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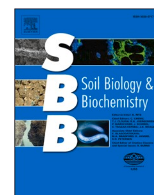

Soil microbial community restructuring and functional changes in ancient kauri (*Agathis australis*) forests impacted by the invasive pathogen *Phytophthora agathidicida*

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ABSTRACT

New Zealand's culturally iconic, ancient kauri (*Agathis australis*) forests are threatened with extinction as a result of dieback caused by an invasive and highly virulent soil-borne pathogen (*Phytophthora agathidicida*). Kauri trees function as a foundation species in their forests, supporting an ecologically distinct plant and soil environment. The impacts of disease outbreak and subsequent tree dieback on kauri forest ecosystems, in particular soil microbial communities, remains unknown. Using high throughput amplicon sequencing and GeoChip 5 S microarray analysis we analysed the differences in diversity, taxonomic composition and functional genes related to C and N cycling of soil microbial communities associated with asymptomatic and symptomatic mature kauri. Our results found significant differences in the fungal diversity and both fungal and bacterial community composition between asymptomatic and symptomatic kauri. Several microbial taxa known in the literature for their roles in disease suppression, such as *Penicillium, Trichoderma, Enterobacteriacae* and *Pseudomonas*, were identified as being significantly higher in asymptomatic kauri soils. These findings have provided a promising direction for the discovery of disease suppressive microbial taxa against kauri dieback. In addition, the composition and abundances of microbial genes related to C and N cycling also differed significantly. These findings highlight the potential long term impacts that dieback disease may have on the health and functioning of kauri forests.

1. Introduction

Globally, large-scale forest disease and dieback events are increasing at alarming rates as a result of biological invasions, climate change and anthropogenic disturbances (Cobb and Metz, 2017; Edburg et al., 2012; Steffen et al., 2015). The implications of this are dire, as these forests have critical roles in maintaining biodiversity, carbon (C) storage and climate regulation (Davidson and Janssens, 2006; Nolan et al., 2018; Hui et al., 2017). Soil-borne pathogens are known to affect soil microbial communities and their functional responses in highly managed monoculture systems (Dignam et al. 2018, 2019), including the cycling of C and nitrogen (N). However, little is known about the impact of invasive soil pathogens on old growth, tree dominated forests. Kauri (*Agathis australis*) are one of New Zealand's most ancient native tree species and are currently under threat from the spread of dieback disease, caused by the invasive soil-borne pathogen *Phytophthora agathidicida* (Waipara et al., 2009; Beever et al., 2009).

Kauri are an iconic and culturally significant tree species for New Zealand, being regarded by the indigenous Māori as their living ancestors (Black et al., 2018). Kauri function as foundation species in their namesake forests, significantly influencing surrounding plant species composition and supporting the most species rich forest type in New Zealand (Wyse et al., 2014; Ogden, 1995). In addition, kauri have a huge presence in their forests, reaching heights of up to 60 m, diameters of up to 5 m and ages of up to 2000 years (Steward and Beveridge, 2010). Kauri forests are incredibly carbon dense and over their long life histories their acidic, tannin rich leaf litter accumulates to form a mor humus layer up to 2 m deep (Wyse et al., 2014; Steward and Beveridge, 2010; Macinnis-Ng and Schwendenmann et al., 2015). Prior to European arrival kauri had an extensive distribution covering over 1 million

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hectares over New Zealand's upper North Island. However, a history of uncontrolled logging for timber and land clearance means now only 7500 ha of primary kauri forest currently remains (Steward and Beveridge, 2010). Therefore, studying the wider secondary impacts of dieback on the kauri forest ecosystem is urgently required for us to effectively manage its spread and protect the remnant ancient kauri forests.

In the face of pathogen invasion, soil microbial communities are readily able to modulate their activity and functional responses to promote plant host health and provide pathogen defence (Raaijmakers and Mazzola, 2016; Desprez-Loustau et al., 2015). For soil-borne pathogens such as *P. agathidicida*, host infection begins at the root zone when motile zoospores encyst at the root surface and colonize the root via penetrative hyphae (Bellgard et al., 2016). Consequently, pathogens must interact with host's associated soil microbial communities prior to and throughout the infection event. The diversity, abundance and functional roles of the indigenous soil microbiota will determine how they interact with invading pathogens. This interaction will be a key factor in determining whether the pathogen can successfully spread, establish, and elicit host infection (Kemen, 2014; Thakur et al., 2019). Studying the shifts in diversity and composition of the soil microbiota associated with host-pathogen infection events can aid the identification of taxa which respond to pathogen invasion and host disease expression. This can guide the discovery of host beneficial microbial taxa which suppress disease development (Penton et al. 2014).

Our study aimed to characterise the differences in soil microbial diversity and taxonomic composition between dieback asymptomatic and symptomatic kauri trees. Our understanding of the soil microbial communities associated with kauri and their response to dieback represents a large knowledge gap which requires more research. There are a limited number of studies published on the fungal and bacterial associations of kauri (Morrison and English, 1967; McKenzie et al., 2002; Padamsee et al., 2016; Byers et al., 2020). However, there are no published studies which have examined the response of the fungal and bacterial soil communities associated with kauri soils to dieback disease expression.

P. agathidicida is a highly virulent pathogen of kauri, with all cases of infection resulting in tree mortality (Horner and Hough, 2014). Aside from initial tree mortality, cascading effects following loss of a foundation species can impact long term ecosystem functioning and biogeochemical cycling (Edburg et al., 2012; Avila et al., 2016; Peltzer et al., 2010; Lovett et al., 2010; Boyd et al., 2013). For example, large stores of C and immobilised N are a notable feature of kauri forest soils (Wyse et al., 2014; Macinnis-Ng and Schwendenmann, 2015) and changes to these soil properties following kauri dieback could greatly impact the long term C and N cycling dynamics of kauri forests. Soil microorganisms play key roles in biogeochemical cycling and ecosystem function, therefore it is important to quantify the impacts of tree dieback on their functional potential (He et al., 2007). Our study also aimed to investigate the secondary impacts of kauri dieback disease on soil microbial function by studying the differences in functional genes related to C and N cycling between asymptomatic and symptomatic kauri trees.

2. Methods

2.1. Soil collection

Field sites were located within Waipoua Forest situated on the west coast of Northland, New Zealand (35° 38′S, 173° 34′E). Waipoua Forest is a ∼13,000 ha designated national conservation area and the largest intact fragment of old growth kauri forest remaining in New Zealand (Steward and Beveridge, 2010). Preliminary ground surveys were conducted across Waipoua Forest in kauri forest stands confirmed to be infected with *P. agathidicida* from the results of kauri dieback surveys (Tom Donovan, personal communications, March 21, 2018) and using local tribal knowledge of Te Roroa, the traditional owners of Waipoua Forest. This was conducted to confirm there were sufficient numbers of mature (over 200 years old) asymptomatic and symptomatic kauri trees in infected forest stands suitable for soil sampling. Four sampling sites were selected to gain an adequate number of symptomatic kauri trees of a similar size, age, canopy dominance and degree of disease expression. Within each site, asymptomatic and symptomatic trees selected for sampling were within a 200 m distance from one another (Fig. S1, supplementary materials). Soil samples were collected from 20 asymptomatic and 20 symptomatic mature kauri trees and trees were diagnosed as being asymptomatic or symptomatic based on classic kauri dieback symptomology (Waipara et al., 2013). Sampling trees at the late stages of disease expression was required, so only trees expressing all the symptoms of lower trunk gummosis, root and wood rot, extensive leaf litter loss and leaf chlorosis were classified as symptomatic trees.

For soil sampling, a 500 g composite soil sample formed of four 125 g subsamples, was taken from the cardinal points around the base of each tree (Waipara et al., 2013). Following the removal of the litter layer, the A horizon or top 10 cm of soil depending on the depth of the A horizon was targeted for sampling. Each soil sample was sieved and transported back to the lab at 4 °C in biohazard-labelled triple-contained packaging to meet with biosecurity requirements. Each soil sample was split into 10 g subsamples stored at −20 °C for DNA analysis, 200 g subsamples stored at 4 °C for pathogen detection assays and 200 g subsamples were retained for chemical analysis (see details below).

2.2. Pathogen bioassays

A soil baiting bioassay (Bellgard et al., 2013) and real time PCR (Than et al., 2013; McDougal et al., 2014) were used to confirm the presence and absence of *P. agathidicida* in each soil sample. Pathogen bioassays were undertaken to confirm that symptomatic soil samples tested positive for *P. agathidicida* and asymptomatic soil samples tested negative for *P. agathidicida*. A full description of these methods and results are provided in the supplementary materials.

2.3. Soil chemical analyses

Soil samples were sent to Hills Laboratories (Christchurch, New Zealand) where they were air dried at 35 °C and sieved < 2 mm prior to determination of pH, total carbon (C), organic matter, total nitrogen (N), bioavailable N (aerobically mineralizable N), and bioavailable phosphorus (P – Olsen P) using established methods (Nelson and Sommers, 1996; Keeney and Bremner, 1966; Hinds and Lowe, 1980; Olsen, 1954).

2.4. Soil DNA extraction and amplicon sequencing

Methods for soil DNA extraction and amplicon sequencing followed those published previously (Byers et al., 2020). Soil DNA was extracted using a DNeasy Powersoil Kit (Qiagen, Germany) following manufacturer's instructions. Three 0.25 g DNA extractions were taken per soil sample and combined to provide a 300 μL DNA extract. DNA samples were quantified using a Quant-iT dsDNA Assay kit on a Qubit 4 Fluorometer (Invitrogen, Massachusetts USA). Sample purity was assessed using a Nanodrop Spectrophotometer to check for A260/A280 ratio of >1.8.

Genomic DNA samples were sent to Novogene Co., Ltd (Hong Kong) for library preparation. For sequencing, the bacterial 16 S rRNA gene region was amplified using primers 341F/806 R to target the V3–V4 gene region (Fadrosh et al., 2014). The fungal ITS gene region was amplified using primers ITS3/ITS4 to target the ITS2 gene region (Yang et al., 2018). Following quality control checks, successful libraries were sequenced on the Illumina HiSeq platform to generate 250bp paired end reads. Following sequencing, raw sequencing reads were assigned to samples based on unique barcodes attached during library preparation. Barcodes and primers were truncated and paired end reads merged using FLASH V1.2.7. These reads were quality filtered using QIIME V1.7.0 to obtain high quality reads and chimera sequences were removed using

UCHIME.

The following steps were performed using Qiime 2 2019.4 (Bolyen et al., 2019). Sequencing reads were clustered into OTUs at 99% sequence similarity and a representative sequence from each OTU was identified taxonomically using reference databases ‘Green Genes 13.8’ for 16 S rRNA classification and ‘UNITE November 18, 2018’ for ITS classification. The resulting OTU cluster, with taxonomic assignment, was used to create OTU and taxonomic frequency tables representing the abundance of identified species in each sample. Frequency tables were filtered to remove non-bacterial or fungal taxa and low abundance taxa of less than 10 read counts. Fungal OTU frequency tables were rarefied to 27,500 counts per sample and bacterial OTU frequency tables were rarefied to 3000 counts per sample. Alpha diversity analyses were performed on rarefied OTU frequency tables using Qiime 2 ‘diversity’ plugin. The Phyloseq R package (McMurdie and Holmes, 2013) was used to analyse and visualise the OTU frequency and taxonomic datasets. Beta diversity analyses were performed and visualised using NMDS plots. Kruskal Wallis tests were used to identify taxonomic classes and orders which had significantly different relative abundances between asymptomatic and symptomatic kauri soils. DESeq2 was used to identify OTUs which were found to have significantly different abundances between asymptomatic and symptomatic kauri soil (Love et al., 2014).

2.5. GeoChip 5s microarray analysis

The GeoChip 5 S microarray was selected to explore the functional attributes of soil microbial communities (Shi et al., 2019). Large stores of carbon and immobilised nitrogen are two highly notable features of kauri forests which occur due to the high biomasses of kauri trees and the strong influences they exert on their surrounding soil environment (Ogden, 1995; Macinnis-Ng and Schwendenmann, 2015). Only genes related to carbon and nitrogen cycling were retained for further analysis to keep the GeoChip 5 S microarray analysis concise and focused on the objectives of this study. Five DNA samples extracted from asymptomatic kauri soils and five DNA samples extracted from symptomatic kauri soils were sent to the Institute for Environmental Genomics (University of Oklahoma, USA) where the microarray analysis was completed. For analysis of the functional gene data, alpha diversity calculations were performed using the Institute of Environmental Genomics ‘MicroAnalysis’ data analysis software. Alike with the amplicon sequencing dataset, beta diversity analyses were performed using the ‘Phyloseq’ R package and differential abundance testing of individual carbon and nitrogen cycling genes was completed using DESeq2 (Love et al., 2014).

3. Results

3.1. Symptomatic kauri soils have increased fungal diversity and altered community composition

For fungal communities, the number of observed OTUs, Shannon diversity and Pielou’s evenness were significantly higher in symptomatic kauri soil than asymptomatic kauri soil (Table 1). For bacterial communities, there were no significant differences in observed OTUs, Shannon diversity, Faith’s phylogenetic diversity or Pielou’s evenness between asymptomatic and symptomatic kauri soils (Table 1).

There was a significant difference in fungal community composition between asymptomatic and symptomatic kauri soils (ANOSIM $R = 0.46$, p-value = 0.001), as illustrated in Fig. 1. There was also a significant difference in bacterial community composition (ANOSIM, $R = 0.17$, p-value = 0.004) between asymptomatic and symptomatic kauri soils (Fig. 1). However, the R test statistic score for fungal communities (0.46) was much higher than bacterial communities (0.17) suggesting that the differences in composition between asymptomatic and symptomatic kauri soils is stronger for fungal communities than bacterial communities. Unweighted and weighted UniFrac distance matrices were also calculated for bacterial communities to measure for differences in phylogenetic beta diversity between asymptomatic and symptomatic soils. There was a significant difference in both unweighted UniFrac distances (ANOSIM $R = 0.18$, p-value = 0.006) and weighted UniFrac distances (ANOSIM $R = 0.16$, p-value = 0.004) between asymptomatic soils.

3.1.1. No significant differences in soil chemical properties between asymptomatic and symptomatic kauri soils

Mantel tests found no significant correlation ($p > 0.05$) between soil chemical properties and differences in fungal or bacterial community composition. Additionally, no significant differences ($p > 0.05$) were found for any of the soil chemical properties between asymptomatic and symptomatic kauri soils (Table S4, supplementary materials).

Although not significant, values for total C, organic matter, total nitrogen, anaerobically mineralizable nitrogen (AM Nitrogen), pH and Olsen P were found higher in symptomatic kauri soils when compared to asymptomatic kauri soils (Table 2). Values for volume weight and C: N ration were found higher in asymptomatic soils when compared to symptomatic soils.

3.2. Differences in taxonomic abundances of microbial communities between asymptomatic and symptomatic kauri soils

3.2.1. Fungal communities

Fungal communities in asymptomatic and symptomatic kauri soils were dominated by two phyla, the Basidiomycota and Ascomycota, which contributed to over 80% relative abundance. At class level (Fig. 2), the Tremellomycetes were a dominant group in asymptomatic kauri soil having a combined relative abundance of 40% which was found to be significantly higher than symptomatic soil ($p < 0.001$). In contrast, the Agaricomycetes were the most dominant group in symptomatic soil, having a relative abundance of 41%, and were found in significantly higher relative abundance when compared to asymptomatic kauri soil ($p < 0.001$).

A total of 39 fungal OTUs were found significantly higher in asymptomatic kauri soil than symptomatic kauri soil (Fig. 3). Fungal OTUs with the highest log 2 fold change values (Fig. 3), i.e. those with the greatest differential abundance in asymptomatic soils, were members of the Xyariales, *Trichosporonaceae, Tricholomataceae*, *Penicillium*, *Trichoderma* and *Auricularia*. In symptomatic kauri soil, 202 fungal OTUs were found significantly higher when compared to asymptomatic kauri soil (Fig. 3). Fungal OTUs with the greatest differential abundance in symptomatic soils included members belonging to the *Hygrophoraceae*, Chaetothyriales, *Agaricaceae, Hydnodontaceae* and Saccharomycetales.

Table 1
Fungal and bacterial alpha diversity values (mean average ± standard error) of symptomatic and asymptomatic kauri soil.

Fungi	Alpha diversity	Asymptomatic	Symptomatic	Kruskal-Wallis test result
	Shannon diversity	5.38 ± 1.09	7.13 ± 0.97	H–value = 13.47, p-value <0.001
	Pielou’s evenness	0.49 ± 0.08	0.59 ± 0.07	H–value = 11.15, p-value <0.001
	Observed OTUs	2103.47 ± 559.41	3799.93 ± 698.13	H–value = 19.15, p-value <0.001
Bacteria	Shannon diversity	9.86 ± 0.46	9.89 ± 0.43	H–value = 0.00, p-value = 0.95
	Pielou’s evenness	0.79 ± 0.01	0.79 ± 0.01	H–value = 0.12, p-value = 0.72
	Observed OTUs	5567.53 ± 935.56	5675.60 ± 1275.65	H–value = 0.02, p-value = 0.88
	Faith’s diversity	55.52 ± 1.52	52.30 ± 1.29	H–value = 2.42, p-value = 0.12

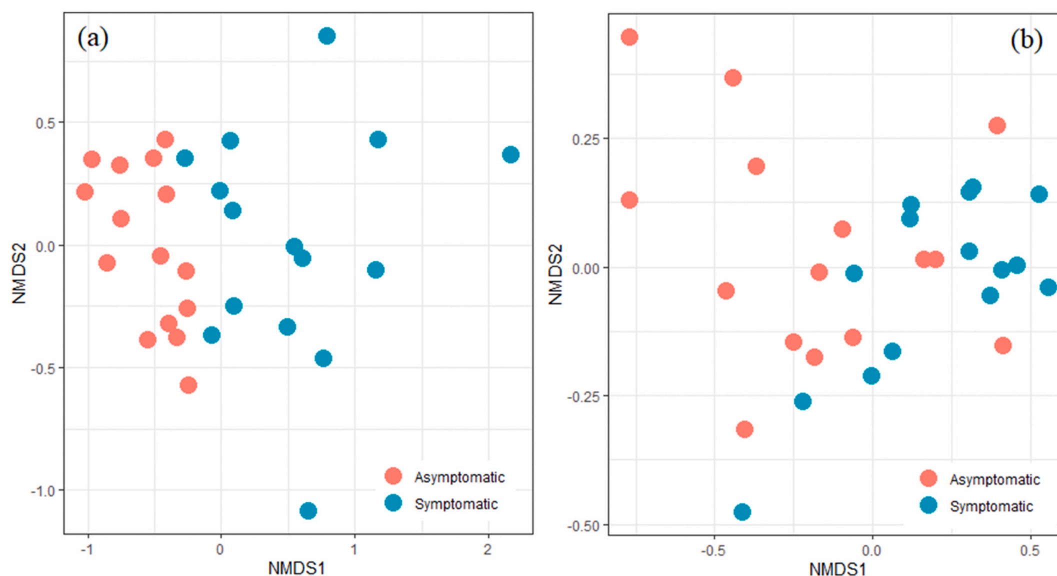

Fig. 1. MDS ordination plot showing differences in fungal (a) and bacterial (b) community composition between asymptomatic and symptomatic kauri soils. Community dissimilarity scores were calculated using Bray Curtis index.

Table 2
Soil chemical properties of symptomatic and asymptomatic kauri soil (mean average ± standard error).

	Symptomatic	Asymptomatic
pH	5.33 ± 0.08	5.23 ± 0.09
Olsen P (mg/L)	6.50 ± 1.19	5.75 ± 0.48
Volume weight (g/mg)	0.57 ± 0.04	0.63 ± 0.05
AM Nitrogen (ug/g)	215.75 ± 8.59	207.25 ± 16.13
Organic matter (%)	31.95 ± 3.18	25.70 ± 2.97
Total C (%)	18.53 ± 1.84	14.90 ± 1.72
Total N (%)	0.70 ± 0.08	0.54 ± 0.08
C:N ratio	27.10 ± 1.56	27.78 ± 0.59

3.2.2. *Bacterial communities*

The phylum Proteobacteria and its class the Gammaproteobacteria were found in significantly higher relative abundance in asymptomatic kauri soils compared to symptomatic kauri soils ($p < 0.05$). In symptomatic kauri soils, the Acidobacteria ($p < 0.001$) and Actinobacteria ($p < 0.05$), Acidimicrobiia ($p < 0.01$) and Solibacteres ($p < 0.05$) were found in significantly higher relative abundance compared to asymptomatic soils (Fig. 4). Dominant bacteria common to both asymptomatic and symptomatic kauri soil included members of the orders Rhizobiales and Rhodospirialles such as *Bradyrhizobiaceae, Rhodoplanes, Acetobacteraceae* and *Rhodospirillaceae.*

A total of 131 bacterial OTUs were found significantly higher in asymptomatic kauri soil compared to symptomatic kauri soil (Fig. 5).

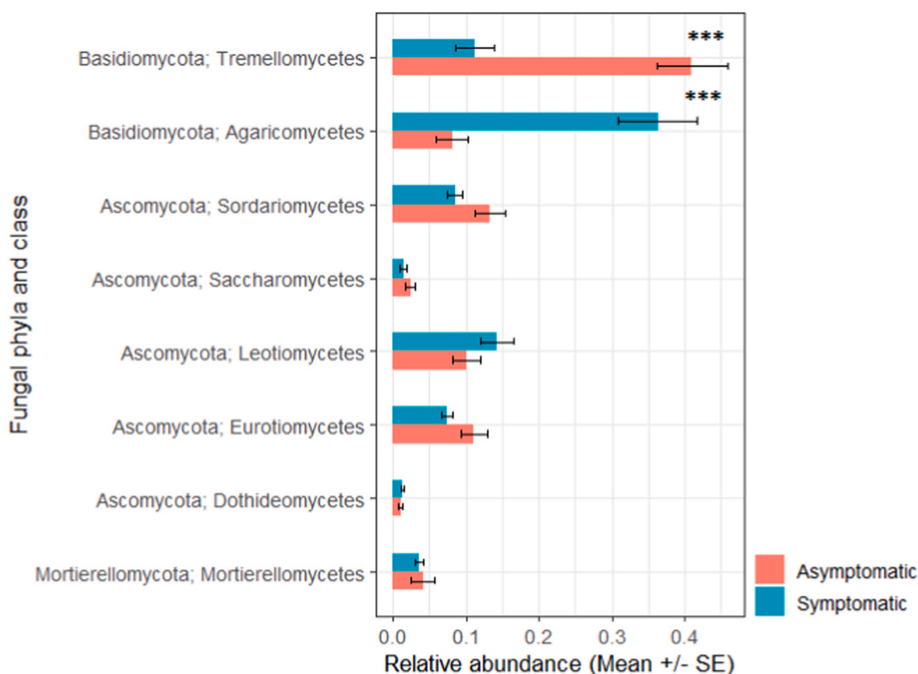

Fig. 2. The relative abundance (mean ± standard error) values of fungal classes in asymptomatic and symptomatic kauri soil. Significant differences in the relative abundances of fungal classes between asymptomatic and symptomatic soils are denoted by *, where p < 0.05 is *, p < 0.01 is ** and p < 0.001 is ***.

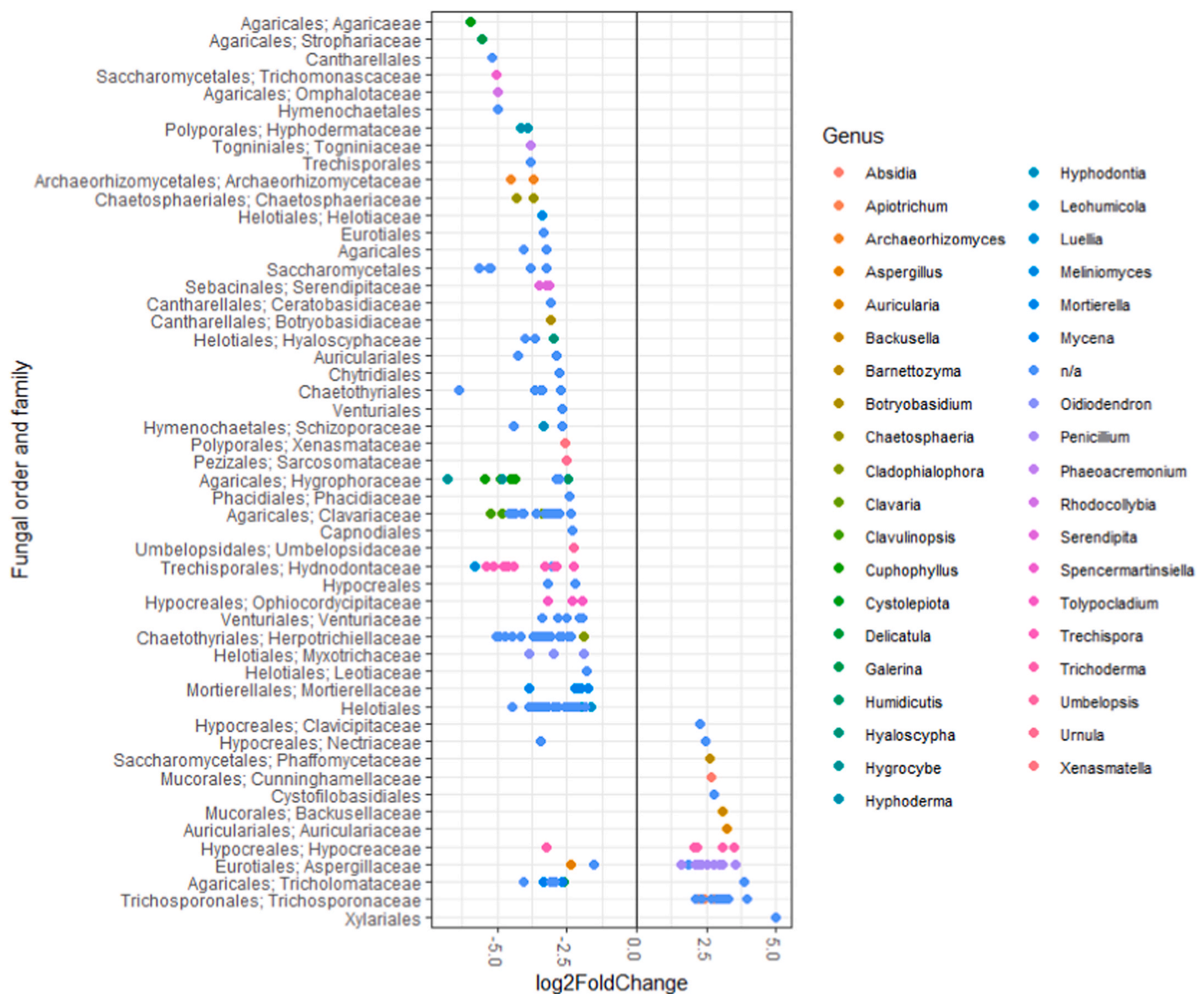

Fig. 3. The fungal OTUs with a significantly different relative abundance between asymptomatic and symptomatic kauri soils. The 'log2FoldChange' values of each fungal OTU, represented by its assigned genus, are split along the *x axis* according to their Genera associated Order and Family. Positive 'log2FoldChange' values are OTUs found significantly higher in asymptomatic soil and negative values are those significantly higher in symptomatic soil.

Bacterial OTUs found with the greatest differential abundance in asymptomatic soils included many members of the *Actinetobacter*, *Enterobacteriaceae*, *Leuconostocaceae* and *Pseudomonas.* A total of 68 bacterial OTUs were found significantly higher in symptomatic kauri soil when compared to asymptomatic kauri soil (Fig. 5). Bacterial OTUs found with the greatest differential abundance in symptomatic soils were members of the *Koribaceraceae*, Pedosphaerales, Solirubrobacterales and *Syntrophobacteraceae*.

3.3. Changes in composition and abundance of microbial genes related to carbon and nitrogen cycling

No significant differences were found in the number of detected probes, Shannon diversity or Pielou's evenness between asymptomatic and symptomatic kauri soil (Table S6, supplementary materials). A significant difference was found in the composition of carbon cycling (ANOSIM $R = 0.304$, p-value $= 0.019$) and nitrogen cycling genes (ANOSIM $R = 0.304$, p-value $= 0.025$) between asymptomatic and symptomatic kauri soil when Bray Curtis dissimilarity scores were tested using ANOSIM (Fig. 6).

In total, there were 17,223 carbon cycling gene probes and 5018 nitrogen cycling gene probes detected by the microarray. DESeq2 analysis found that 518 of these carbon and nitrogen cycling genes had significant differential abundances between asymptomatic and symptomatic kauri soils ($p < 0.05$). The majority of these gene probes were for carbon degradation (281 gene probes), with 182 carbon degradation gene probes being found significantly higher in symptomatic kauri soils and 99 being found higher in asymptomatic kauri soil. Of the 120 nitrogen cycling gene probes significantly different between asymptomatic and symptomatic soil, 47 gene probes were higher in asymptomatic soils and 73 gene probes were higher in symptomatic soils. Gene probes which had the highest differential abundance in asymptomatic kauri soil included those for pectin degradation (*RgaE*), chitin degradation (*acetylglucosaminidase*), tannin degradation (*tannase_Cdeg*), cellulose degradation (*cellobiase*), starch (*amyA*) and hemicellulose degradation (*xyla*). Gene probes with the highest differential abundance in symptomatic soils included those for the reductive tricarboxylic acid cycle (*frdA_rTCA*), ammonification (*gdh*), cellulose degradation (*exoglucanase*), hemicellulose degradation (*xylanase*), lignin degradation (*phenol_oxidase*), agar degradation (*beta_agarase*) and dissimilatory N reduction

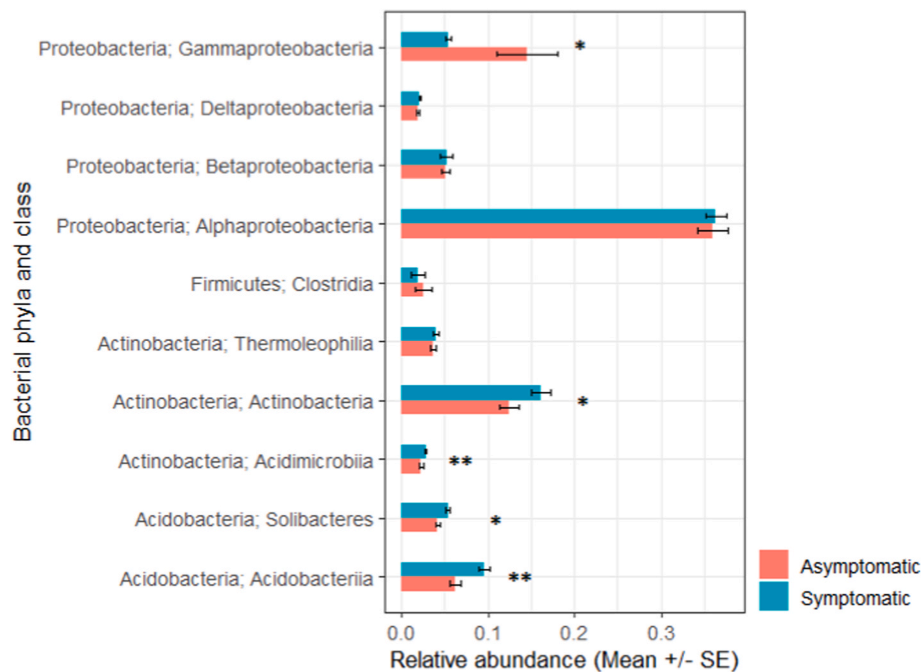

Fig. 4. The relative abundance (mean ± standard error) values of bacterial classes in asymptomatic and symptomatic kauri soil. Significant differences in the relative abundances of bacterial classes between asymptomatic and symptomatic soils are denoted by *, where $p < 0.05$ is *, $p < 0.01$ is ** and $p < 0.001$ is ***.

(*nrfa*). Although there were many gene probes with significantly different abundances between asymptomatic and symptomatic soils, when looking at individual genes grouped according to their functional gene category (Fig. 7), there were few clear trends showing a clear split of gene categories between asymptomatic and symptomatic soils. However, as noted, there was a higher number of carbon degradation genes found significantly higher in symptomatic soils.

4. Discussion

4.1. Changes in microbial diversity in response to tree disease expression

Our study found pronounced and significant differences in the diversity, composition and functional properties of microbial communities between asymptomatic and symptomatic kauri soils. Fungal diversity of symptomatic kauri soils was significantly higher in comparison to asymptomatic kauri soils. This could be the result of a secondary colonisation processes, or secondary disease process, which has been described to follow tree death (Jung et al., 2018). This secondary colonisation of saprophytic fungi and secondary pathogens occurs due to the increased availability of dead plant tissues provided by the diseased host. Fungi vary in their ability to exploit resources and niches meaning colonisation of decayed resources can lead to a change in community composition (Jung et al., 2018; Boddy and Hiscox, 2017). Secondary colonisation of saprophytic fungi can further aid the disease process, by degrading host tissue and releasing *Phytophthora*'s resting structures growing within the host tissue (Jung et al., 2018).

The differences in microbial community composition between asymptomatic and symptomatic kauri soils may reflect their differences in functional composition. Functional differentiation can explain differences in diversity patterns of soil microbial communities because different microbial taxonomic groups exhibit differences in their niche patterns and life history strategies (Schimel and Schaeffer, 2012). Changes to plant inputs can influence microbial community structure because microorganisms vary in their ability to decompose different types of plant material (van der Wal et al., 2013). We observed a clear shift between the Tremellomycetes dominated asymptomatic kauri soil and the Agaricomycetes dominated symptomatic kauri soil. A large proportion of the fungal OTUs significantly higher in symptomatic kauri soils belonged to the Agaricomycetes, a class of mushroom forming fungi which function as saprotrophs and wood decay fungi in forest ecosystems and form a critical role in organic matter decomposition (Rosa and Capelari, 2009; Hibbett et al., 2014). Given their increased dominance in symptomatic kauri soil, in line with the greater number carbon degradation genes found in significantly higher abundance in symptomatic kauri soil, they may be part of the response to the increased volume of necrotic root and wood tissue following disease expression.

4.2. The microbial taxa of interest for their roles in soil disease suppression

One aim of studying soils associated with asymptomatic kauri was to identify if they exhibited any properties of disease suppression mediated by their microbial communities. Our results identified several genera that predominated in asymptomatic kauri soils which have been studied elsewhere for their antagonistic activities against pathogens, such as *Penicillium*, *Trichoderma* and *Pseudomonas* (Garbeva et al., 2004). These may represent taxa associated with good kauri health and with potential roles in providing disease suppression against *P. agathidicida*.

Penicillium is a cosmopolitan genus well adapted to the soil environment, making it a highly competitive fungus which is better able to suppress plant pathogens (Nicoletti and De Stefano, 2012). Numerous *Penicillium* species have reported antagonistic activities against a range of soil borne *Phytophthora* pathogens (Fang and Tsao, 1995; Ma et al., 2008). Fang and Tsao (1995) demonstrated that *Penicillium funiculosum* was able to reduce the symptoms of *Phytophthora* root rot and promote plant growth in azalea and citrus plants following infection with *P. cinnamomi*, *P. parasitica* and *P. citrophthora*. Wakelin et al. (2006) used dual culture assay screenings to identify that *Penicillium radicum* was highly effective at inhibiting *P. cinnamomi.* When grown in dual culture with *P. radicum*, *P. cinnamomi* mycelial growth was inhibited by 72%. This was much higher than the inhibition values found for the three other plant pathogens tested which ranged from 13.01% to 54.54%. Another *Penicillium* species, *P. striatisporum*, has been identified as a promising biocontrol agent against several *Phytophthora* species, effectively inhibiting the growth of *P. capsici, P. infestans, P. drechsleri, P.*

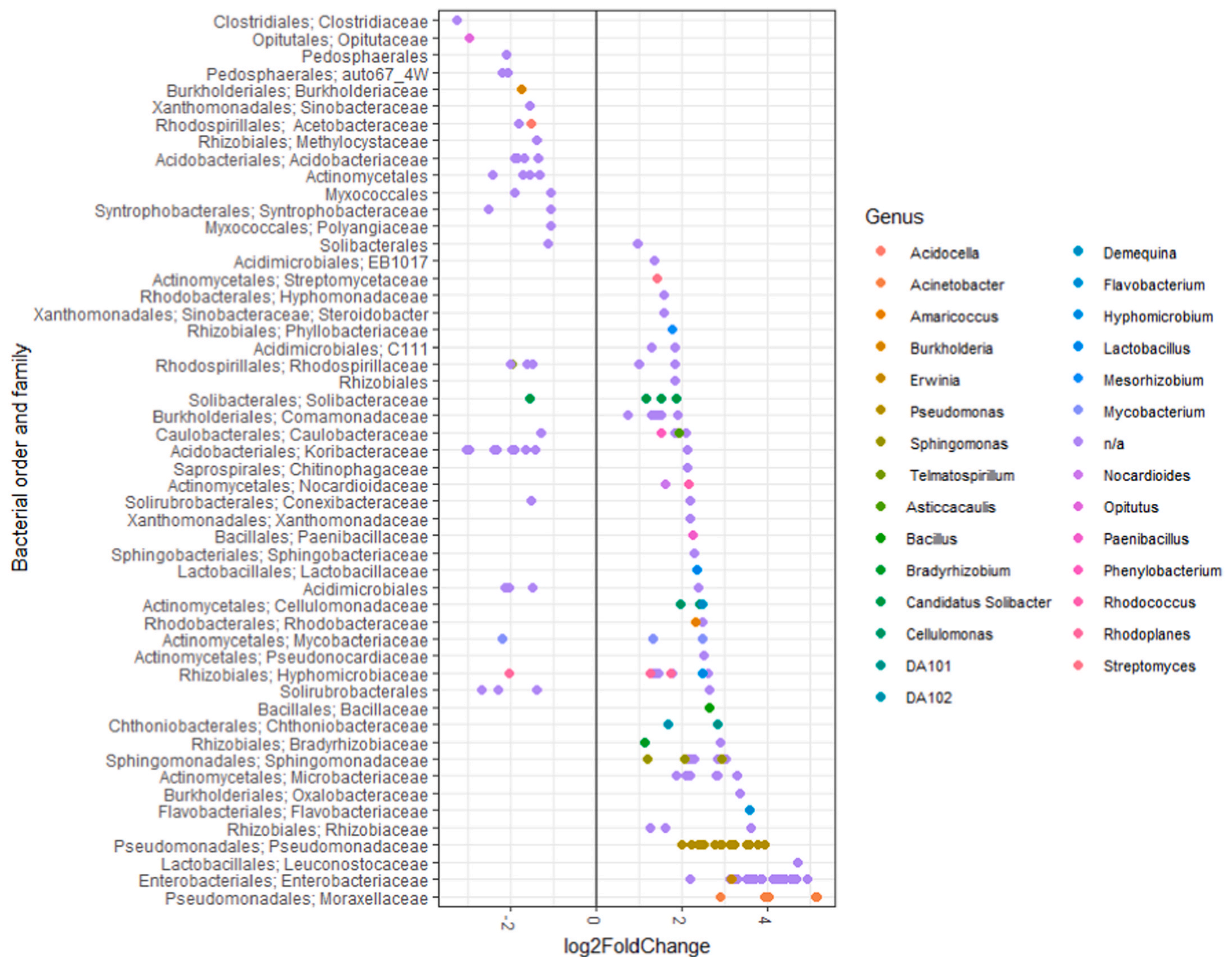

Fig. 5. The bacterial OTUs with a significantly different relative abundance between asymptomatic and symptomatic kauri soils. The 'log2FoldChange' values of each fungal OTU, represented by its assigned genus, are split along the *x axis* according to their Genera associated Order and Family. Positive 'log2FoldChange' values are OTUs found significantly higher in asymptomatic soil, negative values are those significantly higher in symptomatic soil.

megasperma and *P. nicotianae* (Ma et al., 2008). In the same study, pot trials identified that soils amended *P. striatisporum* had significantly reduced incidences of root rot in chilli peppers following soil infestation with *P. capsici*. Although the exact mode of antagonism was not conclusively identified, the efficacy of *P. striatisporum* against *Phytophthora* was attributed to its production of antifungal metabolites.

Trichoderma, which is perhaps the most well studied and widely applied biocontrol fungus (Schuster and Schmoll, 2010), was also found in significantly higher relative abundance in asymptomatic kauri soil. Previous studies have demonstrated *Trichoderma* to antagonise numerous different *Phytophthora* species. For example, *T. harzianum* was demonstrated to reduce disease incidence in pepper plants following infection with *P. capsici* by inducing the plant defence responses (Ahmed et al., 2000). Additionally, Bae et al. (2016) found that metabolites extracted from both *T. atroviride/petersenii* and *T. virens* strongly inhibited the mycelial growth of seven different *Phytophthora* species (*P. melonis, P. cactorum, P. drechsleri, P. sojae, P. capsici, P. nicotianae* and *P. infestans*). These findings then further identified that treatment of pepper leaves with the metabolites of *T. atroviride/petersenii* inhibited *P. capsici* growth via the induction of plant defence responses. Widmer (2014) isolated 16 different *Trichoderma* strains from soil and used dual culture assays to screen them against *Phytopthora ramorum*, the causal agent of sudden oak death. Findings identified two *T. asperellum* isolates which were able to mycoparasitise *P. ramorum* and were then used to successfully remediate *P. ramorum* infested potting mixes.

Several members of the Gammaproteobacteria were found in significantly higher relative abundance in asymptomatic kauri soil; members of this bacterial class have previously been associated with disease suppressive soils (Mendes et al., 2011). Members of the genus *Pseudomonas* are often reported as plant growth promoting rhizobacteria (PGPR) and their disease suppressive properties have been extensively studied, especially with respect to oomycete plant pathogens (Haas and Défago, 2005). Several *in vitro* and *in vivo* studies have confirmed the antagonistic effects of *Pseudomonas* against *Phytophthora* pathogens, including *P. capsici* and *P. infestans* (Zohara et al., 2016; Caulier et al., 2018). Strains of *Pseudomonas fluorescens* have been shown to produce biosurfactants, such as cyclic lipopeptides, which antagonise *Phytophthora*'s sporangia and zoospores whilst promoting induced systemic resistance in plants (Tran et al., 2007). Sang et al. (2018) identified two strains of *Pseudomonas corrugata* which were highly effective root colonisers, thus could competitively exclude *P. capsici* root colonisation and reduce plant disease severity.

Two other members of the Gammaproteobacteria, *Enterobacteraceae* and *Actinetobacter* were also found in significantly higher abundance in

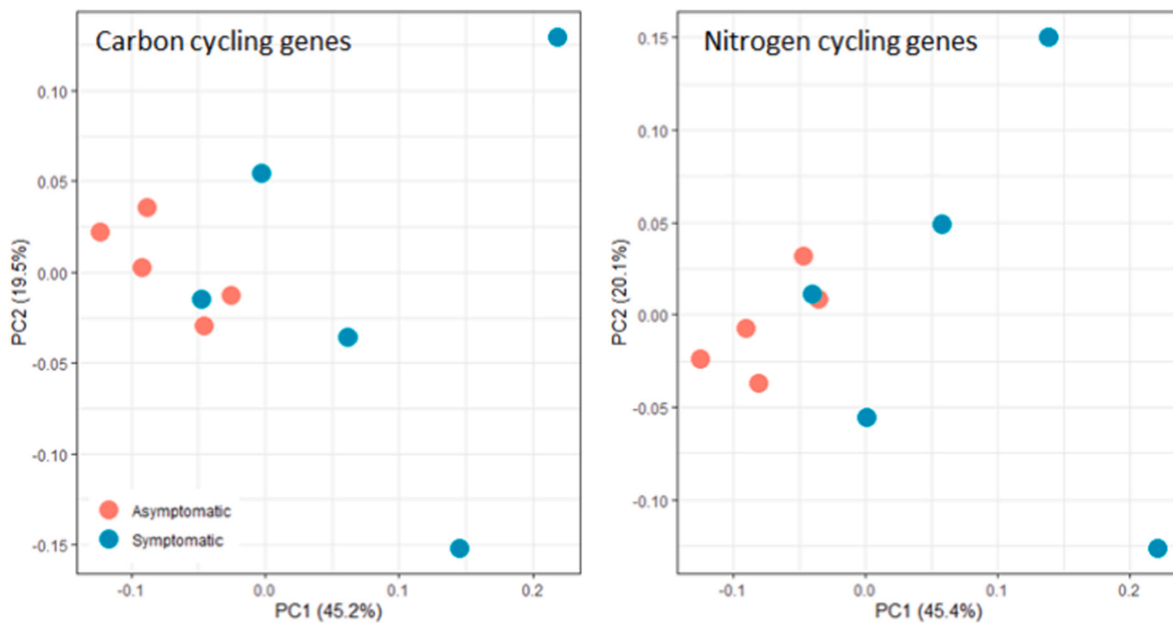

Fig. 6. Principle coordinate analysis (PCoA) illustrating the differences in composition of carbon and nitrogen cycling genes between asymptomatic and symptomatic kauri soils.

asymptomatic kauri soil. In a previous study which aimed to discover potential biocontrol agents against blank shank disease of tobacco, Liu et al. (2020) isolated several bacterial strains, including strains of *Enterobacter* and *Actinetobacter*, which were suppressive against the pathogen *Phytophthora nicotianae*. A previous study has found *E. cloaceae* is able to suppress disease caused by the oomycete pathogen, *Pythium ultimum*, by competing for plant derived fatty acids required by *P. ultimum* for sporangia formation (van Dijk and Nelson, 2000). In addition, *A. baumannii* has been demonstrated to inhibit *P. capsici* growth through the production of anti-fungal metabolites (Xue et al., 2009; Liu et al., 2007).

These findings provide valuable guidance in the search for potential microbial antagonists against *P. agathidicida*. Further *in vivo* and *in vitro* studies are needed to assess the suppressive capabilities of these potential microbial control agents against kauri dieback disease.

4.3. The functional responses of soil microbial communities

4.3.1. Potential long term impacts on carbon and nitrogen cycling dynamics

A series of secondary impacts have been observed to follow the widespread dieback of a large keystone tree species, including reduced carbon uptake, soil nutrient loss, increased plant biomass decomposition and reduced release of plant root exudates (Edburg et al., 2012). Long term these impacts can cascade to alter biogeochemical processes such as C and N cycling, plant species composition and plant productivity (Edburg et al., 2012; Schimel and Schaeffer, 2012). New Zealand's kauri forests are incredibly carbon dense, with kauri trees themselves contributing to most of the forest's carbon inputs (Macinnis-Ng and Schwendenmann, 2015). Forest dieback events often result in forests transitioning from net C sinks to C sources, due to increased C losses and reduced C uptake (Avila et al., 2016). Our study found significant differences in the composition carbon cycling genes of microbial communities between asymptomatic and symptomatic kauri soils, including differences in the gene abundances of many genes related to carbon degradation. These differences may reflect a response of the soil microbial communities to a change in litter quantity and quality following tree dieback. Although not significant, total C (%) and organic matter (%) was higher in symptomatic soils compared to asymptomatic which may suggest an increase in C turnover in symptomatic soils. During the degradation of organic matter, enzymes produced by soil microorganisms break down complex plant carbon polymers into more simple forms for cell uptake and metabolism (Adetunji et al., 2017). Depending on the carbon substrates available and the ability of microbial taxa to produce the appropriate enzyme, different taxonomic groups will specialise on particular substrates (i.e. sucrose, cellulose or lignin). Additionally, the products of enzymatic breakdown attract opportunistic microorganisms which further influence the composition and function of resident microbial communities (Schimel and Schaeffer, 2012). As discussed, the secondary colonisation process, or secondary disease process occurs following tree dieback which leads to the increased abundance of saprophytic fungi which degrade the host's necrotic plant and root tissues (Jung et al., 2018). During this colonisation process, saprophytic fungi will first metabolise more simple carbon forms provided by the decayed plant tissues before then utilising more recalcitrant carbon forms (Boddy and Hiscox, 2017). Symptomatic kauri soil had a greater number of carbon degradation genes that were found to have a significantly higher relative abundance than compared to asymptomatic soil. When considered alongside the shifts in fungal community composition and the increased % of total C in symptomatic soils, these findings may suggest an increase in carbon degradation rates in symptomatic soils.

Kauri forest soils have large stores of immobilised nitrogen and low rates of nitrification, attributed to the low soil pH, slow litter decomposition rate and high tannin content of their leaf litter (Wyse et al., 2014). Therefore, changes to nitrogen cycling dynamics following tree dieback could potentially produce large secondary effects on the surrounding soil and plant environment. Our study found significant differences in the composition of nitrogen cycling genes between asymptomatic and symptomatic kauri soil, as well as significant differences in the abundances of individual genes related to nitrogen cycling. However, in regard to N cycling, there were no clear trends shown by the data to support any reasonable conclusions about what impact tree dieback disease expression has microbial gene function. A previous study has found that increased concentrations of *P. agathidicida* DNA in forest soil was correlated to decreased soil C and N concentrations (Schwendenmann and Michalzik, 2019). This goes against previous findings that soil N concentrations increase following tree dieback, in response to increased inputs of N-rich litter and reduced plant N uptake (Edburg et al., 2012; Xiong et al., 2011).

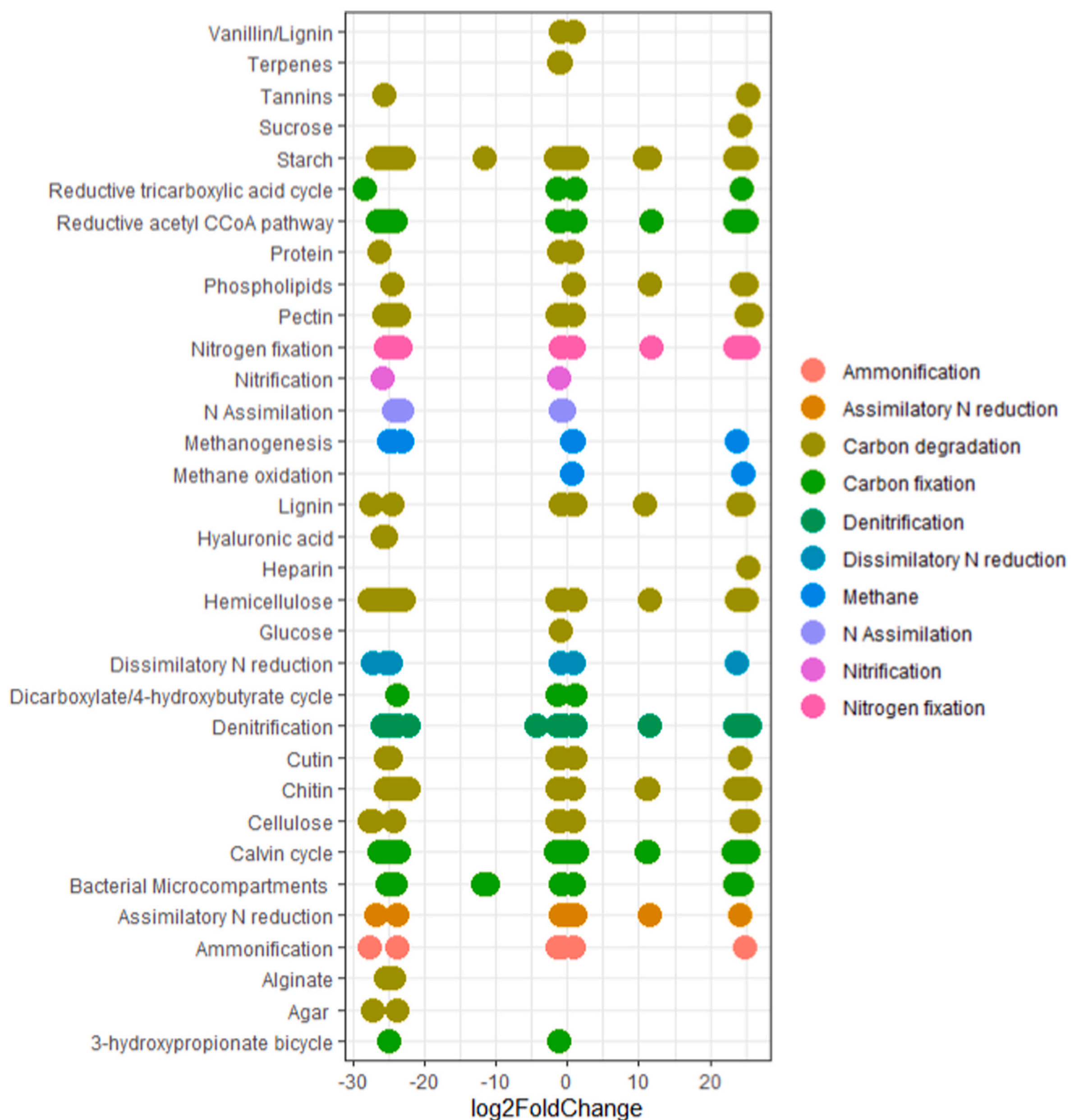

Fig. 7. The carbon and nitrogen cycling genes with a significantly different gene counts between asymptomatic and symptomatic kauri soils. The 'log2FoldChange' values of each gene are represented along the x axis by their broader functional roles. Genes with positive 'log2FoldChange' values were found significantly higher in asymptomatic soil, negative values are those significantly higher in symptomatic soil.

4.4. Study limitations and considerations

A kauri dieback study conducted in a regenerative kauri forest found a significant correlation between decreasing soil C and N concentrations with increasing *P. agathidicida* concentrations (Schwendenmann and Michalzik, 2019). In our study, the values for total C and N were higher in symptomatic soils, although none of these differences were found to be significant. The lack of consistency between the findings of these two studies could be attributed to differences in forest site, age and soil chemical testing methods. Additionally, the lack of observed differences in soil chemical properties in our study could also be due to the time period since disease expression was observed. Typically, symptoms for root and collar rot diseases progress more gradually than other forms of tree mortality such as drought and fire (Anderegg et al., 2013). Consequently, the associated short-term impacts are comparatively small compared to the long-term effects on the forest environment. Slower, selective tree mortality allows for co-occurring tree species to increase in abundance and coverage, thus limiting direct impacts such as reduced plant productivity. However over time, changes to forest species composition can lead to large alterations in C and N cycling dynamics across the forests (Lovett et al., 2010). Indeed, findings from our study have shown significant shifts in the composition of C and N cycling genes between asymptomatic and symptomatic kauri soils. Assessing the impacts of disturbance events on soil ecosystem health using biological indicators, such as changes to microbial composition and function, can serve as a more sensitive and dynamic method to detect short term impacts rather than measuring changes to soil physical and chemical properties (Adetunji et al., 2017). Over time, the changes to soil microbial diversity and function coupled with changes to plant species composition post disease outbreak will cascade to see further changes to soil chemical properties such as pH, total C, total N and organic matter content (Edburg et al., 2012).

5. Conclusion

This study has found significant differences in the fungal diversity of microbial communities between asymptomatic and symptomatic soils, with fungal diversity being significantly higher in symptomatic soils. The composition of fungal and bacterial communities was found to be significantly different between asymptomatic and symptomatic soils. Results of taxonomic analyses identified several microbial taxa significantly higher in asymptomatic kauri soil, such as *Penicillium, Trichoderma, Enterobacteriacae, Actinetobacter* and *Pseudomonas*, which are well studied in the literature for their roles in plant health and disease suppression. Such findings warrant further research which should aim to isolate and screen these microbial taxa against *P. agathidicida* to assess their capabilities as potential biocontrol agents. In addition, the plant beneficial roles that these microbial taxa may possess should be explored, so we can begin to identify microbial taxa that can support kauri health in the face of pathogen attack.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.108016.

References

Adetunji, A., Lewu, F., Mulidzi, R., Ncube, B., 2017. The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review. Journal of Soil Science and Plant Nutrition 17, 794–807.
Ahmed, A.S., Sánchez, C.P., Candela, M.E., 2000. Evaluation of induction of systemic resistance in pepper plants (Capsicum Annuum) to Phytophthora capsici using Trichoderma harzianum and its relation with capsidiol accumulation. European Journal of Plant Pathology 106, 817–824.
Anderegg, W.R.L., Kane, J.M., Anderegg, L.D.L., 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Climate Change 3, 30–36.
Avila, J.M., Gallardo, A., Ibáñez, B., Gómez-Aparicio, L., 2016. Quercus suber dieback alters soil respiration and nutrient availability in Mediterranean forests. Journal of Ecology 104, 1441–1452.
Bae, S.-J., Mohanta, T.K., Chung, J.Y., Ryu, M., Park, G., Shim, S., Hong, S.B., Seo, H., Bae, D.-W., Bae, I., Kim, J.-J., Bae, H., 2016. Trichoderma metabolites as biological control agents against Phytophthora pathogens. Biological Control 92, 128–138.
Beever, R.E., Waipara, N.W., Ramsfield, T.D., Dick, M.A., Horner, I.J., 2009. Kauri (Agathis australis) under threat from Phytophthora. Phytophthoras in Forests and Natural Ecosystems 74, 74–85.
Bellgard, S.E., Padamsee, M., Probst, C.M., Lebel, T., Williams, S.E., Jung, T., 2016. Visualizing the early infection of Agathis australis by Phytophthora agathidicida, using microscopy and fluorescent in situ hybridization. Forest Pathology 46, 622–631.
Bellgard, S.E., Weir, B.S., Pennycook, S.R., Paderes, E.P., Winks, C., Beever, R.E., Than, D.J., Hill, L.M.W., Williams, S.E., 2013. Specialist Phytophthora Research: Biology. Pathology Ecology and Detection of PTA. MPI Contract 11927.
Black, A., Waipara, N., Gerth, M., 2018. Calling time on New Zealand's oldest tree species. Nature 561, 177–178.
Boddy, L., Hiscox, J., 2017. Fungal ecology: principles and mechanisms of colonization and competition by saprotrophic fungi. The Fungal Kingdom 293–308.
Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37, 852–857.
Boyd, I.L., Freer-Smith, P.H., Gilligan, C.A., Godfray, H.C.J., 2013. The consequence of tree pests and diseases for ecosystem services. Science 342, 1235773.
Byers, A.-K., Condron, L., Donavan, T., O'Callaghan, M., Patuawa, T., Waipara, N., Black, A., 2020. Soil microbial diversity in adjacent forest systems- contrasting native, old growth kauri (Agathis australis) forest with exotic pine (Pinus radiata) plantation forest. FEMS Microbiology Ecology 96. https://doi.org/10.1093/femsec/fiaa047.
Caulier, S., Gillis, A., Colau, G., Licciardi, F., Liépin, M., Desoignies, N., et al., 2018. Versatile antagonistic activities of soil-borne Bacillus spp. and Pseudomonas spp. against Phytophthora infestans and other potato pathogens. Frontiers in Microbiology 9, 143.
Cobb, R., Metz, M., 2017. Tree diseases as a cause and consequence of interacting forest disturbances. Forests 8, 147.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.
Desprez-Loustau, M.-L., Aguayo, J., Dutech, C., Hayden, K.J., Husson, C., Jakushkin, B., et al., 2015. An evolutionary ecology perspective to address forest pathology challenges of today and tomorrow. Annals of Forest Science 73, 45–67.
Dignam, B.E., O'Callaghan, M., Condron, L.M., Kowalchuk, G.A., Van Nostrand, J.D., Zhou, J., et al., 2018. Effect of land use and soil organic matter quality on the structure and function of microbial communities in pastoral soils: implications for disease suppression. PloS One 13.
Dignam, B.E., O'Callaghan, M., Condron, L.M., Raaijmakers, J.M., Kowalchuk, G.A., Wakelin, S.A., 2019. Impacts of long-term plant residue management on soil organic matter quality, Pseudomonas community structure and disease suppressiveness. Soil Biology and Biochemistry 135, 396–406.
Edburg, S.L., Hicke, J.A., Brooks, P.D., Pendall, E.G., Ewers, B.E., Norton, U., et al., 2012. Cascading impacts of bark beetle-caused tree mortality on coupled biogeophysical and biogeochemical processes. Frontiers in Ecology and the Environment 10, 416–424.
Fadrosh, D.W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M., et al., 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome 2, 6.
Fang, J., Tsao, P., 1995. Efficacy of Penicillium funiculosum as a biological control agent against Phytophthora root rots of azalea and citrus. Phytopathology 85, 871–878.
Garbeva, P., Van Veen, J., Van Elsas, J., 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annual Review of Phytopathology 42, 243–270.
Haas, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology 3, 307–319.
He, Z., Gentry, T.J., Schadt, C.W., Wu, L., Liebich, J., Chong, S.C., et al., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. The ISME Journal 1, 67–77.
Hibbett, D.S., Bauer, R., Binder, M., Giachini, A., Hosaka, K., Justo, A., et al., 2014. 14 Agaricomycetes. Systematics and Evolution. Springer.
Hinds, A., Lowe, L., 1980. Application of the Berthelot reaction to the determination of ammonium-N in soil extracts and soil digests. Communications in Soil Science and Plant Analysis 11, 469–475.
Horner, I.J., Hough, E.G., 2014. Pathogenicity of four Phytophthora species on kauri: in vitro and glasshouse trials. New Zealand Plant Protection 67, 54–59.
Hui, D., Deng, Qi, Tian, H., Luo, Y., 2017. Climate change and carbon sequestration in forest ecosystems. Handbook of Climate Change Mitigation and Adaptation 555, 594.
Jung, T., Perez-Sierra, A., Duran, A., Horta Jung, M., Balci, Y., Scanu, B., 2018. Canker and decline diseases caused by soil- and airborne Phytophthora species in forests and woodlands. Persoonia 40, 182–220.
Keeney, D., Bremner, J., 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability 1. Agronomy Journal 58, 498–503.
Kemen, E., 2014. Microbe–microbe interactions determine oomycete and fungal host colonization. Current Opinion in Plant Biology 20, 75–81.
Liu, C., Chen, X., Liu, T., Lian, B., Gu, Y., Caer, V., et al., 2007. Study of the antifungal activity of Acinetobacter baumannii LCH001 in vitro and identification of its antifungal components. Applied Microbiology and Biotechnology 76, 459–466.
Liu, T., Xiao, Y., Yin, J., Yi, T., Zhou, Z., Hsiang, T., Tang, Q., Chen, W., 2020. Effects of cultured root and soil microbial communities on the disease of nicotiana tabacum caused by Phytophthora nicotianae. Frontiers in Microbiology 11, 929.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15, 550.
Lovett, G.M., Arthur, M.A., Weathers, K.C., Griffin, J.M., 2010. Long-term changes in forest carbon and nitrogen cycling caused by an introduced pest/pathogen complex. Ecosystems 13, 1188–1200.
Ma, Y., Chang, Z-z., Zhao, J-t., Zhou, M-g., 2008. Antifungal activity of Penicillium striatisporum Pst10 and its biocontrol effect on Phytophthora root rot of chilli pepper. Biological Control 44, 24–31.
Macinnis-Ng, C., Schwendenmann, L., 2015. Litterfall, carbon and nitrogen cycling in a southern hemisphere conifer forest dominated by kauri (Agathis australis) during drought. Plant Ecology 216, 247–262.
McDougal, R., Scott, P., Ganley, B., 2014. Comparison of a real time PCR assay and a soil bioassay technique for detection of Phytophthora taxon Agathis from soil. Kauri Dieback Joint Agency Response.
McKenzie, E., Buchanan, P., Johnston, P., 2002. Checklist of fungi on kauri (Agathis australis) in New Zealand. New Zealand Journal of Botany 40, 269–296.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8.
Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H.M., et al., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332, 1097.

Morrison, T.M., English, D.A., 1967. The significance of mycorrhizal nodules of Agathis australis. New Phytologist 66, 245–250.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. Methods of Soil Analysis: Part 3 Chemical Methods 5, 961–1010.

Nicoletti, R., De Stefano, M., 2012. Penicillium restrictum as an antagonist of plant pathogenic fungi. Dynamic Biochemistry, Process Biotechnology and Molecular Biology 6, 61–69.

Nolan, C., Overpeck, J.T., Allen, J.R.M., Anderson, P.M., Betancourt, J.L., Binney, H.A., et al., 2018. Past and future global transformation of terrestrial ecosystems under climate change. Science 361, 920.

Ogden, J., 1995. The long-term conservation of forest diversity in New Zealand. Pacific Conservation Biology 2, 77–90.

Olsen, S.R., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. US Department of Agriculture.

Padamsee, M., Johansen, R.B., Stuckey, S.A., Williams, S.E., Hooker, J.E., Burns, B.R., et al., 2016. The arbuscular mycorrhizal fungi colonising roots and root nodules of New Zealand kauri Agathis australis. Fungal Biol. 120, 807–817.

Peltzer, D., Allen, R., Lovett, G., Whitehead, D., Wardle, D., 2010. Effects of biological invasions on forest carbon sequestration. Global Change Biology 16, 732–746.

Penton, C.R., Gupta, V., Tiedje, J.M., Neate, S.M., Ophel-Keller, K., Gillings, M., Harvey, P., Pham, A., Roget, D.K., 2014. Fungal community structure in disease suppressive soils assessed by 28S LSU gene sequencing. PLoS one 9, e93893.

Raaijmakers, J.M., Mazzola, M., 2016. Soil immune responses. Science 352, 1392.

Rosa, L.H., Capelari, M., 2009. Agaricales fungi from Atlantic rain forest fragments in Minas Gerais, Brazil. Brazilian Journal of Microbiology 40, 846–851.

Schimel, J., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3, 348.

Schuster, A., Schmoll, M., 2010. Biology and biotechnology of Trichoderma. Applied Microbiology and Biotechnology 87, 787–799.

Schwendenmann, L., Michalzik, B., 2019. Dissolved and particulate carbon and nitrogen fluxes along a Phytophthora agathidicida infection gradient in a kauri (Agathis australis) dominated forest. Fungal Ecol. 42, 100861.

Shi, Z., Yin, H., Van Nostrand, J.D., Voordeckers, J.W., Tu, Q., Deng, Y., et al., 2019. Functional gene array-based ultrasensitive and quantitative detection of microbial populations in complex communities. mSystems 4 e00296-19.

Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., et al., 2015. Planetary boundaries: guiding human development on a changing planet. Science 347, 1259855.

Steward, G.A., Beveridge, A.E., 2010. A review of New Zealand kauri (Agathis australis): its ecology, history, growth and potential for management for timber. New Zealand Journal of Forestry Science 40, 33–59.

Thakur, M.P., van der Putten, W.H., Cobben, M.M., van Kleunen, M., Geisen, S., 2019. Microbial invasions in terrestrial ecosystems. Nature Reviews Microbiology 17, 621–631.

Than, D.J., Hughes, K.J.D., Boonhan, N., Tomlinson, J.A., Woodhall, J.W., Bellgard, S.E., et al., 2013. A TaqMan real-time PCR assay for the detection of Phytophthora 'taxon Agathis' in soil, pathogen of Kauri in New Zealand. Forest Pathology 43, 324–330.

Tran, H., Ficke, A., Asiimwe, T., Höfte, M., Raaijmakers, J.M., 2007. Role of the cyclic lipopeptide massetolide A in biological control of Phytophthora infestans and in colonization of tomato plants by Pseudomonas fluorescens. New Phytologist 175, 731–742.

van der Wal, A., Geydan, T.D., Kuyper, T.W., De Boer, W., 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiology Reviews 37, 477–494.

van Dijk, K., Nelson, E.B., 2000. Fatty acid competition as a mechanism by which Enterobacter cloacae suppresses Pythium ultimum sporangium germination and damping-off. Applied and Environmental Microbiology 66, 5340–5347.

Waipara, N., Craw, J., Davis, A., Meys, J., Sheeran, B., Peart, A., et al., 2009. Management of kauri dieback. New Zealand Plant Protection 62, 407.

Waipara, N.W., Hill, S., Hill, L.M.W., Hough, E.G., Horner, I.J., 2013. Surveillance methods to determine tree health, distribution of kauri dieback disease and associated pathogens. New Zealand Plant Protection 66, 235–241.

Wakelin, S.A., Anstis, S.T., Warren, R.A., et al., 2006. The role of pathogen suppression on the growth promotion of wheat by Penicillium radicum. Australasian Plant Pathology 35, 253–258.

Widmer, T.L., 2014. Screening Trichoderma species for biological control activity against Phytophthora ramorum in soil. Biological Control 79, 43–48.

Wyse, S.V., Burns, B.R., Wright, S.D., 2014. Distinctive vegetation communities are associated with the long-lived conifer Agathis australis (New Zealand kauri, Araucariaceae) in New Zealand rainforests. Austral Ecology 39, 388–400.

Xiong, Y., D'Atri, J.J., Fu, S., Xia, H., Seastedt, T.R., 2011. Rapid soil organic matter loss from forest dieback in a subalpine coniferous ecosystem. Soil Biology and Biochemistry 43, 2450–2456.

Xue, Q.-Y., Chen, Y., Li, S.-M., Chen, L.-F., Ding, G.-C., Guo, D.-W., et al., 2009. Evaluation of the strains of Acinetobacter and Enterobacter as potential biocontrol agents against Ralstonia wilt of tomato. Biological Control 48, 252–258.

Yang, R.-H., Su, J.-H., Shang, J.-J., Wu, Y.-Y., Li, Y., Bao, D.-P., et al., 2018. Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. PloS One 13, e0206428.

Zohara, F., Akanda, M.A.M., Paul, N.C., Rahman, M., Islam, M.T., 2016. Inhibitory effects of Pseudomonas spp. on plant pathogen Phytophthora capsici in vitro and in planta. Biocatal. Agric. Biotechnol. 5, 69–77.