated with denitrification, Napa and Nrfa associated with dissimilatory N reduction, P450nor, nitrate reductase from N assimilation, Amoa, Amoa_quasi, and Hao associated with nitrification, and *Nifh* associated with nitrogen fixation (Figure 5.12).

The Narg gene family was found to be the highest-detected gene family in the N-cycling gene category across all samples. This gene family was detected from 46, 55, and 51 species from archaea and bacteria in ABG, Tairua, and Whangapoa samples, respectively.

Genes related to the nitrogen cycle were found in various organisms present in ABG samples. These organisms include three phyla of archaea, eighteen different bacterial phyla, class Chlorophyta, class Echinodermata, class Ascomycota, and Basidiomycota. The Tairua and Whangapoa samples were found to be associated with three archaeal phyla, fifteen bacterial phyla, one fungal phylum, and one phylum from the kingdom Viridiplantae. Among the fungal divisions, only the Ascomycota class was detected. Bacillariophyta, Bangiophyceae, Basidiomycota, and Phaeophyceae were only found in ABG samples (Figure 5.11).

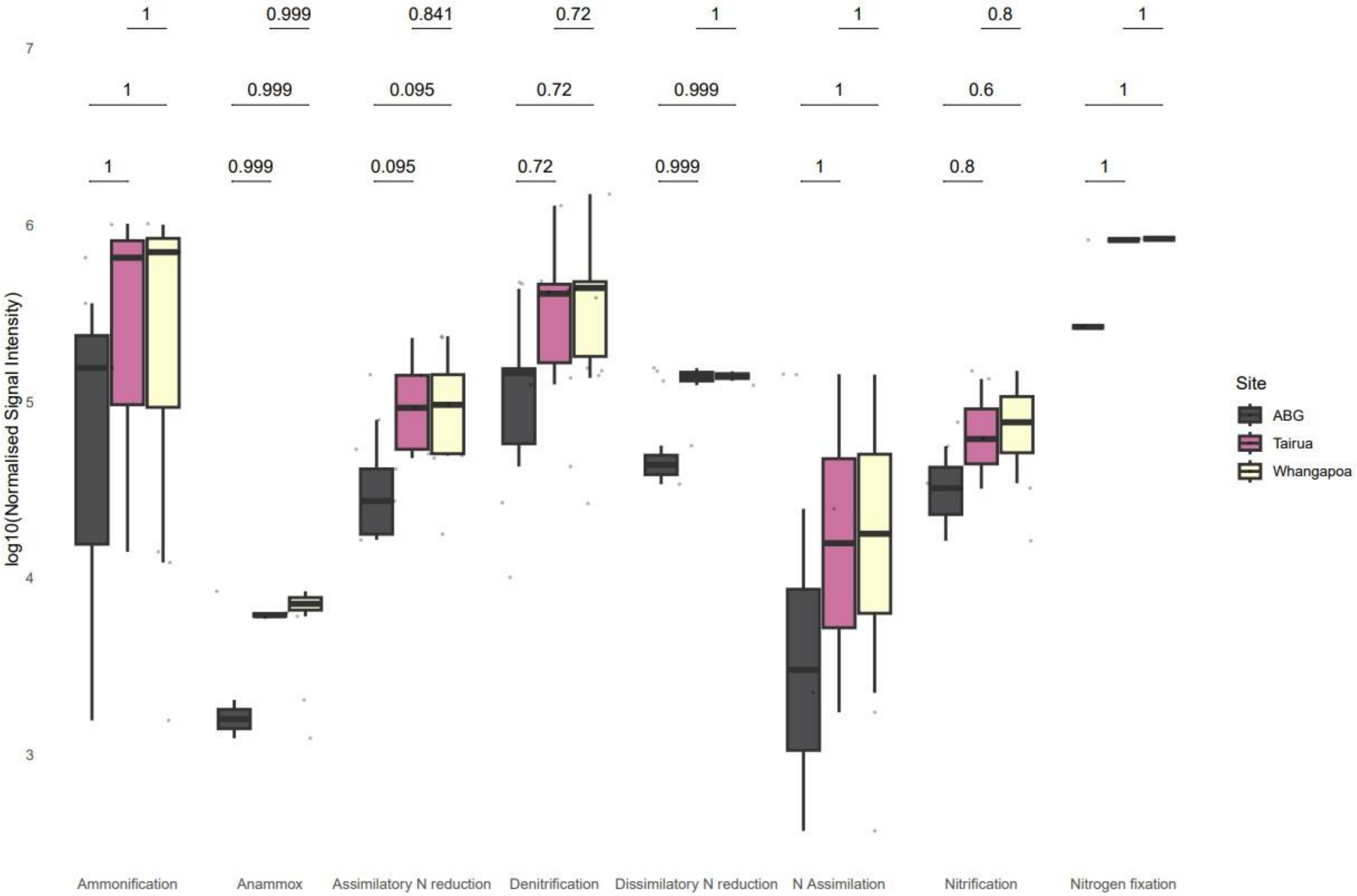


Figure 5.10: Normalised signal intensity (log10) of Nitrogen cycling gene categories detected in ABG, Tairua and Whangapoa kauri forests.

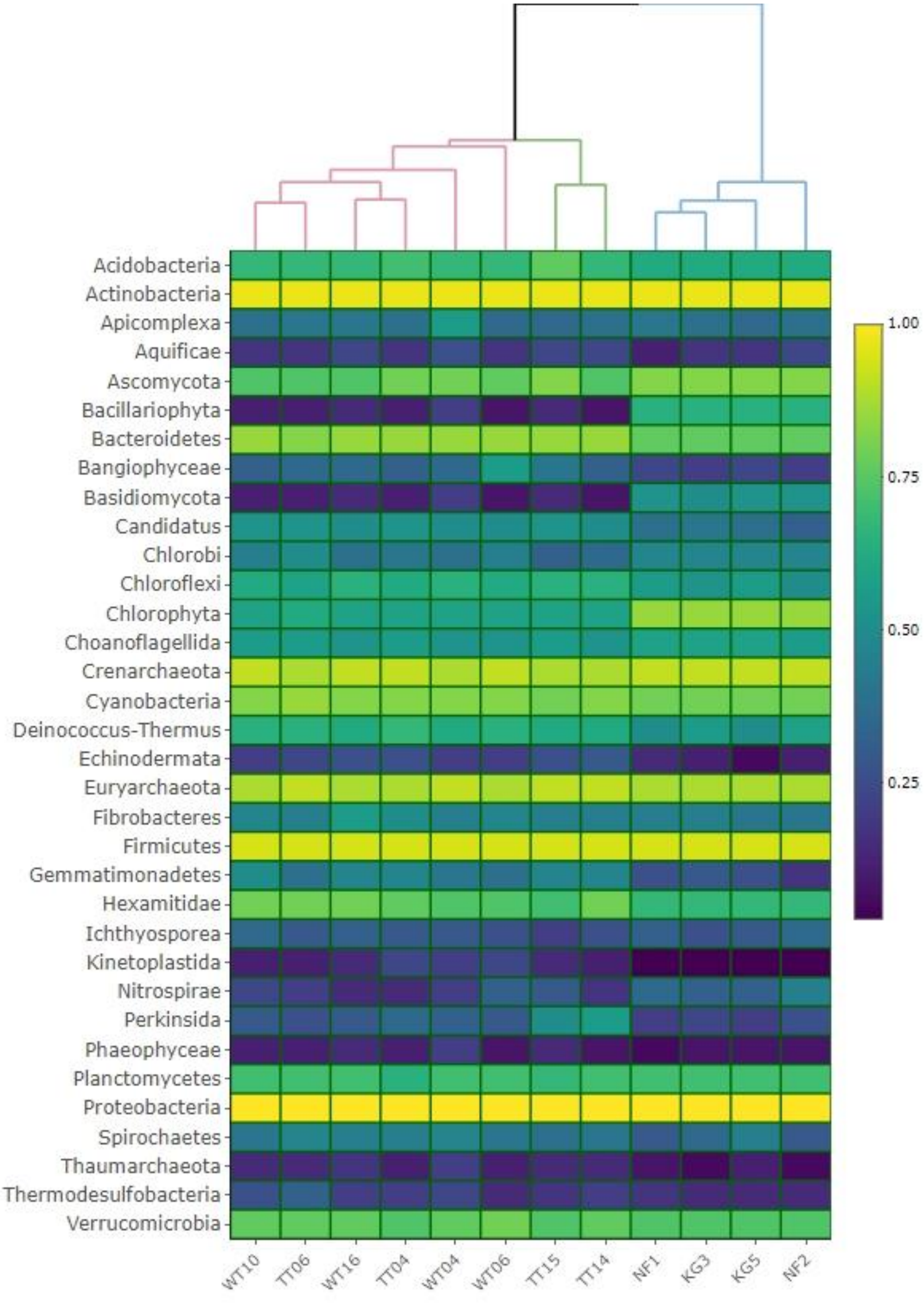


Figure 5.11: Taxonomy of genes detected in Nitrogen cycling genes at ABG, Tairua and Whangaopoa kauri forest. NF1, NF2, KG3, and KG5 represent kauri tree samples from ABG, TT04, TT04, TT14 and TT15 from Tairua and WT04, WT06, WT10 and WT16 and Whangaopa.

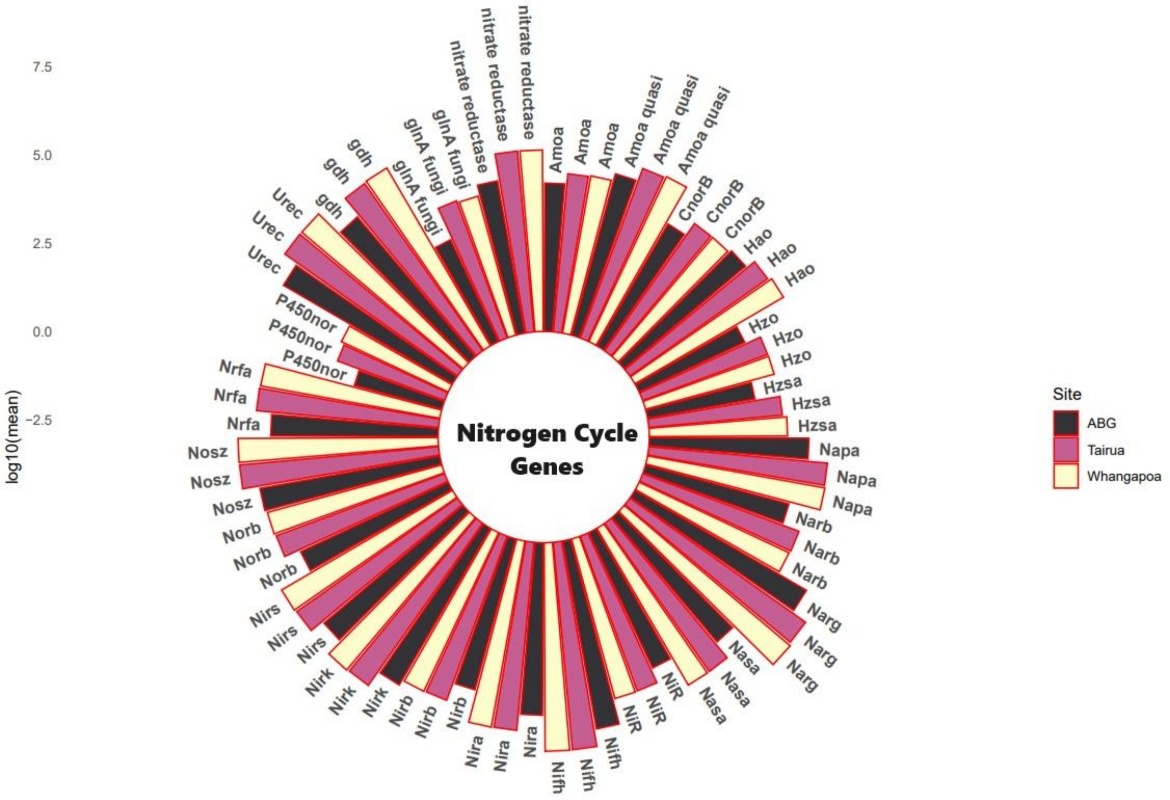


Figure 5.12: The normalized log means signal intensity of nitrogen cycling genes detected GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

5.4.6 Phosphorus Cycling

The presence of genes associated with phosphorus oxidation, phytic acid hydrolysis, polyphosphate degradation, and polyphosphate synthesis was detected in samples collected from ABG, Tairua, and Whangapoa (Figure 5.13a). The 5f1 htxA and 5f1 ptxD gene families were linked to phosphorus oxidation, while phytase was associated in phytic acid hydrolysis. The genes 5f1 ppk2, 5f1 ppn, and ppx were identified relating to polyphosphate degradation, and the ppk gene was detected involving polyphosphate synthesis (figure 5.13b). These genes were present in all the samples analysed, indicating their potential significance in the respective ecosystems. Polyphosphate degradation was the highest detected phosphorus cycling-related gene subcategory in all three samples. Among the gene families, ppx of phosphate degradation was the highest detected gene family in the phosphorus cycling gene category, this gene family was detected from 409, 435, and 403 species from archaea, bacteria, and Ascomycota, respectively. Genes related to the phosphorus cycle were found in samples from ABG, Tairua, and Whangapoa, covering 18 bacterial phyla, one archaeal phylum, as well as Ascomycota and Basidiomycota (Figure 5.14).

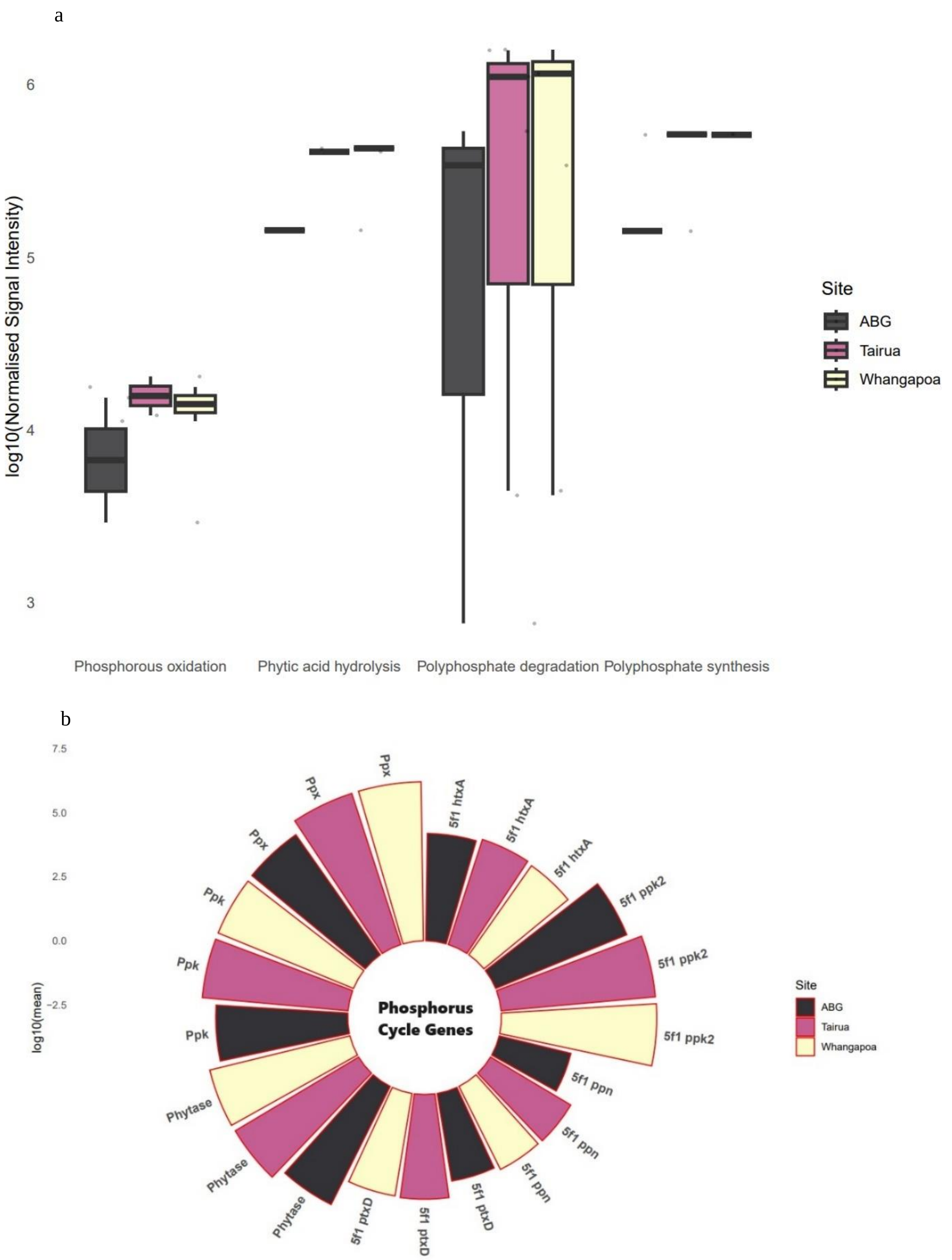


Figure 5.13: The normalized signal intensity of phosphorus cycling gene categories (a) and phosphorus cycling genes (b) detected GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

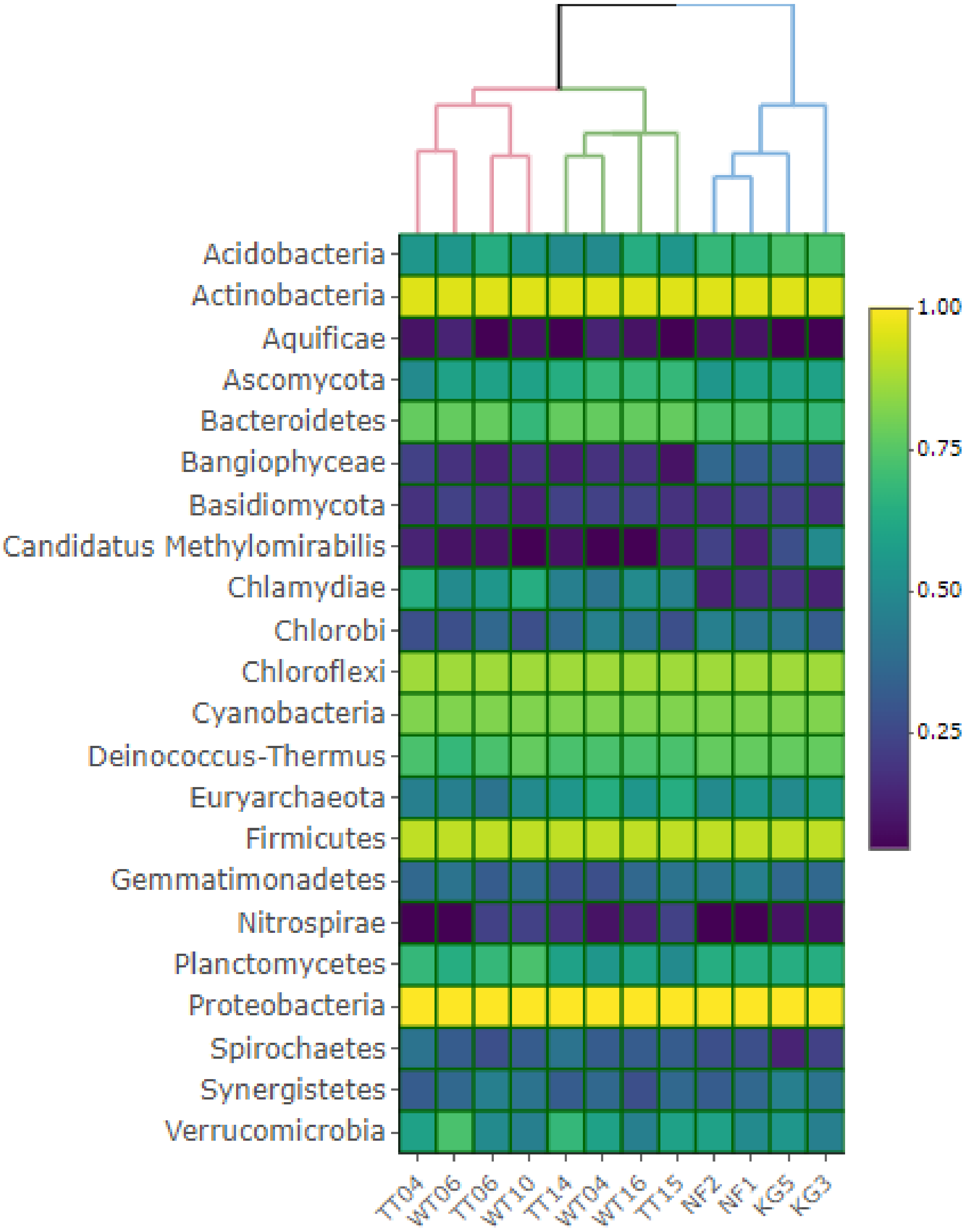


Figure 5.14: Taxonomy of genes detected in Phosphorus cycling genes at ABG, Tairua and Whangaopoa kauri forest. NF1, NF2, KG3, and KG5 represent kauri tree samples from ABG, TT04, TT04, TT14 and TT15 from Tairua and WT04, WT06, WT10 and WT16 and Whangaopa.

5.4.7 Microbial Defence

The ABG, Tairua, and Whangapoa samples detected genes related to antibiotic resistance, antimicrobial biosynthesis, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), and environmental toxins. The number of gene families detected in each subcategory was 18, 10, 48, and 2, respectively (Figure 5.15).

Microbial defence genes detected in samples collected from ABG, Tairua, and Whangapoa were associated with three archaeal phyla, twenty-two bacterial phyla, Ascomycota and Basidiomycota phyla of fungi, Arthropoda, and kingdom Viridiplantae (Figure 5.16).

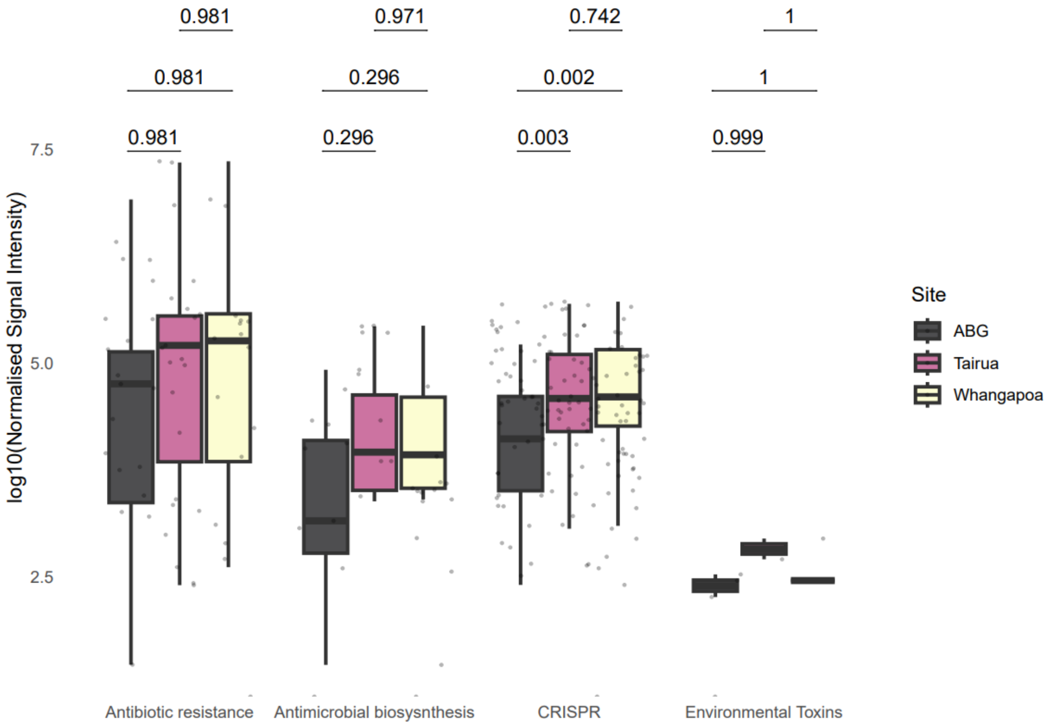


Figure 5.15: Normalised signal intensity (log10) of microbial defence gene categories detected in ABG, Tairua and Whangapoa kauri forests.

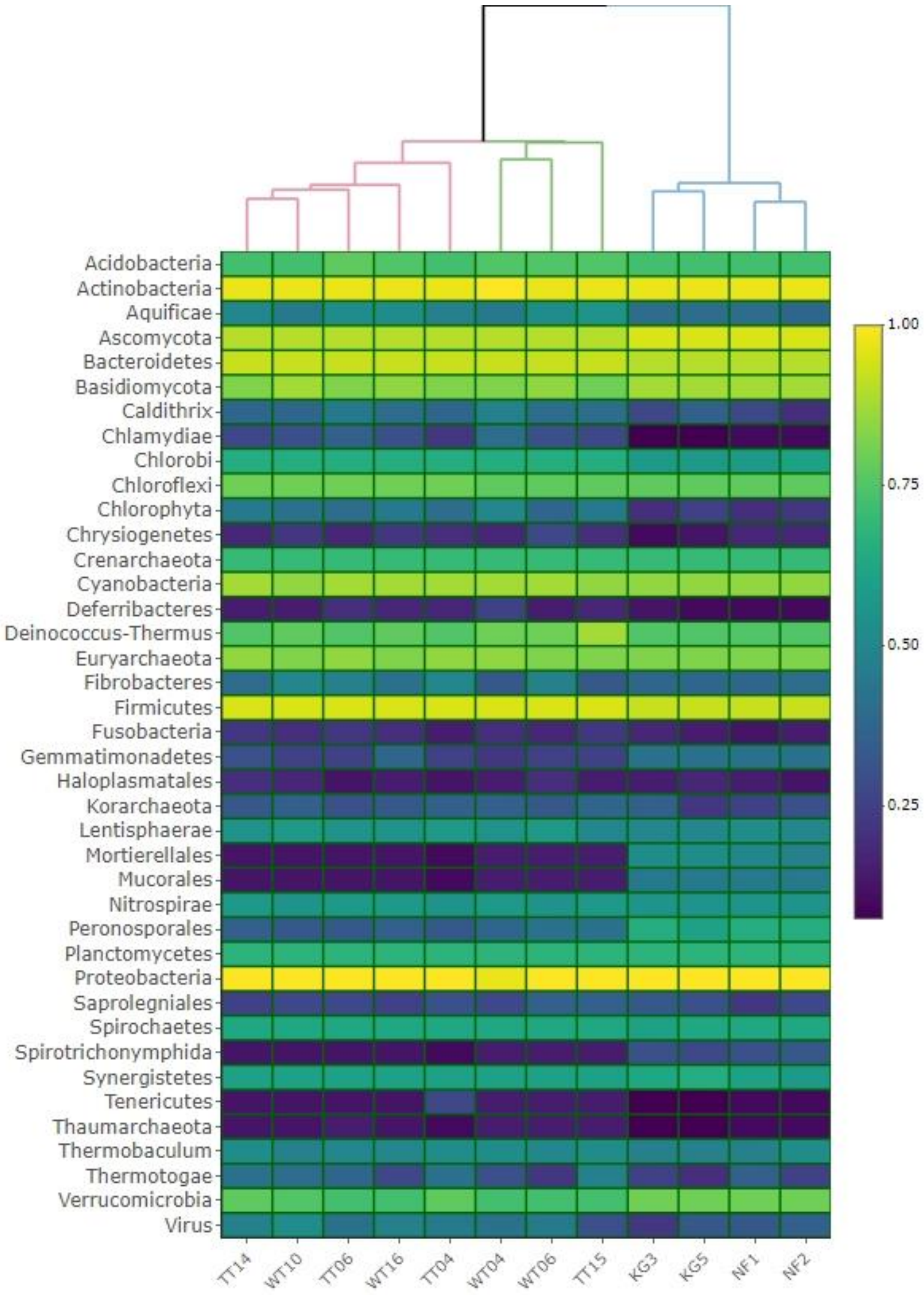


Figure 5.16: Taxonomy of genes detected in Microbial defence genes at ABG, Tairua and Whangaopoa kauri forest. NF1, NF2, KG3, and KG5 represent kauri tree samples from ABG, TT04, TT04, TT14 and TT15 from Tairua and WT04, WT06, WT10 and WT16 and Whangaopa.

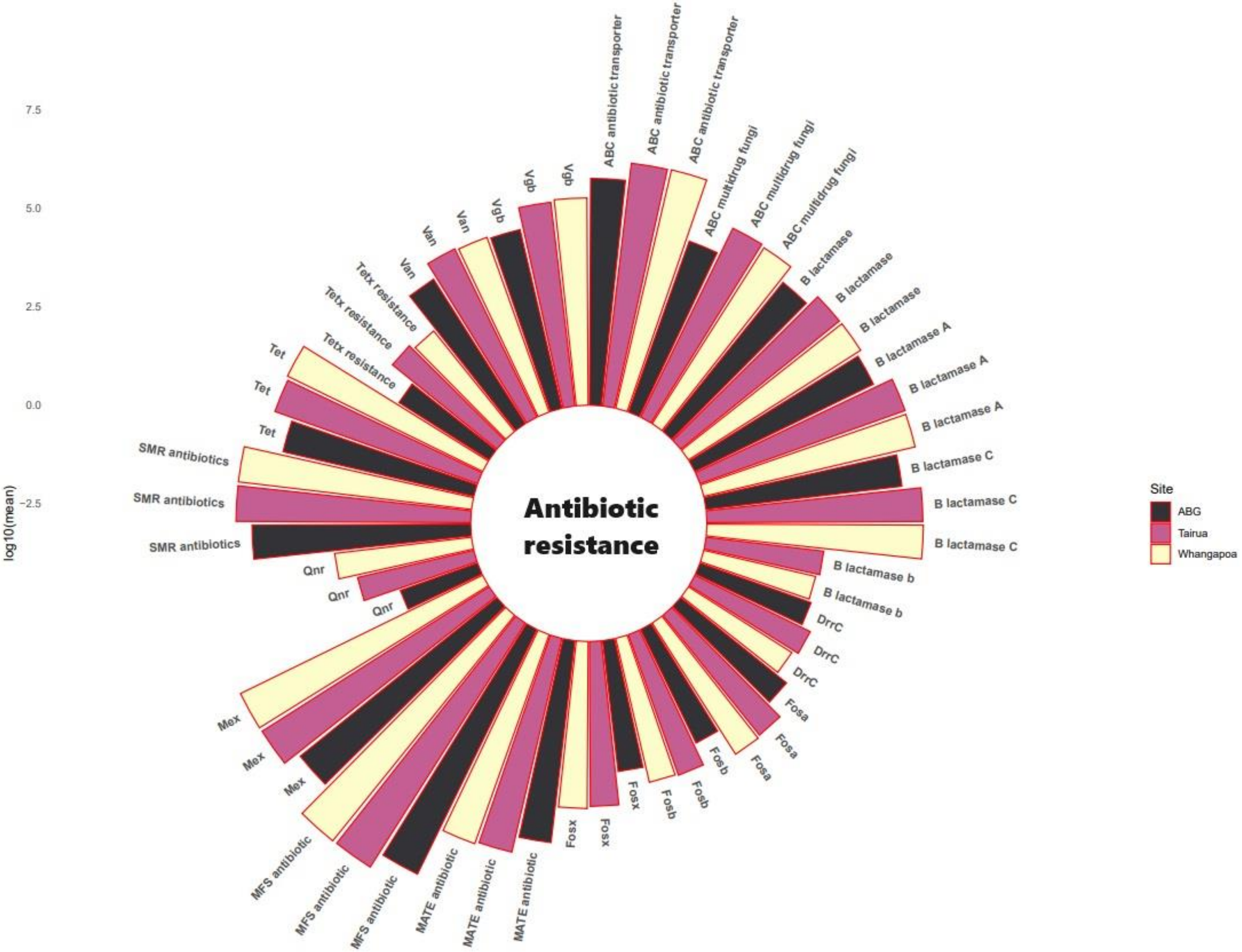


Figure 5.17: The normalized signal intensity of antibiotic genes subcategories detected by GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

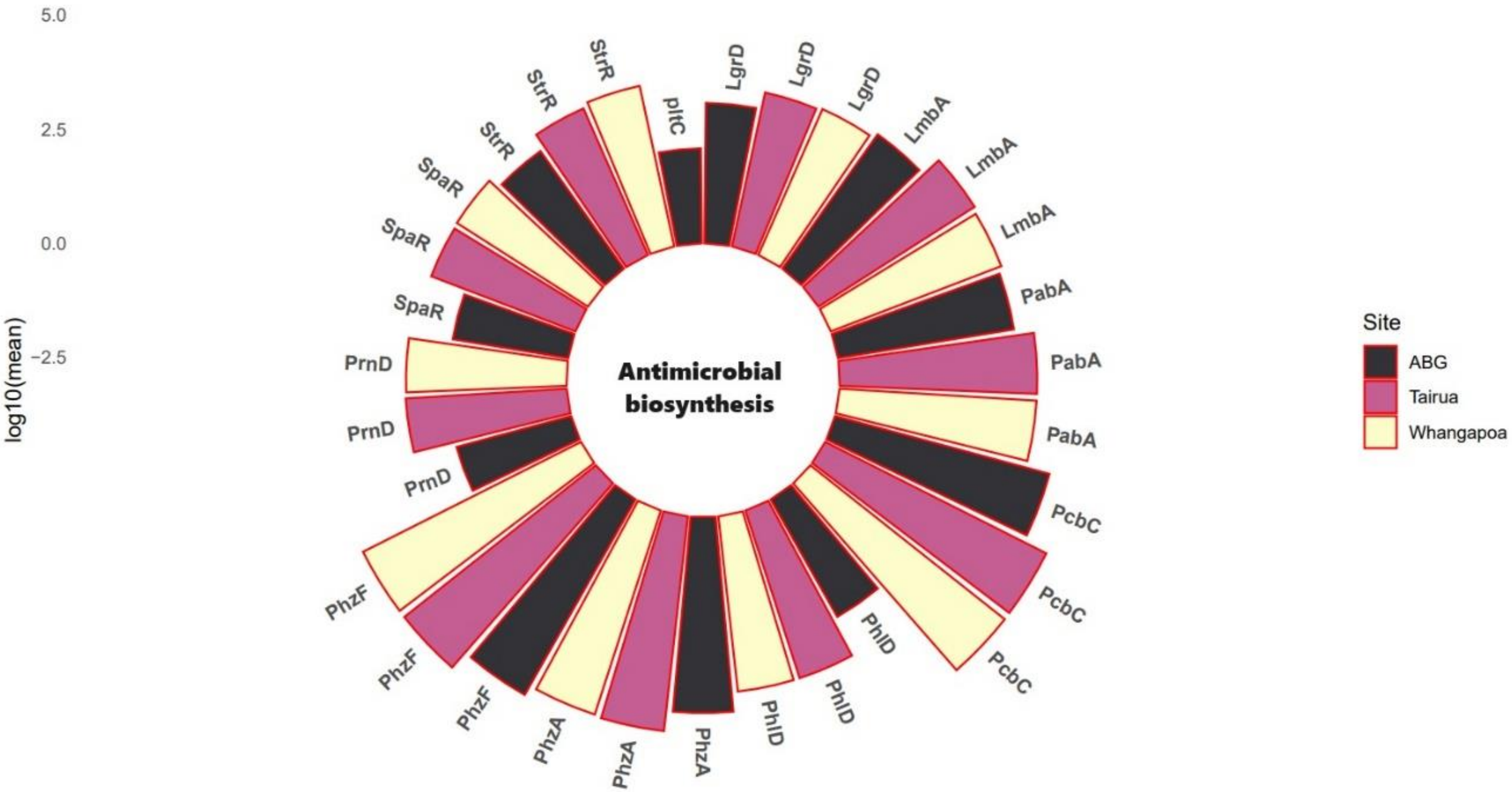


Figure 5.18: The normalized signal intensity of antimicrobial biosynthesis genes subcategories detected by GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

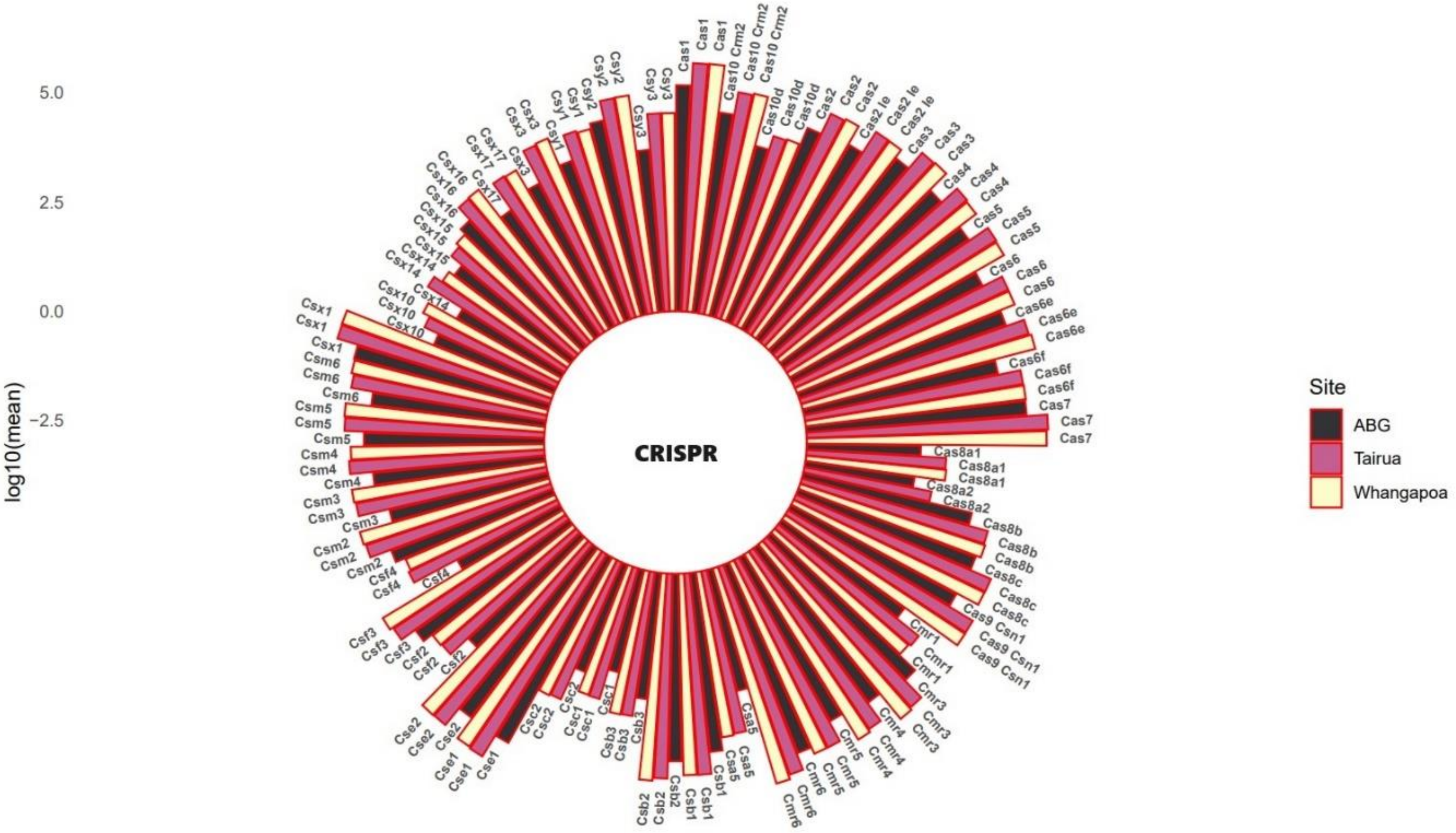


Figure 5.19: The normalized signal intensity of CRISPR genes subcategories detected by GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

5.4.8 Plant growth promotion genes

Antioxidant enzymes, anti-pathogens, disease suppression, drought tolerance, and phytohormones gene subcategories were detected. The anti-pathogen subcategory had the highest number of detected gene families (Figure 5.20). Plant growth promotion genes were identified in the samples from ABG, Tairua, and Whangapoa and were linked with two archaeal phyla, 22 bacterial phyla, Ascomycota, and Basidiomycota phyla of fungi (Figure 5.21).

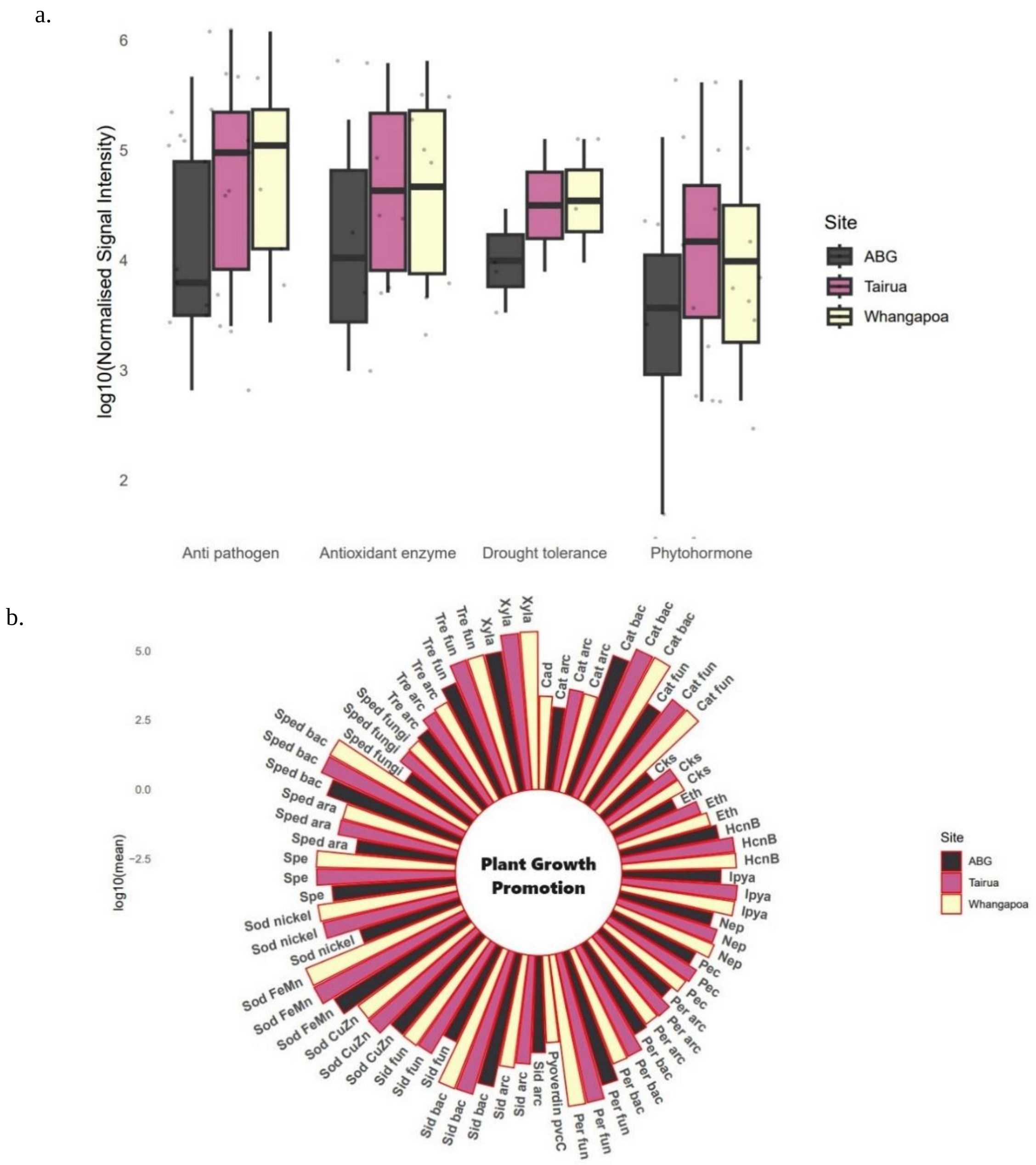


Figure 5.20: The normalized signal intensity of plant growth promotion gene categories (a) and plant growth promotion genes (b) detected GeoChip 5.0 in kauri soil at ABG, Tairua and Whangapoa.

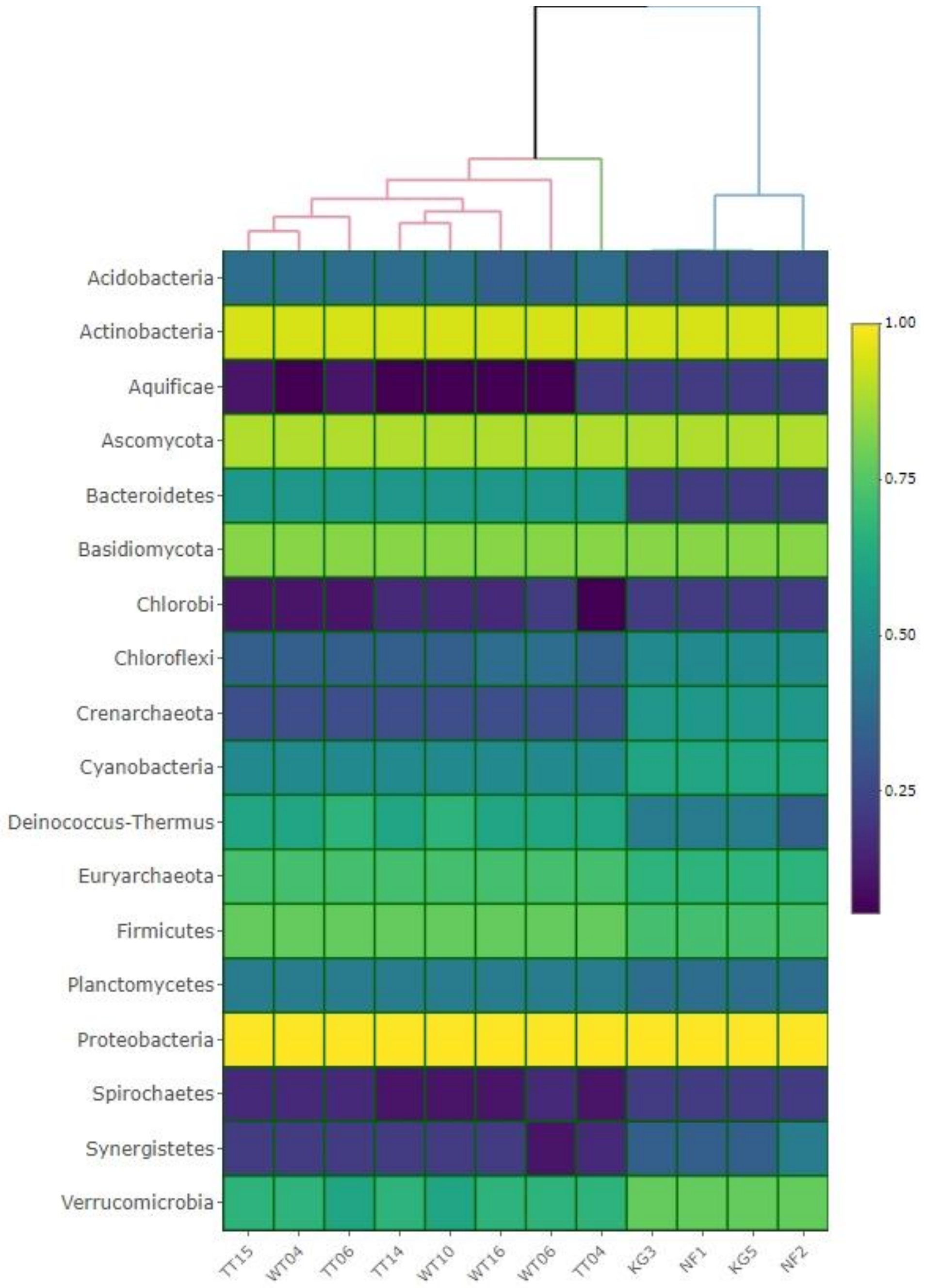


Figure 5.21: Taxonomy of genes detected in Plant growth promotion genes at ABG, Tairua and Whangaopoa kauri forest. NF1, NF2, KG3, and KG5 represent kauri tree samples from ABG, TT04, TT04, TT14 and TT15 from Tairua and WT04, WT06, WT10 and WT16 and Whangaopa.

5.5 Discussion

5.5.1 Carbon cycling genes

The major carbon changes within forest ecosystems are the accumulation of living biomass, dead biomass, herbivore, and respiratory losses (Scott & Giardina, 2008). Litterfall is the major input of organic material to the forest floor (Berg & Meentemeyer, 2001) and constitutes a significant proportion of forest carbon deposits (Clark et al., 2016). Since a large proportion of nutrients are tied up in living organic matter, litterfall and decomposition are vital for regulating nutrient availability and ecosystem productivity (Cuevas & Medina, 1986). Litterfall dynamics influence all nutrient cycles because the quantity, type and quality of material reaching the forest floor in different seasons are strong determinants of element cycling (Prescott, 2002).

Kauri forests are known to store vast amounts of carbon (Silvester & Orchard, 1999b) and nitrogen (Silvester & Orchard, 1999a) and are ranked as one of the most carbon-dense ecosystems in the world. New Zealand's kauri forests are incredibly carbon-dense, with kauri trees themselves contributing to most of the forest's carbon inputs (Macinnis-Ng & Schwendenmann, 2015). Depending on the carbon substrates available and the ability of microbial taxa to produce the appropriate enzyme, different taxonomic groups will specialise substrates (i.e., sucrose, cellulose, or lignin). Additionally, the products of enzymatic breakdown attract opportunistic microorganisms, further influencing the composition and function of resident microbial communities (Schimel & Schaeffer, 2012). Our study showed significantly higher carbon cycling genes in natural kauri forests than in ABG. This may be explained by the higher carbon litter present in the natural kauri forest, and the kauri trees in the ABG site are only 20 years old, so litterfall may have been lesser than forest kauri trees. Similarly, Waipoua kauri soil had a greater number of carbon degradation genes due to the presence of high litter in the kauri forests (Byers, Condron, O'Callaghan, et al., 2020c).

Our study detected that the overall C cycling functional genes included C fixation genes, C degradation genes, and methane metabolism genes. The main functional gene families of C fixation found in this study included RubisCO, pcc, and CODH in the Calvin cycle, indicating that the Calvin cycle may be the most important C fixation pathway in the kauri soil. This finding follows those of previous studies (Tahon et al., 2018; Xiao et al., 2014) showing that the Calvin cycle is the most important pathway for autotrophic microorganisms to fix CO_2 from the atmosphere in forest ecosystems (Jansson et al., 2010).

Soil microbial functional genes responsible for degrading starch, hemicellulose, cellulose, and lignin are major biogeochemical processes in the soil ecosystem. These genes were detected in ABG, Tairua, and Whangapoa kauri soil. The C degradation genes in the soil established a dominant community, with the highest signal intensity from Proteobacteria and Actinobacteria, which may be the most prevalent bacteria in kauri soil. Soil bacteria play a crucial role in degrading starch, chitin, and hemicellulose. Both soil fungi and bacteria function in cellulose degradation, while soil fungi are the major group involved in lignin degradation (Janusz et al., 2017). The findings align with the results of previous research, indicating that fungi have a vital function in decomposing resistant carbon, such as lignin, in the soil with the help of their oxidase activities and hyphal structures.

Meanwhile, bacteria are generally responsible for decomposing labile organic matter, such as starch, in the soil (Moreno-Espíndola et al., 2018). The genes *amyA*, *ara*, *glx* and enzymes glucoamylase, xylanase, and phenol-oxidase are linked to Proteobacteria, Actinobacteria and Firmicutes in bacteria, and Ascomycota and Basidiomycota in fungi. These genes are essential predictors of enzyme activity to use moderately labile and recalcitrant forms of carbon sources (Trivedi et al., 2017).

5.5.2 Nitrogen and Phosphorus Cycling genes

Nitrogen and phosphorus concentrations were lower in the kauri soil (Silvester, 2000; Thomas & Spurway, 1993). Kauri seedlings have low nutrient requirements, and it is thought that kauri seedlings are better able to compete with faster-growing angiosperm seedlings on sites of low fertility than on sites of higher fertility (Burns & Leathwick, 1996; Pook, 1979). Therefore, a decrease in nutrient availability caused by the accumulation of kauri litter will improve the conditions for kauri regeneration relative to other species. The thickness of the organic layer and kauri diameter were positively correlated, suggesting that organic material accumulates with the increasing age of the kauri tree. This may indicate in our study that the availability of carbon cycling genes and signal intensity is higher than nitrogen and phosphorus cycling genes. However, the availability of gene families of ammonification, anammox, assimilation, assimilatory N reduction, denitrification, dissimilatory N reduction, N assimilation, nitrification, nitrogen fixation phosphorus oxidation, phytic acid hydrolysis, polyphosphate degradation, and polyphosphate synthesis in kauri soil microbial community suggest that cycling of nitrogen and phosphorus is evidenced.

Kauri forest soils have low nitrification rates due to their acidic soil, slow litter decomposition rate, and high tannin content in leaf litter, which results in large stores of immobilised nitrogen (Wyse et al., 2014). Our study evidenced this as the higher signal intensity and potential gene families of denitrification genes were detected compared to nitrification genes in kauri soil.

A previous study has found that increased concentrations of *P. agathidicida* DNA in forest soil were connected to reduced soil C and N concentrations (Schwendenmann & Michalzik, 2021). This contradicts previous findings that soil N concentrations increase after the death of the tree due to increased inputs of N-rich litter and reduced plant N uptake (Edburg et al., 2012; Xiong et al., 2011). However, the study showed no significant changes in the potential N cycling gene signal intensities between kauri dieback symptomatic and non-symptomatic trees.

5.5.3 Microbial Defense

Very few studies were reported on microbial defence genes and plant growth-promoting genes related to kauri soil. 80 gene families of antibiotic resistance, antimicrobial biosynthesis, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), and environmental toxins were detected in ABG, Tairua and Whangapoa kauri soil. This indicates kauri soil potentially consists of microbes with the potential to resist microbial pathogens. According to a recent study (Zhang et al., 2022), *Pseudomonas aurantiaca ST-TJ4* is capable of suppressing *Phytophthora cinnamomi* by producing phenazine antimicrobial and volatile organic compounds (VOCs) (Y. Zhang et al., 2022). The current study also identified the antimicrobial biosynthesis gene *PhID*, involved in producing phenazine, from *Streptomyces cattleya* and *Saccharo polyspora*. *Lysobacter enzymogenes* has the potential to produce chitinase, a carbon degradation enzyme that was detected in our analysis that can control a variety of plant *Phytophthora* diseases caused by three significant Oomycetes (*P. sojae, P. capsici and P. infestans). Lysobacter enzymogenes* inhibit mycelial growth, suppressing cyst germination, and eliciting plant immune responses against *Phytophthora* (Lin et al., 2023).

5.5.4 Plant Growth Promotion

The detection of plant growth promotion genes indicates that kauri soil consists of potential microorganisms that can enhance the growth of kauri. Plant growth-promoting microorganisms (PGPM) can establish symbiotic associations and protect plants against plant pathogens by having anti-pathogen genes or promote tolerance to abiotic stresses, such as salinity or drought tolerance (Dhawi, 2023; Qiu et al., 2019). PGPM may serve as biofertilisers for promoting plant growth through several mechanisms, such as increasing nutrient availability by solubilisation, phosphate and zinc or sequestration of iron by siderophores (H. Rodriguez et al., 2004). Other mechanisms include changes in phytohormone levels by producing indole-acetic acid (IAA) or modulation of ethylene levels by ACC deaminases (Ali & Kim, 2018; Idris et al., 2009). Additionally, bacteria can produce volatiles (e.g., volatile organic compounds/VOCs, sulphur-containing compounds, or indole) that are used to communicate with other microbe or to promote growth and/or stress tolerance (Weisskopf et al., 2021). The generation of oxygen radicals can be further worsened during environmental adversity, and consequently, superoxide dismutase SOD enzymes have been proposed to be essential for plant stress tolerance. Three forms of the enzyme detected in kauri soil of ABG, Tairua and Whangapoa, which are classified by their active site metal ion: copper/zinc (SodCuZn), manganese (SodFeMn), iron and Sodnickel nickel forms.

5.6 Conclusion

GeoChip 5.0M was used to analyze the functional genes of soil microbes found in kauri soils at the Auckland Botanic Gardens (ABG), Tairua, and Whangapoa kauri forests in Aotearoa New Zealand. The findings suggest that there are significant differences in the signal intensities of carbon cycling genes and microbial defence genes between the ABG region and both Tairua and Whangapoa. Moreover, the normalised signal intensity of carbon degradation genes in Tairua and Whangapoa kauri soil was significantly higher than in ABG kauri soil due to litter formation in the natural kauri forests. The detected genes involved in various processes of the nitrogen cycle in kauri soil, such as ammonification, Anammox, assimilation, assimilatory N reduction, denitrification, dissimilatory N reduction, N assimilation, nitrification, and nitrogen fixation. Additionally, genes involved in phosphorus cycle processes such as phosphorus oxidation, phytic acid hydrolysis, polyphosphate degradation, and polyphosphate synthesis were also detected. Several critical genes, such as *PhID*, have been detected related to microbial defence. These genes produce antimicrobial substances that can suppress the growth of several *Phytophthora* species. The kauri soil has been found to contain several plant growth-promoting genes, such as pathogen, phytohormones, drought tolerance, and stress tolerance genes. This is the first step towards understanding how microbial functional gene potentials could explain above ground conditions such as plant health. Further research is necessary to analyse the expressed genes during different seasons and in various environments, as well as the kauri dieback health status.

Chapter 6

Screening of *Agathis australis* endophytes as biological control agents against kauri dieback pathogen *Phytophthora agathidicida*

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In this chapter, the potential of fungal endophytes isolated from kauri roots to affect the growth of *P. agathidicida* was investigated. Seventeen endophytic fungal isolates were examined for their morphology and ability to antagonise the pathogen *P. agathidicida*. Five fungal endophytes exhibited growth suppression of the pathogen in dual culture. *Pezicula* sp. partially inhibited the growth of *P. agathidicida*, whereas *Coprinellus micaceus* and *Ilyonectria mors-panacis* completely inhibited the growth of the pathogen. No oospores were observed in the dual culture, nor was the *P. agathidicida* viable when transferred into fresh culture media. Chapter 6 has been published in the Springer Journal of Plant Pathology (Lawrence et al., 2024).

Screening of *Agathis australis* endophytes as biological control agents against kauri dieback pathogen *Phytophthora agathidicida*

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6.1 Abstract

Agathis australis (Kauri) is a significant and iconic native tree of Aotearoa, New Zealand. A plant disease known as kauri-dieback is currently affecting kauri trees. The aetiology of the disease has been associated with *Phytophthora agathidicida* (PA) which poses severe threat to kauri at individual and population levels. Currently, the only treatment available is the injection of phosphonate into the trees.

We investigated the potential of fungal endophytes to influence the growth of PA. Seventeen previously isolated endophytic fungi from kauri roots were examined morphologically and the antagonistic effects against the plant pathogen. Five of the 17 fungal endophytes tested demonstrated growth suppression of the pathogen in dual culture. *Pezicula* sp. partly inhibited the growth of *P. agathidicida* whilst *Coprinellus micaceus* and *Ilyonectria mors-panacis* completely inhibited the growth of the pathogen. Oospores were not observed in the dual culture nor was the *P. agathidicida* viable when transferred into fresh culture media.

This study illustrates that naturally occurring fungal species in kauri roots can suppress the growth of PA. This finding offers a possibility of a biological control for dieback disease in kauri which may contribute to the mitigation of natural disease management and biocontrol of plant diseases without compromising the natural ecosystem.

Key words: biocontrol, fungi, plant, pathogen, dual-culture

6.2 Introduction

Agathis australis (kauri) is endemic to Aotearoa, New Zealand and belongs to the ancient family of coniferous trees, Araucariaceae. Māori have a significant relationship with kauri as it is considered as one of their taonga (treasure) which is also evident in their Māori traditions, artwork, folktales, and legends. Virgin kauri forests then covered more than 1.5 million hectares of Aotearoa/New Zealand (Halkett, J., 1980). Subsequently, due to its timber appearance and working properties the kauri timber and gum industries were established (Cheeseman et al., 2011; Steward, 2011). This led to the harmful misuse of the species, completely changing the upper North Island's forest landscape (Steward, 2011). Large proportion of the kauri forest was also transformed into farmlands. To date, only 7,500 hectares of primary virgin kauri forests remains, and 60,000 hectares of secondary kauri forests, or savannah containing regenerating kauri. Much of these forests are restricted to remote locations on the Great Barrier Island, Coromandel Peninsula, and the North Auckland Peninsula (Halkett, J., 1980).

At present, a *Phytophthora* disease, known as kauri dieback, is threatening the existence of kauri. *Phytophthora agathidicida* is the primary causal agent of the kauri-dieback disease throughout lowland stands in Northern New Zealand (Beever et al., 2007; Weir et al., 2015). *P. agathidicida* infects the roots and damages the tissues that distribute nutrients within the tree to cause collar rot in all age groups of kauri trees (Beever et al., 2007). Several symptoms arise as a result, including the characteristic root and collar rot, resin exuding lesions, severe chlorosis, canopy thinning and widespread tree mortality (Bassett et al., 2017; Waipara et al., 2013). Considering the current distribution of kauri forests and the widespread dispersal of *P. agathidicida* throughout major kauri stands (e.g. Waitakere Ranges and Waipoua Forest) in Northern New Zealand, this pathogen poses a significant threat to the long-term survival of this iconic tree species (Bassett et al., 2017; Waipara et al., 2013).

Control agents such as phosphonate effectively control many plant diseases caused by *Phytophthora* spp. (Horner & Hough, 2013b; Ij & Eg, 2014). Likewise, in recent glasshouse studies on 2-year-old kauri seedlings, phosphonate protected *P. agathidicida*; phosphonate injection into the trunk resulted in a 100% survival rate following soil inoculation. Despite the evidence for phosphonate protection against *P. agathidicida*, there are ongoing concerns with phytotoxicity and interrogations about the commercial practicability of widespread use of phosphonate as a control measure. Phytotoxic symptoms (e.g., leaf yellowing) were observed in 20% of phosphonate-injected individuals (Ij & Eg, 2014). Furthermore, applying phosphonate to entire kauri forests was recognized as unsustainable given the huge costs that would be involved (Ij & Eg, 2014). Therefore, fungicide usage (i.e., phosphonate) will likely only supplement other disease management strategies currently in place to mitigate kauri dieback, with a specific focus on shielding the largest and most notable kauri individuals.

One approach gaining more attention is the role of endophytes in plant health. Endophytes are microorganisms that live inside plant tissues and are not harmful to the host plant (Hallmann et al., 1997). Endophytes inhabit most plant and have been isolated from various plants species (Ziadi et al., 2016). They live in intra- and intercellular spaces of the plant tissue interacting with the hosts, and a diverse array of species has been reported to be endophytic (Elbeltagy et al., 2001). Colonization may take place at the local tissue level or throughout the plant, with microbial colonies and biofilms residing latently in the intercellular spaces and inside the vascular tissues (Gage et al., 1996; Gopalaswamy et al., 2000; Hinton & Bacon, 1995; Sessitsch et al., 2002). (Sturz & Nowak, 2000)), proposed that these endophytes originated from the rhizosphere or phylloplane micro-flora and observed that many rhizosphere bacteria could penetrate and colonize root tissue, providing a route into the xylem. In this vascular tissue, the microbes could transport themselves throughout the plant and colonize it systemically. Once inside the plant, endophytic populations have been observed to grow between 2.0 and 7.0 log10 cells per gram of fresh tissue (McInroy & Kloepper, 2007; Shishido et al., 1999).

Specific endophytes have demonstrated to enhance plant health. The fungal endophyte from Yew tree *Paraconiothrium* SSM001 increase plant resistance to pathogens (Soliman et al., 2015). Norway spruce root endophyte *Phialocephala sphareoides* was able to inhibit phytopathogens *Heterobasidion parviporum*, *Phytophthora pini*, *Botrytis cinereal* under in vitro conditions and promote root shoot ratio (Terhonen et al., 2016). *Colletotrichum tofieldiae*, a fungal endophyte of *Arabidopsis thaliana*, transfers macronutrient-phosphorus to shoots and promotes plant growth (Hiruma et al., 2016) (Azevedo et al., 2000; Bacilio-Jiménez et al., 2001; Iwai et al., 2010; James, 2000; Shishido et al., 1999; Sturz & Nowak, 2000).

Dark septate endophytes (DSE), belonging to the class 4 endophytes, colonize their hosts' roots (R. J. Rodriguez et al., 2009). They are conidial or sterile septate fungal endophytes that form melanized structures, including inter- and intracellular hyphae and microsclerotia in the roots (Jumpponen & Trappe, 1998; Tellenbach et al., 2013). These assemblages were found in almost all natural ecosystems, particularly stressful environments. In some cases, several DSEs have been reported to exhibit tolerance to stressors under *in vitro* culture conditions (Berthelot et al., 2016; Likar & Regvar, 2013; Zhan et al., 2015)

Biological control agents (BCAs), whose primary mode of action is through competition, sustaining high environmental population levels is essential to suppress target pathogens (Alabouvette et al., 2006). Moreover, the ecotoxicological risk and associated risk assessments required to facilitate their application are much lower as they inhibit pathogens through general ecological processes rather than the production of antimicrobial compounds (Köhl et al., 2019).

The diversity and the distribution of endophytes with the potential to be BCAs living in *A. australis* roots are poorly understood. In this study, we demonstrated formerly isolated fungal endophytes from *A. australis* roots to suppress the growth of *P. agathidicida*. This study is an initiative to screen endophytes for inhibiting the growth of *P. agathidicida* and highlights the need to further investigate their potential as biological control agents for the kauri dieback pathogen.

6.3 Materials and Methods

Endophytes evaluated in this study were previously isolated from *A. australis* root tissues. (NSF Award Search: Award # 1613884 - EAPSI: Identifying Fungi Associated with the Ancient Conifer Agathis Australis and Testing their Potential as Biological Control Agents against Harmful Pathogen). Root samples were taken from rickers (age range from 150 years to 300 years) located at Waitakere ranges, and no tap roots were sampled in this study; fine roots were collected (approximately 3g per sample). Roots were washed, surface-sterilized, and cultured in Potato Dextrose Agar (PDA). All plates were incubated in ambient conditions at 20°C, and any growth was subcultured to obtain pure cultures for sequencing. The full ITS gene sequence data were submitted to GenBank with accession numbers listed in **Table 6.1**.

6.3.1 *In vitro Screening of endophytes for bioactivity against* Phytophthora agathidicida

Endophyte isolates from kauri roots were cultured individually in clarified V8 juice Agar (cV8A). Media was prepared by adding 200ml of Clarified V8 juice, 15g Bacto agar (Difco) and 800ml of distilled water and autoclaved at 121°C for 15 minutes (Tuite, 1969). cV8A was selected for this experiment due to its ability to induce the sporulation of *Phytophthora* species (Weir et al., 2015).

The 17 endophytes (**Table 6.1**) and *P. agathidicida* were grown individually in cV8A, at 20°C, in the dark in triplicates to obtain their growth rate. Radial growth (radius) of mycelia in millimetres was recorded every 24 hours for 30 days. Endophyte cultures which were slow growing (less than 20mm in 14 days) in cV8A were not considered in the dual-culture experiment. The growth rate of *Phytophthora agathidicida* was determined in cV8A culture media.

An *in vitro* dual culture method was employed in which an endophyte isolate, and PA were inoculated in the same Petri plate using agar plugs collected from eight to ten days old fungal cultures grown in cV8A. Endophyte isolate and PA were grown side by side in the same Petri plate using 5mm diameter plugs. The *in vitro* screening was conducted in a growth chamber set at 20 +/- 2 °C in triplicate. Control treatments had plugs of uncolonized media. The growth rate was measured every day for 35 days, and the endophyte growth inhibition effect on the pathogen were assessed using the formula:

$$\text{Growth inhibition \%} = \frac{R2 - R1}{R2} \times 100\ \%$$

in which R2 is PA mycelia radius in control plate (mm), R1 is PA mycelia radius in PA-endophyte dual culture (mm). Five endophytes in **Figure 6.1** were screened for potential as biological agents (BCAs) against kauri dieback pathogen *Phytophthora agathidicida.*

Microscopic examination of the fungal structure was carried out using a compound light microscope (Leica DM750, image captured by Leica ICC50 HD camera and analyzed using Leica LAZ EZ software manufactured by Leica Microsystems (Schweiz) AG Max Schmidheiny-Str. 201 9435

Heerbrugg Switzerland). The viability and formation of oospore by the *P. agathidicida* in the dual culture was examined by taking an agar plug from the dual culture plate at the antagonistic zone where the endophyte (macroconidia) and pathogen (oospores) were present. The agar plug was inoculated on fresh cV8A plate and incubated at 20°C, in the dark for 35 days to determine viability after dual culture with endophyte. Microscopic examination of the hyphae and fruiting bodies were carried out using compound light microscope.

6.3.2 Growth rate data analysis

The mean growth rate and percentage of growth inhibition among treatments and control were compared using the Student T-test at the 5% level (p=0.05) of significance and presented as the mean values +/- standard deviation (SD).

6.3.3 Phylogenetic analysis

The full ITS gene region was used in the analysis to determine the taxonomic identity and phylogenetic placement of the endophyte isolates. The National Centre for Biotechnology Information (NCBI) BLASTn tool (https:// blast.ncbi.nlm.nih.gov/Blast.cgi) was used to infer the taxonomic identity of the isolates. Phylogenetic analyses were performed using the MEGA v11.0 software. The alignment was carried out using the Muscle algorithm of consensus ITS1, 5.8S rRNA gene, ITS 2 and large subunit of rRNA and was inspected in MEGA11 to ensure consistent reading frame, accurate gap placement, and reduction of sequence overhangs.

The model test module of MEGA 11 was used to ensure the accurate choice of substitution model and the evolutionary history was inferred using the maximum likelihood (ML) method (Harris and Stocker, 1998) based on a suitable substitution model (SYM+I+G4; corrected Akaike Information Criterion scores & weights) with 1000 bootstrap replicates. FigTree v1.4.4 was used to visualise and edit the ML tree.

The full ITS gene sequence data used in this study were deposited in the GenBank nucleotide database with the accession numbers provided in **Table 6.1**.

6.4 Results

Seventeen endophyte isolates from the research, NSF Award Search: Award # 1613884 - EAPSI: Identifying Fungi Associated with the Ancient Conifer *Agathis australis* and Testing their Potential as Biological Control Agents against Harmful Pathogen, were considered in this study (**Table 6.1**). Ten of the 17 grew on the cV8A media, and only five of these isolates were optimally grown (>20mm radial growth after 14 days at 20°C in the dark) in the cV8A, which allowed the examination of their ability to inhibit the growth of the kauri dieback pathogen *Phytophthora agathidicida* in vitro **(Figure 6.1)**.

The taxonomic assignment of each fungal isolate was determined based on the closest full ITS gene region match using the NCBI BLASTn database (**Table 6.1**). The phylogenetic analyses further support the delineation of these different isolate with >50% bootstrap support (**Figure 6.2**).

Isolates ICMP 21398 and ICMP 21347 could be the same species of *Pezicula* with 100% bootstrap support. Whilst ES107 and ICMP 21343 are identical isolates which are closely associated with the *P. melanigena* and *P. radicicola* with 99% support. ES91 and ICMP 21339 are grouped within the *Ilyonectria* sp. clade. ICMP 21358 is closely associated with *Pestalotiopsis parva* with 81% bootstrap support. ICMP 21377 has a closest BLAST match of *Sphaerostilbella novae-zealadiae* but also closely associated with *Trichoderma* sp. and *Hypomyces* sp. ICMP 21382 is associated with genus *Pencillium* sp., and ICMP 21392 is closely associated with *Leptodophora echinate* and *Cadophora constrictospora*. ICMP 21455 and LJ215 are within the Basidiomycota clade, particularly closely associate with *Coprinellus ovatus*, *Mycena seminau* and *Mysena sinar*, respectively. Lastly isolates ICMP 21345 and LJ 235; ICMP 21447 and LJ 308 are closely related with 100% and 97% bootstrap support, and all are associated with *Neolauriomyces eucalypti*, *Lareunionomyces eucalypticola* and *Hyphodiscus brachyconius*.

In vitro Screening of endophytes for bioactivity against pathogen PA

All seventeen endophyte isolates were initially grown in cV8A media. Ten endophytes were able to grow in cV8A media at 20°C in the dark. The remaining seven endophyte isolates did not grow in cV8A and were not considered in the dual-culture experiment. ICMP 21455 was the fastest growing (65mm) endophyte in cV8A, and ICMP 21398 was the slowest (13mm). ES107, ES91, ICMP 21339, and ICMP 21347 had mean growth of 41mm, 38mm, 51mm, and 38mm, and respectively, on day 14 (**Figure. 6.1**). Only the endophyte isolates that grew >20mm on day 14 were selected for the dual culture experiment with pathogen PA (**Figure. 6.1**). *Phytophthora agathidicida* grew well more than 4.5cm by day 14. The mean growth of PA in cV8A on day 14 incubation was 59mm with a growth rate of 4.21mm per day which gradually decreased from day 16 to 32mm with an average growth rate of 1.33mm per day.

Table 6.1: Fungal endophyte isolates from *Agathis australis* roots and the closest BLASTn match.

Culture Number (Landcare Research Institute)	**NCBI Accession Number**	**Isolate identity**	**Closest Match**	**Percentage of match (%)**
ES107*	MN 121358*	*Pezicula* sp. 6 CC-2015*	*Pezicula melanigena* (NR 155611)	99.63
ICMP 21447	MN 094300	Hyaloscyphaceae III GK-2010	*Xenopolyscytalum pinea* (NR 156543)	89.85
ES91*	MN 080334*	*Ilyonectria mors-panacis**	*Ilyonectria cyclaminicola* (NR 121495)	99.48
ICMP 21345	MN 094250	Hyaloscyphaceae	*Neolauriomyces eucalypti* (NR 160353)	88.95
ICMP 21339*	MN 080374*	*Ilyonectria mors-panacis**	*Ilyonectria leucospermi* (NR 152889)	99.81
ICMP 21343	MN 121356	*Pezicula* sp. 6 CC-2015	*Pezicula ericae* (NR 155653)	98.66
ICMP 21347*	MN 094296*	*Pezicula* sp.*	*Pezicula neosporulosa* (NR 138003)	96.48
ICMP 21358	MN 218809	*Pestalotiopsis neglecta*	*Pestalotiopsis grevilleae* (NR 147548)	99.66
ICMP 21375	MN 218802	*Metapochonia bulbillosa*	*Metapochonia bulbillosa* (NR 154142)	100.00
ICMP 21377	MN 148684	*Hypomyces* sp.	*Sphaerostilbella novae-zelandiae* (NR 164433)	94.12
ICMP 21382	MN 864193	*Penicillium kapuscinskii*	*Penicillium cosmopolitanum* (NR 163689)	99.61
ICMP 21392	MN 218806	*Cadophora* sp.	*Leptodophora echinate* (NR 170730)	98.13
ICMP 21398	MN 094276	*Pezicula* sp.	*Pezicula neosporulosa* (NR 138003)	96.80
ICMP 21455*	MN 218798*	*Coprinellus micaceus**	*Coprinellus ovatus* (NR 171838)	97.00
LJ215	MN 218791	*Mycena* sp.	*Mycena sinar* (NR 154169)	88.46
LJ235	MN 094220	Hyaloscyphaceae	*Xenopolyscytalum pinea* (NR 156543)	88.64
LJ308	MN 094305	Hyaloscyphaceae *III GK-2010*	*Neofabraea inaequalis* (NR 155470)	88.87

Notes * Indicates the cultures examined for antagonism (biocontrol analysis) against PA.

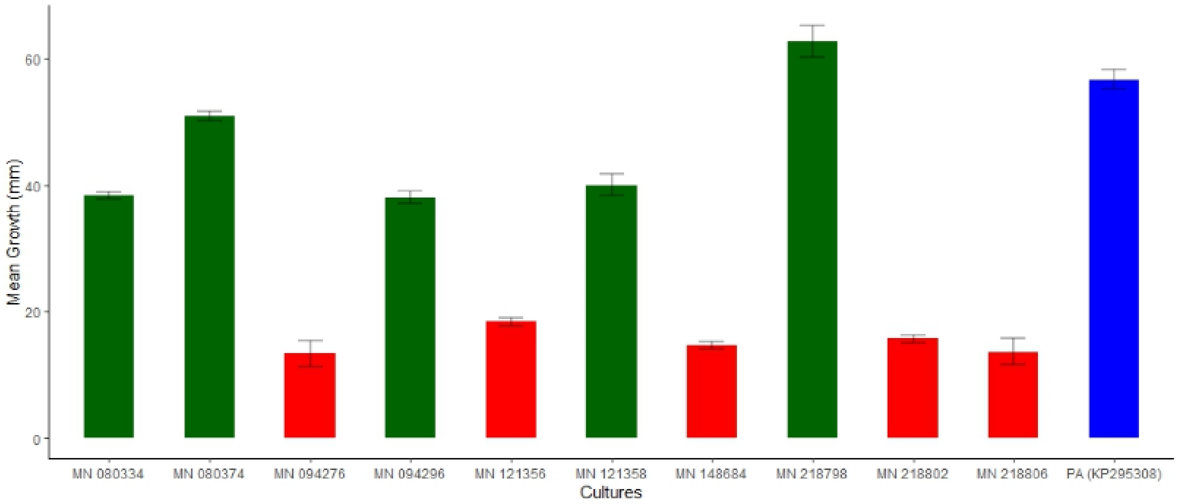

Figure. 6.1 Radial mean growth of endophyte isolates from *Agathis australis* roots and pathogen *Phytophthora agathidicida* in cV8A media at 20°C in the dark on day 14. Red bars indicate the endophyte isolates which did not grew above 20mm on day 14. Green bars indicate the endophytes isolates which grew above 20mm on day 14. Blue bar is the growth of PA.

Five endophytes were employed for the in vitro dual culture with the pathogen *Phytophthora agathidicida* (**Figure. 6.3**). The five endophyte isolates exhibited various levels of inhibition against PA in dual cultures (**Figure. 6.3b**). *Coprinellus micaceus* (ICMP 21455) was most effective in suppressing PA growth with 100% growth coverage of the plate on day 24. *Ilyonectria mors-panacis* (ICMP 21339) suppressed the mycelial growth of PA and grew over the pathogen zone on day 31. *Pezicula* sp. (ES107) 6 CC-2015 inhibited the growth of the pathogen and occupied 69.30% of the plate. *Ilyonectria mors-panacis* (ES91) and *Pezicula* sp. (ICMP 21347) occupied 48.2% and 38.7% of the plate, respectively (**Figure. 6.3b**).

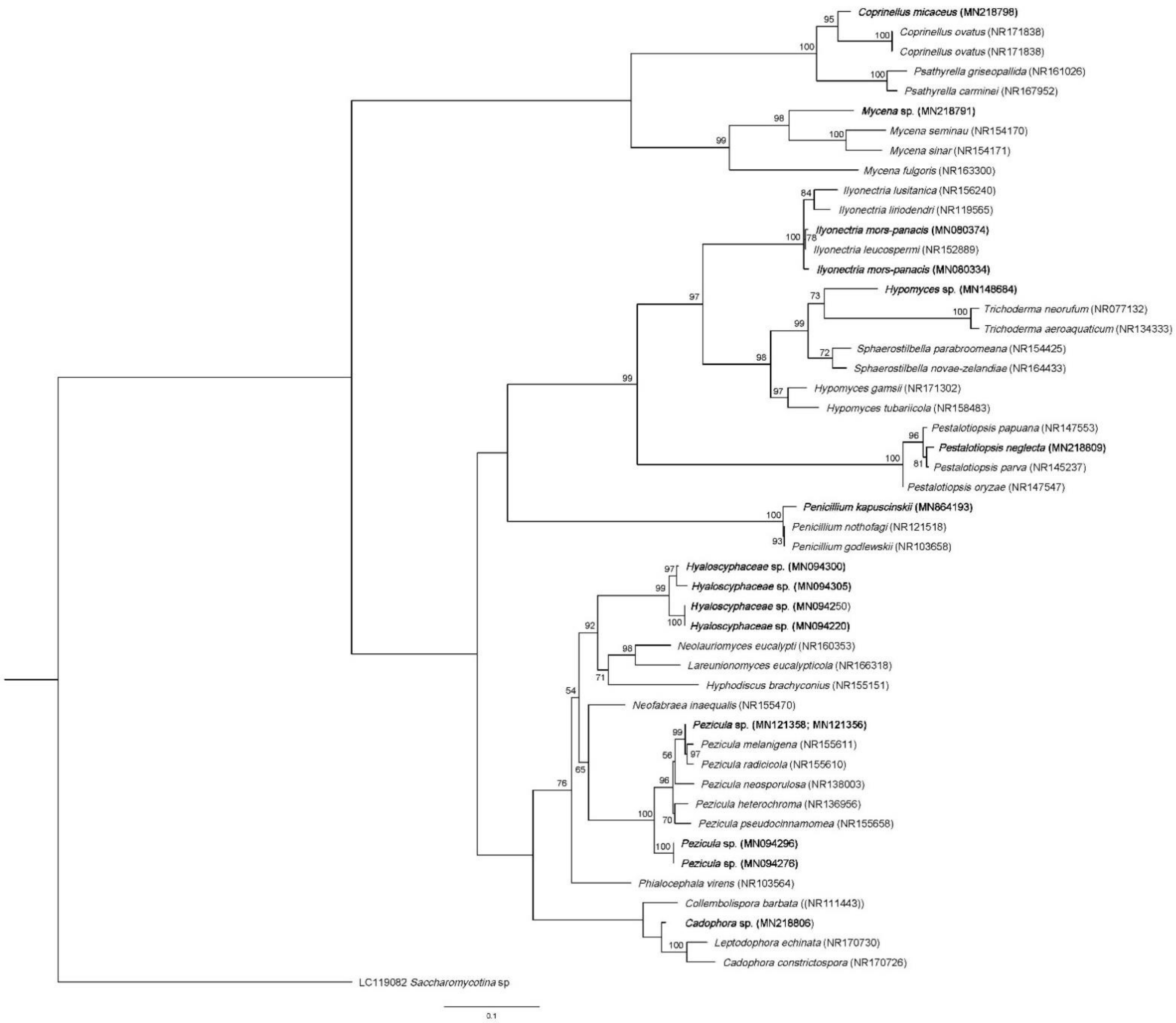

Figure. 6.2 Phylogenetic relationships among fungal ITS gene region. Fungal endophyte isolated from kauri roots are in bold. The tree topology is supported by bootstrap values for 1000 replications, shown for branches supported by more than 50% of the trees. The scale bars represent the nucleotide change per position.

Furthermore, the growth of the pathogen was not altered for the first eight days in dual culture, although growth suppression became evident when the PA hyphae were in contact with the endophyte hyphae (**Figure. 6.3a**). C*oprinellus micaceus* (ICMP 21455), *Ilyonectria mors-panacis* (ICMP 21339) and *Pezicula* sp. (6 CC-2015) were found to penetrate the pathogen zone and suppressed its growth (**Appendix D**).

Coprinellus micaceus (ICMP 21455) and *Ilyonectria mors-panacis* (ICMP 21339) covered the entire pathogen zone and suppressed the PA growth entirely by day 24 and 31 (**Appendix D**). All isolates examined in dual cultures inhibited the growth of the pathogen significantly with a *p*-value < 0.001 using the two-sample student t-tests (**Table 6.2**).

Table 6.1: The mycelial inhibition values of *P. agathidicida* cultures in dual culture.

Fungal Isolate	Dual Culture Bioassay	
	Mycelial Inhibition %	Significance - *p*-value
ICMP 21455	100%	0.00054
ICMP 21347	37.83%	0.00032
ES91	47.88%	0.00014
ICMP 21339	100%	0.00044
ES107	69.30%	0.00044

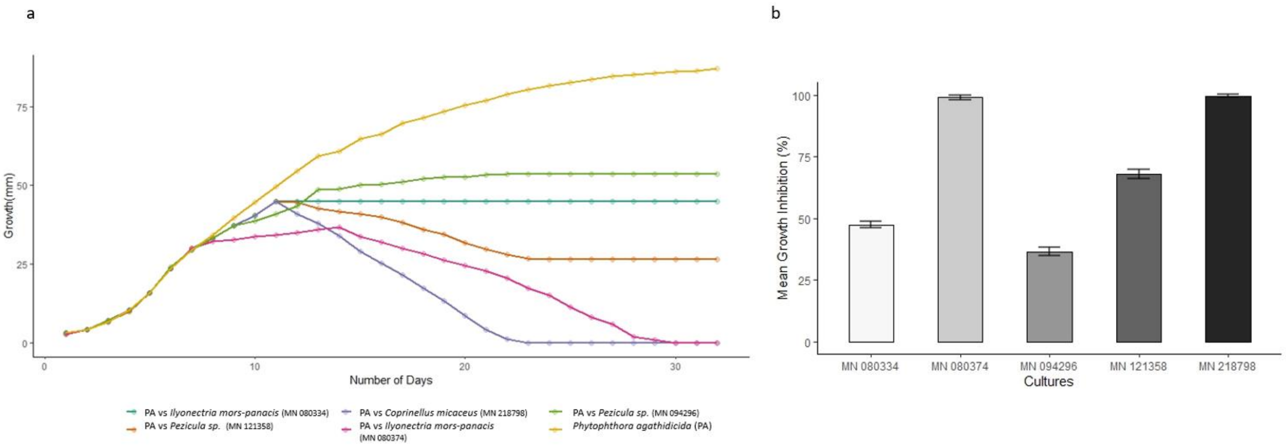

Figure. 6.3 Mean growth of *Phytophthora agathidicida* in the presence of endophyte isolate (a). Mycelial growth inhibition of *Phytophthora agathidicida* on day 32 in dual culture experiment (b).

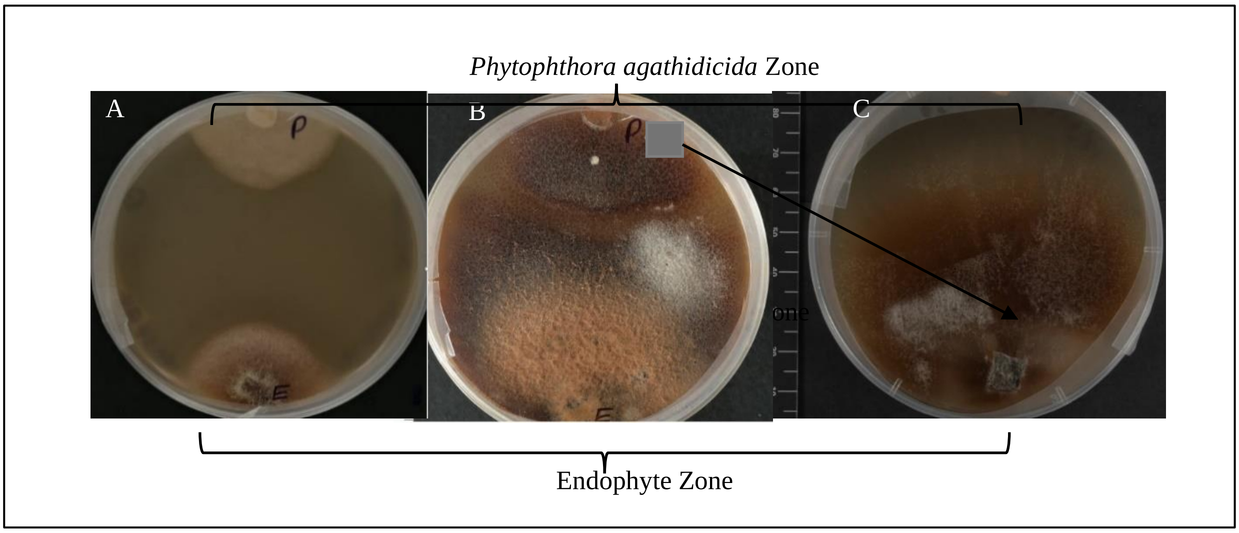

Figure. 6.4 Viability and induce oospore formation test. *P. agathidicida* and endophyte *Ilyonectria mors-panacis* in dual-culture experiment (A). Endophyte over growing on *P. agathidicida* (B), where agar plug was isolated and inoculated in fresh cV8A culture media incubated for 40 days until the culture plate was saturated with hyphae (C).

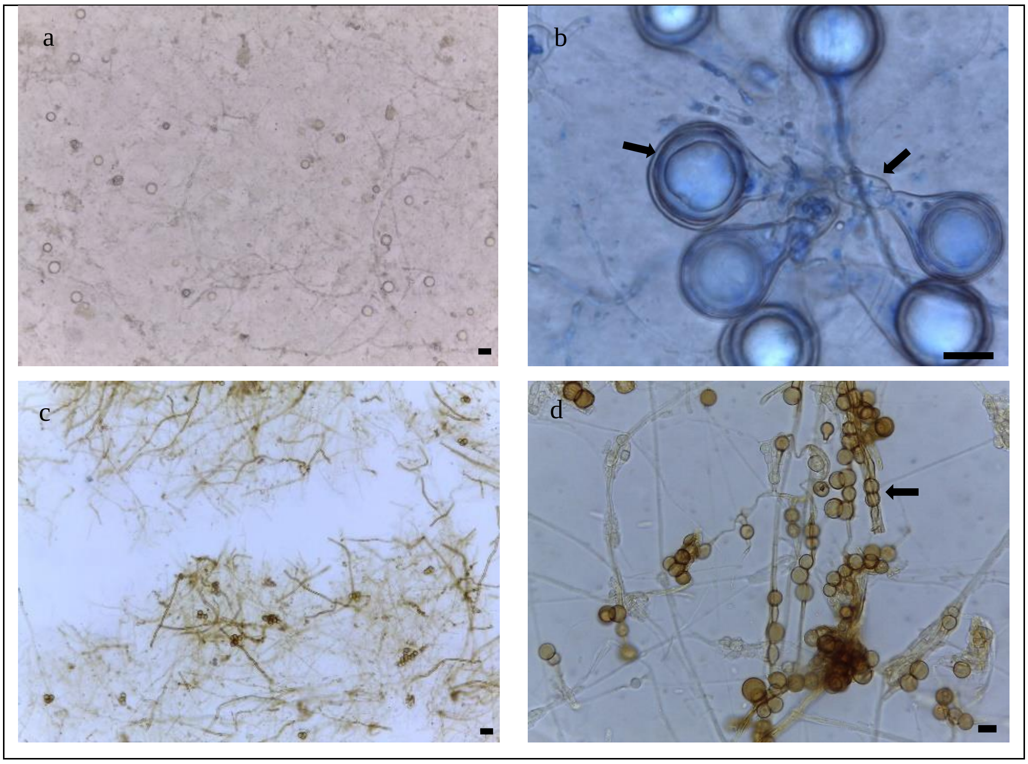

Figure. 6.5 *Phytophthora agathidicida* mycelia grown in pure culture at 100X magnification (a), oogonia with amphigynous, sub-globose antheridia stained with bromophenol blue at 1000X magnification (b). Dual culture of *P. agathidicida* and *Ilyonectria mors-pancis* mycelia at 100X magnification (c), specimen stained with bromophenol blue with no evidence of *P. agathidicida* oogonia only chlamydospore of *Ilyonectria* at 400X magnification (d).

To determine the viability of the PA in the dual culture after the growth suppression of the endophyte isolate, an agar plug was obtained from the pathogen zone and cultured in fresh cV8A plate. Hyphae growth of endophyte was evident on day 2. The culture was allowed to saturate and incubated at optimum condition for 40 days or until the available nutrient has depleted. PA under nutrient depletion stress produce oospores. Microscopic examination of slides stained with bromophenol blue did not reveal observable oospores (**Figure. 6.4** and **6.5**).

PA oogonium ornamentation is irregular and slightly raised projections larger than the oospore diameter. PA is homothallic, with in vitro isolates forming oogonia rapidly (3–4 days) and abundantly on cV8A in 3-4 days (Weir et al., 2015). Oogonia are globose with a width ranging between (22 - 45) µm. Oogonium wall ornamentation is mildly stipulate. Oospores nearly fill the oogonia with a width ranging between 19–35µm. Sporangia are globose to ovoid-ellipsoid, papillate, borne terminally from long thin branched. Sporangia are non-caducous. Sporangia have a width ranging between 12.4 – 50µm, and PA lacks chlamydospores in culture (**Figure. 6.6**) (Weir et al., 2015).

Coprinellus micacues was microscopically examined from the dual culture. The culture had high density of mycelia covering the entire plate, and air hyphae were also observed. Clamp cells and some septa were also observed but overall occurred at very sparse. (**Figure. 6.7**).

Furthermore, microscopic examination of *Ilyonectria mors panacis* isolate on dual culture showed simple sporodochial conidiophores which were loosely aggregated, unbranched or lightly branched, with 1 to 3 septate, and cylindrical to subulate. Some complex conidiophores accumulated in small sporodochia and occasionally branched. Macroconidia were predominant with 1 to 3 septate, straight, cylindrical with nearly rounded ends and mainly without a hilum. Microconidia were almost straight and spheroidal to subcylindrical (**Figure. 6.8**). The chlamydospores observed were spherical or subspherical and 8–16 µm in diameter. Chlamydospores are thick-walled, found in chains or clumps and medium brown in colour (**Figure. 6.8 e - f**).

6.5 Discussion

Most plant species studied up to date have been found to host endophytes. Interactions between plants and endophytes may be symbiotic, mutualistic, and other types of relationships without causing symptoms or causing harm to their host. Endophytes colonize the same environment as plant pathogens and share an intimate relationship with the host plants. Thus, endophytes are a valuable natural resource for potential utilization as biological control agents (Schulz et al., 2002; Stone et al., 2000; Tan & Zou, 2001; Zilber-Rosenberg & Rosenberg, 2008). In our *in vitro* dual culture assay, root endophytes of *Agathis australis* exhibited evident growth suppression of kauri dieback pathogen *Phytophthora agathidicida*.

Clarified V8 juice agar was selected for the experiment as it induces sporulation and is favourable to *Phytophthora agathidicida*. Moreover, endophytes were selected based on the growth rate in cV8A media to study the endophyte behaviour in a favourable pathogen environment. Six slow growing endophyte isolates in cV8A did not show visible hyphae until day 14.

Even though ICMP 21382 (*Pencillium kapuscinskii*) grew in cV8A, it did not exhibit the radial growth pattern. The growth pattern was significant in measuring the growth rate of both endophyte and pathogen. Ten endophytes had significant growth in cV8A. The endophytes with radial mycelial growth of >20mm on day 14 were considered for the dual culture as these endophytes had relatively similar growth rate compared to PA. According to Duncan (1988), nutrient content is significant in the expression of fungal reproductive structures in culture media. A high concentration of carbohydrates presents in the medium or the absence of some minerals may contribute to the development of specific fungal species. The presence of specific ions and nutrient depletion in axenic culture led to the production of sporangia by *Phytophthora cinnamomi.* Nevertheless, it is not known whether the poor performance of other endophyte isolates could be attributed to the high concentration of

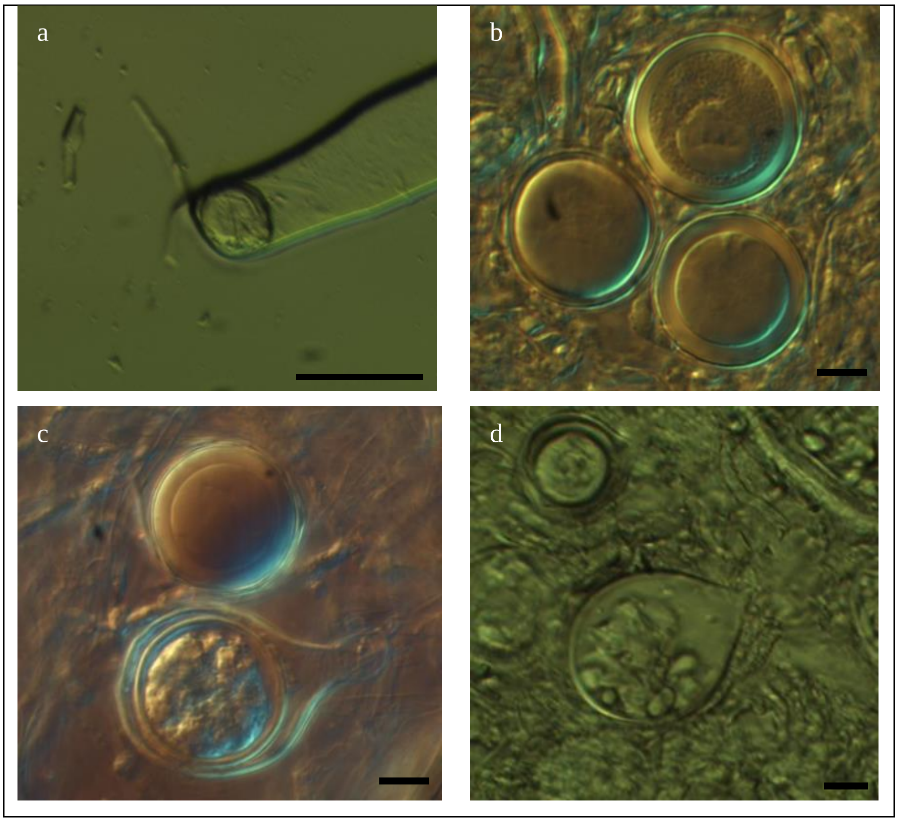

Figure. 6.6 Life cycle stages of *Phytophthora agathidicida.* Biflagellate zoospore (a), oospores (b) sporangia (c) and oogonia (d). Scale bar is 10µm.

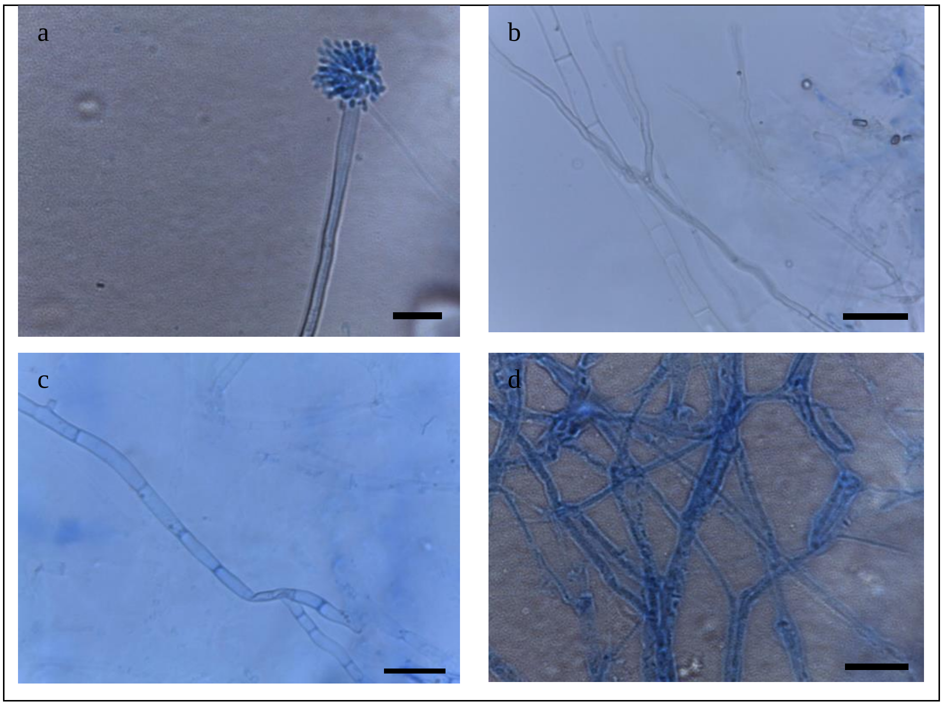

Figure. 6.7 *Coprinellus micacues* odiophores (a), hyphae (b,c,d) stained with bromophenol blue. Scale bar is 10µm.

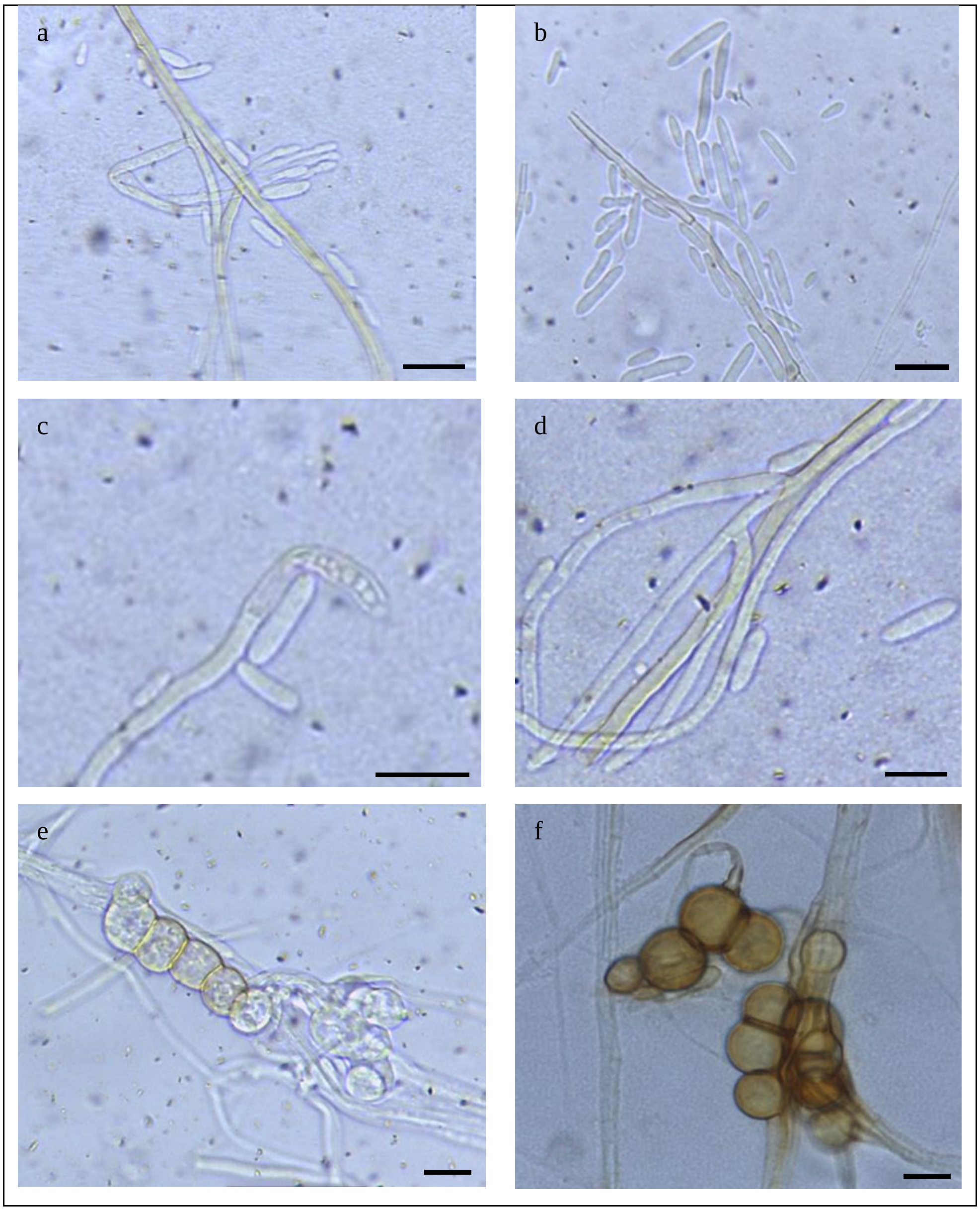

Figure. 6.8 *Ilyonectria mors-panacis* simple conidiophores with aerial mycelium (a), micro and macro conidia (a, b, c, d), chlamydophores on mycelium (e and f). Scale bar is 10µm.

carbohydrates or the lack of some minerals in the cV8A culture media. However, the role of nutrients in the fungal growth *in vivo* could be investigated.

Four isolates from Ascomycota and one isolate from Basidiomycota were able to grow >20mm on day 14. The Ascomycota isolates are *Ilyonectria mors-panacis* and *Pezicula* sp. *Ilyonectria* species generally had a reasonable growth rate in cV8A (Goh et al., 2022).

One Basidiomycota isolate, *Coprinellus micacues* has ecological significance because it can live in poor nutrient ecosystems and create interactions with bacteria to overcome nutrient stress and feasible environmental conditions (Marini-Macouzet et al., 2020; Velez et al., 2018). *Coprinus* species are known for degrading monomeric phenolic and chloroaromatic compounds (Guiraud et al., 1999). Moreover, *Coprinus* species were reported to exhibit high tolerance to high nitrite concentration, ammonium and urea, and were only inhibited at high $NaNO_2$ concentrations (Hintikka & Niemi, 1999).

Two isolates, *Coprinellus micacues* (ICMP 21455) which was fast growing compared to PA (72mm in 14 days), and *Ilyonectria mors-panacis* (ICMP 21339) overgrew the mycelium of *P. agathidicida* in dual culture. These isolates were most effective in suppressing *P. agathidicida* growth with 100% growth coverage of the plate on day 24 and day 31, respectively, suggesting that these isolates have a faster growth rate and were not inhibited by the presence of *P. agathidicida*. Furthermore, these endophytes outcompeted the PA on the cV8A dual culture. Micaceol a sterol and (*Z*,*Z*)-4-oxo-2,5-hetpadienedioic acid were extracted from *Coprinus micaceus* from the Canadian Prairie region that demonstrated antimicrobial activity against pathogens *Corynebacterium xerosis* and *Staphylococcus aureus* and exhibited glutathione S-transferase inhibition (Zahid et al., 2006). Furthermore, gas chromatography and mass spectrometry studies of *C. micaceus* extracts in ethanol, chloroform and distilled water were reported to exhibit antimicrobial activities (Avcı et al., 2014). The culture filtrates of *Coprinus comatus* effectively reduced the disease severity of *Phytophthora* blight of pepper (J. Chen & Huang, 2011). These studies suggests that *C. micaceus* isolated from kauri roots may produce chemical compounds that are pathogen inhibitors.

C. micacues and *I. mors-panacis* outcompeted the pathogen in cV8A dual culture set up which could present competition for space and nutrition. Further microscopic examination of the pathogen region in the dual culture plate showed no evidence of *P. agathidicida* oospores in the presence of *Ilyonectria mors-panacis.* This indicates that *I. mors-panacis* inhibits the regeneration of PA oospores. In an Australian shrub study, *Ilyonectria* species also exhibited antagonistic qualities against *Phytophthora cinnamomi* in *in vitro* dual culture (Andres et al., 2022).

However, *in vitro* results do not always translate the disease suppression in plants. Studies suggested that *in vitro* assays can be used as a rapid technique for selecting promising biological control agents (BCA) (Rajkumar M et al., 2005). According to (Latha et al., 2009) and (Pal & McSpadden Gardener, 2006), the most effective BCA inhibit plant pathogens by multiple mechanisms of action, including the production of antimicrobial compounds, competition for space and nutrients, plant growth promotion and induced systemic resistance. (Byers1 et al., 2021)identified *Penicillium* and *Burkholderia* soil isolates demonstrating inhibition towards *Phytophthora agathidicida* and suggesting these species were promising BCA. Our dual culture results suggest that *Coprinellus micacues* and *Ilyonectria mors-panacis* may have multiple modes of action in pathogen suppression, such as competition for space or substrate or nutrients or production of metabolites antagonistic to *Phytophthora* or production of antimicrobial substance. Further studies are needed on the endophyte's mechanism of action is supressing pathogens. Future research should measure the impact of endophytes on *P. agathidicida* on sporangia production and zoospore release to quantify their inhibitory potential better and specific research is required to characterize the metabolites with potential antimicrobial action, as it is essential to fully understand their mode of action before they can be applied as BCA (Spadaro & Gullino, 2005).

6.6 Conclusion

Kauri roots harbour endophytic fungi that can suppress growth and reproductive metabolism of plant pathogen such as *P. agathidicida*. In this study we identified and successfully cultured ten isolates in cV8A media that favours the growth of the plant pathogen. Five out of the ten isolates demonstrated different level of Pa growth inhibition in the dual culture experiment. Of the 17 endophytes we identified four Ascomycota and one Basidiomycota endophytic isolates that can significantly inhibit the growth of *Phytophthora agathidicida* in *in vitro* dual cultures. *Coprinellus micaceus* and *Ilyonectria mors-panacis* not only inhibited the growth of Pa it also suppressed the zoospore formation of *P. agathidicida*. Further studies need to be conducted on the mechanism of inhibition and possible metabolite production of these isolates as they can be considered potential biocontrol agent for plant pathogen such as *P. agathidicida* in kauri.

Chapter 7

General Discussion

7.1 Introduction

The main aim of the research was to characterize the soil microbiota and the functional potential of soil samples collected from both anthropogenic and forest environments and screen fungal kauri root endophyte isolates for biocontrol potentials. The literature review provides a comprehensive summary of the background for this research highlighting the research gaps and importance of the study. The research study investigated the microbial community structures using molecular-based and culture-based methods to identify taxa and their functional gene potential related to dieback disease biocontrol. Chapter three establishes the baseline microbial communities in a controlled environment, ABG. It is shown that cardinal point sampling did not significantly vary the community. In chapter four, the microbial community in a natural kauri forest was analysed while considering the tree health and phosphonate treatment. The results showed significant differences in bacterial and fungal diversity among trees with different health statuses. Additionally, there were significant differences in the beta diversity of soil bacteria and fungi between the phosphonate-treated and non-treated trees.

In Chapter five, the functional genes of soil microbes found in kauri trees at the Auckland Botanic Gardens (ABG) and the Tairua and Whangapoa kauri forests in the Waikato region of New Zealand were elucidated. The results showed significant differences in carbon cycling and microbial defence genes among the ABG region, Tairua, and Whangapoa. The normalised signal intensity of carbon degradation genes was higher in Tairua and Whangapoa kauri soil due to litter formation. Genes involved in nitrogen and phosphorus cycle processes were also identified. PhID and other microbial defence genes were found to produce antimicrobial substances against *Phytophthora* species. Plant growth-promoting genes that has the potential to enhance kauri development were also detected in the kauri soil samples.

Chapter six reveals that certain kauri root endophytes and soil microorganisms exhibit antagonistic behaviour towards the pathogen *Phytophthora agathidicida*. This discovery presents a potentially promising solution for managing kauri dieback. Additionally, the results identified key soil microbial organisms that are well-studied and have the potential to suppress the kauri dieback pathogen. These findings provide valuable insights for future research endeavours in this field. They will facilitate future research that assesses the influence of biological control on the spread of kauri dieback across fragmented kauri forests in Aotearoa New Zealand.

The final chapter outlines the main findings and implications of this research. In addition, the limitations of the scope of this research were discussed, and recommendations for future work were proposed. Overall, this research sheds new light on the complex interactions between kauri trees and their soil microbiota and provides a solid foundation for developing effective strategies for the management and preservation of this iconic species.

7.2 Summary of Findings

7.2.1 Analysis of Kauri Soil

Soil samples were collected from the top 10 cm depth at four cardinal points of the tree 100 cm away from the trunk at ABG. In Tairua, samples were collected in the four cardinal points, and collected from the surface and 10cm depth. The results showed there were no significant differences between cardinal points and surface and 10 cm depth samples. Hence the Whangapoa samples were collected at four cardinal points but later pooled to one sample per tree. Studies carried out in the Eurasian Steppe (Inner Mongolia, China) (Zhao et al., 2021) and in a forested montane watershed in Colorado, USA (Eilers et al., 2012) showed that soil samples taken at the surface and a depth of 10cm did not differ significantly. However, both cases noted significant differences beyond a depth of 20cm. Similarly, the kauri forest soil showed no significant differences between surface and 10cm depth soil samples. The kauri lateral roots spread out more than five meters from the trunk, creating a similar ecosystem surrounding the tree (Padamsee et al., 2016; Yang Jaynie, 2022). This may lead to no significant differences being observed among the soil samples taken from the four cardinal points. Therefore, samples collected at one meter from the trunk may be representative of the kauri root environment. However, this may vary depending on the specific environment, plants surrounding the kauri, and the slope of the cardinal point. Some interesting observations were made regarding the microbial community of samples taken from the North and South cardinal points of the Native Forest tree 2 in ABG, which was located on a slope. These observations were found to be different compared to the ones taken from the west and east cardinal points located at a plane. The relative abundance of phylum Actinobacteria was higher at the slope cardinal points compared to the cardinal points on the plane with 12% and 6.2% on the slope and plane, respectively. On the other hand, phyla Myxococcota, Bacteroidota, and Planctomycetota had lower relative abundance at the slope cardinal points compared to the plane, with 0.1%, 2.5%, and 2.2% on the slope, and 8.2%, 8.7%, and 8.2% on the plane, respectively. This might be due to kauri roots not being evenly spread on the slope compared to the plane. Further research needs to be conducted to identify the impact of ground slope and kauri rhizosphere.

7.2.2 Key Microorganisms detected in kauri in anthropogenic environments.

The ABG kauri soil key genera identified that are known to have plant growth-enhancing potential include *Flavobacterium, Brevundimonas, Streptomyces, Nitrospira, Nitrobacter, Candidatus, Luteibacter, Sphingobium, Penicillium, and Arthrobacter.* These genera are highly researched for their role in plant growth promotion and disease suppression in plants. *Nitrospira* and *Nitrobacter* genera were identified as having the potential to play a key role in nitrogen cycles (Daims et al., 2015). *Arthrobacter* has been found to promote growth in various plants, potential beneficial effects of this bacterial genus on plant growth and yield have been suggested and *Arthrobacter* species are known to produce different carotenoids (Y. chen Sun et al., 2022). *Streptomyces* is a microorganism that is widely researched due to its ability to produce various bioactive compounds (Olanrewaju & Babalola, 2019). *Penicillium* spp. interact with the roots of plants, leading to improved plant growth. And promotes plant growth and also plays a role in phosphate solubilization, which could be a factor in their ability to enhance plant growth. *Bdellovibrio* rhizosphere soil controls bacteria, supports nutrient cycling and fosters microbial communities (Senthil Kumar et al., 2023). Certain species of *Penicillium* have been found to produce antibiotics that can resist pathogens and activate multiple defence signals in plants, thus increasing their resistance of plants (Radhakrishnan et al., 2014). These microorganisms, which are abundant in kauri soil, need to be further studied on their role in the development of kauri.

7.2.3 Key Microorganisms detected in kauri roots

ABG kauri roots were detected with a high presence of Proteobacteria in their microbiome, which is similar to the root microbiomes of other conifer species such as Brazilian Araucaria, *Araucaria angustifolia*, *Pinus thunbergii*, *Pinus flexilis*, and *Araucaria araucana*. These findings suggest that Proteobacteria may have a significant role in the microbiome of Araucariaceae and conifer tree species (Carper et al., 2018; Ma et al., 2020). (Neroni & Cardoso, 2007) (Lammel et al., 2013)(Jaime Alarcón et al., 2020). *Pseudomonas, Rhizobium*, Enterobacteriaceae, *Staphylococcus* and *Burkholderia* were highly studied as bacterial endophytes were detected in kauri root at ABG. Pseudomonadaceae are well-known as plant growth-promoting bacteria, while some of them are known as plant pathogens (McSpadden

Gardener, 2007). There are only a handful of studies conducted on kauri root endophytes, further research on endophytes may enable to identify resistance species to kauri dieback pathogen *Phytophthora agathidicida.*

Limitations and recommendations for future research

Chapter 3 utilized high-throughput sequencing of microbial DNA to detect variations in the composition and relative abundance of microbial taxa present in kauri at ABG and kauri forest soils. However, although this method was efficient for the primary objectives of the study, it did not provide quantitative measurements of the absolute abundance of the active members of the soil microbiota, which is a limitation shared by the studies presented in Chapters 3 and 4 that also employed high-throughput sequencing of microbial DNA (Bang-Andreasen et al., 2020; Cox et al., 2017). To address this issue, future studies could opt for high-throughput sequencing of microbial RNA, which would more accurately quantify differences in the taxonomic abundance of metabolically active soil microbial communities. This approach has been recommended in previous studies, including Cox et al. (2017), Tviet et al. (2014), and Young et al. (2018).

7.2.3 Significant differences were found in the soil microbial communities associated with symptomatic and non-symptomatic kauri trees

Chapter 4 identified significant differences in the composition of fungal and bacterial communities between dieback disease symptomatic and non-symptomatic kauri soils. According to the present study, trees showing signs of canopy thinning (canopy score > 2.5) had a higher bacterial Shannon diversity index compared to non-symptomatic (canopy score < 2.5). This might be correlated with the presence of the dead kauri tree, as previous studies have indicated that bacteria play a crucial role in decomposing dead plant biomass and significantly contribute to decomposition processes in litter and soil (López-Mondéjar et al., 2016; Štursová et al., 2012). In the study, trees with symptoms (canopy score > 2.5) were observed to have lower fungal Shannon diversity index compared to those less symptoms (canopy score < 2.5). However, the NMDS plots showed that bacterial communities had lesser clustering between samples compared to fungal communities. This was evident in both Tairua and Whangapoa forest. This finding was similar to what was observed in a *Picea abies* tree dieback and significant changes in the fungal community (Štursová et al., 2014). The changes included a decrease in biomass, disappearance of fungi that were symbiotic with tree roots, and a relative increase in saprotrophic taxa. This observation was also evident in the current study where fungal diversity in the surface samples of symptomatic tree (TT11) at Tairua showing lower fungal diversity. Furthermore, the number of fungal OTUs shared by healthy *Pinus bungeana* soil were significantly higher than those of unhealthy plants, indicating that the health conditions of the plants had a pronounced effect on the fungal community (J. Yang et al., 2022) Colonization of decomposed resources may alter community composition (Jung et al., 2018). In symptomatic soil, saprotrophic fungi such as *Mycena, Trichorderma, Umbelopsis, Cantharellales, Chaetothyriales, Mortierella, Leohumicola*, and *Apiotrichum* were detected. The accumulation of acidic podzols in the soil is caused by increased litter layer due to the dieback symptoms in kauri trees (Steward and Beveridge, 2010). The fungal Shannon diversity index is significantly low compared to slightly thinning and non-symptomatic trees. This might be due to loss of symbiotic fungi of the dead tree and kauri dieback symptomatic status. Further research needs to conduct to identify the cause of the difference in bacterial and alpha and beta diversities.

7.2.4 Impact of Phosphonate treatment

The differential abundance test results showed that certain bacterial genera, namely *Rhodomicrobium*, *Rhizobium*, and *Rugosimonospora, c*lass Pseudomonadaceae were significantly positively associated with phosphonate treated kauri soil. Similarly, fungal genera such as *Dendrochytridium, Phlalocephala, Infundichalara, Cryptosporiopsis, Cercophora, Malasuezia, Agaricas,* and *Mycena* were also found to be significantly positively associated with phosphonate treated kauri soil. Phosphonate activates plant defense mechanisms (Ramezani et al., 2018; Mohammadi et al., 2020) and also has a direct effect on the pathogen (Eshraghi et al., 2014; Achary et al., 2017; Gill et al., 2018). There are, however, some concerns about phytotoxicity and the practicality of using phosphonate as a control measure to kauri dieback. Further research and evaluation can help address these issues and enhance its effectiveness in kauri protection.

Limitations and recommendations for future research

Chapter 4 analysed soils collected from two kauri forest (Tairua and Whangapoa) in Aotearoa New Zealand (Waikato Region, New Zealand). Findings should be validated by characterising the soil microbiota of dieback disease symptomatic and non-symptomatic kauri sampled from several different kauri forests across Aotearoa New Zealand. Future studies could sample kauri soils from various kauri forest regions to identify if the differences found in the soil microbiota between symptomatic and non-symptomatic kauri is consistent across a larger geographical scale. Additionally, only one sampling point was performed and sampling at different time points could validate the findings of the first sampling round. Further sampling from the same symptomatic and non-symptomatic kauri is needed with samples analysed using the same methods presented in Chapter 3 and 4. The spread and expression of *Phytophthora* diseases can be influenced by environmental differences across seasons, such as temperature and rainfall (Burgess et al., 2019; Sánchez et al., 2002). Furthermore, the composition of fungal (Voříšková et al., 2014) and bacterial (López-Mondéjar et al., 2015) soil communities can be influenced by seasonality. Therefore, sampling should be repeated during the same season as the first sampling round (autumn) to avoid differences in seasonality limiting comparison between repeated sampling rounds. However, once the findings of the Chapter 3 and 4 have been validated, future studies may wish to perform soil sampling across different seasons to investigate how differences in environmental conditions impact the response of the soil microbiota to kauri dieback disease.

7.2.5 Functional gene potential of kauri soils

GeoChip 5.0M was used to analyse the functional genes present in the soil microbes found in kauri trees at the Auckland Botanic Gardens (ABG), Tairua and Whangapoa kauri forests in the Waikato region of Aotearoa New Zealand. The study revealed significant differences in the signal intensities of carbon cycling genes and microbial defense genes between the ABG region and both Tairua and Whangapoa. The normalised signal intensity of carbon degradation genes in Tairua and Whangapoa kauri soil was significantly higher than in ABG kauri soil due to litter formation in the natural kauri forests.

Additionally, the research detected genes involved in various nitrogen cycle processes in kauri soil, such as ammonification, Anammox, assimilation, assimilatory N reduction, denitrification, dissimilatory N reduction, N assimilation, nitrification, and nitrogen fixation. Genes that play a role in phosphorus cycle processes such as phosphorus oxidation, phytic acid hydrolysis, polyphosphate degradation, and polyphosphate synthesis were also identified.

Several critical genes related to microbial defense, such as PhID, have been detected. These genes produce antimicrobial substances that can suppress the growth of several *Phytophthora* species. The kauri soil has been found to contain several plant growth-promoting genes, such as pathogen, phytohormones, drought tolerance, and stress tolerance genes. This indicates that microbial genes could enhance kauri development.

However, further research is necessary to analyse the expressed genes during different seasons and in various environments, as well as the kauri dieback health status. This study gives us the first step towards understanding how microbial genes could enhance kauri development.

7.2.6 Screening of kauri endophytes against PA

The potential of kauri root endophytic fungi to affect the growth of PA was explored. Seventeen endophytic fungi isolated from kauri roots were cultured and examined their morphological characteristics as well as their antagonistic effects against the kauri dieback pathogen. Out of the seventeen fungi tested, five showed suppressions of the pathogen growth in dual culture. *Pezicula* sp. partially inhibited the growth of *P. agathidicida,* while *Coprinellus micaceus* and *Ilyonectria mors-panacis* completely inhibited the growth of the pathogen. In the dual culture, oospores were not observed, and when the *P. agathidicida* was transferred into fresh culture media, it was found to be non-viable.

Limitations and recommendations for future research

The growth inhibition of *P. agathidicida* by each endophyte was evaluated by assessing their impact on the mycelial growth of *P. agathidicida*. However, their potential to inhibit sporangia production in *P. agathidicida* and zoospore release needs to be quantified. Such measurements are required because it is the motile zoospores released by *Phytophthora* pathogens that are responsible for plant infection (Bellgard et al., 2016).

Effective biocontrol agents should ideally be highly host specific to minimise their impacts on nontarget organisms and prevent their efficacy being compromised by alternate hosts (Stiling & Cornelissen, 2005). Therefore, the potential deleterious effects of the microbial strains on kauri and the co-occurring plant species of kauri forests when applied to soils in larger loads needs to be evaluated. Many of the antagonistic traits of biocontrol agents against soil pathogens, such as competition for nutrients and the release of anti-microbial metabolites, can also impact the activity and function of the resident soil microbiota (Cordier & Alabouvette, 2009; Scherwinski et al., 2008). However, in vivo, studies need to be performed to identify if these endophytes can effectively suppress pathogens. Several bacterial species, including *Pseudomonas*, *Rhizobium,* Enterobacteriaceae, *Staphylococcus* and Burkholderia, have been extensively studied as bacterial endophytes, which were found in the kauri root at ABG also need to be studied invitro and in vivo.

7.3 Conclusion

The study was conducted on kauri soil at the Auckland Botanic Gardens, Tairua and Whangapoa kauri forests in Aotearoa New Zealand. The study found that bacterial orders Acidobacteriales and fungal orders Mortierellales were dominant in the ABG kauri soil. Significant genera including *Penicillium, Mortierella, Streptomyces, Nitrospira,* and *Flavobacterium* were identified. Additionally, bacterial endophytes *Pseudomonas, Rhizobium,* *Enterobacteriaceae, Staphylococcus,* and *Burkholderia* were detected in kauri roots. The study found no significant difference between cardinal points. The study also found significant variations in the diversity of bacterial and fungal communities in dieback non-symptomatic and symptomatic kauri soil. Microbial taxa that suppress diseases were positively correlated with non-symptomatic kauri soil. This information may help identify microbial antagonists to counteract kauri dieback disease. However, further investigations are necessary to evaluate the efficacy of potential microbial control agents. Geochip 5.0M was used to analyse the functional genes of soil microbes found in kauri trees at the Auckland Botanic Gardens and two kauri forests in the Waikato region of Aotearoa New Zealand. The study found significant differences in carbon cycling and microbial defence genes between the Auckland Botanic Gardens, Tairua, and Whangapoa kauri soil. Carbon degradation genes' normalized signal intensity was higher in Tairua and Whangapoa kauri soil due to litter formation in natural kauri soil than planted kauri soil. The study also identified genes involved in nitrogen and phosphorus cycles, microbial defence, and plant growth promotion. Further research is necessary to analyse gene expression in different seasons and environments, as well as to investigate kauri dieback health status. One of the key findings was that naturally occurring fungal species in kauri roots can effectively suppress the growth of PA. This suggests the possibility of using biological control to manage dieback disease in kauri without harming the natural ecosystem. This finding may contribute to mitigating natural disease management and biocontrol of plant diseases. More research is needed to understand how climate change, age, and physiochemical characteristics affect microbial communities in kauri soil.

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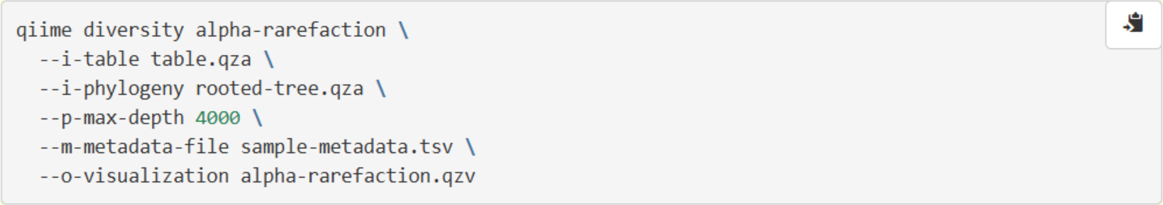
Appendix A

A1. Centred Log Ratio

Centred log ration converts the observed abundance to log ratio within each sample. The centre of mass of all taxa is the reference. Within each sample, for each taxon, the log ratio is computed relative to the geometric mean of all taxa. Centred log ratio is used in the heatmaps to clearly differentiate the most abundant taxa.

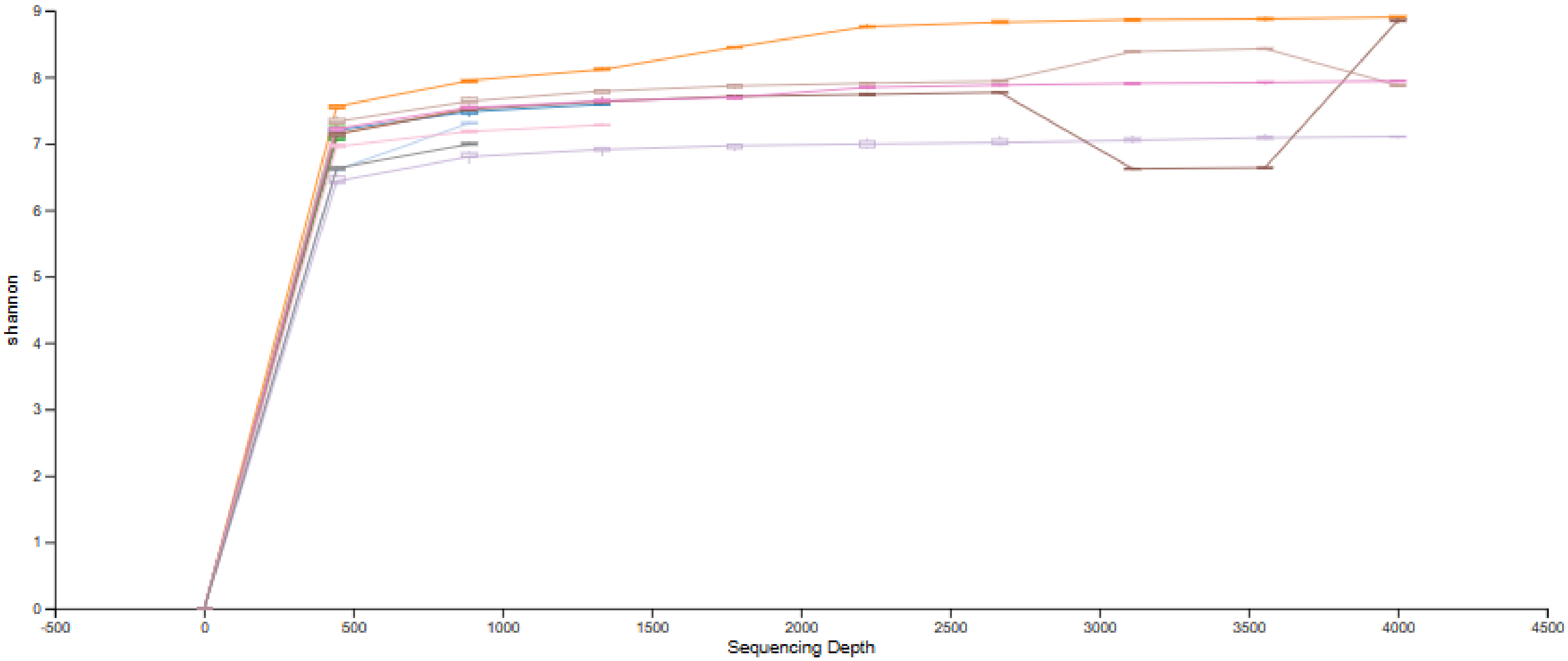
A2. Determining the rarefied sample depth

Alpha rarefactions plots were derived using QIIME 2 2022.2. Sequencing depths were plotted against observed ASVs and alpha diversity metrics. Samples were rarefied and 500 amplicon reads were considered for each sample in determining the alpha and beta diversity. At 500 sequencing depth, the sampling depth starts to plateau suggesting that the diversity of the samples have been fully observed across all samples. However, soil sample were expected to have high biomass hence the alpha and beta diversity were determined using 1000 sequencing depth per sample. Alpha and beta diversity were determined with both 500 and 1000 sequencing depth per samples and results show no significant differences between the two sampling depths hence 500 sequencing depth per sample was used as the rarefied depth to retain as many samples as possible for all downstream analyses.

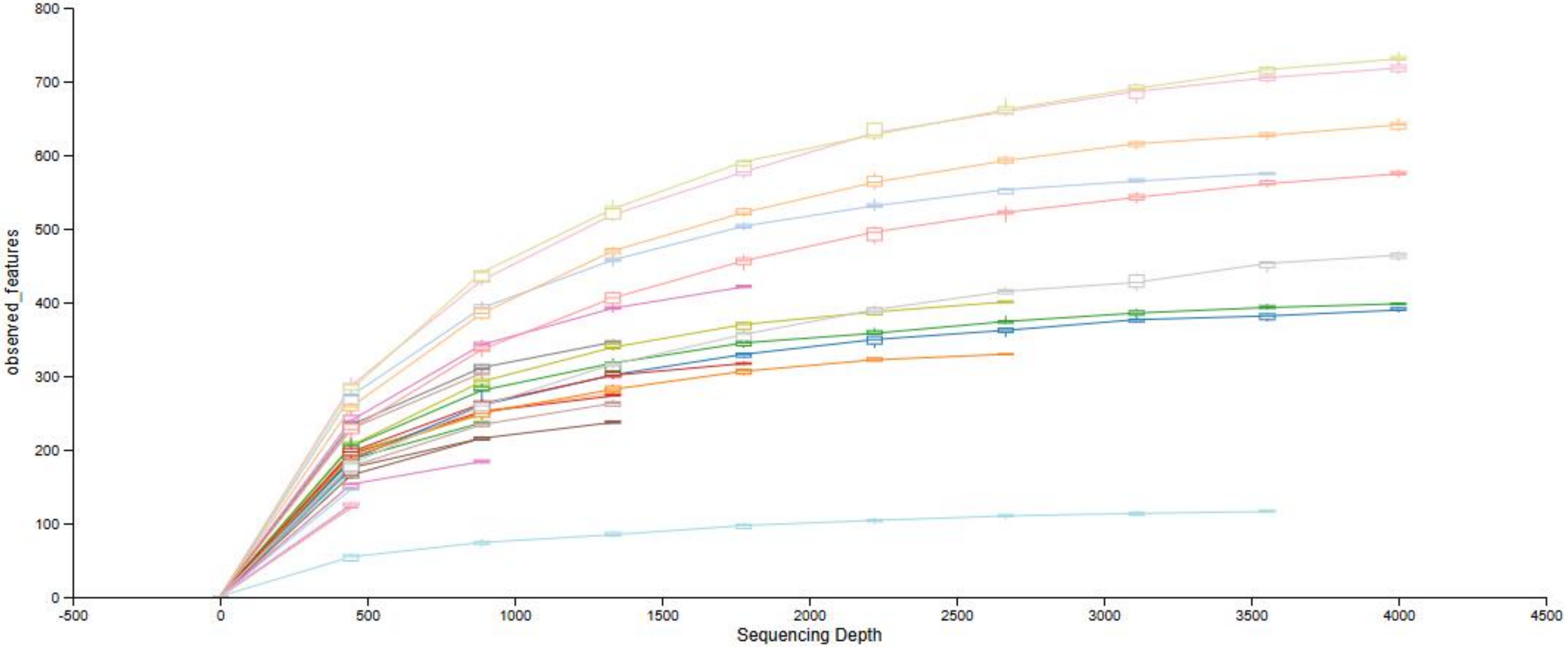


```
qiime diversity alpha-rarefaction \
  --i-table table.qza \
  --i-phylogeny rooted-tree.qza \
  --p-max-depth 4000 \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization alpha-rarefaction.qzv
```

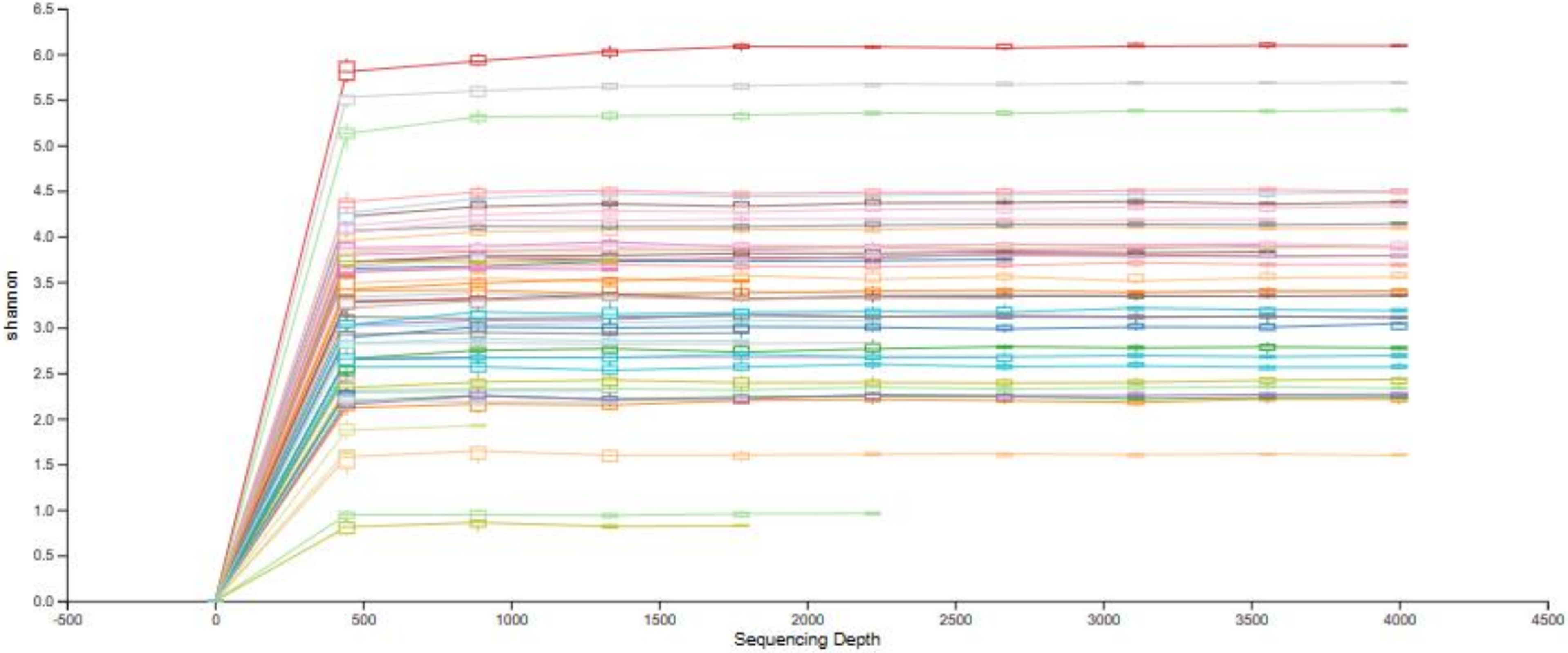
A2.1 Alpha rarefaction plots of 16S rRNA gene reads against Shannon Diversity of ABG



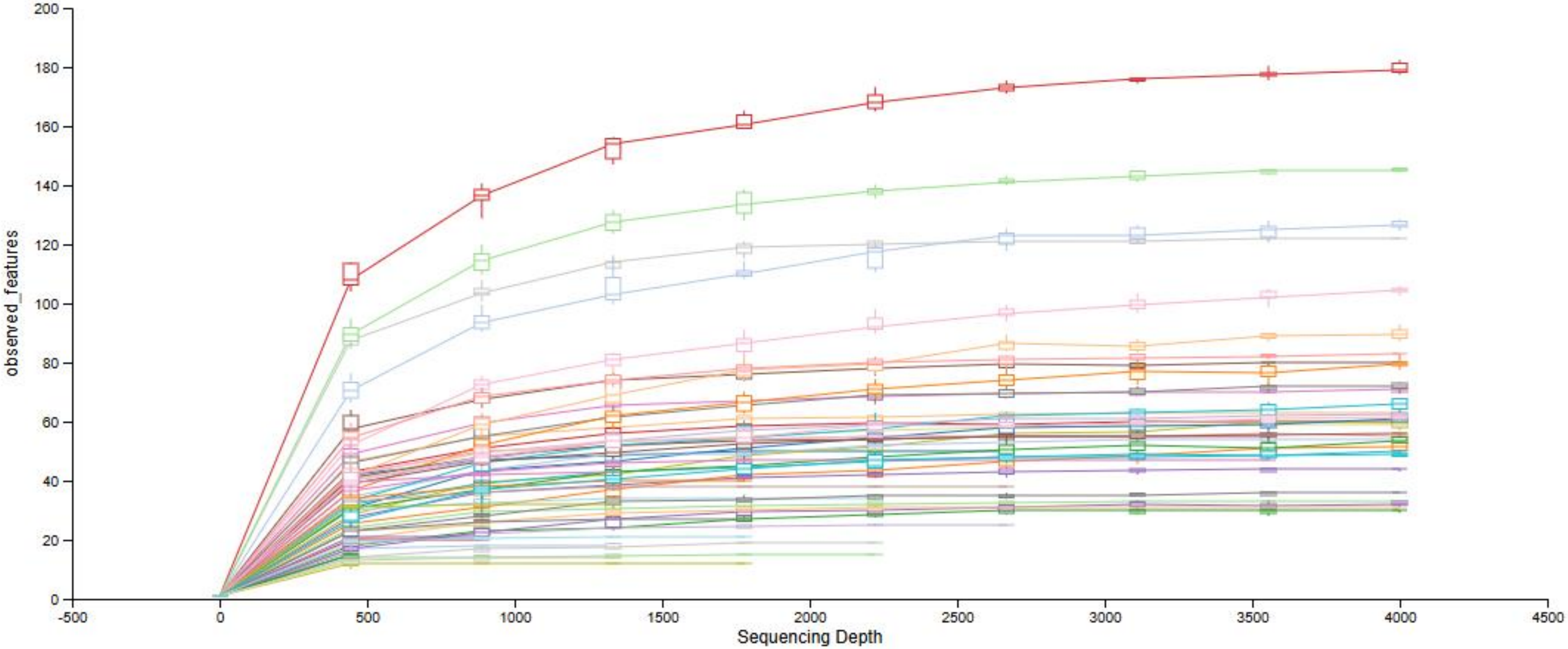
A2.2 Alpha rarefaction plots of 16S rRNA gene against observed ASVs of ABG



A2.3 Alpha rarefaction plots of ITS1 region reads against Shannon Diversity of ABG



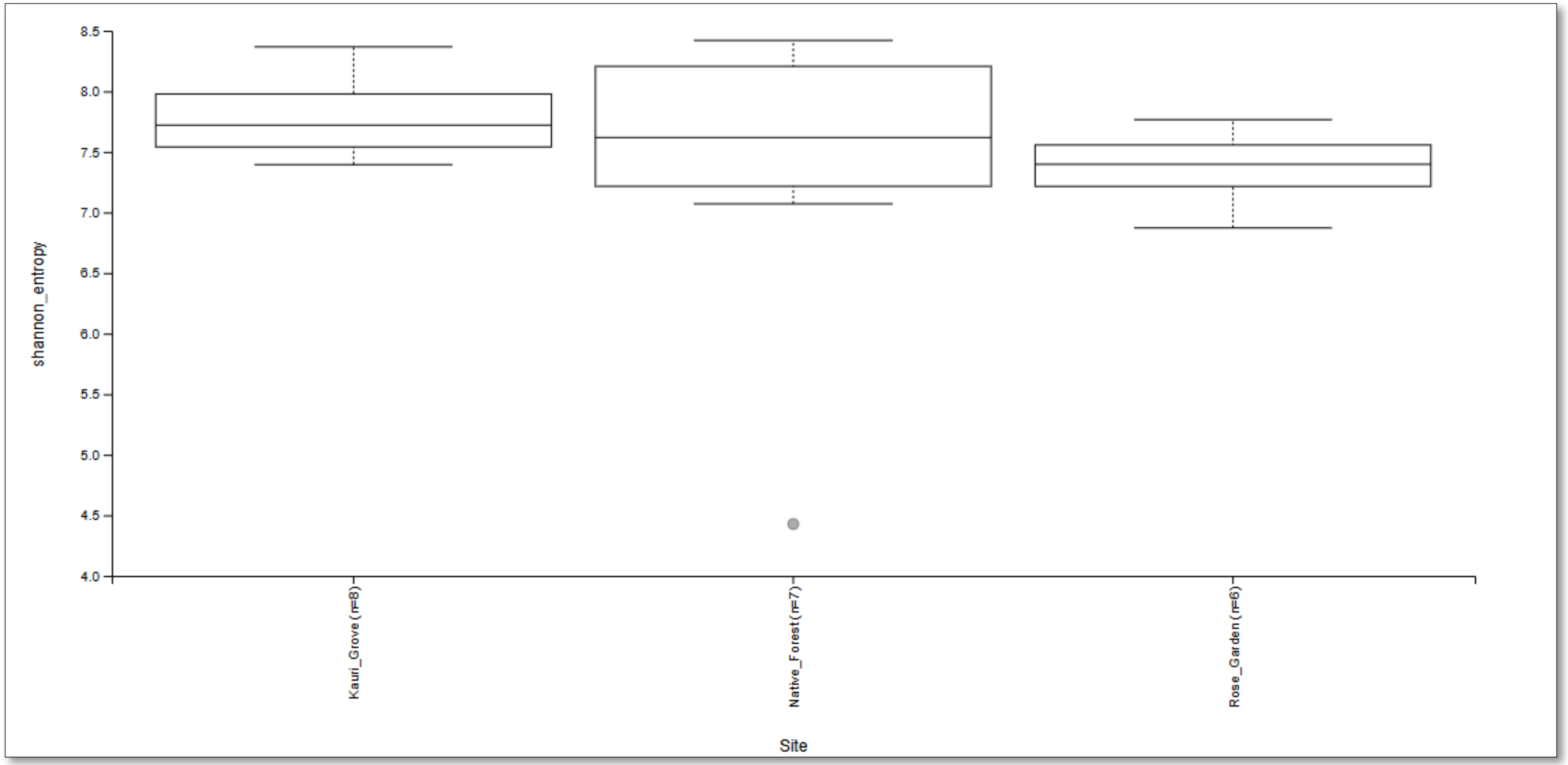
A2.4 Alpha rarefaction plots of 16S rRNA gene against observed ASVs of ABG



Alpha and Best diversity analysis performed with 500 and 1000 sequences per sample sampling depth. However, no significant differences were observed.

A3. Analysis of Independence between groups.

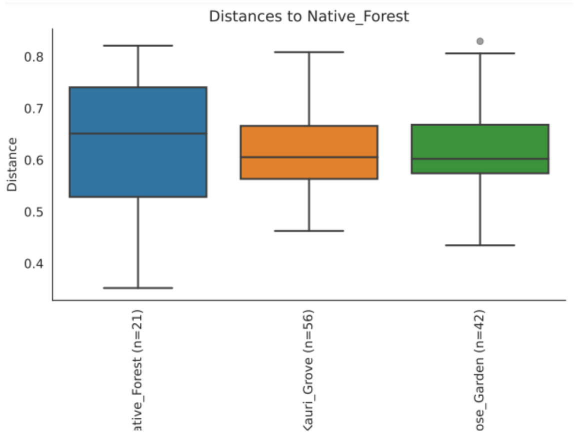
Shannon diversity analysis between gardens at ABG

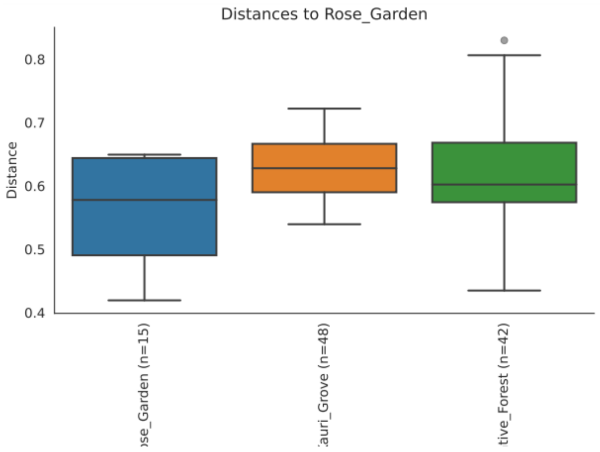


Kruskal-Wallis (Between Gardens at ABG)

H	2.906771799
p-value	0.2337773996

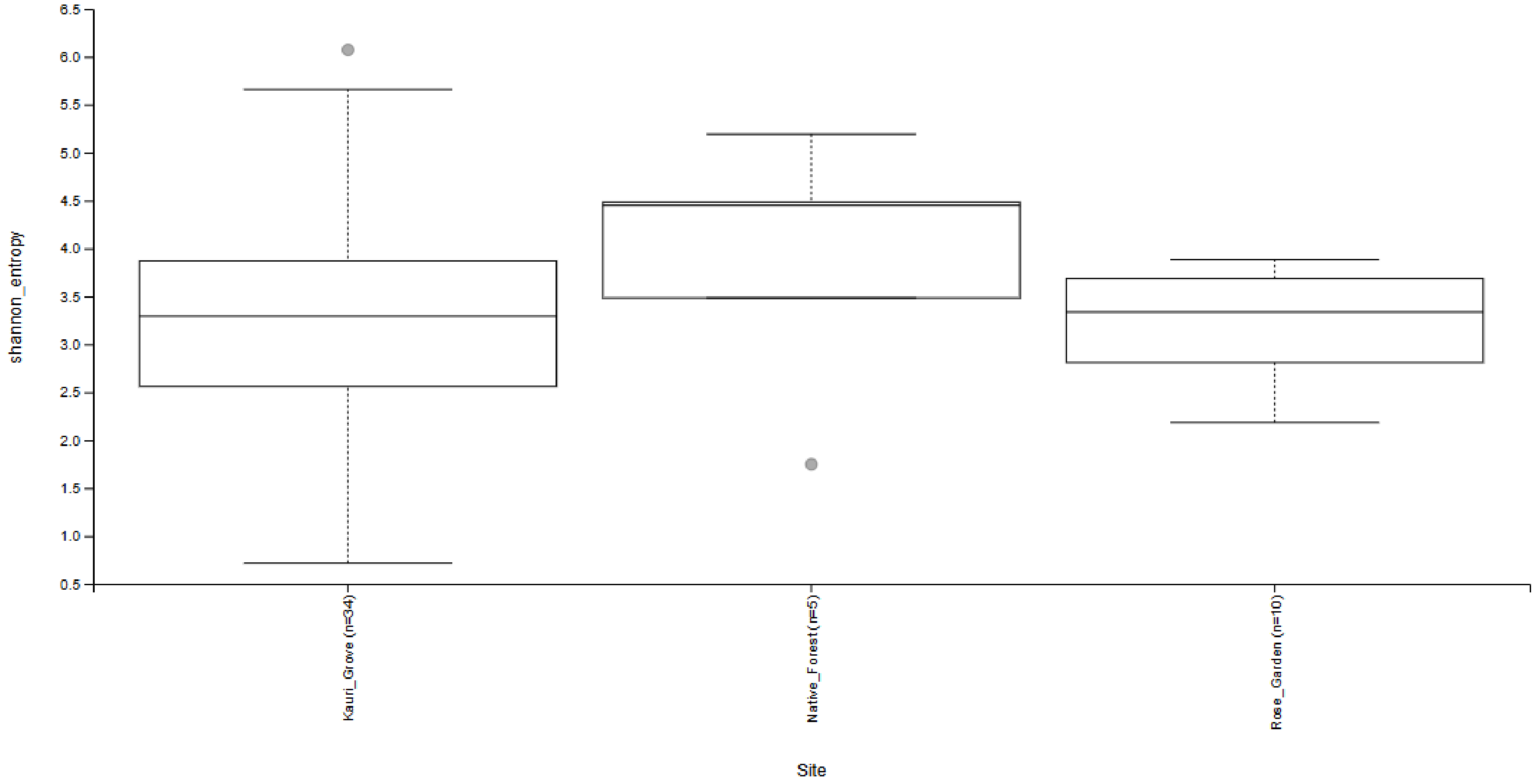
Group 1	Group 2	H	p-value	q-value
Kauri Grove (n=8)	Native Forest (n=7)	0.053571	0.816961	0.816961
Kauri Grove (n=8)	Rose Garden (n=6)	3.75	0.052808	0.158423
Native Forest (n=7)	Rose Garden (n=6)	0.734694	0.391366	0.587049





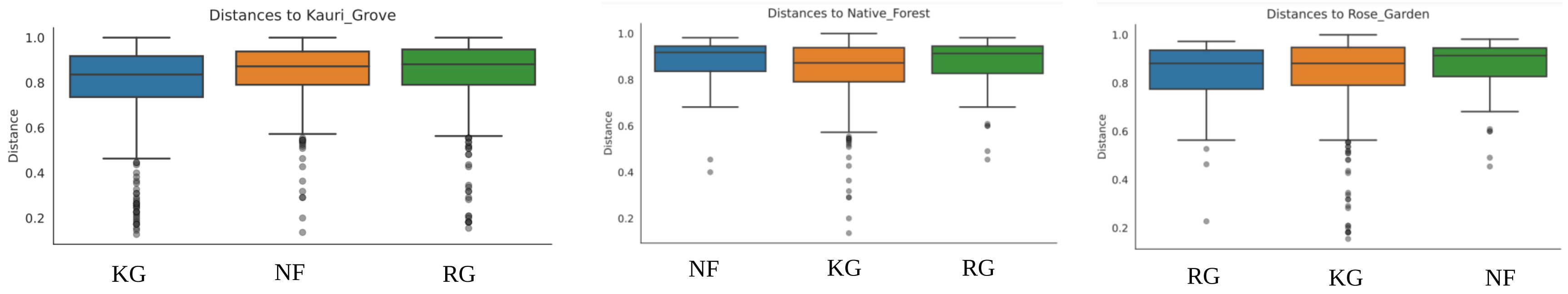
Method name	PERMANOVA
test statistic name	pseudo-F
Sample size	21
Number of groups	3
test statistic	3.219295
p-value	0.001
Number of permutations	999

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
Kauri Grove	Native Forest	15	999	3.555919	0.001	0.003
Kauri Grove	Rose Garden	14	999	5.142207	0.003	0.0045
Native Forest	Rose Garden	13	999	1.536103	0.065	0.065


6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
shannon_entropy
Kauri_Grove (n=34)
Native_Forest (n=5)
Rose_Garden (n=10)
Site

Kruskal-Wallis (all groups)	
H	2.143058823529401
p-value	0.3424843171180072

Group 1	Group 2	H	p-value	q-value
Kauri Grove (n=8)	Native Forest (n=7)	1.921765	0.165662	0.266864
Kauri Grove (n=8)	Rose Garden (n=6)	0.028235	0.866557	0.866557
Native Forest (n=7)	Rose Garden (n=6)	1.815000	0.177910	0.266864

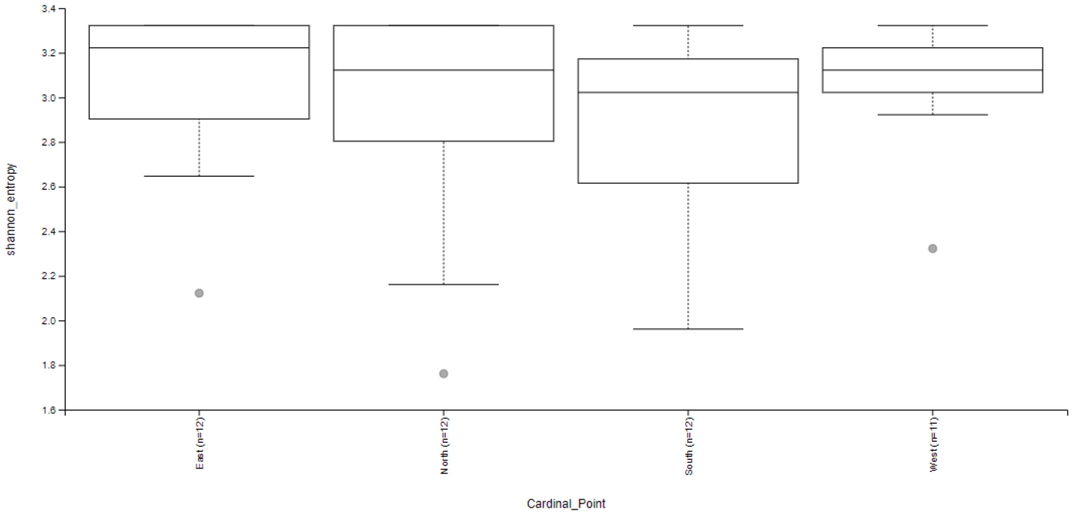


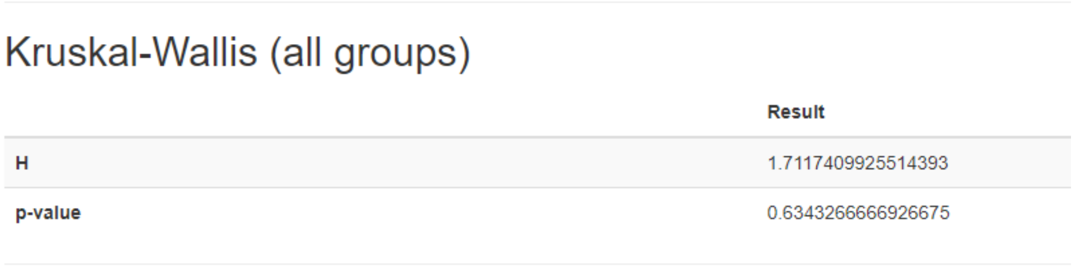
Method name	PERMANOVA
test statistic name	pseudo-F
Sample size	60
Number of groups	3
test statistic	1.661148
p-value	0.01
Number of permutations	999

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
Kauri Grove	Native Forest	15	999	1.288937	0.198	0.1980
Kauri Grove	Rose Garden	14	999	2.072102	0.009	0.0270
Native Forest	Rose Garden	13	999	1.494648	0.079	0.1185

A4. Cardinal point analysis

A4.1 Alpha diversity boxplots of cardinal points of bacterial reads in ABG soil samples



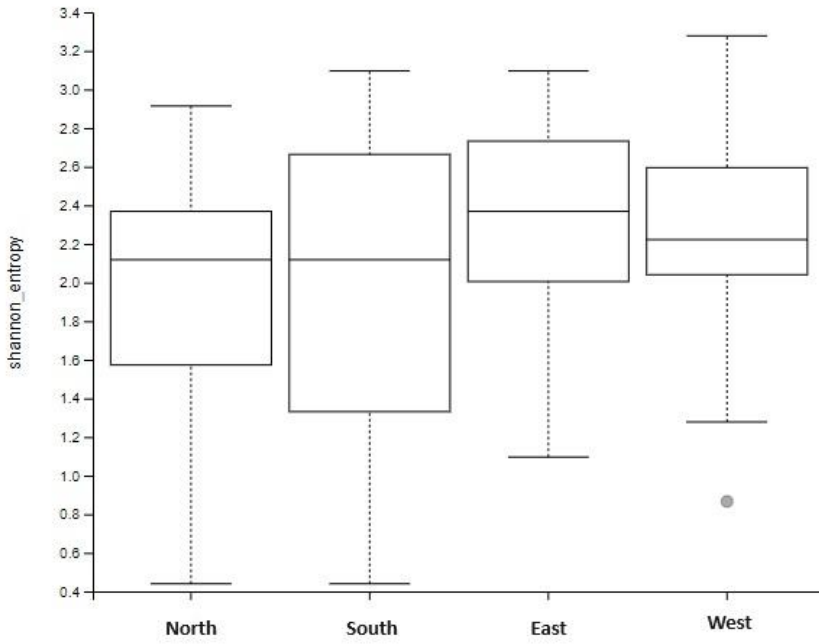


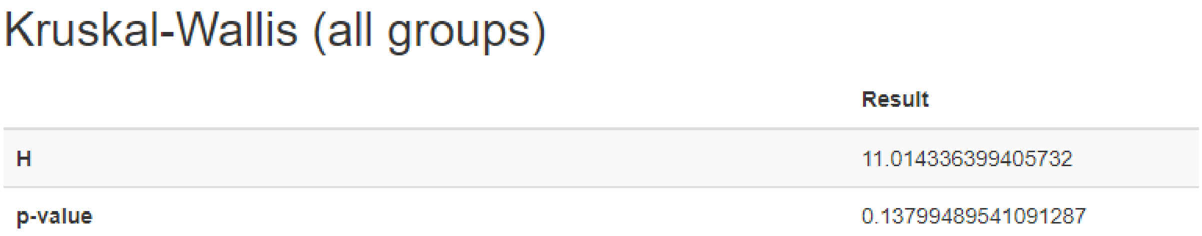
Kruskal-Wallis (all groups)

	Result
H	1.7117409925514393
p-value	0.6343266666926675

No significant difference was observed.

A4.2 Alpha diversity boxplots of cardinal points of fungal reads in ABG soil samples





Kruskal-Wallis (all groups)

	Result
H	11.014336399405732
p-value	0.13799489541091287

No significant difference was observed.

A5. Differential Abundance Results

A5.1 Bacterial Differential Abundance Results

A5.1.1 Microbial taxa positively correlated to KG have a positive estimate value

term	taxon	rank	formula	estimate	std.error	statistic	p.value
KG	P: Bacteroidetes	Phylum	`P: Bacteroidetes`~KG	1.215509964	0.706468556	1.720543617	0.093468405
KG	P: Nitrospirae	Phylum	`P: Nitrospirae`~KG	-1.319913872	0.498037555	-2.650229602	0.011659767
KG	P: Actinobacteria	Phylum	`P: Actinobacteria`~KG	1.900780351	0.652371656	2.913646439	0.005955415
KG	P: Acidobacteria	Phylum	`P: Acidobacteria`~KG	1.248362374	0.372472828	3.351552867	0.001827446
KG	P: Firmicutes	Phylum	`P: Firmicutes`~KG	-0.481121387	0.237762835	-2.023534868	0.050092517
KG	P: Verrucomicrobia	Phylum	`P: Verrucomicrobia`~KG	-0.086627486	0.449407416	-0.192759361	0.848173964
KG	P: Planctomycetes	Phylum	`P: Planctomycetes`~KG	1.629262456	0.824163023	1.976869151	0.055345954
KG	P: Proteobacteria	Phylum	`P: Proteobacteria`~KG	-0.792332251	0.620175913	-1.277592751	0.209143439
KG	C: Sphingobacteriia	Class	`C: Sphingobacteriia`~KG	2.149135457	1.12335708	1.913136522	0.063287614
KG	C: Flavobacteriia	Class	`C: Flavobacteriia`~KG	-2.571502565	1.07269549	-2.397234433	0.021541427
KG	C: [Saprospirae]	Class	`C: [Saprospirae]`~KG	1.007396079	0.765934264	1.315251355	0.196306925
KG	C: Nitrospira	Class	`C: Nitrospira`~KG	-1.748315778	0.639893195	-2.732199359	0.009492483
KG	C: Thermoleophilia	Class	`C: Thermoleophilia`~KG	3.262006075	0.83051669	3.927682745	0.00034976
KG	C: Actinobacteria	Class	`C: Actinobacteria`~KG	1.727099311	0.936740513	1.84373291	0.073030629
KG	C: Acidobacteriia	Class	`C: Acidobacteriia`~KG	1.248362374	0.372472828	3.351552867	0.001827446
KG	C: Bacilli	Class	`C: Bacilli`~KG	-0.766722658	0.362711683	-2.113862593	0.041147559
KG	C: [Spartobacteria]	Class	`C: [Spartobacteria]`~KG	-0.158027804	0.591238058	-0.267282868	0.79069671
KG	C: Planctomycetia	Class	`C: Planctomycetia`~KG	2.057664363	1.030142382	1.997456272	0.052972148
KG	C: Deltaproteobacteria	Class	`C: Deltaproteobacteria`~KG	-0.248273192	1.305005248	-0.190246891	0.85012837
KG	C: Betaproteobacteria	Class	`C: Betaproteobacteria`~KG	-1.381326655	1.011490505	-1.365634822	0.180083097
KG	C: Gammaproteobacteria	Class	`C: Gammaproteobacteria`~KG	-2.007324327	1.467139971	-1.368188698	0.17928915
KG	C: Alphaproteobacteria	Class	`C: Alphaproteobacteria`~KG	-0.273456847	0.651253333	-0.419893201	0.676928172
KG	O: Sphingobacteriales	Order	`O: Sphingobacteriales`~KG	2.149135457	1.12335708	1.913136522	0.063287614
KG	O: Flavobacteriales	Order	`O: Flavobacteriales`~KG	-2.571502565	1.07269549	-2.397234433	0.021541427
KG	O: [Saprospirales]	Order	`O: [Saprospirales]`~KG	1.007396079	0.765934264	1.315251355	0.196306925

KG	O: Nitrospirales	Order	`O: Nitrospirales`~KG	-1.748315778	0.639893195	-2.732199359	0.009492483
KG	O: Gaiellales	Order	`O: Gaiellales`~KG	3.262006075	0.83051669	3.927682745	0.00034976
KG	O: Actinomycetales	Order	`O: Actinomycetales`~KG	1.727099311	0.936740513	1.84373291	0.073030629
KG	O: Acidobacteriales	Order	`O: Acidobacteriales`~KG	1.248362374	0.372472828	3.351552867	0.001827446
KG	O: Bacillales	Order	`O: Bacillales`~KG	-0.766722658	0.362711683	-2.113862593	0.041147559
KG	O: [Chthoniobacterales]	Order	`O: [Chthoniobacterales]`~KG	-0.158027804	0.591238058	-0.267282868	0.79069671
KG	O: Pirellulales	Order	`O: Pirellulales`~KG	2.923582909	0.941519983	3.105173508	0.003585231
KG	O: Gemmatales	Order	`O: Gemmatales`~KG	-1.050980556	0.504421666	-2.083535713	0.043980257
KG	O: Myxococcales	Order	`O: Myxococcales`~KG	-3.304682255	0.975379798	-3.388097911	0.001650528
KG	O: Bdellovibrionales	Order	`O: Bdellovibrionales`~KG	3.056409064	0.866990743	3.525307611	0.001121657
KG	O: Burkholderiales	Order	`O: Burkholderiales`~KG	-1.381326655	1.011490505	-1.365634822	0.180083097
KG	O: Xanthomonadales	Order	`O: Xanthomonadales`~KG	-0.991659887	1.508832925	-0.657236378	0.514990294
KG	O: Enterobacteriales	Order	`O: Enterobacteriales`~KG	-1.516793318	1.108726094	-1.368050528	0.179332035
KG	O: Rickettsiales	Order	`O: Rickettsiales`~KG	-1.945031042	0.642495831	-3.02730531	0.004414821
KG	O: Rhodospirillales	Order	`O: Rhodospirillales`~KG	1.692657948	1.040324686	1.627047758	0.111992046
KG	O: Caulobacterales	Order	`O: Caulobacterales`~KG	-1.63004232	1.216139216	-1.340341877	0.188093401
KG	O: Rhodobacterales	Order	`O: Rhodobacterales`~KG	-1.356358559	0.851475933	-1.592949966	0.119456764
KG	O: Rhizobiales	Order	`O: Rhizobiales`~KG	0.163583493	1.041129485	0.157121179	0.875981225
KG	O: Sphingomonadales	Order	`O: Sphingomonadales`~KG	-3.599323186	1.239040935	-2.904926774	0.006092379
KG	F: Sphingobacteriaceae	Family	`F: Sphingobacteriaceae`~KG	2.149135457	1.12335708	1.913136522	0.063287614
KG	F: Flavobacteriaceae	Family	`F: Flavobacteriaceae`~KG	-2.571502565	1.07269549	-2.397234433	0.021541427
KG	F: Chitinophagaceae	Family	`F: Chitinophagaceae`~KG	1.007396079	0.765934264	1.315251355	0.196306925
KG	F: Nitrospiraceae	Family	`F: Nitrospiraceae`~KG	-1.748315778	0.639893195	-2.732199359	0.009492483
KG	F: Gaiellaceae	Family	`F: Gaiellaceae`~KG	3.262006075	0.83051669	3.927682745	0.00034976
KG	F: Micromonosporaceae	Family	`F: Micromonosporaceae`~KG	-1.836964431	0.70964703	-2.588560726	0.013581781
KG	F: Nocardioidaceae	Family	`F: Nocardioidaceae`~KG	-2.477265388	0.872334176	-2.839812374	0.007212274
KG	F: Thermomonosporaceae	Family	`F: Thermomonosporaceae`~KG	3.676728071	0.964361168	3.812604856	0.000490318
KG	F: Micrococcaceae	Family	`F: Micrococcaceae`~KG	-2.23242899	0.775523255	-2.878609991	0.006523841
KG	F: Streptomycetaceae	Family	`F: Streptomycetaceae`~KG	-2.205185278	1.157235642	-1.905562877	0.064293548
KG	F: Sporichthyaceae	Family	`F: Sporichthyaceae`~KG	-0.98484553	0.840334179	-1.171968907	0.248505149

KG	F: Acidobacteriaceae	Family	`F: Acidobacteriaceae`~KG	1.360980346	0.410180066	3.318007041	0.00200567
KG	F: Koribacteraceae	Family	`F: Koribacteraceae`~KG	-1.512696132	1.187254672	-1.274112596	0.210360922
KG	F: Planococcaceae	Family	`F: Planococcaceae`~KG	-0.766722658	0.362711683	-2.113862593	0.041147559
KG	F: [Chthoniobacteraceae]	Family	`F: [Chthoniobacteraceae]`~KG	-0.158027804	0.591238058	-0.267282868	0.79069671
KG	F: Pirellulaceae	Family	`F: Pirellulaceae`~KG	2.923582909	0.941519983	3.105173508	0.003585231
KG	F: Isosphaeraceae	Family	`F: Isosphaeraceae`~KG	-1.050980556	0.504421666	-2.083535713	0.043980257
KG	F: Cystobacteraceae	Family	`F: Cystobacteraceae`~KG	-3.304682255	0.975379798	-3.388097911	0.001650528
KG	F: Bdellovibrionaceae	Family	`F: Bdellovibrionaceae`~KG	3.056409064	0.866990743	3.525307611	0.001121657
KG	F: Comamonadaceae	Family	`F: Comamonadaceae`~KG	0.090393672	0.511763367	0.176631775	0.86073568
KG	F: Burkholderiaceae	Family	`F: Burkholderiaceae`~KG	-1.046547741	0.486835927	-2.149692911	0.038008902
KG	F: Oxalobacteraceae	Family	`F: Oxalobacteraceae`~KG	-0.787353485	0.963981293	-0.816772577	0.419149677
KG	F: Xanthomonadaceae	Family	`F: Xanthomonadaceae`~KG	-0.991659887	1.508832925	-0.657236378	0.514990294
KG	F: Enterobacteriaceae	Family	`F: Enterobacteriaceae`~KG	-1.516793318	1.108726094	-1.368050528	0.179332035
KG	F: mitochondria	Family	`F: mitochondria`~KG	-1.945031042	0.642495831	-3.02730531	0.004414821
KG	F: Rhodospirillaceae	Family	`F: Rhodospirillaceae`~KG	1.646088549	1.016837949	1.618830759	0.11375502
KG	F: Acetobacteraceae	Family	`F: Acetobacteraceae`~KG	1.236801396	1.02638774	1.205004062	0.235652693
KG	F: Caulobacteraceae	Family	`F: Caulobacteraceae`~KG	-1.63004232	1.216139216	-1.340341877	0.188093401
KG	F: Hyphomonadaceae	Family	`F: Hyphomonadaceae`~KG	-1.356358559	0.851475933	-1.592949966	0.119456764
KG	F: Beijerinckiaceae	Family	`F: Beijerinckiaceae`~KG	0.163583493	1.041129485	0.157121179	0.875981225
KG	F: Sphingomonadaceae	Family	`F: Sphingomonadaceae`~KG	-3.34384073	1.223842056	-2.732248589	0.009491302
KG	F: Erythrobacteraceae	Family	`F: Erythrobacteraceae`~KG	-4.040022442	1.107255607	-3.648680952	0.00078851
KG	G: Mucilaginibacter	Genus	`G: Mucilaginibacter`~KG	2.589714907	1.155792847	2.240639328	0.030973681
KG	G: Solitalea	Genus	`G: Solitalea`~KG	-1.57061201	0.57624961	-2.725575833	0.009652674
KG	G: Flavobacterium	Genus	`G: Flavobacterium`~KG	-2.571502565	1.07269549	-2.397234433	0.021541427
KG	G: Trachelomonas	Genus	`G: Trachelomonas`~KG	0.908685143	0.962956685	0.943640723	0.351314612
KG	G: Ferruginibacter	Genus	`G: Ferruginibacter`~KG	0.421647474	1.230470666	0.342671699	0.7337338
KG	G: Lacibacter	Genus	`G: Lacibacter`~KG	-1.626390326	0.745992119	-2.180170922	0.035507677
KG	G: Filimonas	Genus	`G: Filimonas`~KG	-0.308496869	0.842966479	-0.365965761	0.7164202
KG	G: Nitrospira	Genus	`G: Nitrospira`~KG	-1.748315778	0.639893195	-2.732199359	0.009492483
KG	G: Gaiella	Genus	`G: Gaiella`~KG	3.262006075	0.83051669	3.927682745	0.00034976

KG	G: Rugosimonospora	Genus	`G: Rugosimonospora`~KG	-1.836964431	0.70964703	-2.588560726	0.013581781
KG	G: Nocardioides	Genus	`G: Nocardioides`~KG	-2.477265388	0.872334176	-2.839812374	0.007212274
KG	G: Actinomadura	Genus	`G: Actinomadura`~KG	3.676728071	0.964361168	3.812604856	0.000490318
KG	G: Arthrobacter	Genus	`G: Arthrobacter`~KG	-2.23242899	0.775523255	-2.878609991	0.006523841
KG	G: Streptomyces	Genus	`G: Streptomyces`~KG	-2.205185278	1.157235642	-1.905562877	0.064293548
KG	G: Sporichthya	Genus	`G: Sporichthya`~KG	-0.98484553	0.840334179	-1.171968907	0.248505149
KG	G: Telmatobacter	Genus	`G: Telmatobacter`~KG	2.359889263	0.746354845	3.16188644	0.003076268
KG	G: Candidatus Koribacter	Genus	`G: Candidatus Koribacter`~KG	-1.512696132	1.187254672	-1.274112596	0.210360922
KG	G: Granulicella	Genus	`G: Granulicella`~KG	2.313796904	0.790189043	2.928156147	0.005733879
KG	G: Acidopila	Genus	`G: Acidopila`~KG	3.565176081	1.034818825	3.445217651	0.001406405
KG	G: Acidicapsa	Genus	`G: Acidicapsa`~KG	0.415215532	1.03924037	0.399537532	0.691734032
KG	G: Bryocella	Genus	`G: Bryocella`~KG	0.427410808	1.209382194	0.353412519	0.725732405
KG	G: Terriglobus	Genus	`G: Terriglobus`~KG	2.909594941	0.844732937	3.444396228	0.001409657
KG	G: Staphylococcus	Genus	`G: Staphylococcus`~KG	-0.766722658	0.362711683	-2.113862593	0.041147559
KG	G: Ellin506	Genus	`G: Ellin506`~KG	-0.158027804	0.591238058	-0.267282868	0.79069671
KG	G: planctomycete	Genus	`G: planctomycete`~KG	2.923582909	0.941519983	3.105173508	0.003585231
KG	G: Nostocoida	Genus	`G: Nostocoida`~KG	-1.050980556	0.504421666	-2.083535713	0.043980257
KG	G: Cystobacter	Genus	`G: Cystobacter`~KG	-3.304682255	0.975379798	-3.388097911	0.001650528
KG	G: Bdellovibrio	Genus	`G: Bdellovibrio`~KG	3.056409064	0.866990743	3.525307611	0.001121657
KG	G: Sphaerotilus	Genus	`G: Sphaerotilus`~KG	0.090393672	0.511763367	0.176631775	0.86073568
KG	G: Burkholderia	Genus	`G: Burkholderia`~KG	-1.046547741	0.486835927	-2.149692911	0.038008902
KG	G: Pseudoburkholderia	Genus	`G: Pseudoburkholderia`~KG	-0.753652379	0.850176166	-0.886466133	0.380942346
KG	G: Massilia	Genus	`G: Massilia`~KG	0.404155263	0.626210647	0.645398262	0.522547253
KG	G: Luteibacter	Genus	`G: Luteibacter`~KG	-0.897350898	1.253896982	-0.71564962	0.47858238
KG	G: Rhodanobacter	Genus	`G: Rhodanobacter`~KG	0.490968445	1.268582588	0.38702127	0.700899521
KG	G: Gynumella	Genus	`G: Gynumella`~KG	1.19591407	0.810921903	1.474758623	0.148515101
KG	G: Lysobacter	Genus	`G: Lysobacter`~KG	-1.523835255	1.047020669	-1.455401311	0.153770369
KG	G: Thermomonas	Genus	`G: Thermomonas`~KG	-2.520999871	0.914435712	-2.756891312	0.00891678
KG	G: Enterobacteriaceae - Genus	Genus	`G: Enterobacteriaceae - Genus`~KG	-1.516793318	1.108726094	-1.368050528	0.179332035

KG	G: Nageia	Genus	`G: Nageia`~KG	-1.945031042	0.642495831	-3.02730531	0.004414821
KG	G: Reyranella	Genus	`G: Reyranella`~KG	-0.730348051	1.10680609	-0.659869925	0.513317208
KG	G: Rhodopila	Genus	`G: Rhodopila`~KG	1.236801396	1.02638774	1.205004062	0.235652693
KG	G: Dongia	Genus	`G: Dongia`~KG	-2.16506746	0.921028228	-2.350706954	0.024029127
KG	G: Skermanella	Genus	`G: Skermanella`~KG	6.510739071	0.817213213	7.967001714	1.25894E-09
KG	G: Stella	Genus	`G: Stella`~KG	-1.79664346	0.717225404	-2.504991388	0.01665146
KG	G: Caulobacter	Genus	`G: Caulobacter`~KG	-0.590883271	1.151420717	-0.513177557	0.610797912
KG	G: Nitrobacteria	Genus	`G: Nitrobacteria`~KG	-2.499031292	0.731029922	-3.418507529	0.001515915
KG	G: Brevundimonas	Genus	`G: Brevundimonas`~KG	-3.035789331	0.785691229	-3.863845262	0.000422022
KG	G: Arthrospira	Genus	`G: Arthrospira`~KG	0.531791881	0.798493757	0.665993788	0.50943814
KG	G: Asticcacaulis	Genus	`G: Asticcacaulis`~KG	0.163793723	0.662326331	0.247300636	0.806006877
KG	G: Woodsholea	Genus	`G: Woodsholea`~KG	-1.356358559	0.851475933	-1.592949966	0.119456764
KG	G: Methylocapsa	Genus	`G: Methylocapsa`~KG	0.163583493	1.041129485	0.157121179	0.875981225
KG	G: Sphingobium	Genus	`G: Sphingobium`~KG	-2.005501134	1.167304444	-1.718061765	0.093924911
KG	G: Sphingomonas	Genus	`G: Sphingomonas`~KG	0.468610214	1.288303074	0.363742215	0.718066519
KG	G: Sphingosinicella	Genus	`G: Sphingosinicella`~KG	-3.679970575	1.37085473	-2.684435115	0.010705073
KG	G: Porphyrobacter	Genus	`G: Porphyrobacter`~KG	-4.040022442	1.107255607	-3.648680952	0.00078851

A5.1.2 Microbial taxa positively correlated to RG have a positive estimate value

term	taxon	rank	formula	estimate	std.error	statistic	p.value
RG	P: Bacteroidetes	Phylum	`P: Bacteroidetes`~RG	-0.18511	0.799718	-0.23147	0.818194
RG	P: Nitrospirae	Phylum	`P: Nitrospirae`~RG	1.488492	0.539964	2.756651	0.008922
RG	P: Actinobacteria	Phylum	`P: Actinobacteria`~RG	-2.46837	0.677867	-3.64138	0.000805
RG	P: Acidobacteria	Phylum	`P: Acidobacteria`~RG	-1.81508	0.356772	-5.08751	1.01E-05
RG	P: Firmicutes	Phylum	`P: Firmicutes`~RG	-0.0167	0.273027	-0.06116	0.951555
RG	P: Verrucomicrobia	Phylum	`P: Verrucomicrobia`~RG	-0.43732	0.485427	-0.90089	0.373319
RG	P: Planctomycetes	Phylum	`P: Planctomycetes`~RG	-1.97158	0.888534	-2.21891	0.03254
RG	P: Proteobacteria	Phylum	`P: Proteobacteria`~RG	0.377997	0.688321	0.549157	0.58611
RG	C: Sphingobacteriia	Class	`C: Sphingobacteriia`~RG	-1.92719	1.244692	-1.54833	0.129833

RG	C: Flavobacteriia	Class	`C: Flavobacteriia`~RG	2.235443	1.202293	1.859316	0.070738
RG	C: [Saprospirae]	Class	`C: [Saprospirae]`~RG	-0.1046	0.854345	-0.12243	0.903202
RG	C: Nitrospira	Class	`C: Nitrospira`~RG	1.862494	0.701376	2.655486	0.011508
RG	C: Thermoleophilia	Class	`C: Thermoleophilia`~RG	-3.63924	0.897757	-4.0537	0.000241
RG	C: Actinobacteria	Class	`C: Actinobacteria`~RG	-2.59915	0.979945	-2.65234	0.011599
RG	C: Acidobacteriia	Class	`C: Acidobacteriia`~RG	-1.81508	0.356772	-5.08751	1.01E-05
RG	C: Bacilli	Class	`C: Bacilli`~RG	-0.0507	0.418292	-0.1212	0.90417
RG	C: [Spartobacteria]	Class	`C: [Spartobacteria]`~RG	-0.52232	0.640116	-0.81597	0.4196
RG	C: Planctomycetia	Class	`C: Planctomycetia`~RG	-2.61758	1.10257	-2.37407	0.022749
RG	C: Deltaproteobacteria	Class	`C: Deltaproteobacteria`~RG	-1.2668	1.409655	-0.89866	0.374495
RG	C: Betaproteobacteria	Class	`C: Betaproteobacteria`~RG	-1.12525	1.115548	-1.00869	0.319502
RG	C: Gammaproteobacteria	Class	`C: Gammaproteobacteria`~RG	1.386419	1.624238	0.853581	0.398686
RG	C: Alphaproteobacteria	Class	`C: Alphaproteobacteria`~RG	-0.16085	0.711744	-0.226	0.822412
RG	O: Sphingobacteriales	Order	`O: Sphingobacteriales`~RG	-1.92719	1.244692	-1.54833	0.129833
RG	O: Flavobacteriales	Order	`O: Flavobacteriales`~RG	2.235443	1.202293	1.859316	0.070738
RG	O: [Saprospirales]	Order	`O: [Saprospirales]`~RG	-0.1046	0.854345	-0.12243	0.903202
RG	O: Nitrospirales	Order	`O: Nitrospirales`~RG	1.862494	0.701376	2.655486	0.011508
RG	O: Gaiellales	Order	`O: Gaiellales`~RG	-3.63924	0.897757	-4.0537	0.000241
RG	O: Actinomycetales	Order	`O: Actinomycetales`~RG	-2.59915	0.979945	-2.65234	0.011599
RG	O: Acidobacteriales	Order	`O: Acidobacteriales`~RG	-1.81508	0.356772	-5.08751	1.01E-05
RG	O: Bacillales	Order	`O: Bacillales`~RG	-0.0507	0.418292	-0.1212	0.90417
RG	O: [Chthoniobacterales]	Order	`O: [Chthoniobacterales]`~RG	-0.52232	0.640116	-0.81597	0.4196
RG	O: Pirellulales	Order	`O: Pirellulales`~RG	-1.99906	1.103593	-1.81141	0.077987
RG	O: Gemmatales	Order	`O: Gemmatales`~RG	-0.7507	0.568049	-1.32154	0.194223
RG	O: Myxococcales	Order	`O: Myxococcales`~RG	0.916353	1.205245	0.760304	0.451765
RG	O: Bdellovibrionales	Order	`O: Bdellovibrionales`~RG	-2.18315	1.030574	-2.11838	0.040739
RG	O: Burkholderiales	Order	`O: Burkholderiales`~RG	-1.12525	1.115548	-1.00869	0.319502
RG	O: Xanthomonadales	Order	`O: Xanthomonadales`~RG	0.481276	1.65376	0.291019	0.772619
RG	O: Enterobacteriales	Order	`O: Enterobacteriales`~RG	1.696362	1.208209	1.40403	0.16843
RG	O: Rickettsiales	Order	`O: Rickettsiales`~RG	0.573717	0.775427	0.739872	0.463925

RG	O: Rhodospirillales	Order	`O: Rhodospirillales`~RG	-1.39913	1.15181	-1.21473	0.231965
RG	O: Caulobacterales	Order	`O: Caulobacterales`~RG	-0.75199	1.35243	-0.55603	0.581451
RG	O: Rhodobacterales	Order	`O: Rhodobacterales`~RG	0.669284	0.953392	0.702003	0.486955
RG	O: Rhizobiales	Order	`O: Rhizobiales`~RG	-1.65515	1.104157	-1.49901	0.142133
RG	O: Sphingomonadales	Order	`O: Sphingomonadales`~RG	2.445589	1.440873	1.697296	0.097818
RG	F: Sphingobacteriaceae	Family	`F: Sphingobacteriaceae`~RG	-1.92719	1.244692	-1.54833	0.129833
RG	F: Flavobacteriaceae	Family	`F: Flavobacteriaceae`~RG	2.235443	1.202293	1.859316	0.070738
RG	F: Chitinophagaceae	Family	`F: Chitinophagaceae`~RG	-0.1046	0.854345	-0.12243	0.903202
RG	F: Nitrospiraceae	Family	`F: Nitrospiraceae`~RG	1.862494	0.701376	2.655486	0.011508
RG	F: Gaiellaceae	Family	`F: Gaiellaceae`~RG	-3.63924	0.897757	-4.0537	0.000241
RG	F: Micromonosporaceae	Family	`F: Micromonosporaceae`~RG	0.016333	0.83978	0.019449	0.984585
RG	F: Nocardioidaceae	Family	`F: Nocardioidaceae`~RG	2.58287	0.960527	2.689014	0.010583
RG	F: Thermomonosporaceae	Family	`F: Thermomonosporaceae`~RG	-4.79868	0.961591	-4.99036	1.37E-05
RG	F: Micrococcaceae	Family	`F: Micrococcaceae`~RG	2.347357	0.852717	2.752798	0.00901
RG	F: Streptomycetaceae	Family	`F: Streptomycetaceae`~RG	0.548753	1.318597	0.416164	0.679631
RG	F: Sporichthyaceae	Family	`F: Sporichthyaceae`~RG	1.662592	0.893483	1.860799	0.070524
RG	F: Acidobacteriaceae	Family	`F: Acidobacteriaceae`~RG	-1.90842	0.403088	-4.7345	3.03E-05
RG	F: Koribacteraceae	Family	`F: Koribacteraceae`~RG	0.028552	1.322773	0.021585	0.982892
RG	F: Planococcaceae	Family	`F: Planococcaceae`~RG	-0.0507	0.418292	-0.1212	0.90417
RG	F: [Chthoniobacteraceae]	Family	`F: [Chthoniobacteraceae]`~RG	-0.52232	0.640116	-0.81597	0.4196
RG	F: Pirellulaceae	Family	`F: Pirellulaceae`~RG	-1.99906	1.103593	-1.81141	0.077987
RG	F: Isosphaeraceae	Family	`F: Isosphaeraceae`~RG	-0.7507	0.568049	-1.32154	0.194223
RG	F: Cystobacteraceae	Family	`F: Cystobacteraceae`~RG	0.916353	1.205245	0.760304	0.451765
RG	F: Bdellovibrionaceae	Family	`F: Bdellovibrionaceae`~RG	-2.18315	1.030574	-2.11838	0.040739
RG	F: Comamonadaceae	Family	`F: Comamonadaceae`~RG	-0.74833	0.545259	-1.37244	0.177974
RG	F: Burkholderiaceae	Family	`F: Burkholderiaceae`~RG	-0.74753	0.549328	-1.36082	0.181589
RG	F: Oxalobacteraceae	Family	`F: Oxalobacteraceae`~RG	-0.25561	1.060172	-0.2411	0.810773
RG	F: Xanthomonadaceae	Family	`F: Xanthomonadaceae`~RG	0.481276	1.65376	0.291019	0.772619
RG	F: Enterobacteriaceae	Family	`F: Enterobacteriaceae`~RG	1.696362	1.208209	1.40403	0.16843
RG	F: mitochondria	Family	`F: mitochondria`~RG	0.573717	0.775427	0.739872	0.463925

RG	F: Rhodospirillaceae	Family	`F: Rhodospirillaceae`~RG	-1.34064	1.126274	-1.19033	0.241301
RG	F: Acetobacteraceae	Family	`F: Acetobacteraceae`~RG	-0.93343	1.130985	-0.82532	0.41434
RG	F: Caulobacteraceae	Family	`F: Caulobacteraceae`~RG	-0.75199	1.35243	-0.55603	0.581451
RG	F: Hyphomonadaceae	Family	`F: Hyphomonadaceae`~RG	0.669284	0.953392	0.702003	0.486955
RG	F: Beijerinckiaceae	Family	`F: Beijerinckiaceae`~RG	-1.65515	1.104157	-1.49901	0.142133
RG	F: Sphingomonadaceae	Family	`F: Sphingomonadaceae`~RG	2.069009	1.42152	1.45549	0.153746
RG	F: Erythrobacteraceae	Family	`F: Erythrobacteraceae`~RG	4.446337	1.204415	3.691699	0.000697
RG	G: Mucilaginibacter	Genus	`G: Mucilaginibacter`~RG	-2.60788	1.273357	-2.04804	0.047512
RG	G: Solitalea	Genus	`G: Solitalea`~RG	1.02289	0.667129	1.533272	0.133493
RG	G: Flavobacterium	Genus	`G: Flavobacterium`~RG	2.235443	1.202293	1.859316	0.070738
RG	G: Trachelomonas	Genus	`G: Trachelomonas`~RG	-0.52029	1.059554	-0.49105	0.626216
RG	G: Ferruginibacter	Genus	`G: Ferruginibacter`~RG	0.196411	1.344249	0.146112	0.884605
RG	G: Lacibacter	Genus	`G: Lacibacter`~RG	-1.06919	0.845748	-1.26419	0.21386
RG	G: Filimonas	Genus	`G: Filimonas`~RG	0.21745	0.920696	0.23618	0.814562
RG	G: Nitrospira	Genus	`G: Nitrospira`~RG	1.862494	0.701376	2.655486	0.011508
RG	G: Gaiella	Genus	`G: Gaiella`~RG	-3.63924	0.897757	-4.0537	0.000241
RG	G: Rugosimonospora	Genus	`G: Rugosimonospora`~RG	0.016333	0.83978	0.019449	0.984585
RG	G: Nocardioides	Genus	`G: Nocardioides`~RG	2.58287	0.960527	2.689014	0.010583
RG	G: Actinomadura	Genus	`G: Actinomadura`~RG	-4.79868	0.961591	-4.99036	1.37E-05
RG	G: Arthrobacter	Genus	`G: Arthrobacter`~RG	2.347357	0.852717	2.752798	0.00901
RG	G: Streptomyces	Genus	`G: Streptomyces`~RG	0.548753	1.318597	0.416164	0.679631
RG	G: Sporichthya	Genus	`G: Sporichthya`~RG	1.662592	0.893483	1.860799	0.070524
RG	G: Telmatobacter	Genus	`G: Telmatobacter`~RG	-2.31445	0.834658	-2.77294	0.00856
RG	G: Candidatus Koribacter	Genus	`G: Candidatus Koribacter`~RG	0.028552	1.322773	0.021585	0.982892
RG	G: Granulicella	Genus	`G: Granulicella`~RG	-3.24045	0.796695	-4.06737	0.000231
RG	G: Acidopila	Genus	`G: Acidopila`~RG	-4.37588	1.081257	-4.04703	0.000246
RG	G: Acidicapsa	Genus	`G: Acidicapsa`~RG	-2.56655	1.057257	-2.42756	0.020048
RG	G: Bryocella	Genus	`G: Bryocella`~RG	-2.76034	1.243546	-2.21974	0.032479
RG	G: Terriglobus	Genus	`G: Terriglobus`~RG	-4.03496	0.828411	-4.87072	1.99E-05
RG	G: Staphylococcus	Genus	`G: Staphylococcus`~RG	-0.0507	0.418292	-0.1212	0.90417

RG	G: Ellin506	Genus	`G: Ellin506`~RG	-0.52232	0.640116	-0.81597	0.4196
RG	G: planctomycete	Genus	`G: planctomycete`~RG	-1.99906	1.103593	-1.81141	0.077987
RG	G: Nostocoida	Genus	`G: Nostocoida`~RG	-0.7507	0.568049	-1.32154	0.194223
RG	G: Cystobacter	Genus	`G: Cystobacter`~RG	0.916353	1.205245	0.760304	0.451765
RG	G: Bdellovibrio	Genus	`G: Bdellovibrio`~RG	-2.18315	1.030574	-2.11838	0.040739
RG	G: Sphaerotilus	Genus	`G: Sphaerotilus`~RG	-0.74833	0.545259	-1.37244	0.177974
RG	G: Burkholderia	Genus	`G: Burkholderia`~RG	-0.74753	0.549328	-1.36082	0.181589
RG	G: Pseudoburkholderia	Genus	`G: Pseudoburkholderia`~RG	-0.68393	0.93057	-0.73496	0.466875
RG	G: Massilia	Genus	`G: Massilia`~RG	0.11557	0.686731	0.16829	0.867248
RG	G: Luteibacter	Genus	`G: Luteibacter`~RG	1.319554	1.360566	0.969857	0.338251
RG	G: Rhodanobacter	Genus	`G: Rhodanobacter`~RG	-0.95395	1.378202	-0.69217	0.493041
RG	G: Gynumella	Genus	`G: Gynumella`~RG	-0.87705	0.898562	-0.97606	0.335208
RG	G: Lysobacter	Genus	`G: Lysobacter`~RG	2.830991	1.080232	2.620725	0.012546
RG	G: Thermomonas	Genus	`G: Thermomonas`~RG	3.486349	0.935262	3.727673	0.000628
RG	G: Enterobacteriaceae - Genus	Genus	`G: Enterobacteriaceae - Genus`~RG	1.696362	1.208209	1.40403	0.16843
RG	G: Nageia	Genus	`G: Nageia`~RG	0.573717	0.775427	0.739872	0.463925
RG	G: Reyranella	Genus	`G: Reyranella`~RG	0.338275	1.213283	0.27881	0.781903
RG	G: Rhodopila	Genus	`G: Rhodopila`~RG	-0.93343	1.130985	-0.82532	0.41434
RG	G: Dongia	Genus	`G: Dongia`~RG	1.713561	1.038968	1.649292	0.107332
RG	G: Skermanella	Genus	`G: Skermanella`~RG	-5.4629	1.156587	-4.7233	3.14E-05
RG	G: Stella	Genus	`G: Stella`~RG	0.457837	0.84143	0.544117	0.58954
RG	G: Caulobacter	Genus	`G: Caulobacter`~RG	-0.68689	1.255714	-0.54701	0.587567
RG	G: Nitrobacteria	Genus	`G: Nitrobacteria`~RG	2.271708	0.83429	2.722924	0.009717
RG	G: Brevundimonas	Genus	`G: Brevundimonas`~RG	-1.41699	0.98528	-1.43816	0.158573
RG	G: Arthrospira	Genus	`G: Arthrospira`~RG	-0.42578	0.873572	-0.4874	0.628774
RG	G: Asticcacaulis	Genus	`G: Asticcacaulis`~RG	0.52345	0.718236	0.728799	0.470593
RG	G: Woodsholea	Genus	`G: Woodsholea`~RG	0.669284	0.953392	0.702003	0.486955
RG	G: Methylocapsa	Genus	`G: Methylocapsa`~RG	-1.65515	1.104157	-1.49901	0.142133
RG	G: Sphingobium	Genus	`G: Sphingobium`~RG	3.038644	1.226851	2.476784	0.017823
RG	G: Sphingomonas	Genus	`G: Sphingomonas`~RG	-0.74479	1.402906	-0.53089	0.598582

RG	G: Sphingosinicella	Genus	`G: Sphingosinicella`~RG	2.381426	1.584994	1.502483	0.141238
RG	G: Porphyrobacter	Genus	`G: Porphyrobacter`~RG	4.446337	1.204415	3.691699	0.000697

A5.1.3 Microbial taxa positively correlated to NF have a positive estimate value

term	taxon	rank	formula	estimate	std.error	statistic	p.value
NF	P: Bacteroidetes	Phylum	`P: Bacteroidetes`~NF	-1.65628	0.876584	-1.88947	0.066477
NF	P: Nitrospirae	Phylum	`P: Nitrospirae`~NF	0.108719	0.677413	0.160492	0.873344
NF	P: Actinobacteria	Phylum	`P: Actinobacteria`~NF	0.269769	0.900903	0.299443	0.766234
NF	P: Acidobacteria	Phylum	`P: Acidobacteria`~NF	0.431729	0.525309	0.821857	0.416285
NF	P: Firmicutes	Phylum	`P: Firmicutes`~NF	0.773667	0.286525	2.700178	0.01029
NF	P: Verrucomicrobia	Phylum	`P: Verrucomicrobia`~NF	0.709336	0.550128	1.289401	0.205052
NF	P: Planctomycetes	Phylum	`P: Planctomycetes`~NF	0.04197	1.081861	0.038794	0.969258
NF	P: Proteobacteria	Phylum	`P: Proteobacteria`~NF	0.741898	0.782493	0.948122	0.349058
NF	C: Sphingobacteriia	Class	`C: Sphingobacteriia`~NF	-0.82859	1.46411	-0.56593	0.574767
NF	C: Flavobacteriia	Class	`C: Flavobacteriia`~NF	1.083954	1.427905	0.759122	0.452463
NF	C: [Saprospirae]	Class	`C: [Saprospirae]`~NF	-1.43677	0.950817	-1.51109	0.139038
NF	C: Nitrospira	Class	`C: Nitrospira`~NF	0.28722	0.873669	0.328751	0.744149
NF	C: Thermoleophilia	Class	`C: Thermoleophilia`~NF	-0.32038	1.229867	-0.2605	0.795882
NF	C: Actinobacteria	Class	`C: Actinobacteria`~NF	0.712789	1.216695	0.58584	0.561447
NF	C: Acidobacteriia	Class	`C: Acidobacteriia`~NF	0.431729	0.525309	0.821857	0.416285
NF	C: Bacilli	Class	`C: Bacilli`~NF	1.264545	0.43319	2.919147	0.00587
NF	C: [Spartobacteria]	Class	`C: [Spartobacteria]`~NF	0.932462	0.724111	1.287733	0.205626
NF	C: Planctomycetia	Class	`C: Planctomycetia`~NF	0.220471	1.353119	0.162935	0.871433
NF	C: Deltaproteobacteria	Class	`C: Deltaproteobacteria`~NF	2.050597	1.597772	1.28341	0.20712
NF	C: Betaproteobacteria	Class	`C: Betaproteobacteria`~NF	3.635209	1.152959	3.152938	0.003152
NF	C: Gammaproteobacteria	Class	`C: Gammaproteobacteria`~NF	1.316769	1.866369	0.705524	0.484787
NF	C: Alphaproteobacteria	Class	`C: Alphaproteobacteria`~NF	0.638398	0.809354	0.788774	0.435138
NF	O: Sphingobacteriales	Order	`O: Sphingobacteriales`~NF	-0.82859	1.46411	-0.56593	0.574767
NF	O: Flavobacteriales	Order	`O: Flavobacteriales`~NF	1.083954	1.427905	0.759122	0.452463

NF	O: [Saprospirales]	Order	`O: [Saprospirales]`~NF	-1.43677	0.950817	-1.51109	0.139038
NF	O: Nitrospirales	Order	`O: Nitrospirales`~NF	0.28722	0.873669	0.328751	0.744149
NF	O: Gaiellales	Order	`O: Gaiellales`~NF	-0.32038	1.229867	-0.2605	0.795882
NF	O: Actinomycetales	Order	`O: Actinomycetales`~NF	0.712789	1.216695	0.58584	0.561447
NF	O: Acidobacteriales	Order	`O: Acidobacteriales`~NF	0.431729	0.525309	0.821857	0.416285
NF	O: Bacillales	Order	`O: Bacillales`~NF	1.264545	0.43319	2.919147	0.00587
NF	O: [Chthoniobacterales]	Order	`O: [Chthoniobacterales]`~NF	0.932462	0.724111	1.287733	0.205626
NF	O: Pirellulales	Order	`O: Pirellulales`~NF	-1.94433	1.279477	-1.51962	0.136883
NF	O: Gemmatales	Order	`O: Gemmatales`~NF	2.627451	0.511186	5.139913	8.57E-06
NF	O: Myxococcales	Order	`O: Myxococcales`~NF	3.960853	1.233981	3.209817	0.0027
NF	O: Bdellovibrionales	Order	`O: Bdellovibrionales`~NF	-1.91026	1.209369	-1.57955	0.1225
NF	O: Burkholderiales	Order	`O: Burkholderiales`~NF	3.635209	1.152959	3.152938	0.003152
NF	O: Xanthomonadales	Order	`O: Xanthomonadales`~NF	0.917794	1.890878	0.48538	0.630194
NF	O: Enterobacteriales	Order	`O: Enterobacteriales`~NF	0.143514	1.419436	0.101106	0.919998
NF	O: Rickettsiales	Order	`O: Rickettsiales`~NF	2.286108	0.814263	2.807579	0.007835
NF	O: Rhodospirillales	Order	`O: Rhodospirillales`~NF	-0.80841	1.338531	-0.60396	0.549461
NF	O: Caulobacterales	Order	`O: Caulobacterales`~NF	3.533926	1.446214	2.443571	0.019298
NF	O: Rhodobacterales	Order	`O: Rhodobacterales`~NF	1.240875	1.08072	1.148193	0.258066
NF	O: Rhizobiales	Order	`O: Rhizobiales`~NF	1.916779	1.264155	1.516253	0.137731
NF	O: Sphingomonadales	Order	`O: Sphingomonadales`~NF	2.414107	1.666767	1.448377	0.155713
NF	F: Sphingobacteriaceae	Family	`F: Sphingobacteriaceae`~NF	-0.82859	1.46411	-0.56593	0.574767
NF	F: Flavobacteriaceae	Family	`F: Flavobacteriaceae`~NF	1.083954	1.427905	0.759122	0.452463
NF	F: Chitinophagaceae	Family	`F: Chitinophagaceae`~NF	-1.43677	0.950817	-1.51109	0.139038
NF	F: Nitrospiraceae	Family	`F: Nitrospiraceae`~NF	0.28722	0.873669	0.328751	0.744149
NF	F: Gaiellaceae	Family	`F: Gaiellaceae`~NF	-0.32038	1.229867	-0.2605	0.795882
NF	F: Micromonosporaceae	Family	`F: Micromonosporaceae`~NF	2.84882	0.843832	3.376052	0.001707
NF	F: Nocardioidaceae	Family	`F: Nocardioidaceae`~NF	0.480711	1.198026	0.401252	0.690482
NF	F: Thermomonosporaceae	Family	`F: Thermomonosporaceae`~NF	0.553383	1.414534	0.391212	0.697825
NF	F: Micrococcaceae	Family	`F: Micrococcaceae`~NF	0.407265	1.06785	0.381388	0.70504
NF	F: Streptomycetaceae	Family	`F: Streptomycetaceae`~NF	2.725364	1.448095	1.882034	0.067507

NF	F: Sporichthyaceae	Family	`F: Sporichthyaceae`~NF	-0.64333	1.064128	-0.60456	0.549063
NF	F: Acidobacteriaceae	Family	`F: Acidobacteriaceae`~NF	0.378267	0.579037	0.653269	0.517516
NF	F: Koribacteraceae	Family	`F: Koribacteraceae`~NF	2.326113	1.467705	1.584864	0.121285
NF	F: Planococcaceae	Family	`F: Planococcaceae`~NF	1.264545	0.43319	2.919147	0.00587
NF	F: [Chthoniobacteraceae]	Family	`F: [Chthoniobacteraceae]`~NF	0.932462	0.724111	1.287733	0.205626
NF	F: Pirellulaceae	Family	`F: Pirellulaceae`~NF	-1.94433	1.279477	-1.51962	0.136883
NF	F: Isosphaeraceae	Family	`F: Isosphaeraceae`~NF	2.627451	0.511186	5.139913	8.57E-06
NF	F: Cystobacteraceae	Family	`F: Cystobacteraceae`~NF	3.960853	1.233981	3.209817	0.0027
NF	F: Bdellovibrionaceae	Family	`F: Bdellovibrionaceae`~NF	-1.91026	1.209369	-1.57955	0.1225
NF	F: Comamonadaceae	Family	`F: Comamonadaceae`~NF	0.840948	0.625258	1.344962	0.18661
NF	F: Burkholderiaceae	Family	`F: Burkholderiaceae`~NF	2.616369	0.484995	5.394627	3.85E-06
NF	F: Oxalobacteraceae	Family	`F: Oxalobacteraceae`~NF	1.565727	1.188674	1.317205	0.195658
NF	F: Xanthomonadaceae	Family	`F: Xanthomonadaceae`~NF	0.917794	1.890878	0.48538	0.630194
NF	F: Enterobacteriaceae	Family	`F: Enterobacteriaceae`~NF	0.143514	1.419436	0.101106	0.919998
NF	F: mitochondria	Family	`F: mitochondria`~NF	2.286108	0.814263	2.807579	0.007835
NF	F: Rhodospirillaceae	Family	`F: Rhodospirillaceae`~NF	-0.81243	1.307519	-0.62135	0.538079
NF	F: Acetobacteraceae	Family	`F: Acetobacteraceae`~NF	-0.70738	1.302221	-0.54321	0.590158
NF	F: Caulobacteraceae	Family	`F: Caulobacteraceae`~NF	3.533926	1.446214	2.443571	0.019298
NF	F: Hyphomonadaceae	Family	`F: Hyphomonadaceae`~NF	1.240875	1.08072	1.148193	0.258066
NF	F: Beijerinckiaceae	Family	`F: Beijerinckiaceae`~NF	1.916779	1.264155	1.516253	0.137731
NF	F: Sphingomonadaceae	Family	`F: Sphingomonadaceae`~NF	2.509177	1.623074	1.545942	0.130407
NF	F: Erythrobacteraceae	Family	`F: Erythrobacteraceae`~NF	0.476718	1.606485	0.296746	0.768276
NF	G: Mucilaginibacter	Genus	`G: Mucilaginibacter`~NF	-0.62359	1.533887	-0.40654	0.686627
NF	G: Solitalea	Genus	`G: Solitalea`~NF	1.111538	0.76666	1.449845	0.155306
NF	G: Flavobacterium	Genus	`G: Flavobacterium`~NF	1.083954	1.427905	0.759122	0.452463
NF	G: Trachelomonas	Genus	`G: Trachelomonas`~NF	-0.73694	1.211835	-0.60812	0.546729
NF	G: Ferruginibacter	Genus	`G: Ferruginibacter`~NF	-0.91661	1.53327	-0.59782	0.553509
NF	G: Lacibacter	Genus	`G: Lacibacter`~NF	3.944547	0.754217	5.229991	6.46E-06
NF	G: Filimonas	Genus	`G: Filimonas`~NF	0.196624	1.055081	0.186359	0.853155
NF	G: Nitrospira	Genus	`G: Nitrospira`~NF	0.28722	0.873669	0.328751	0.744149

NF	G: Gaiella	Genus	`G: Gaiella`~NF	-0.32038	1.229867	-0.2605	0.795882
NF	G: Rugosimonospora	Genus	`G: Rugosimonospora`~NF	2.84882	0.843832	3.376052	0.001707
NF	G: Nocardioides	Genus	`G: Nocardioides`~NF	0.480711	1.198026	0.401252	0.690482
NF	G: Actinomadura	Genus	`G: Actinomadura`~NF	0.553383	1.414534	0.391212	0.697825
NF	G: Arthrobacter	Genus	`G: Arthrobacter`~NF	0.407265	1.06785	0.381388	0.70504
NF	G: Streptomyces	Genus	`G: Streptomyces`~NF	2.725364	1.448095	1.882034	0.067507
NF	G: Sporichthya	Genus	`G: Sporichthya`~NF	-0.64333	1.064128	-0.60456	0.549063
NF	G: Telmatobacter	Genus	`G: Telmatobacter`~NF	-0.64961	1.043202	-0.62271	0.537198
NF	G: Candidatus Koribacter	Genus	`G: Candidatus Koribacter`~NF	2.326113	1.467705	1.584864	0.121285
NF	G: Granulicella	Genus	`G: Granulicella`~NF	0.637786	1.088601	0.585877	0.561422
NF	G: Acidopila	Genus	`G: Acidopila`~NF	0.172749	1.481571	0.116598	0.907792
NF	G: Acidicapsa	Genus	`G: Acidicapsa`~NF	2.719829	1.224725	2.220767	0.032403
NF	G: Bryocella	Genus	`G: Bryocella`~NF	2.955123	1.436323	2.057422	0.046555
NF	G: Terriglobus	Genus	`G: Terriglobus`~NF	0.749643	1.20344	0.622917	0.53706
NF	G: Staphylococcus	Genus	`G: Staphylococcus`~NF	1.264545	0.43319	2.919147	0.00587
NF	G: Ellin506	Genus	`G: Ellin506`~NF	0.932462	0.724111	1.287733	0.205626
NF	G: planctomycete	Genus	`G: planctomycete`~NF	-1.94433	1.279477	-1.51962	0.136883
NF	G: Nostocoida	Genus	`G: Nostocoida`~NF	2.627451	0.511186	5.139913	8.57E-06
NF	G: Cystobacter	Genus	`G: Cystobacter`~NF	3.960853	1.233981	3.209817	0.0027
NF	G: Bdellovibrio	Genus	`G: Bdellovibrio`~NF	-1.91026	1.209369	-1.57955	0.1225
NF	G: Sphaerotilus	Genus	`G: Sphaerotilus`~NF	0.840948	0.625258	1.344962	0.18661
NF	G: Burkholderia	Genus	`G: Burkholderia`~NF	2.616369	0.484995	5.394627	3.85E-06
NF	G: Pseudoburkholderia	Genus	`G: Pseudoburkholderia`~NF	2.075244	1.019508	2.035535	0.048814
NF	G: Massilia	Genus	`G: Massilia`~NF	-0.78318	0.77672	-1.00831	0.319681
NF	G: Luteibacter	Genus	`G: Luteibacter`~NF	-0.3298	1.576991	-0.20914	0.83546
NF	G: Rhodanobacter	Genus	`G: Rhodanobacter`~NF	0.484916	1.586902	0.305574	0.761597
NF	G: Gynumella	Genus	`G: Gynumella`~NF	-0.71749	1.035737	-0.69273	0.49269
NF	G: Lysobacter	Genus	`G: Lysobacter`~NF	-1.33468	1.327214	-1.00563	0.320955
NF	G: Thermomonas	Genus	`G: Thermomonas`~NF	-0.63677	1.247881	-0.51028	0.612806
NF	G: Enterobacteriaceae - Genus	Genus	`G: Enterobacteriaceae - Genus`~NF	0.143514	1.419436	0.101106	0.919998

NF	G: Nageia	Genus	`G: Nageia`~NF	2.286108	0.814263	2.807579	0.007835
NF	G: Reyranella	Genus	`G: Reyranella`~NF	0.697183	1.386808	0.502725	0.618059
NF	G: Rhodopila	Genus	`G: Rhodopila`~NF	-0.70738	1.302221	-0.54321	0.590158
NF	G: Dongia	Genus	`G: Dongia`~NF	1.133869	1.218346	0.930662	0.357904
NF	G: Skermanella	Genus	`G: Skermanella`~NF	-3.00297	1.596616	-1.88083	0.067674
NF	G: Stella	Genus	`G: Stella`~NF	2.206345	0.899106	2.453931	0.018826
NF	G: Caulobacter	Genus	`G: Caulobacter`~NF	1.824803	1.413592	1.290898	0.204538
NF	G: Nitrobacteria	Genus	`G: Nitrobacteria`~NF	0.923119	1.034105	0.892674	0.37765
NF	G: Brevundimonas	Genus	`G: Brevundimonas`~NF	6.603226	0.442786	14.91291	1.82E-17
NF	G: Arthrospira	Genus	`G: Arthrospira`~NF	-0.27209	1.002955	-0.27129	0.787638
NF	G: Asticcacaulis	Genus	`G: Asticcacaulis`~NF	-0.94296	0.814331	-1.15795	0.25411
NF	G: Woodsholea	Genus	`G: Woodsholea`~NF	1.240875	1.08072	1.148193	0.258066
NF	G: Methylocapsa	Genus	`G: Methylocapsa`~NF	1.916779	1.264155	1.516253	0.137731
NF	G: Sphingobium	Genus	`G: Sphingobium`~NF	-0.85462	1.508384	-0.56658	0.574329
NF	G: Sphingomonas	Genus	`G: Sphingomonas`~NF	0.245339	1.612689	0.15213	0.879889
NF	G: Sphingosinicella	Genus	`G: Sphingosinicella`~NF	2.624333	1.819865	1.442048	0.157481
NF	G: Porphyrobacter	Genus	`G: Porphyrobacter`~NF	0.476718	1.606485	0.296746	0.768276

A5.2 Fungal (ITS1) Differential Abundance Results

A5.2.1 Microbial taxa positively correlated to KG have a positive estimate value

term	taxon	rank	formula	estimate	std.error	statistic	p.value
KG	P: Glomeromycota	Phylum	`P: Glomeromycota`~KG	0.17327	0.876645	0.197651	0.844371
KG	P: Basidiomycota	Phylum	`P: Basidiomycota`~KG	0.831907	0.471815	1.763206	0.085906
KG	P: Ascomycota	Phylum	`P: Ascomycota`~KG	0.154642	0.469076	0.329674	0.743457
KG	P: Mortierellomycota	Phylum	`P: Mortierellomycota`~KG	-0.37845	0.257265	-1.47103	0.149515
KG	P: Olpidiomycota	Phylum	`P: Olpidiomycota`~KG	-0.00287	0.489902	-0.00586	0.995359

KG	C: Glomeromycetes	Class	`C: Glomeromycetes`~KG	0.225666	0.618767	0.364703	0.717355
KG	C: Microbotryomycetes	Class	`C: Microbotryomycetes`~KG	1.699654	0.608413	2.793587	0.008121
KG	C: Tremellomycetes	Class	`C: Tremellomycetes`~KG	0.827699	0.470574	1.758913	0.086643
KG	C: Saccharomycetes	Class	`C: Saccharomycetes`~KG	-0.62496	0.446145	-1.40081	0.169384
KG	C: Wallemiomycetes	Class	`C: Wallemiomycetes`~KG	-0.0293	0.41783	-0.07012	0.944469
KG	C: Leotiomycetes	Class	`C: Leotiomycetes`~KG	1.520898	1.357454	1.120405	0.269573
KG	C: Eurotiomycetes	Class	`C: Eurotiomycetes`~KG	2.102398	0.64386	3.265303	0.00232
KG	C: Geoglossomycetes	Class	`C: Geoglossomycetes`~KG	0.770747	1.144869	0.673219	0.504882
KG	C: Sordariomycetes	Class	`C: Sordariomycetes`~KG	0.026784	0.796815	0.033614	0.973361
KG	C: Archaeorhizomycetes	Class	`C: Archaeorhizomycetes`~KG	0.811797	1.121193	0.724047	0.473471
KG	C: Agaricomycetes	Class	`C: Agaricomycetes`~KG	0.241483	0.53855	0.448395	0.656414
KG	C: Archaeosporomycetes	Class	`C: Archaeosporomycetes`~KG	-0.18961	0.775118	-0.24462	0.808066
KG	C: Mortierellomycetes	Class	`C: Mortierellomycetes`~KG	-0.37845	0.257265	-1.47103	0.149515
KG	C: Olpidiomycetes	Class	`C: Olpidiomycetes`~KG	-0.00287	0.489902	-0.00586	0.995359
KG	O: Gigasporales	Order	`O: Gigasporales`~KG	0.536834	0.499356	1.075052	0.28913
KG	O: Glomerales	Order	`O: Glomerales`~KG	0.241505	0.539752	0.447437	0.6571
KG	O: Microbotryomycetes_ord_Incertae_sedis	Order	`O: Microbotryomycetes_ord_Incertae_sedis`~KG	0.928152	0.616015	1.506704	0.140155
KG	O: Cystofilobasidiales	Order	`O: Cystofilobasidiales`~KG	1.793755	0.774096	2.317225	0.025977
KG	O: Saccharomycetales	Order	`O: Saccharomycetales`~KG	-0.86271	0.611258	-1.41137	0.166273
KG	O: Wallemiales	Order	`O: Wallemiales`~KG	-0.0293	0.567967	-0.05158	0.959133
KG	O: Helotiales	Order	`O: Helotiales`~KG	1.937025	1.559964	1.241711	0.221953
KG	O: Thelebolales	Order	`O: Thelebolales`~KG	1.04054	0.488806	2.12874	0.039818
KG	O: Eurotiales	Order	`O: Eurotiales`~KG	2.736383	0.810397	3.376594	0.001704
KG	O: Geoglossales	Order	`O: Geoglossales`~KG	0.849995	1.335575	0.636426	0.528314
KG	O: Hypocreales	Order	`O: Hypocreales`~KG	0.331144	0.816848	0.405392	0.687463
KG	O: Sordariales	Order	`O: Sordariales`~KG	0.066594	1.317766	0.050535	0.959961
KG	O: Xylariales	Order	`O: Xylariales`~KG	1.699539	0.527171	3.223886	0.002598
KG	O: Archaeorhizomycetales	Order	`O: Archaeorhizomycetales`~KG	1.12879	1.351475	0.835228	0.40881
KG	O: Sebacinales	Order	`O: Sebacinales`~KG	0.018145	0.562368	0.032265	0.974429

KG	O: Sporidiobolales	Order	`O: Sporidiobolales`~KG	0.372238	0.469992	0.792009	0.433272
KG	O: Leucosporidiales	Order	`O: Leucosporidiales`~KG	1.68039	0.675294	2.488382	0.017332
KG	O: Tremellales	Order	`O: Tremellales`~KG	1.255717	0.52382	2.397229	0.021542
KG	O: Trichosporonales	Order	`O: Trichosporonales`~KG	0.719568	1.016435	0.707933	0.483307
KG	O: Filobasidiales	Order	`O: Filobasidiales`~KG	3.078073	0.931657	3.30387	0.002086
KG	O: Archaeosporales	Order	`O: Archaeosporales`~KG	-0.18961	0.947767	-0.20006	0.842501
KG	O: Diversisporales	Order	`O: Diversisporales`~KG	0.065949	0.711969	0.092629	0.926685
KG	O: Mortierellales	Order	`O: Mortierellales`~KG	-0.37845	0.257265	-1.47103	0.149515
KG	O: Cantharellales	Order	`O: Cantharellales`~KG	0.348962	0.614307	0.568058	0.573337
KG	O: Olpidiales	Order	`O: Olpidiales`~KG	-0.00287	0.632927	-0.00453	0.996408
KG	F: Gigasporaceae	Family	`F: Gigasporaceae`~KG	0.536834	0.499356	1.075052	0.28913
KG	F: Glomeraceae	Family	`F: Glomeraceae`~KG	0.241505	0.539752	0.447437	0.6571
KG	F: Microbotryomycetes_fam_Incertae_sedis	Family	`F: Microbotryomycetes_fam_Incertae_sedis`~KG	0.928152	0.616015	1.506704	0.140155
KG	F: Cystofilobasidiaceae	Family	`F: Cystofilobasidiaceae`~KG	1.793755	0.774096	2.317225	0.025977
KG	F: Saccharomycetales_fam_Incertae_sedis	Family	`F: Saccharomycetales_fam_Incertae_sedis`~KG	-0.86271	0.611258	-1.41137	0.166273
KG	F: Wallemiaceae	Family	`F: Wallemiaceae`~KG	-0.0293	0.567967	-0.05158	0.959133
KG	F: Leotiaceae	Family	`F: Leotiaceae`~KG	0.335642	0.461638	0.727067	0.471641
KG	F: Pseudeurotiaceae	Family	`F: Pseudeurotiaceae`~KG	1.04054	0.488806	2.12874	0.039818
KG	F: Helotiaceae	Family	`F: Helotiaceae`~KG	0.450445	1.043854	0.431521	0.668528
KG	F: Vibrisseaceae	Family	`F: Vibrisseaceae`~KG	0.611482	0.657592	0.92988	0.358303
KG	F: Helotiales_fam_Incertae_sedis	Family	`F: Helotiales_fam_Incertae_sedis`~KG	-0.3209	0.973544	-0.32963	0.743493
KG	F: Myxotrichaceae	Family	`F: Myxotrichaceae`~KG	1.819905	1.581663	1.150627	0.257075
KG	F: Dermateaceae	Family	`F: Dermateaceae`~KG	-0.38474	0.689488	-0.558	0.580114
KG	F: Aspergillaceae	Family	`F: Aspergillaceae`~KG	2.736383	0.810397	3.376594	0.001704
KG	F: Geoglossaceae	Family	`F: Geoglossaceae`~KG	0.849995	1.335575	0.636426	0.528314
KG	F: Nectriaceae	Family	`F: Nectriaceae`~KG	1.150726	1.208945	0.951843	0.347192
KG	F: Cordycipitaceae	Family	`F: Cordycipitaceae`~KG	0.533997	0.914375	0.584002	0.56267
KG	F: Hypocreaceae	Family	`F: Hypocreaceae`~KG	1.703208	0.965401	1.764249	0.085728

KG	F: Bionectriaceae	Family	`F: Bionectriaceae`~KG	-0.77134	1.0246	-0.75282	0.456194
KG	F: Chaetomiaceae	Family	`F: Chaetomiaceae`~KG	0.167864	0.450608	0.372527	0.71157
KG	F: Lasiosphaeriaceae	Family	`F: Lasiosphaeriaceae`~KG	0.056554	1.313111	0.043069	0.965872
KG	F: Sporocadaceae	Family	`F: Sporocadaceae`~KG	1.699539	0.527171	3.223886	0.002598
KG	F: Chaetomellaceae	Family	`F: Chaetomellaceae`~KG	0.186923	0.948085	0.197159	0.844754
KG	F: Archaeorhizomycetaceae	Family	`F: Archaeorhizomycetaceae`~KG	1.12879	1.351475	0.835228	0.40881
KG	F: Sebacinaceae	Family	`F: Sebacinaceae`~KG	0.018145	0.562368	0.032265	0.974429
KG	F: Sporidiobolaceae	Family	`F: Sporidiobolaceae`~KG	0.372238	0.469992	0.792009	0.433272
KG	F: Leucosporidiaceae	Family	`F: Leucosporidiaceae`~KG	1.68039	0.675294	2.488382	0.017332
KG	F: Trimorphomycetaceae	Family	`F: Trimorphomycetaceae`~KG	1.261938	0.522968	2.413029	0.020751
KG	F: Rhynchogastremataceae	Family	`F: Rhynchogastremataceae`~KG	0.038427	0.631094	0.06089	0.951766
KG	F: Bulleribasidiaceae	Family	`F: Bulleribasidiaceae`~KG	-0.01089	0.469927	-0.02318	0.981627
KG	F: Trichosporonaceae	Family	`F: Trichosporonaceae`~KG	0.719568	1.016435	0.707933	0.483307
KG	F: Filobasidiaceae	Family	`F: Filobasidiaceae`~KG	-0.32816	0.413765	-0.7931	0.432642
KG	F: Piskurozymaceae	Family	`F: Piskurozymaceae`~KG	3.522009	0.920988	3.824163	0.000474
KG	F: Archaeosporaceae	Family	`F: Archaeosporaceae`~KG	-0.18961	0.947767	-0.20006	0.842501
KG	F: Diversisporaceae	Family	`F: Diversisporaceae`~KG	0.712294	0.590532	1.20619	0.235201
KG	F: Mortierellaceae	Family	`F: Mortierellaceae`~KG	-0.37845	0.257265	-1.47103	0.149515
KG	F: Acaulosporaceae	Family	`F: Acaulosporaceae`~KG	-0.64634	0.44585	-1.44969	0.155348
KG	F: Clavulinaceae	Family	`F: Clavulinaceae`~KG	0.348962	0.614307	0.568058	0.573337
KG	F: Olpidiaceae	Family	`F: Olpidiaceae`~KG	-0.00287	0.632927	-0.00453	0.996408
KG	G: Scutellospora	Genus	`G: Scutellospora`~KG	0.536834	0.499356	1.075052	0.28913
KG	G: Rhizoglomus	Genus	`G: Rhizoglomus`~KG	0.241505	0.539752	0.447437	0.6571
KG	G: Curvibasidium	Genus	`G: Curvibasidium`~KG	0.229808	0.492982	0.466158	0.643764
KG	G: Cystofilobasidium	Genus	`G: Cystofilobasidium`~KG	1.793755	0.774096	2.317225	0.025977
KG	G: Candida	Genus	`G: Candida`~KG	-0.86271	0.611258	-1.41137	0.166273
KG	G: Wallemia	Genus	`G: Wallemia`~KG	-0.0293	0.567967	-0.05158	0.959133
KG	G: Alatospora	Genus	`G: Alatospora`~KG	0.335642	0.461638	0.727067	0.471641
KG	G: Pseudeurotium	Genus	`G: Pseudeurotium`~KG	1.04054	0.488806	2.12874	0.039818
KG	G: Infundichalara	Genus	`G: Infundichalara`~KG	0.450445	1.043854	0.431521	0.668528

KG	G: Phialocephala	Genus	`G: Phialocephala`~KG	0.611482	0.657592	0.92988	0.358303
KG	G: Mycoarthris	Genus	`G: Mycoarthris`~KG	-0.3209	0.973544	-0.32963	0.743493
KG	G: Oidiodendron	Genus	`G: Oidiodendron`~KG	1.819905	1.581663	1.150627	0.257075
KG	G: Cryptosporiopsis	Genus	`G: Cryptosporiopsis`~KG	-0.38474	0.689488	-0.558	0.580114
KG	G: Penicillium	Genus	`G: Penicillium`~KG	2.736383	0.810397	3.376594	0.001704
KG	G: Trichoglossum	Genus	`G: Trichoglossum`~KG	0.849995	1.335575	0.636426	0.528314
KG	G: Ilyonectria	Genus	`G: Ilyonectria`~KG	2.184727	1.24338	1.757087	0.086958
KG	G: Mariannaea	Genus	`G: Mariannaea`~KG	0.586139	1.112708	0.526768	0.601416
KG	G: Leptobacillium	Genus	`G: Leptobacillium`~KG	0.533997	0.914375	0.584002	0.56267
KG	G: Trichoderma	Genus	`G: Trichoderma`~KG	1.703208	0.965401	1.764249	0.085728
KG	G: Clonostachys	Genus	`G: Clonostachys`~KG	-0.77134	1.0246	-0.75282	0.456194
KG	G: Thelonectria	Genus	`G: Thelonectria`~KG	0.175485	0.624953	0.280797	0.780389
KG	G: Fusarium	Genus	`G: Fusarium`~KG	1.982748	1.226585	1.616478	0.114264
KG	G: Humicola	Genus	`G: Humicola`~KG	0.167864	0.450608	0.372527	0.71157
KG	G: Cercophora	Genus	`G: Cercophora`~KG	0.056554	1.313111	0.043069	0.965872
KG	G: Pestalotiopsis	Genus	`G: Pestalotiopsis`~KG	1.699539	0.527171	3.223886	0.002598
KG	G: Sphaerographium	Genus	`G: Sphaerographium`~KG	0.186923	0.948085	0.197159	0.844754
KG	G: Archaeorhizomyces	Genus	`G: Archaeorhizomyces`~KG	1.12879	1.351475	0.835228	0.40881
KG	G: Sebacina	Genus	`G: Sebacina`~KG	0.018145	0.562368	0.032265	0.974429
KG	G: Rhodotorula	Genus	`G: Rhodotorula`~KG	0.372238	0.469992	0.792009	0.433272
KG	G: Leucosporidium	Genus	`G: Leucosporidium`~KG	1.68039	0.675294	2.488382	0.017332
KG	G: Colacogloea	Genus	`G: Colacogloea`~KG	0.657228	0.483264	1.359977	0.181852
KG	G: Saitozyma	Genus	`G: Saitozyma`~KG	1.261938	0.522968	2.413029	0.020751
KG	G: Papiliotrema	Genus	`G: Papiliotrema`~KG	0.038427	0.631094	0.06089	0.951766
KG	G: Vishniacozyma	Genus	`G: Vishniacozyma`~KG	-0.01089	0.469927	-0.02318	0.981627
KG	G: Apiotrichum	Genus	`G: Apiotrichum`~KG	0.719568	1.016435	0.707933	0.483307
KG	G: Naganishia	Genus	`G: Naganishia`~KG	-0.32816	0.413765	-0.7931	0.432642
KG	G: Solicoccozyma	Genus	`G: Solicoccozyma`~KG	3.522009	0.920988	3.824163	0.000474
KG	G: Archaeospora	Genus	`G: Archaeospora`~KG	-0.18961	0.947767	-0.20006	0.842501
KG	G: Diversispora	Genus	`G: Diversispora`~KG	0.712294	0.590532	1.20619	0.235201

KG	G: Mortierella	Genus	`G: Mortierella`~KG	-0.37845	0.257265	-1.47103	0.149515
KG	G: Acaulospora	Genus	`G: Acaulospora`~KG	-0.64634	0.44585	-1.44969	0.155348
KG	G: Clavulina	Genus	`G: Clavulina`~KG	0.348962	0.614307	0.568058	0.573337
KG	G: Olpidium	Genus	`G: Olpidium`~KG	-0.00287	0.632927	-0.00453	0.996408

A5.2.2 Microbial taxa positively correlated to RG have a positive estimate value

term	taxon	rank	formula	estimate	std.error	statistic	p.value
RG	P: Glomeromycota	Phylum	`P: Glomeromycota`~RG	0.322756	0.955557	0.337767	0.737398
RG	P: Basidiomycota	Phylum	`P: Basidiomycota`~RG	-1.07119	0.506455	-2.11508	0.041037
RG	P: Ascomycota	Phylum	`P: Ascomycota`~RG	-0.47639	0.506676	-0.94023	0.353039
RG	P: Mortierellomycota	Phylum	`P: Mortierellomycota`~RG	0.480231	0.277867	1.728274	0.092058
RG	P: Olpidiomycota	Phylum	`P: Olpidiomycota`~RG	0.437236	0.5298	0.825285	0.414361
RG	C: Glomeromycetes	Class	`C: Glomeromycetes`~RG	0.527486	0.670875	0.786265	0.436588
RG	C: Microbotryomycetes	Class	`C: Microbotryomycetes`~RG	-0.76024	0.718308	-1.05837	0.296568
RG	C: Tremellomycetes	Class	`C: Tremellomycetes`~RG	-1.07786	0.504489	-2.13653	0.039136
RG	C: Saccharomycetes	Class	`C: Saccharomycetes`~RG	-0.28856	0.496995	-0.58061	0.56493
RG	C: Wallemiomycetes	Class	`C: Wallemiomycetes`~RG	0.428774	0.450583	0.9516	0.347314
RG	C: Leotiomycetes	Class	`C: Leotiomycetes`~RG	-2.11994	1.46556	-1.4465	0.156235
RG	C: Eurotiomycetes	Class	`C: Eurotiomycetes`~RG	-1.50171	0.756734	-1.98447	0.05446
RG	C: Geoglossomycetes	Class	`C: Geoglossomycetes`~RG	0.076684	1.25652	0.061029	0.951656
RG	C: Sordariomycetes	Class	`C: Sordariomycetes`~RG	-0.22789	0.868623	-0.26236	0.794461
RG	C: Archaeorhizomycetes	Class	`C: Archaeorhizomycetes`~RG	-1.08101	1.219185	-0.88666	0.380837
RG	C: Agaricomycetes	Class	`C: Agaricomycetes`~RG	0.307844	0.587038	0.524403	0.603044
RG	C: Archaeosporomycetes	Class	`C: Archaeosporomycetes`~RG	-0.18803	0.845839	-0.2223	0.825268
RG	C: Mortierellomycetes	Class	`C: Mortierellomycetes`~RG	0.480231	0.277867	1.728274	0.092058

RG	C: Olpidiomycetes	Class	`C: Olpidiomycetes`~RG	0.437236	0.5298	0.825285	0.414361
RG	O: Gigasporales	Order	`O: Gigasporales`~RG	-0.14373	0.552574	-0.26011	0.796183
RG	O: Glomerales	Order	`O: Glomerales`~RG	0.309025	0.588335	0.525254	0.602458
RG	O: Microbotryomycetes_ord_Incertae_sedis	Order	`O: Microbotryomycetes_ord_Incertae_sedis`~RG	-0.32217	0.689936	-0.46696	0.643193
RG	O: Cystofilobasidiales	Order	`O: Cystofilobasidiales`~RG	-0.72272	0.894661	-0.80781	0.424229
RG	O: Saccharomycetales	Order	`O: Saccharomycetales`~RG	-0.3829	0.681369	-0.56196	0.577442
RG	O: Wallemiales	Order	`O: Wallemiales`~RG	0.579723	0.612548	0.946414	0.349917
RG	O: Helotiales	Order	`O: Helotiales`~RG	-2.60586	1.684001	-1.54742	0.130051
RG	O: Thelebolales	Order	`O: Thelebolales`~RG	-0.74324	0.551203	-1.3484	0.185511
RG	O: Eurotiales	Order	`O: Eurotiales`~RG	-1.95456	0.957018	-2.04234	0.048101
RG	O: Geoglossales	Order	`O: Geoglossales`~RG	0.057816	1.464948	0.039466	0.968725
RG	O: Hypocreales	Order	`O: Hypocreales`~RG	-0.23995	0.89233	-0.2689	0.789459
RG	O: Sordariales	Order	`O: Sordariales`~RG	-0.44308	1.436051	-0.30854	0.759356
RG	O: Xylariales	Order	`O: Xylariales`~RG	-1.21396	0.618508	-1.96272	0.057031
RG	O: Archaeorhizomycetales	Order	`O: Archaeorhizomycetales`~RG	-1.38291	1.471046	-0.94008	0.353113
RG	O: Sebacinales	Order	`O: Sebacinales`~RG	0.524895	0.607666	0.863789	0.393123
RG	O: Sporidiobolales	Order	`O: Sporidiobolales`~RG	0.008606	0.517017	0.016645	0.986807
RG	O: Leucosporidiales	Order	`O: Leucosporidiales`~RG	-0.95849	0.779211	-1.23008	0.226227
RG	O: Tremellales	Order	`O: Tremellales`~RG	-1.83431	0.536196	-3.42098	0.001505
RG	O: Trichosporonales	Order	`O: Trichosporonales`~RG	-0.98758	1.104755	-0.89394	0.376983
RG	O: Filobasidiales	Order	`O: Filobasidiales`~RG	-0.93635	1.143269	-0.81901	0.417888
RG	O: Archaeosporales	Order	`O: Archaeosporales`~RG	-0.1503	1.034355	-0.1453	0.885239
RG	O: Diversisporales	Order	`O: Diversisporales`~RG	0.424144	0.773857	0.548091	0.586835
RG	O: Mortierellales	Order	`O: Mortierellales`~RG	0.480231	0.277867	1.728274	0.092058
RG	O: Cantharellales	Order	`O: Cantharellales`~RG	0.266898	0.67171	0.397342	0.693339
RG	O: Olpidiales	Order	`O: Olpidiales`~RG	0.588185	0.683957	0.859973	0.395197
RG	F: Gigasporaceae	Family	`F: Gigasporaceae`~RG	-0.14373	0.552574	-0.26011	0.796183
RG	F: Glomeraceae	Family	`F: Glomeraceae`~RG	0.309025	0.588335	0.525254	0.602458

RG	F: Microbotryomycetes_fam_Incertae_sedis	Family	`F: Microbotryomycetes_fam_Incertae_sedis`~RG	-0.32217	0.689936	-0.46696	0.643193
RG	F: Cystofilobasidiaceae	Family	`F: Cystofilobasidiaceae`~RG	-0.72272	0.894661	-0.80781	0.424229
RG	F: Saccharomycetales_fam_Incertae_sedis	Family	`F: Saccharomycetales_fam_Incertae_sedis`~RG	-0.3829	0.681369	-0.56196	0.577442
RG	F: Wallemiaceae	Family	`F: Wallemiaceae`~RG	0.579723	0.612548	0.946414	0.349917
RG	F: Leotiaceae	Family	`F: Leotiaceae`~RG	0.046302	0.507124	0.091304	0.927731
RG	F: Pseudeurotiaceae	Family	`F: Pseudeurotiaceae`~RG	-0.74324	0.551203	-1.3484	0.185511
RG	F: Helotiaceae	Family	`F: Helotiaceae`~RG	-0.75098	1.135207	-0.66154	0.512261
RG	F: Vibrisseaceae	Family	`F: Vibrisseaceae`~RG	-0.44294	0.722043	-0.61346	0.54323
RG	F: Helotiales_fam_Incertae_sedis	Family	`F: Helotiales_fam_Incertae_sedis`~RG	0.781359	1.056162	0.73981	0.463962
RG	F: Myxotrichaceae	Family	`F: Myxotrichaceae`~RG	-1.4503	1.739705	-0.83365	0.409689
RG	F: Dermateaceae	Family	`F: Dermateaceae`~RG	-0.52648	0.750525	-0.70148	0.487275
RG	F: Aspergillaceae	Family	`F: Aspergillaceae`~RG	-1.95456	0.957018	-2.04234	0.048101
RG	F: Geoglossaceae	Family	`F: Geoglossaceae`~RG	0.057816	1.464948	0.039466	0.968725
RG	F: Nectriaceae	Family	`F: Nectriaceae`~RG	-1.0581	1.323616	-0.7994	0.429028
RG	F: Cordycipitaceae	Family	`F: Cordycipitaceae`~RG	-0.31122	1.00086	-0.31096	0.757533
RG	F: Hypocreaceae	Family	`F: Hypocreaceae`~RG	0.319574	1.094402	0.292008	0.771869
RG	F: Bionectriaceae	Family	`F: Bionectriaceae`~RG	2.011937	1.077907	1.866521	0.069699
RG	F: Chaetomiaceae	Family	`F: Chaetomiaceae`~RG	0.272244	0.490566	0.554959	0.582175
RG	F: Lasiosphaeriaceae	Family	`F: Lasiosphaeriaceae`~RG	-0.45796	1.430829	-0.32007	0.750671
RG	F: Sporocadaceae	Family	`F: Sporocadaceae`~RG	-1.21396	0.618508	-1.96272	0.057031
RG	F: Chaetomellaceae	Family	`F: Chaetomellaceae`~RG	-0.80284	1.026747	-0.78192	0.439104
RG	F: Archaeorhizomycetaceae	Family	`F: Archaeorhizomycetaceae`~RG	-1.38291	1.471046	-0.94008	0.353113
RG	F: Sebacinaceae	Family	`F: Sebacinaceae`~RG	0.524895	0.607666	0.863789	0.393123
RG	F: Sporidiobolaceae	Family	`F: Sporidiobolaceae`~RG	0.008606	0.517017	0.016645	0.986807
RG	F: Leucosporidiaceae	Family	`F: Leucosporidiaceae`~RG	-0.95849	0.779211	-1.23008	0.226227
RG	F: Trimorphomycetaceae	Family	`F: Trimorphomycetaceae`~RG	-1.84432	0.534759	-3.44888	0.001392
RG	F: Rhynchogastremataceae	Family	`F: Rhynchogastremataceae`~RG	-0.17425	0.688034	-0.25326	0.801436
RG	F: Bulleribasidiaceae	Family	`F: Bulleribasidiaceae`~RG	0.51346	0.505926	1.014893	0.316576

RG	F: Trichosporonaceae	Family	`F: Trichosporonaceae`~RG	-0.98758	1.104755	-0.89394	0.376983
RG	F: Filobasidiaceae	Family	`F: Filobasidiaceae`~RG	0.836089	0.434498	1.924262	0.061834
RG	F: Piskurozymaceae	Family	`F: Piskurozymaceae`~RG	-1.69965	1.149947	-1.47803	0.147642
RG	F: Archaeosporaceae	Family	`F: Archaeosporaceae`~RG	-0.1503	1.034355	-0.1453	0.885239
RG	F: Diversisporaceae	Family	`F: Diversisporaceae`~RG	-0.19962	0.655743	-0.30442	0.762471
RG	F: Mortierellaceae	Family	`F: Mortierellaceae`~RG	0.480231	0.277867	1.728274	0.092058
RG	F: Acaulosporaceae	Family	`F: Acaulosporaceae`~RG	0.623763	0.489381	1.274597	0.210191
RG	F: Clavulinaceae	Family	`F: Clavulinaceae`~RG	0.266898	0.67171	0.397342	0.693339
RG	F: Olpidiaceae	Family	`F: Olpidiaceae`~RG	0.588185	0.683957	0.859973	0.395197
RG	G: Scutellospora	Genus	`G: Scutellospora`~RG	-0.14373	0.552574	-0.26011	0.796183
RG	G: Rhizoglomus	Genus	`G: Rhizoglomus`~RG	0.309025	0.588335	0.525254	0.602458
RG	G: Curvibasidium	Genus	`G: Curvibasidium`~RG	0.153527	0.538848	0.284917	0.777254
RG	G: Cystofilobasidium	Genus	`G: Cystofilobasidium`~RG	-0.72272	0.894661	-0.80781	0.424229
RG	G: Candida	Genus	`G: Candida`~RG	-0.3829	0.681369	-0.56196	0.577442
RG	G: Wallemia	Genus	`G: Wallemia`~RG	0.579723	0.612548	0.946414	0.349917
RG	G: Alatospora	Genus	`G: Alatospora`~RG	0.046302	0.507124	0.091304	0.927731
RG	G: Pseudeurotium	Genus	`G: Pseudeurotium`~RG	-0.74324	0.551203	-1.3484	0.185511
RG	G: Infundichalara	Genus	`G: Infundichalara`~RG	-0.75098	1.135207	-0.66154	0.512261
RG	G: Phialocephala	Genus	`G: Phialocephala`~RG	-0.44294	0.722043	-0.61346	0.54323
RG	G: Mycoarthris	Genus	`G: Mycoarthris`~RG	0.781359	1.056162	0.73981	0.463962
RG	G: Oidiodendron	Genus	`G: Oidiodendron`~RG	-1.4503	1.739705	-0.83365	0.409689
RG	G: Cryptosporiopsis	Genus	`G: Cryptosporiopsis`~RG	-0.52648	0.750525	-0.70148	0.487275
RG	G: Penicillium	Genus	`G: Penicillium`~RG	-1.95456	0.957018	-2.04234	0.048101
RG	G: Trichoglossum	Genus	`G: Trichoglossum`~RG	0.057816	1.464948	0.039466	0.968725
RG	G: Ilyonectria	Genus	`G: Ilyonectria`~RG	-2.30468	1.360229	-1.69433	0.098384
RG	G: Mariannaea	Genus	`G: Mariannaea`~RG	0.196923	1.218069	0.161668	0.872424
RG	G: Leptobacillium	Genus	`G: Leptobacillium`~RG	-0.31122	1.00086	-0.31096	0.757533
RG	G: Trichoderma	Genus	`G: Trichoderma`~RG	0.319574	1.094402	0.292008	0.771869
RG	G: Clonostachys	Genus	`G: Clonostachys`~RG	2.011937	1.077907	1.866521	0.069699
RG	G: Thelonectria	Genus	`G: Thelonectria`~RG	0.616516	0.67522	0.913059	0.366969

RG	G: Fusarium	Genus	`G: Fusarium`~RG	-0.42537	1.381841	-0.30783	0.759896
RG	G: Humicola	Genus	`G: Humicola`~RG	0.272244	0.490566	0.554959	0.582175
RG	G: Cercophora	Genus	`G: Cercophora`~RG	-0.45796	1.430829	-0.32007	0.750671
RG	G: Pestalotiopsis	Genus	`G: Pestalotiopsis`~RG	-1.21396	0.618508	-1.96272	0.057031
RG	G: Sphaerographium	Genus	`G: Sphaerographium`~RG	-0.80284	1.026747	-0.78192	0.439104
RG	G: Archaeorhizomyces	Genus	`G: Archaeorhizomyces`~RG	-1.38291	1.471046	-0.94008	0.353113
RG	G: Sebacina	Genus	`G: Sebacina`~RG	0.524895	0.607666	0.863789	0.393123
RG	G: Rhodotorula	Genus	`G: Rhodotorula`~RG	0.008606	0.517017	0.016645	0.986807
RG	G: Leucosporidium	Genus	`G: Leucosporidium`~RG	-0.95849	0.779211	-1.23008	0.226227
RG	G: Colacogloea	Genus	`G: Colacogloea`~RG	-0.21472	0.53884	-0.39849	0.692499
RG	G: Saitozyma	Genus	`G: Saitozyma`~RG	-1.84432	0.534759	-3.44888	0.001392
RG	G: Papiliotrema	Genus	`G: Papiliotrema`~RG	-0.17425	0.688034	-0.25326	0.801436
RG	G: Vishniacozyma	Genus	`G: Vishniacozyma`~RG	0.51346	0.505926	1.014893	0.316576
RG	G: Apiotrichum	Genus	`G: Apiotrichum`~RG	-0.98758	1.104755	-0.89394	0.376983
RG	G: Naganishia	Genus	`G: Naganishia`~RG	0.836089	0.434498	1.924262	0.061834
RG	G: Solicoccozyma	Genus	`G: Solicoccozyma`~RG	-1.69965	1.149947	-1.47803	0.147642
RG	G: Archaeospora	Genus	`G: Archaeospora`~RG	-0.1503	1.034355	-0.1453	0.885239
RG	G: Diversispora	Genus	`G: Diversispora`~RG	-0.19962	0.655743	-0.30442	0.762471
RG	G: Mortierella	Genus	`G: Mortierella`~RG	0.480231	0.277867	1.728274	0.092058
RG	G: Acaulospora	Genus	`G: Acaulospora`~RG	0.623763	0.489381	1.274597	0.210191
RG	G: Clavulina	Genus	`G: Clavulina`~RG	0.266898	0.67171	0.397342	0.693339
RG	G: Olpidium	Genus	`G: Olpidium`~RG	0.588185	0.683957	0.859973	0.395197

A5.2.2 Microbial taxa positively correlated to RG have a positive estimate value

term	**taxon**	**rank**	**formula**	**estimate**	**std.error**	**statistic**	**p.value**
RG	P: Glomeromycota	Phylum	`P: Glomeromycota`~RG	0.322756	0.955557	0.337767	0.737398

RG	P: Basidiomycota	Phylum	`P: Basidiomycota`~RG	-1.07119	0.506455	-2.11508	0.041037
RG	P: Ascomycota	Phylum	`P: Ascomycota`~RG	-0.47639	0.506676	-0.94023	0.353039
RG	P: Mortierellomycota	Phylum	`P: Mortierellomycota`~RG	0.480231	0.277867	1.728274	0.092058
RG	P: Olpidiomycota	Phylum	`P: Olpidiomycota`~RG	0.437236	0.5298	0.825285	0.414361
RG	C: Glomeromycetes	Class	`C: Glomeromycetes`~RG	0.527486	0.670875	0.786265	0.436588
RG	C: Microbotryomycetes	Class	`C: Microbotryomycetes`~RG	-0.76024	0.718308	-1.05837	0.296568
RG	C: Tremellomycetes	Class	`C: Tremellomycetes`~RG	-1.07786	0.504489	-2.13653	0.039136
RG	C: Saccharomycetes	Class	`C: Saccharomycetes`~RG	-0.28856	0.496995	-0.58061	0.56493
RG	C: Wallemiomycetes	Class	`C: Wallemiomycetes`~RG	0.428774	0.450583	0.9516	0.347314
RG	C: Leotiomycetes	Class	`C: Leotiomycetes`~RG	-2.11994	1.46556	-1.4465	0.156235
RG	C: Eurotiomycetes	Class	`C: Eurotiomycetes`~RG	-1.50171	0.756734	-1.98447	0.05446
RG	C: Geoglossomycetes	Class	`C: Geoglossomycetes`~RG	0.076684	1.25652	0.061029	0.951656
RG	C: Sordariomycetes	Class	`C: Sordariomycetes`~RG	-0.22789	0.868623	-0.26236	0.794461
RG	C: Archaeorhizomycetes	Class	`C: Archaeorhizomycetes`~RG	-1.08101	1.219185	-0.88666	0.380837
RG	C: Agaricomycetes	Class	`C: Agaricomycetes`~RG	0.307844	0.587038	0.524403	0.603044
RG	C: Archaeosporomycetes	Class	`C: Archaeosporomycetes`~RG	-0.18803	0.845839	-0.2223	0.825268
RG	C: Mortierellomycetes	Class	`C: Mortierellomycetes`~RG	0.480231	0.277867	1.728274	0.092058
RG	C: Olpidiomycetes	Class	`C: Olpidiomycetes`~RG	0.437236	0.5298	0.825285	0.414361
RG	O: Gigasporales	Order	`O: Gigasporales`~RG	-0.14373	0.552574	-0.26011	0.796183
RG	O: Glomerales	Order	`O: Glomerales`~RG	0.309025	0.588335	0.525254	0.602458
RG	O: Microbotryomycetes_ord_Incertae_sedis	Order	`O: Microbotryomycetes_ord_Incertae_sedis`~RG	-0.32217	0.689936	-0.46696	0.643193
RG	O: Cystofilobasidiales	Order	`O: Cystofilobasidiales`~RG	-0.72272	0.894661	-0.80781	0.424229
RG	O: Saccharomycetales	Order	`O: Saccharomycetales`~RG	-0.3829	0.681369	-0.56196	0.577442
RG	O: Wallemiales	Order	`O: Wallemiales`~RG	0.579723	0.612548	0.946414	0.349917
RG	O: Helotiales	Order	`O: Helotiales`~RG	-2.60586	1.684001	-1.54742	0.130051
RG	O: Thelebolales	Order	`O: Thelebolales`~RG	-0.74324	0.551203	-1.3484	0.185511
RG	O: Eurotiales	Order	`O: Eurotiales`~RG	-1.95456	0.957018	-2.04234	0.048101
RG	O: Geoglossales	Order	`O: Geoglossales`~RG	0.057816	1.464948	0.039466	0.968725
RG	O: Hypocreales	Order	`O: Hypocreales`~RG	-0.23995	0.89233	-0.2689	0.789459

RG	O: Sordariales	Order	`O: Sordariales`~RG	-0.44308	1.436051	-0.30854	0.759356
RG	O: Xylariales	Order	`O: Xylariales`~RG	-1.21396	0.618508	-1.96272	0.057031
RG	O: Archaeorhizomycetales	Order	`O: Archaeorhizomycetales`~RG	-1.38291	1.471046	-0.94008	0.353113
RG	O: Sebacinales	Order	`O: Sebacinales`~RG	0.524895	0.607666	0.863789	0.393123
RG	O: Sporidiobolales	Order	`O: Sporidiobolales`~RG	0.008606	0.517017	0.016645	0.986807
RG	O: Leucosporidiales	Order	`O: Leucosporidiales`~RG	-0.95849	0.779211	-1.23008	0.226227
RG	O: Tremellales	Order	`O: Tremellales`~RG	-1.83431	0.536196	-3.42098	0.001505
RG	O: Trichosporonales	Order	`O: Trichosporonales`~RG	-0.98758	1.104755	-0.89394	0.376983
RG	O: Filobasidiales	Order	`O: Filobasidiales`~RG	-0.93635	1.143269	-0.81901	0.417888
RG	O: Archaeosporales	Order	`O: Archaeosporales`~RG	-0.1503	1.034355	-0.1453	0.885239
RG	O: Diversisporales	Order	`O: Diversisporales`~RG	0.424144	0.773857	0.548091	0.586835
RG	O: Mortierellales	Order	`O: Mortierellales`~RG	0.480231	0.277867	1.728274	0.092058
RG	O: Cantharellales	Order	`O: Cantharellales`~RG	0.266898	0.67171	0.397342	0.693339
RG	O: Olpidiales	Order	`O: Olpidiales`~RG	0.588185	0.683957	0.859973	0.395197
RG	F: Gigasporaceae	Family	`F: Gigasporaceae`~RG	-0.14373	0.552574	-0.26011	0.796183
RG	F: Glomeraceae	Family	`F: Glomeraceae`~RG	0.309025	0.588335	0.525254	0.602458
RG	F: Microbotryomycetes_fam_Incertae_sedis	Family	`F: Microbotryomycetes_fam_Incertae_sedis`~RG	-0.32217	0.689936	-0.46696	0.643193
RG	F: Cystofilobasidiaceae	Family	`F: Cystofilobasidiaceae`~RG	-0.72272	0.894661	-0.80781	0.424229
RG	F: Saccharomycetales_fam_Incertae_sedis	Family	`F: Saccharomycetales_fam_Incertae_sedis`~RG	-0.3829	0.681369	-0.56196	0.577442
RG	F: Wallemiaceae	Family	`F: Wallemiaceae`~RG	0.579723	0.612548	0.946414	0.349917
RG	F: Leotiaceae	Family	`F: Leotiaceae`~RG	0.046302	0.507124	0.091304	0.927731
RG	F: Pseudeurotiaceae	Family	`F: Pseudeurotiaceae`~RG	-0.74324	0.551203	-1.3484	0.185511
RG	F: Helotiaceae	Family	`F: Helotiaceae`~RG	-0.75098	1.135207	-0.66154	0.512261
RG	F: Vibrisseaceae	Family	`F: Vibrisseaceae`~RG	-0.44294	0.722043	-0.61346	0.54323
RG	F: Helotiales_fam_Incertae_sedis	Family	`F: Helotiales_fam_Incertae_sedis`~RG	0.781359	1.056162	0.73981	0.463962
RG	F: Myxotrichaceae	Family	`F: Myxotrichaceae`~RG	-1.4503	1.739705	-0.83365	0.409689
RG	F: Dermateaceae	Family	`F: Dermateaceae`~RG	-0.52648	0.750525	-0.70148	0.487275
RG	F: Aspergillaceae	Family	`F: Aspergillaceae`~RG	-1.95456	0.957018	-2.04234	0.048101

RG	F: Geoglossaceae	Family	\`F: Geoglossaceae\`~RG	0.057816	1.464948	0.039466	0.968725
RG	F: Nectriaceae	Family	\`F: Nectriaceae\`~RG	-1.0581	1.323616	-0.7994	0.429028
RG	F: Cordycipitaceae	Family	\`F: Cordycipitaceae\`~RG	-0.31122	1.00086	-0.31096	0.757533
RG	F: Hypocreaceae	Family	\`F: Hypocreaceae\`~RG	0.319574	1.094402	0.292008	0.771869
RG	F: Bionectriaceae	Family	\`F: Bionectriaceae\`~RG	2.011937	1.077907	1.866521	0.069699
RG	F: Chaetomiaceae	Family	\`F: Chaetomiaceae\`~RG	0.272244	0.490566	0.554959	0.582175
RG	F: Lasiosphaeriaceae	Family	\`F: Lasiosphaeriaceae\`~RG	-0.45796	1.430829	-0.32007	0.750671
RG	F: Sporocadaceae	Family	\`F: Sporocadaceae\`~RG	-1.21396	0.618508	-1.96272	0.057031
RG	F: Chaetomellaceae	Family	\`F: Chaetomellaceae\`~RG	-0.80284	1.026747	-0.78192	0.439104
RG	F: Archaeorhizomycetaceae	Family	\`F: Archaeorhizomycetaceae\`~RG	-1.38291	1.471046	-0.94008	0.353113
RG	F: Sebacinaceae	Family	\`F: Sebacinaceae\`~RG	0.524895	0.607666	0.863789	0.393123
RG	F: Sporidiobolaceae	Family	\`F: Sporidiobolaceae\`~RG	0.008606	0.517017	0.016645	0.986807
RG	F: Leucosporidiaceae	Family	\`F: Leucosporidiaceae\`~RG	-0.95849	0.779211	-1.23008	0.226227
RG	F: Trimorphomycetaceae	Family	\`F: Trimorphomycetaceae\`~RG	-1.84432	0.534759	-3.44888	0.001392
RG	F: Rhynchogastremataceae	Family	\`F: Rhynchogastremataceae\`~RG	-0.17425	0.688034	-0.25326	0.801436
RG	F: Bulleribasidiaceae	Family	\`F: Bulleribasidiaceae\`~RG	0.51346	0.505926	1.014893	0.316576
RG	F: Trichosporonaceae	Family	\`F: Trichosporonaceae\`~RG	-0.98758	1.104755	-0.89394	0.376983
RG	F: Filobasidiaceae	Family	\`F: Filobasidiaceae\`~RG	0.836089	0.434498	1.924262	0.061834
RG	F: Piskurozymaceae	Family	\`F: Piskurozymaceae\`~RG	-1.69965	1.149947	-1.47803	0.147642
RG	F: Archaeosporaceae	Family	\`F: Archaeosporaceae\`~RG	-0.1503	1.034355	-0.1453	0.885239
RG	F: Diversisporaceae	Family	\`F: Diversisporaceae\`~RG	-0.19962	0.655743	-0.30442	0.762471
RG	F: Mortierellaceae	Family	\`F: Mortierellaceae\`~RG	0.480231	0.277867	1.728274	0.092058
RG	F: Acaulosporaceae	Family	\`F: Acaulosporaceae\`~RG	0.623763	0.489381	1.274597	0.210191
RG	F: Clavulinaceae	Family	\`F: Clavulinaceae\`~RG	0.266898	0.67171	0.397342	0.693339
RG	F: Olpidiaceae	Family	\`F: Olpidiaceae\`~RG	0.588185	0.683957	0.859973	0.395197
RG	G: Scutellospora	Genus	\`G: Scutellospora\`~RG	-0.14373	0.552574	-0.26011	0.796183
RG	G: Rhizoglomus	Genus	\`G: Rhizoglomus\`~RG	0.309025	0.588335	0.525254	0.602458
RG	G: Curvibasidium	Genus	\`G: Curvibasidium\`~RG	0.153527	0.538848	0.284917	0.777254
RG	G: Cystofilobasidium	Genus	\`G: Cystofilobasidium\`~RG	-0.72272	0.894661	-0.80781	0.424229
RG	G: Candida	Genus	\`G: Candida\`~RG	-0.3829	0.681369	-0.56196	0.577442

RG	G: Wallemia	Genus	`G: Wallemia`~RG	0.579723	0.612548	0.946414	0.349917
RG	G: Alatospora	Genus	`G: Alatospora`~RG	0.046302	0.507124	0.091304	0.927731
RG	G: Pseudeurotium	Genus	`G: Pseudeurotium`~RG	-0.74324	0.551203	-1.3484	0.185511
RG	G: Infundichalara	Genus	`G: Infundichalara`~RG	-0.75098	1.135207	-0.66154	0.512261
RG	G: Phialocephala	Genus	`G: Phialocephala`~RG	-0.44294	0.722043	-0.61346	0.54323
RG	G: Mycoarthris	Genus	`G: Mycoarthris`~RG	0.781359	1.056162	0.73981	0.463962
RG	G: Oidiodendron	Genus	`G: Oidiodendron`~RG	-1.4503	1.739705	-0.83365	0.409689
RG	G: Cryptosporiopsis	Genus	`G: Cryptosporiopsis`~RG	-0.52648	0.750525	-0.70148	0.487275
RG	G: Penicillium	Genus	`G: Penicillium`~RG	-1.95456	0.957018	-2.04234	0.048101
RG	G: Trichoglossum	Genus	`G: Trichoglossum`~RG	0.057816	1.464948	0.039466	0.968725
RG	G: Ilyonectria	Genus	`G: Ilyonectria`~RG	-2.30468	1.360229	-1.69433	0.098384
RG	G: Mariannaea	Genus	`G: Mariannaea`~RG	0.196923	1.218069	0.161668	0.872424
RG	G: Leptobacillium	Genus	`G: Leptobacillium`~RG	-0.31122	1.00086	-0.31096	0.757533
RG	G: Trichoderma	Genus	`G: Trichoderma`~RG	0.319574	1.094402	0.292008	0.771869
RG	G: Clonostachys	Genus	`G: Clonostachys`~RG	2.011937	1.077907	1.866521	0.069699
RG	G: Thelonectria	Genus	`G: Thelonectria`~RG	0.616516	0.67522	0.913059	0.366969
RG	G: Fusarium	Genus	`G: Fusarium`~RG	-0.42537	1.381841	-0.30783	0.759896
RG	G: Humicola	Genus	`G: Humicola`~RG	0.272244	0.490566	0.554959	0.582175
RG	G: Cercophora	Genus	`G: Cercophora`~RG	-0.45796	1.430829	-0.32007	0.750671
RG	G: Pestalotiopsis	Genus	`G: Pestalotiopsis`~RG	-1.21396	0.618508	-1.96272	0.057031
RG	G: Sphaerographium	Genus	`G: Sphaerographium`~RG	-0.80284	1.026747	-0.78192	0.439104
RG	G: Archaeorhizomyces	Genus	`G: Archaeorhizomyces`~RG	-1.38291	1.471046	-0.94008	0.353113
RG	G: Sebacina	Genus	`G: Sebacina`~RG	0.524895	0.607666	0.863789	0.393123
RG	G: Rhodotorula	Genus	`G: Rhodotorula`~RG	0.008606	0.517017	0.016645	0.986807
RG	G: Leucosporidium	Genus	`G: Leucosporidium`~RG	-0.95849	0.779211	-1.23008	0.226227
RG	G: Colacogloea	Genus	`G: Colacogloea`~RG	-0.21472	0.53884	-0.39849	0.692499
RG	G: Saitozyma	Genus	`G: Saitozyma`~RG	-1.84432	0.534759	-3.44888	0.001392
RG	G: Papiliotrema	Genus	`G: Papiliotrema`~RG	-0.17425	0.688034	-0.25326	0.801436
RG	G: Vishniacozyma	Genus	`G: Vishniacozyma`~RG	0.51346	0.505926	1.014893	0.316576
RG	G: Apiotrichum	Genus	`G: Apiotrichum`~RG	-0.98758	1.104755	-0.89394	0.376983

RG	G: Naganishia	Genus	`G: Naganishia`~RG	0.836089	0.434498	1.924262	0.061834
RG	G: Solicoccozyma	Genus	`G: Solicoccozyma`~RG	-1.69965	1.149947	-1.47803	0.147642
RG	G: Archaeospora	Genus	`G: Archaeospora`~RG	-0.1503	1.034355	-0.1453	0.885239
RG	G: Diversispora	Genus	`G: Diversispora`~RG	-0.19962	0.655743	-0.30442	0.762471
RG	G: Mortierella	Genus	`G: Mortierella`~RG	0.480231	0.277867	1.728274	0.092058
RG	G: Acaulospora	Genus	`G: Acaulospora`~RG	0.623763	0.489381	1.274597	0.210191
RG	G: Clavulina	Genus	`G: Clavulina`~RG	0.266898	0.67171	0.397342	0.693339
RG	G: Olpidium	Genus	`G: Olpidium`~RG	0.588185	0.683957	0.859973	0.395197

A5.2.2 Microbial taxa positively correlated to NF have a positive estimate value

term	taxon	rank	formula	estimate	std.error	statistic	p.value
NF	P: Glomeromycota	Phylum	`P: Glomeromycota`~NF	-0.69435	1.090568	-0.63669	0.528145
NF	P: Basidiomycota	Phylum	`P: Basidiomycota`~NF	0.106086	0.613179	0.173011	0.863561
NF	P: Ascomycota	Phylum	`P: Ascomycota`~NF	0.383636	0.583876	0.65705	0.515109
NF	P: Mortierellomycota	Phylum	`P: Mortierellomycota`~NF	-0.03898	0.330551	-0.11793	0.906748
NF	P: Olpidiomycota	Phylum	`P: Olpidiomycota`~NF	-0.56939	0.605371	-0.94056	0.35287
NF	C: Glomeromycetes	Class	`C: Glomeromycetes`~NF	-1.04493	0.756041	-1.38211	0.17501
NF	C: Microbotryomycetes	Class	`C: Microbotryomycetes`~NF	-1.6579	0.790466	-2.09737	0.042668
NF	C: Tremellomycetes	Class	`C: Tremellomycetes`~NF	0.121407	0.611377	0.19858	0.84365
NF	C: Saccharomycetes	Class	`C: Saccharomycetes`~NF	1.355243	0.527954	2.566975	0.014321
NF	C: Wallemiomycetes	Class	`C: Wallemiomycetes`~NF	-0.51699	0.515545	-1.0028	0.322298
NF	C: Leotiomycetes	Class	`C: Leotiomycetes`~NF	0.406015	1.723358	0.235595	0.815012
NF	C: Eurotiomycetes	Class	`C: Eurotiomycetes`~NF	-1.314	0.885468	-1.48396	0.146068
NF	C: Geoglossomycetes	Class	`C: Geoglossomycetes`~NF	-1.30494	1.423945	-0.91643	0.365223
NF	C: Sordariomycetes	Class	`C: Sordariomycetes`~NF	0.257259	0.995158	0.25851	0.797408

NF	C: Archaeorhizomycetes	Class	\`C: Archaeorhizomycetes\`~NF	0.15039	1.410915	0.106591	0.915675
NF	C: Agaricomycetes	Class	\`C: Agaricomycetes\`~NF	-0.78136	0.662957	-1.1786	0.245884
NF	C: Archaeosporomycetes	Class	\`C: Archaeosporomycetes\`~NF	0.543058	0.96565	0.562376	0.577163
NF	C: Mortierellomycetes	Class	\`C: Mortierellomycetes\`~NF	-0.03898	0.330551	-0.11793	0.906748
NF	C: Olpidiomycetes	Class	\`C: Olpidiomycetes\`~NF	-0.56939	0.605371	-0.94056	0.35287
NF	O: Gigasporales	Order	\`O: Gigasporales\`~NF	-0.65016	0.624776	-1.04062	0.304627
NF	O: Glomerales	Order	\`O: Glomerales\`~NF	-0.78295	0.664434	-1.17837	0.245977
NF	O: Microbotryomycetes_ord_Incertae_sedis	Order	\`O: Microbotryomycetes_ord_Incertae_sedis\`~NF	-1.02738	0.774967	-1.32571	0.19285
NF	O: Cystofilobasidiales	Order	\`O: Cystofilobasidiales\`~NF	-1.85418	0.988998	-1.87481	0.068521
NF	O: Saccharomycetales	Order	\`O: Saccharomycetales\`~NF	1.850544	0.72408	2.555718	0.01472
NF	O: Wallemiales	Order	\`O: Wallemiales\`~NF	-0.71511	0.700442	-1.02094	0.313738
NF	O: Helotiales	Order	\`O: Helotiales\`~NF	0.393586	1.988097	0.197971	0.844123
NF	O: Thelebolales	Order	\`O: Thelebolales\`~NF	-0.65034	0.637746	-1.01974	0.314298
NF	O: Eurotiales	Order	\`O: Eurotiales\`~NF	-1.71024	1.121194	-1.52537	0.135447
NF	O: Geoglossales	Order	\`O: Geoglossales\`~NF	-1.404	1.662817	-0.84435	0.403758
NF	O: Hypocreales	Order	\`O: Hypocreales\`~NF	-0.20248	1.022738	-0.19798	0.844119
NF	O: Sordariales	Order	\`O: Sordariales\`~NF	0.47749	1.64544	0.29019	0.773249
NF	O: Xylariales	Order	\`O: Xylariales\`~NF	-1.06221	0.723401	-1.46836	0.150237
NF	O: Archaeorhizomycetales	Order	\`O: Archaeorhizomycetales\`~NF	0.05133	1.704759	0.03011	0.976137
NF	O: Sebacinales	Order	\`O: Sebacinales\`~NF	-0.71728	0.693273	-1.03462	0.307385
NF	O: Sporidiobolales	Order	\`O: Sporidiobolales\`~NF	-0.59292	0.584458	-1.01447	0.316773
NF	O: Leucosporidiales	Order	\`O: Leucosporidiales\`~NF	-1.36759	0.88285	-1.54906	0.129657
NF	O: Tremellales	Order	\`O: Tremellales\`~NF	0.445479	0.698816	0.637477	0.527637
NF	O: Trichosporonales	Order	\`O: Trichosporonales\`~NF	0.171875	1.278591	0.134425	0.893776
NF	O: Filobasidiales	Order	\`O: Filobasidiales\`~NF	-3.58053	1.186773	-3.01703	0.004537
NF	O: Archaeosporales	Order	\`O: Archaeosporales\`~NF	0.493528	1.182625	0.417315	0.678796
NF	O: Diversisporales	Order	\`O: Diversisporales\`~NF	-0.65973	0.883604	-0.74664	0.459875
NF	O: Mortierellales	Order	\`O: Mortierellales\`~NF	-0.03898	0.330551	-0.11793	0.906748
NF	O: Cantharellales	Order	\`O: Cantharellales\`~NF	-0.89556	0.757329	-1.18252	0.244345

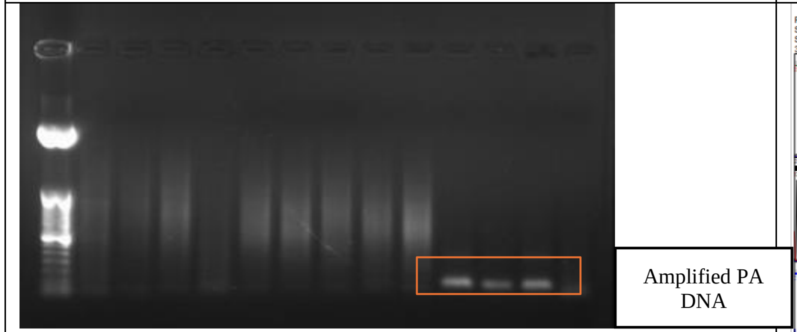
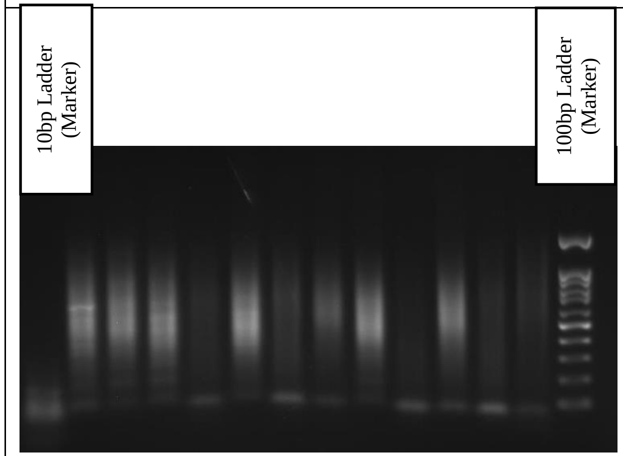
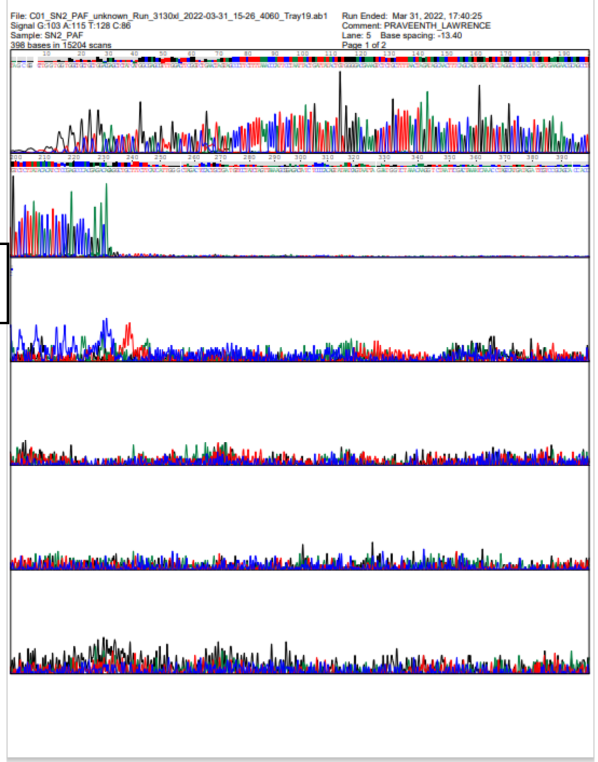
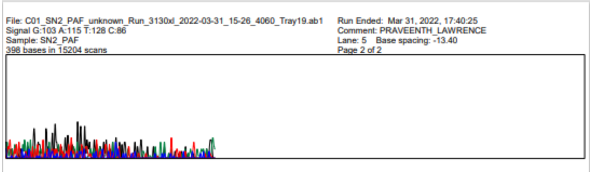
NF	O: Olpidiales	Order	`O: Olpidiales`~NF	-0.76751	0.781301	-0.98235	0.332141
NF	F: Gigasporaceae	Family	`F: Gigasporaceae`~NF	-0.65016	0.624776	-1.04062	0.304627
NF	F: Glomeraceae	Family	`F: Glomeraceae`~NF	-0.78295	0.664434	-1.17837	0.245977
NF	F: Microbotryomycetes_fam_Incertae_sedis	Family	`F: Microbotryomycetes_fam_Incertae_sedis`~NF	-1.02738	0.774967	-1.32571	0.19285
NF	F: Cystofilobasidiaceae	Family	`F: Cystofilobasidiaceae`~NF	-1.85418	0.988998	-1.87481	0.068521
NF	F: Saccharomycetales_fam_Incertae_sedis	Family	`F: Saccharomycetales_fam_Incertae_sedis`~NF	1.850544	0.72408	2.555718	0.01472
NF	F: Wallemiaceae	Family	`F: Wallemiaceae`~NF	-0.71511	0.700442	-1.02094	0.313738
NF	F: Leotiaceae	Family	`F: Leotiaceae`~NF	-0.58521	0.57324	-1.02088	0.313765
NF	F: Pseudeurotiaceae	Family	`F: Pseudeurotiaceae`~NF	-0.65034	0.637746	-1.01974	0.314298
NF	F: Helotiaceae	Family	`F: Helotiaceae`~NF	0.28184	1.307211	0.215604	0.830449
NF	F: Vibrisseaceae	Family	`F: Vibrisseaceae`~NF	-0.37408	0.829072	-0.4512	0.654408
NF	F: Helotiales_fam_Incertae_sedis	Family	`F: Helotiales_fam_Incertae_sedis`~NF	-0.52412	1.215699	-0.43113	0.668812
NF	F: Myxotrichaceae	Family	`F: Myxotrichaceae`~NF	-0.94008	2.005436	-0.46877	0.641915
NF	F: Dermateaceae	Family	`F: Dermateaceae`~NF	1.292159	0.839613	1.538993	0.132092
NF	F: Aspergillaceae	Family	`F: Aspergillaceae`~NF	-1.71024	1.121194	-1.52537	0.135447
NF	F: Geoglossaceae	Family	`F: Geoglossaceae`~NF	-1.404	1.662817	-0.84435	0.403758
NF	F: Nectriaceae	Family	`F: Nectriaceae`~NF	-0.40926	1.527648	-0.2679	0.790225
NF	F: Cordycipitaceae	Family	`F: Cordycipitaceae`~NF	-0.42589	1.146006	-0.37163	0.712234
NF	F: Hypocreaceae	Family	`F: Hypocreaceae`~NF	-3.0807	1.151422	-2.67556	0.010945
NF	F: Bionectriaceae	Family	`F: Bionectriaceae`~NF	-1.43544	1.269079	-1.13109	0.265105
NF	F: Chaetomiaceae	Family	`F: Chaetomiaceae`~NF	-0.61961	0.555263	-1.11588	0.27148
NF	F: Lasiosphaeriaceae	Family	`F: Lasiosphaeriaceae`~NF	0.512708	1.63932	0.312756	0.756176
NF	F: Sporocadaceae	Family	`F: Sporocadaceae`~NF	-1.06221	0.723401	-1.46836	0.150237
NF	F: Chaetomellaceae	Family	`F: Chaetomellaceae`~NF	0.761658	1.179256	0.64588	0.522239
NF	F: Archaeorhizomycetaceae	Family	`F: Archaeorhizomycetaceae`~NF	0.05133	1.704759	0.03011	0.976137
NF	F: Sebacinaceae	Family	`F: Sebacinaceae`~NF	-0.71728	0.693273	-1.03462	0.307385
NF	F: Sporidiobolaceae	Family	`F: Sporidiobolaceae`~NF	-0.59292	0.584458	-1.01447	0.316773
NF	F: Leucosporidiaceae	Family	`F: Leucosporidiaceae`~NF	-1.36759	0.88285	-1.54906	0.129657

NF	F: Trimorphomycetaceae	Family	`F: Trimorphomycetaceae`~NF	0.44889	0.698223	0.642904	0.524147
NF	F: Rhynchogastremataceae	Family	`F: Rhynchogastremataceae`~NF	0.168658	0.788432	0.213916	0.831756
NF	F: Bulleribasidiaceae	Family	`F: Bulleribasidiaceae`~NF	-0.6569	0.577667	-1.13715	0.262594
NF	F: Trichosporonaceae	Family	`F: Trichosporonaceae`~NF	0.171875	1.278591	0.134425	0.893776
NF	F: Filobasidiaceae	Family	`F: Filobasidiaceae`~NF	-0.58462	0.512773	-1.14011	0.261374
NF	F: Piskurozymaceae	Family	`F: Piskurozymaceae`~NF	-3.27235	1.246436	-2.62536	0.012402
NF	F: Archaeosporaceae	Family	`F: Archaeosporaceae`~NF	0.493528	1.182625	0.417315	0.678796
NF	F: Diversisporaceae	Family	`F: Diversisporaceae`~NF	-0.85096	0.739387	-1.1509	0.256965
NF	F: Mortierellaceae	Family	`F: Mortierellaceae`~NF	-0.03898	0.330551	-0.11793	0.906748
NF	F: Acaulosporaceae	Family	`F: Acaulosporaceae`~NF	0.191224	0.571675	0.334497	0.739844
NF	F: Clavulinaceae	Family	`F: Clavulinaceae`~NF	-0.89556	0.757329	-1.18252	0.244345
NF	F: Olpidiaceae	Family	`F: Olpidiaceae`~NF	-0.76751	0.781301	-0.98235	0.332141
NF	G: Scutellospora	Genus	`G: Scutellospora`~NF	-0.65016	0.624776	-1.04062	0.304627
NF	G: Rhizoglomus	Genus	`G: Rhizoglomus`~NF	-0.78295	0.664434	-1.17837	0.245977
NF	G: Curvibasidium	Genus	`G: Curvibasidium`~NF	-0.56058	0.611259	-0.91709	0.364881
NF	G: Cystofilobasidium	Genus	`G: Cystofilobasidium`~NF	-1.85418	0.988998	-1.87481	0.068521
NF	G: Candida	Genus	`G: Candida`~NF	1.850544	0.72408	2.555718	0.01472
NF	G: Wallemia	Genus	`G: Wallemia`~NF	-0.71511	0.700442	-1.02094	0.313738
NF	G: Alatospora	Genus	`G: Alatospora`~NF	-0.58521	0.57324	-1.02088	0.313765
NF	G: Pseudeurotium	Genus	`G: Pseudeurotium`~NF	-0.65034	0.637746	-1.01974	0.314298
NF	G: Infundichalara	Genus	`G: Infundichalara`~NF	0.28184	1.307211	0.215604	0.830449
NF	G: Phialocephala	Genus	`G: Phialocephala`~NF	-0.37408	0.829072	-0.4512	0.654408
NF	G: Mycoarthris	Genus	`G: Mycoarthris`~NF	-0.52412	1.215699	-0.43113	0.668812
NF	G: Oidiodendron	Genus	`G: Oidiodendron`~NF	-0.94008	2.005436	-0.46877	0.641915
NF	G: Cryptosporiopsis	Genus	`G: Cryptosporiopsis`~NF	1.292159	0.839613	1.538993	0.132092
NF	G: Penicillium	Genus	`G: Penicillium`~NF	-1.71024	1.121194	-1.52537	0.135447
NF	G: Trichoglossum	Genus	`G: Trichoglossum`~NF	-1.404	1.662817	-0.84435	0.403758
NF	G: Ilyonectria	Genus	`G: Ilyonectria`~NF	-0.38874	1.614899	-0.24072	0.811066
NF	G: Mariannaea	Genus	`G: Mariannaea`~NF	-1.1743	1.382894	-0.84916	0.401108
NF	G: Leptobacillium	Genus	`G: Leptobacillium`~NF	-0.42589	1.146006	-0.37163	0.712234

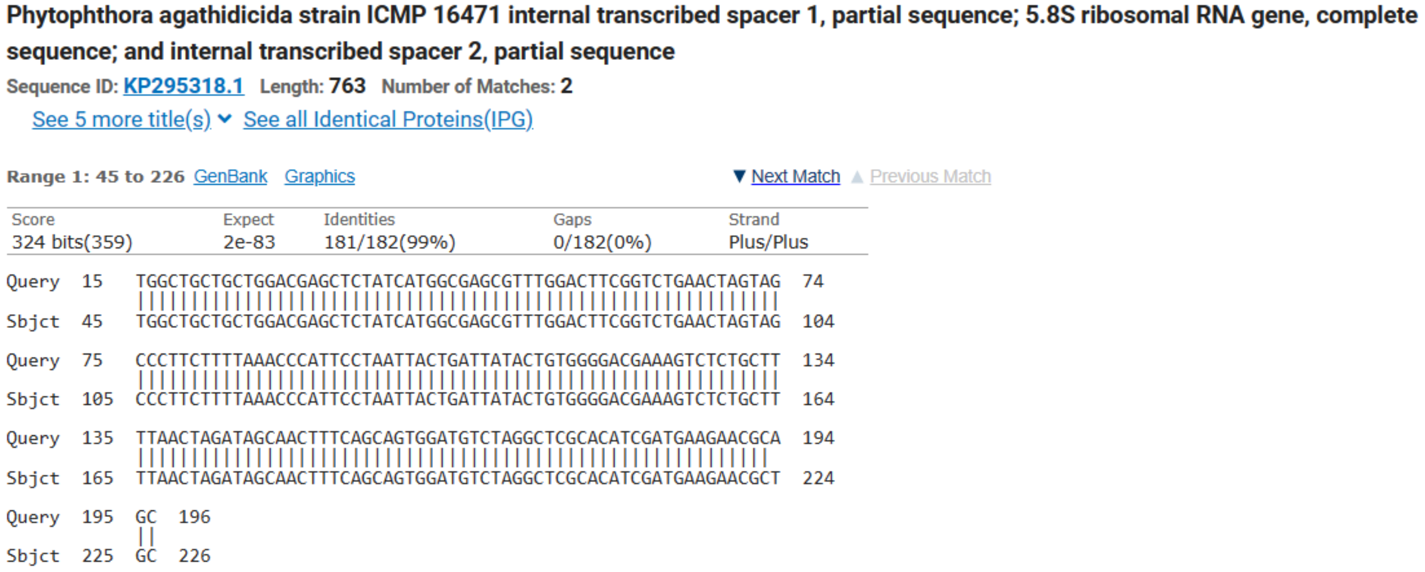
NF	G: Trichoderma	Genus	`G: Trichoderma`~NF	-3.0807	1.151422	-2.67556	0.010945
NF	G: Clonostachys	Genus	`G: Clonostachys`~NF	-1.43544	1.269079	-1.13109	0.265105
NF	G: Thelonectria	Genus	`G: Thelonectria`~NF	-1.08337	0.761997	-1.42175	0.163254
NF	G: Fusarium	Genus	`G: Fusarium`~NF	-2.53975	1.530589	-1.65933	0.105282
NF	G: Humicola	Genus	`G: Humicola`~NF	-0.61961	0.555263	-1.11588	0.27148
NF	G: Cercophora	Genus	`G: Cercophora`~NF	0.512708	1.63932	0.312756	0.756176
NF	G: Pestalotiopsis	Genus	`G: Pestalotiopsis`~NF	-1.06221	0.723401	-1.46836	0.150237
NF	G: Sphaerographium	Genus	`G: Sphaerographium`~NF	0.761658	1.179256	0.64588	0.522239
NF	G: Archaeorhizomyces	Genus	`G: Archaeorhizomyces`~NF	0.05133	1.704759	0.03011	0.976137
NF	G: Sebacina	Genus	`G: Sebacina`~NF	-0.71728	0.693273	-1.03462	0.307385
NF	G: Rhodotorula	Genus	`G: Rhodotorula`~NF	-0.59292	0.584458	-1.01447	0.316773
NF	G: Leucosporidium	Genus	`G: Leucosporidium`~NF	-1.36759	0.88285	-1.54906	0.129657
NF	G: Colacogloea	Genus	`G: Colacogloea`~NF	-0.7451	0.606683	-1.22815	0.226945
NF	G: Saitozyma	Genus	`G: Saitozyma`~NF	0.44889	0.698223	0.642904	0.524147
NF	G: Papiliotrema	Genus	`G: Papiliotrema`~NF	0.168658	0.788432	0.213916	0.831756
NF	G: Vishniacozyma	Genus	`G: Vishniacozyma`~NF	-0.6569	0.577667	-1.13715	0.262594
NF	G: Apiotrichum	Genus	`G: Apiotrichum`~NF	0.171875	1.278591	0.134425	0.893776
NF	G: Naganishia	Genus	`G: Naganishia`~NF	-0.58462	0.512773	-1.14011	0.261374
NF	G: Solicoccozyma	Genus	`G: Solicoccozyma`~NF	-3.27235	1.246436	-2.62536	0.012402
NF	G: Archaeospora	Genus	`G: Archaeospora`~NF	0.493528	1.182625	0.417315	0.678796
NF	G: Diversispora	Genus	`G: Diversispora`~NF	-0.85096	0.739387	-1.1509	0.256965
NF	G: Mortierella	Genus	`G: Mortierella`~NF	-0.03898	0.330551	-0.11793	0.906748
NF	G: Acaulospora	Genus	`G: Acaulospora`~NF	0.191224	0.571675	0.334497	0.739844
NF	G: Clavulina	Genus	`G: Clavulina`~NF	-0.89556	0.757329	-1.18252	0.244345
NF	G: Olpidium	Genus	`G: Olpidium`~NF	-0.76751	0.781301	-0.98235	0.332141

Appendix B

B1. Sequence alignment of the end point PCR to detect Phytophthora agathidicida (PA) in the soil samples.

Amplified DNA using PA specific primers (Than et al., 2013).	Sanger sequencing results
  	 File: C01_SN2_PAF_unknown_Run_3130xl_2022-03-31_15-26_4060_Tray19.ab1 Signal G:103 A:115 T:128 C:86 Sample: SN2_PAF 398 bases in 15204 scans Run Ended: Mar 31, 2022, 17:40:25 Comment: PRAVEENTH_LAWRENCE Lane: 5 Base spacing: -13.40 Page 1 of 2  File: C01_SN2_PAF_unknown_Run_3130xl_2022-03-31_15-26_4060_Tray19.ab1 Signal G:103 A:115 T:128 C:86 Sample: SN2_PAF 398 bases in 15204 scans Run Ended: Mar 31, 2022, 17:40:25 Comment: PRAVEENTH_LAWRENCE Lane: 5 Base spacing: -13.40 Page 2 of 2

B2. NCBI blast results



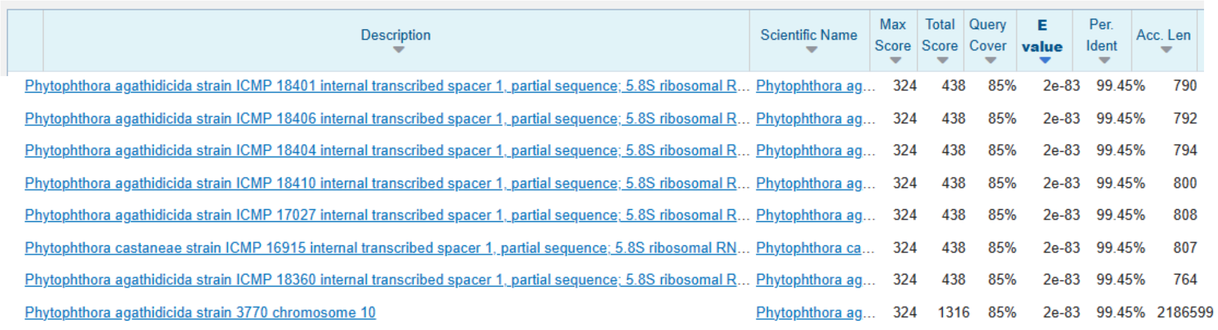
Phytophthora agathidicida strain ICMP 16471 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: KP295318.1 Length: 763 Number of Matches: 2

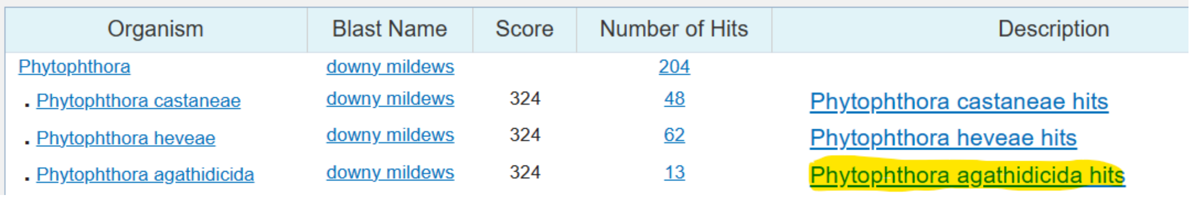
Range 1: 45 to 226

Score	Expect	Identities	Gaps	Strand
324 bits(359)	2e-83	181/182(99%)	0/182(0%)	Plus/Plus

```
Query  15   TGGCTGCTGCTGGACGAGCTCTATCATGGCGAGCGTTTGGACTTCGGTCTGAACTAGTAG  74
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  45   TGGCTGCTGCTGGACGAGCTCTATCATGGCGAGCGTTTGGACTTCGGTCTGAACTAGTAG  104

Query  75   CCCTTCTTTTAAACCCATTCCTAATTACTGATTATACTGTGGGGACGAAAGTCTCTGCTT  134
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  105  CCCTTCTTTTAAACCCATTCCTAATTACTGATTATACTGTGGGGACGAAAGTCTCTGCTT  164

Query  135  TTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCA  194
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 
Sbjct  165  TTAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCT  224

Query  195  GC  196
            ||
Sbjct  225  GC  226
```



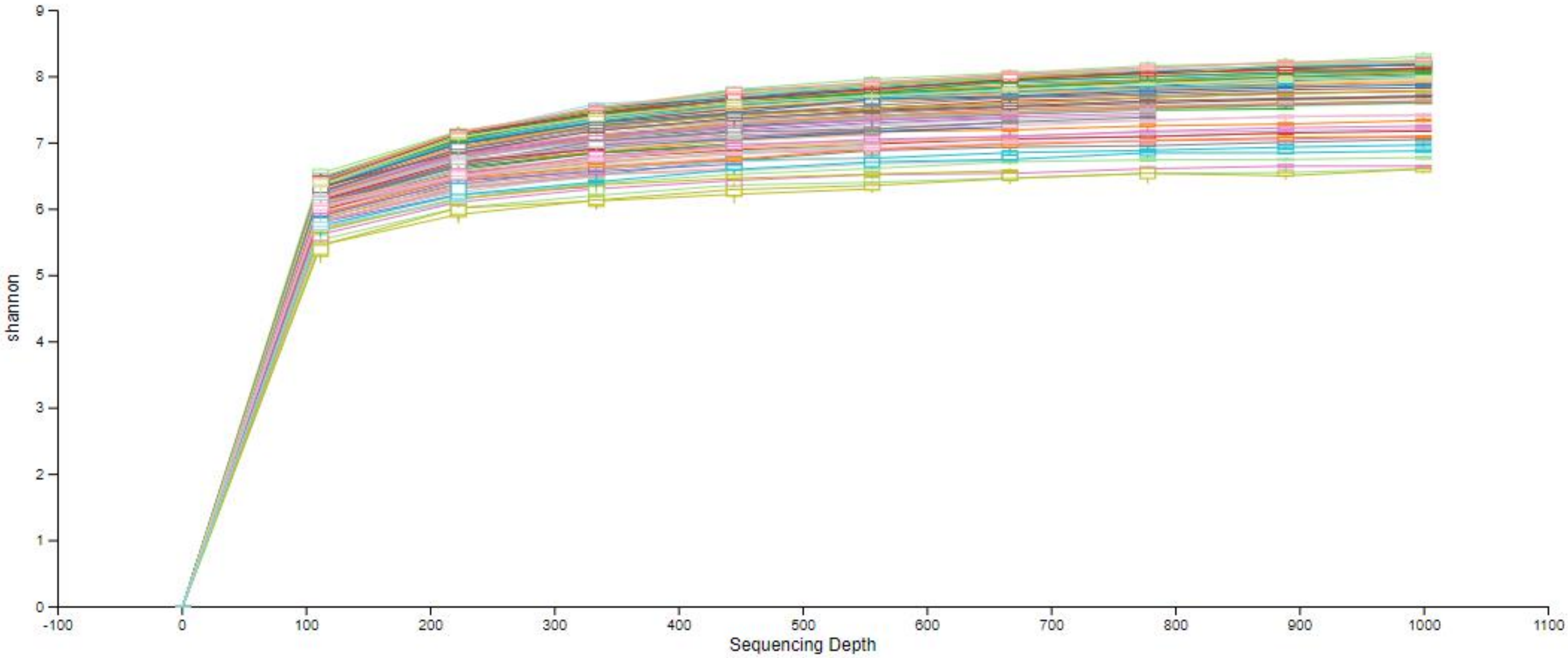
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len
	Phytophthora agathidicida strain ICMP 18401 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...	Phytophthora ag...	324	438	85%	2e-83	99.45%	790
	Phytophthora agathidicida strain ICMP 18406 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...	Phytophthora ag...	324	438	85%	2e-83	99.45%	792
	Phytophthora agathidicida strain ICMP 18404 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...	Phytophthora ag...	324	438	85%	2e-83	99.45%	794
	Phytophthora agathidicida strain ICMP 18410 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...	Phytophthora ag...	324	438	85%	2e-83	99.45%	800
	Phytophthora agathidicida strain ICMP 17027 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...	Phytophthora ag...	324	438	85%	2e-83	99.45%	808
	Phytophthora castaneae strain ICMP 16915 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...	Phytophthora ca...	324	438	85%	2e-83	99.45%	807
	Phytophthora agathidicida strain ICMP 18360 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...	Phytophthora ag...	324	438	85%	2e-83	99.45%	764
	Phytophthora agathidicida strain 3770 chromosome 10	Phytophthora ag...	324	1316	85%	2e-83	99.45%	2186599



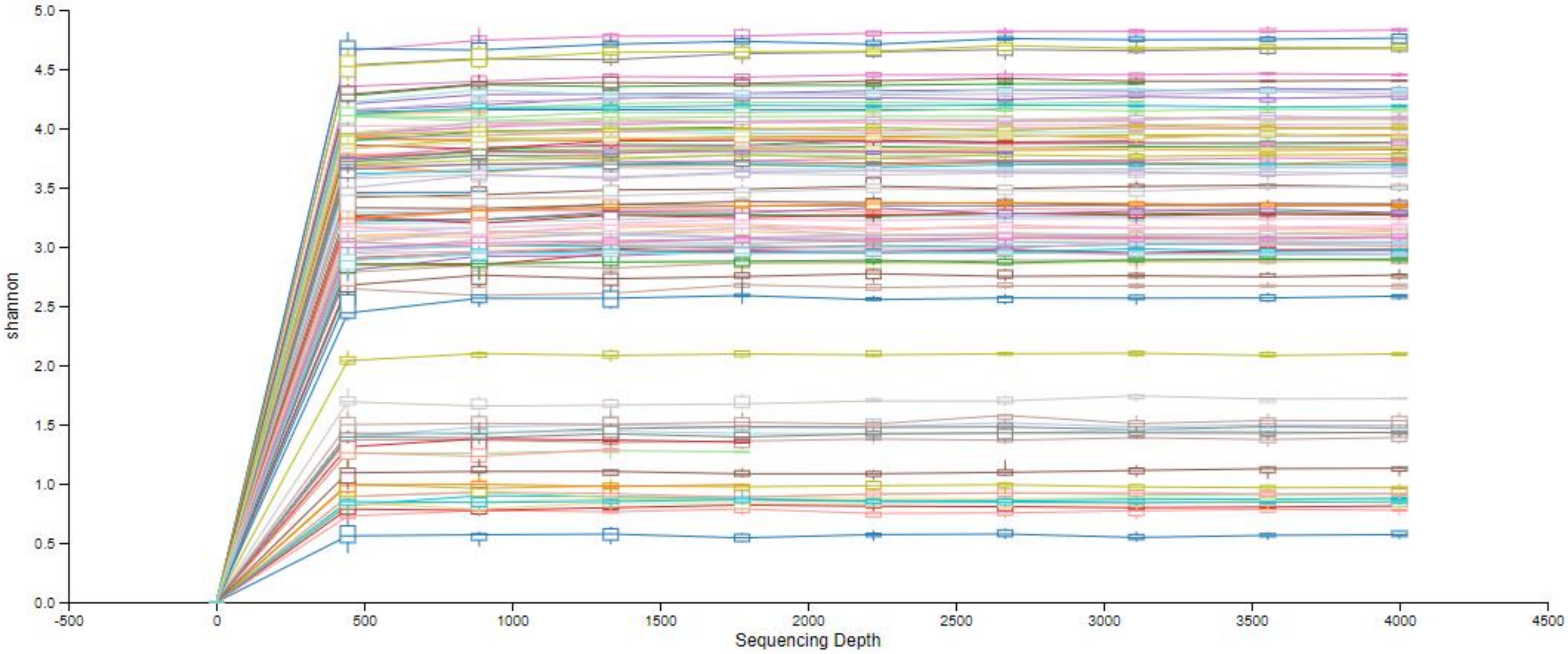
Organism	Blast Name	Score	Number of Hits	Description
Phytophthora	downy mildews		204	
. Phytophthora castaneae	downy mildews	324	48	Phytophthora castaneae hits
. Phytophthora heveae	downy mildews	324	62	Phytophthora heveae hits
. Phytophthora agathidicida	downy mildews	324	13	Phytophthora agathidicida hits

B3. Determining the rarefied sample depth

B2.1 Alpha rarefaction plot of 16S rRNA gene reads of Whangapoa

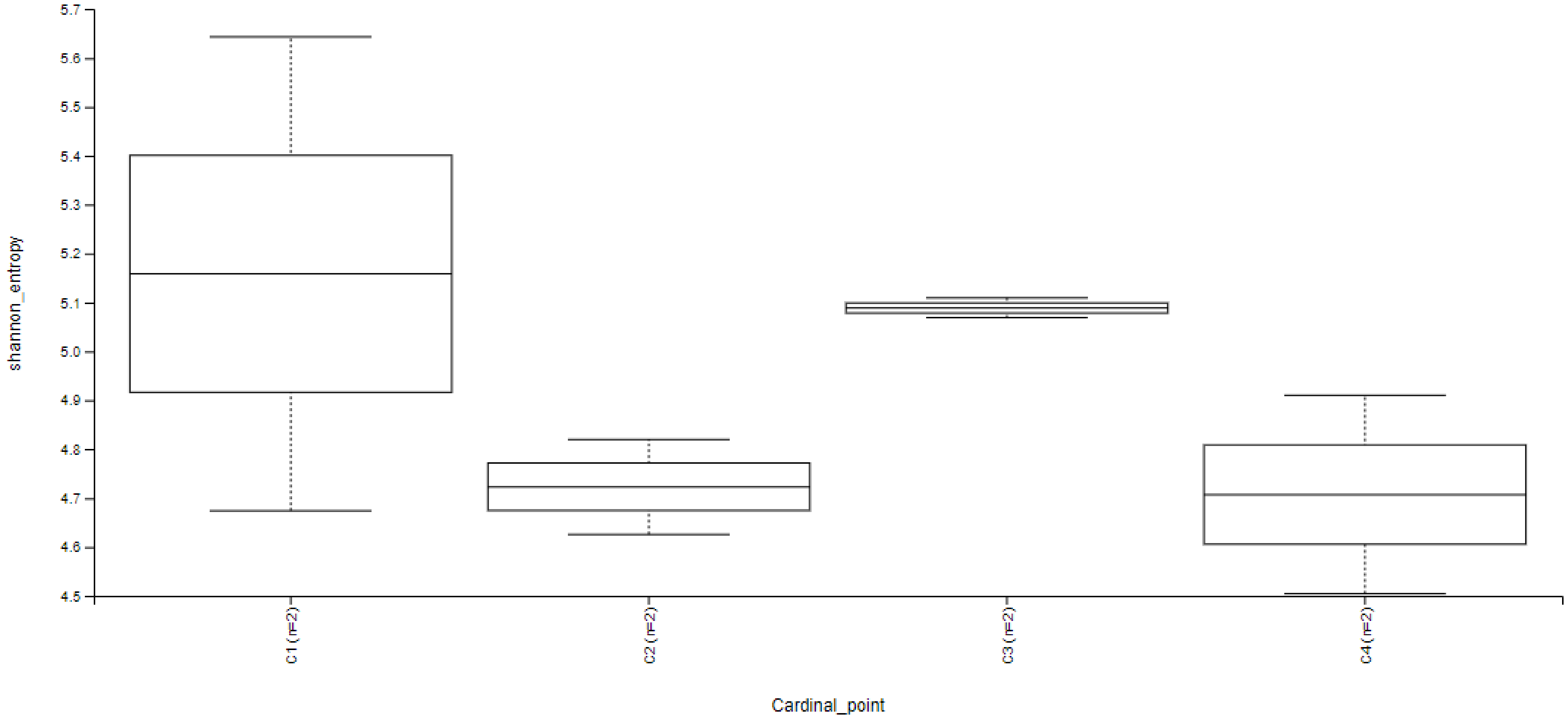


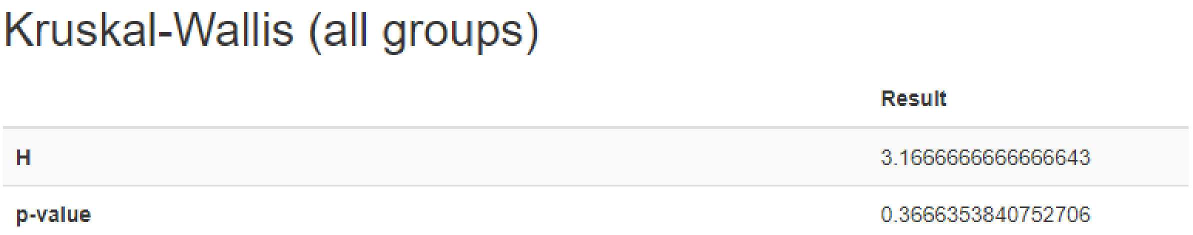
B2.2 Alpha rarefaction plot of ITS1 region reads of Whangapoa



B3. Cardinal Point Analysis

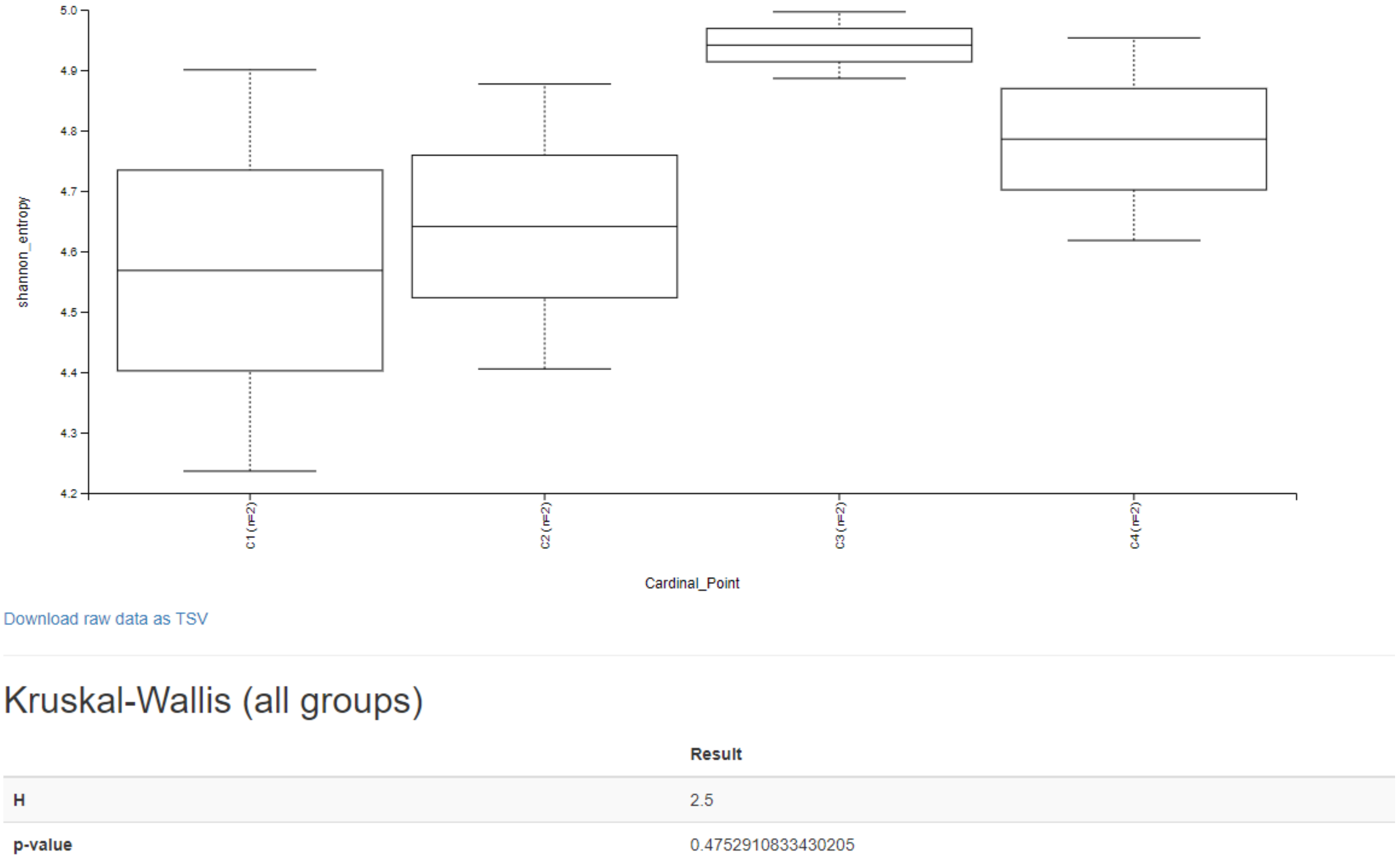
B3.1 Alpha diversity boxplots of cardinal points of bacterial reads in Tairua soil samples




Kruskal-Wallis (all groups)

	Result
H	3.1666666666666643
p-value	0.3666353840752706

B3.2 Alpha diversity boxplots of cardinal points of fungal reads in Tairua soil samples



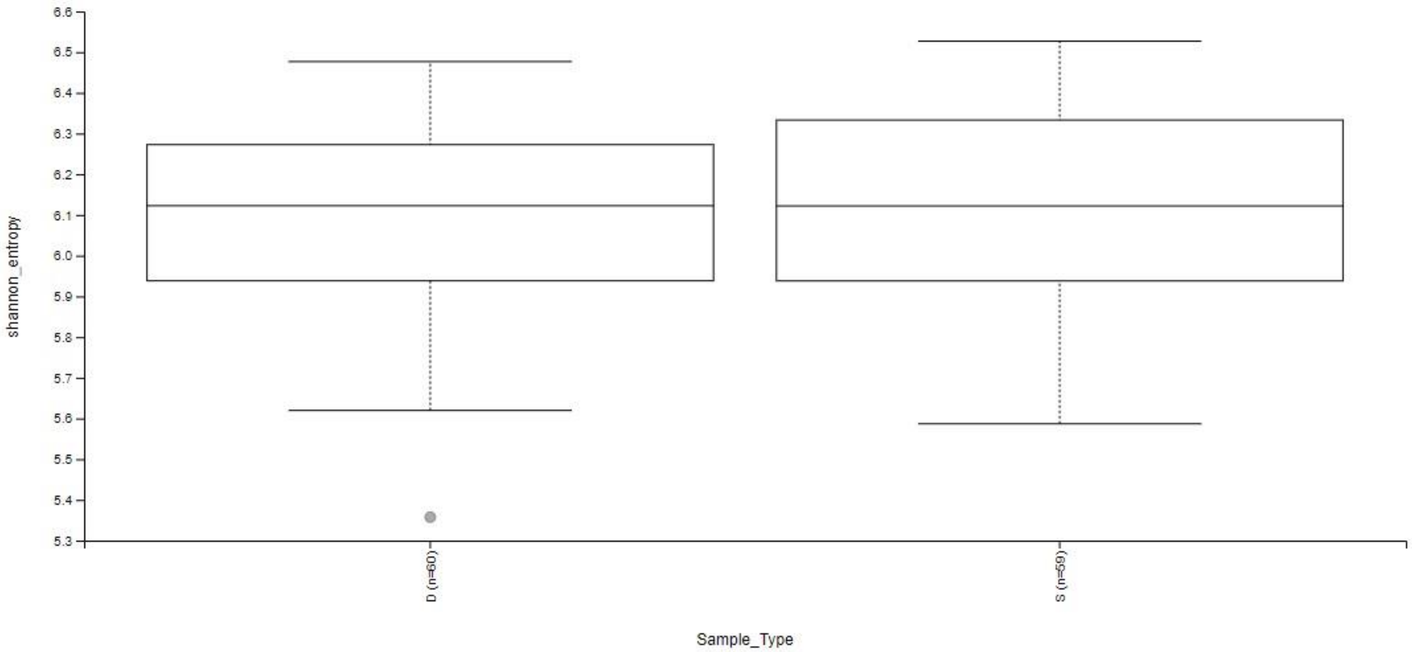
Download raw data as TSV

Kruskal-Wallis (all groups)

	Result
H	2.5
p-value	0.4752910833430205

B4. Soil Depth Analysis (Surface vs 10cm depth)

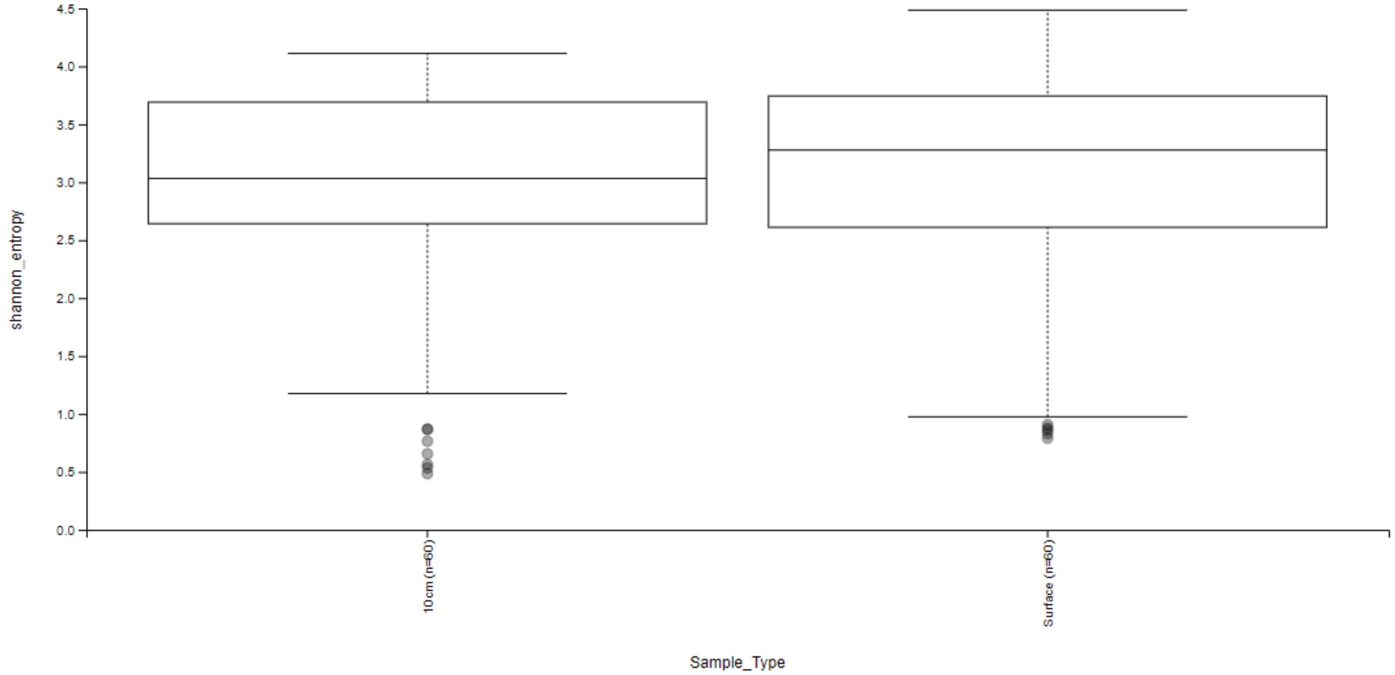
B4.1 Boxplots comparing bacterial reads' alpha diversity in Tairua soil samples between surface and 10cm depth samples.

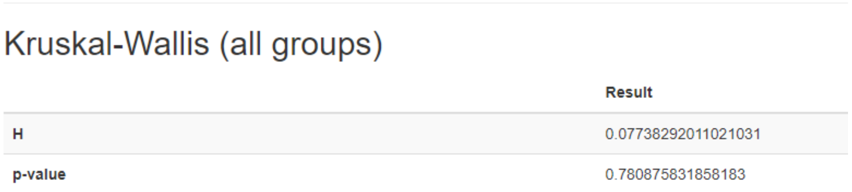


Kruskal-Wallis (all groups)

	Result
H	0.7644165637014122
p-value	0.3819498946857136

B4.2 Boxplots comparing bacterial reads' alpha diversity in Tairua soil samples between surface and 10cm depth samples.





Kruskal-Wallis (all groups)

	Result
H	0.07738292011021031
p-value	0.780875831858183

B5. Sample information of Waikato Kauri soil

B5.1 Sample information of Tairua kauri soil

Tree Number	Terrain	Tree health	Phosphite treatment	Symptom (Basal bleeding)	Canopy Health score	CBH (Inch)	Comments	PA presence
Tree 1	Gully	Non-Symptomatic	no	no	1	66	Healthy site at bottom of gully, good biodiversity around tree, lots of seedlings, below positive tree sites	Negative
Tree 4	Ridge	Non-Symptomatic	no	no	1	9.6	On ridge healthy, bottom of PA positive tree stands no disease expression, lots of seedlings within 2 m of tree	Negative
Tree 7	Ridge	Non-Symptomatic	no	no	1	33	Triple leaders, top of ridge, above pa positive site. Various plant species	Negative
Tree 11	Ridge	Symptomatic	no	no	3	12.8	Top of ridge, top of pa positive site -	Negative
Tree 14	Gully	Symptomatic	no	no	3	19.5	10m from PA positive tree, in gully	Negative
Tree 2	Ridge	Slightly Thinning	no	no	2	79	Thinning slightly	Positive
Tree 3	Ridge	Slightly Thinning	no	no	2	16	Yellowing slightly	Positive
Tree 5	Ridge	Slightly Thinning	no	no	2	73	Thinning slightly	Negative
Tree 6	Ridge	Slightly Thinning	no	no	2	18		Negative
Tree 8	Ridge	Slightly Thinning	no	no	2	26	Leader off to side 500mm up main trunk	Negative
Tree 9	Ridge	Slightly Thinning	no	no	2	41		Negative
Tree 10	Ridge	Slightly Thinning	no	no	2	77		Positive
Tree 12	Ridge	Slightly Thinning	no	no	2	12		Positive
Tree 13	Gully	Symptomatic	no	no	2.5	18	10m from PA positive dead tree, in gully	Negative
Tree 15	Gully	Symptomatic	no	no	2.5	8	1.5m from the dead tree (Pa positive – Confirmed by council using baiting method)	Positive

B5.2: Whangapoa kauri sample information

Tree Number (ID)	Terrain	Tree health	Phosphonate treatment	Symptom (Basal bleeding)	Canopy Health score	CBH (Inch)	Comments	PA presence
4	Gully	Symptomatic	No	No	3	24.8	Dense kauri stand, ridge, within half a metre of another kauri	Positive*
6	Gully	Symptomatic	No	Yes	4	23.2	Active bleeding, tan opaque orange peppermint bleed. On ridge, typical v bleed	Negative
7	Gully	Symptomatic	No	Yes	3	56.1	Old bleed, typical v shape.	Positive*
10	Gully	Non symptomatic	No	No	1	5.8	In a symptomatic area, on a ridge line	Negative
11	Ridge	Symptomatic	No	No	2.5	43.1	Close to Phosphonate treated trees 4.5m away. On a slope.	Negative
12	Ridge	Symptomatic	No	No	2.5	46.9	Bleeding all around but looks like drip bleeding. On a sloping ridge	Negative
16	Ridge	Symptomatic	Yes	Yes	2.5	42	Drip bleeding from above	Negative
17	Ridge	Symptomatic	Yes	Yes	4.5	53	Tree almost dead. Severe bleeding around majority of tree	Negative
18	Ridge	Symptomatic	Yes	Yes	3	36	Small area of bleeding around base of tree	Positive*
19	Ridge	Symptomatic	Yes	Yes	3	91	Bleeding dried up	Negative
20	Ridge	Symptomatic	Yes	Yes	3	50	No base bleeding was noted when phosphonate was first delivered in 2019. Some fresh peppermint opaque bleeding	Negative
21	Ridge	Symptomatic	Yes	Yes	2.5	35		Negative
22	Ridge	Symptomatic	Yes	Yes	3.5	48	Fresh bleeding at base of tree on a lateral root. Opaque colour.	Negative
23	Ridge	Symptomatic	Yes	Yes	3	55	Hardening and fresh in places	Positive*

24	Ridge	Symptomatic	Yes	Yes	2.5	18	Tree 40cm from another tree showing infection and treated large stand of kauri infected. Old bleed base of tree v shape, active bleed on root on tree - large blob approximately size of apple.	Negative
25	Ridge	Symptomatic	Yes	No	3	17		Positive***
26	Ridge	Symptomatic	Yes	No	5-dead	28	Tree is close to death, no live roots found. On a sloping ridge, dense kauri stand. Another dead tree within 3m. cracking on trunk off. A lot of bleeding around base of tree. Typical v bleed.	Positive*
27	Ridge	Symptomatic	Yes	Yes	5-dead	48.8	1m from tree that has died, 4m from tree close to death. On a ridge slope. Substantial old bleeding around base of tree	Negative
28	Ridge	Symptomatic	Yes	Yes	3	56.2	On slope half a metre from another large tree with large DBH and Phosphonate treated. In large kauri stand. Old bleed.	Negative
29	Ridge	Symptomatic	Yes	Yes	3	57		Positive*
30	Ridge	Symptomatic	Yes	Yes	2.5	18	Old bleed	Positive*
31	Ridge	Symptomatic	Yes	Yes		63	Small amount	Positive***
32	Ridge	Symptomatic	No	Yes	3.5	45		Positive**
33	Ridge	Symptomatic	No	No	3.5	40		Positive*
34	Ridge	Symptomatic	No	No	4	20	Rata vine on tree	Positive***
35	Ridge	Symptomatic	No	Yes	3.5	34	No bleeding at base, on slope - within 10m of trees which have received Phosphonate treatment. Bleeding on lateral root.	Negative
36	Ridge	Symptomatic	No	No	4	28	Large stand of kauri - Phosphonate delivery trees within 5 metres of this tree.	Negative
37	Ridge	Symptomatic	No	No	3	29.2	On slope, next to area that’s had Phosphonate - approx. 15m	Negative
39	Ridge	Symptomatic	No	No	3	37.7	Another kauri within 1.5m which is expressing disease	Negative

Note: * PA positive in the surface soil sample, **PA positive in the 10cm depth soil sample ***PA positive in both surface and 10cm depth soil samples, CBH – Circumference at breast height

Appendix C

C1. ABG Geochip data
ABG_Geochip_Only _data.xlsx

C2. Whangapoa Geochip data
Waikato_GC_Only_Data.xlsx

C3. Article published in Elsevier Data in Brief
Lawrence, P., Padamsee, M., Lee, K., & Lacap-Bugler, D. C. (2023). Soil microbial functional gene dataset associated with Agathis australis. *Data in Brief*, *51*, 109791. https://doi.org/10.1016/j.dib.2023.109791

Appendix D

D1. Table: Clarified V8 juice Agar (cV8A)

Item	Quantity
Distilled water	800ml
Clarified V8 juice	200ml
Bacto agar (Difco)	15g
Note. Table content for 1000ml media preparation	

D2. Table: Mycelial mean growth in millimetres of endophyte isolates of Agathis australis and Phytophthora agathidicida in cV8A media at 20°C in the dark.

Days	Endophyte isolates *of Agathis australis*										
	ES107	ICMP 21343	ES91	ICMP 21339	ICMP 21347	ICMP 21375	ICMP 21377	ICMP 21392	ICMP 21398	ICMP 21455	Pa (ICMP 17027Q4)
0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	3	0	0	0	0	5	2
2	3	2	2	4	6	2	5	2	3	10	3
3	7	5	6	8	9	5	9	4	6	16	4
4	11	7	10	12	12	8	12	6	9	21	7
5	15	9	14	17	14	10	13	8	11	25	10
6	20	10	19	23	16	11	13	10	11	29	16
7	25	11	24	28	18	12	13	11	12	33	24
8	30	12	28	32	20	13	14	12	12	36	30
9	33	13	32	36	22	14	14	13	12	39	33
10	35	14	34	40	26	15	14	14	13	43	37
11	37	15	35	43	28	15	14	15	13	47	40
12	39	16	36	46	32	16	15	15	13	52	45
13	40	17	37	49	35	16	15	16	13	61	47

14	41	18	38	51	38	16	15	16	13	65	49

D3. Table: *Phytophthora agathidicida* (PA) mycelial radial growth (mm) against endophytes in dual culture on cV8A

		Mean PA growth in presence of endophyte isolates (dual culture) in cV8A media at 20°C in the dark				
Days	PA* (mm)	21455 (mm)	21347 (mm)	ES91 (mm)	21339 (mm)	ES109 (mm)
0	0	0	0	0	0	0
1	3	3	3	3	3	3
2	4	4	4	4	4	4
3	7	7	7	7	7	7
4	10	11	10	10	10	10
5	16	16	16	16	16	16
6	24	24	24	24	24	24
7	30	30	30	30	30	30
8	34	33	33	33	32	33
9	40	37	37	37	33	37
10	45	40	39	40	34	40
11	50	45	41	45	34	45
12	55	41	43	45	35	45
13	59	38	49	45	36	43
14	61	34	49	45	37	42
15	65	29	50	45	34	41
16	66	25	50	45	32	40
17	70	22	51	45	30	38
18	71	17	52	45	28	36
19	73	13	52	45	26	35
20	75	9	53	45	25	32
21	77	4	53	45	23	30
22	79	1	54	45	21	28
23	80	0	54	45	17	27
24	82	0	54	45	15	27
25	83	0	54	45	12	27
26	84	0	54	45	8	27
27	85	0	54	45	6	27
28	85	0	54	45	2	27
29	86	0	54	45	1	27
30	86	0	54	45	0	27
31	86	0	54	45	0	27
32	87	0	54	45	0	27

Note. *PA mean growth without the presence of endophyte

D4: Dual cultures of fungal endophytes with *Phytophthora agathidicida* on cV8A medium

Dual cultures of fungal endophytes with *Phytophthora agathidicida* on cV8A medium. a-d are *Coprinellus micaceus* (ICMP 21455) with *Phytophthora agathidicida*, e-h *Pezicula* sp. (ICMP 21347) with *Phytophthora agathidicida*, i-l *Ilyonectria mors-panacis* (ES91) with *Phytophthora agathidicida*, m-p *Ilyonectria mors-panacis* (21339) with *Phytophthora agathidicida*, q-t *Pezicula* sp. 6 cc-2015 with *Phytophthora agathidicida.* The diameter of the petri dish is 9cm. Scale bar is 4.5cm.