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Posted Date: 11 March 2024

doi: 10.20944/preprints202403.0643.v1

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Article

Phytophthora Communities Associated with *Agathis australis* (Kauri) in Te Wao Nui o Tiriwa/Waitākere Ranges, New Zealand

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Abstract: Studies of *Phytophthora* impacts in forests generally focus on individual species without recognition that *Phytophthora* occur in multispecies communities. We investigated community structure of *Phytophthora* species in the rhizosphere of *Agathis australis* (kauri) in Te Wao Nui o Tiriwa / Waitākere Ranges, New Zealand, in the context of kauri dieback disease expression. Soil sampling and tree health monitoring was conducted on 767 randomly selected mature kauri trees. *Phytophthora* species were detected using both soil baiting and DNA metabarcoding of eDNA. Four species were detected with soil baiting (*P. agathidicida, P. cinnamomi, P. multivora,* and *P. pseudocryptogea*) and an additional three species with metabarcoding (*P. kernoviae*, *P. cactorum*/*P. aleatoria* and an unknown clade 7 species). *Phytophthora cinnamomi* was the most abundant species and was distributed throughout the forest. Both *P. multivora* and *P. agathidicida* were limited to forest edges, suggesting more recent arrivals. *P. agathidicida* presence was strongly correlated with declining canopy health, confirming its role as the main river of kauri dieback. The limited distribution of *P. agathidicida* and infrequent detections (11.0% samples) suggests that that this species is spreading as an introduced invasive pathogen and provide hope that with strategic management uninfected areas of the forest can be protected. The frequent detections of *P.cinnamomi* and *P. multivora*, from symptomatic trees in the absence of *P. agathidicida* suggest more research is needed to understand their roles in kauri forest health.

Keywords: *Phytophthora*; *Agathis australis*; kauri dieback; community structure; soil baiting; metabarcoding; New Zealand; epidemiology; invasive pathogen; forest management

1. Introduction

Phytophthora species are increasingly associated with the decline of forest ecosystems around the world and are often responsible for causing serious levels of plant disease [1–3]. Considerable research on these issues has been focussed on the single and invasive species implicated in these diseases, however, *Phytophthora* typically occur in forests as multispecies communities often including both native and invasive species [4,5]. Despite increasing knowledge around the diversity and cooccurrence of *Phytophthora* species in forest ecosystems [6–10], little is known about how these species might act together in communities.

 doi:10.20944/preprints202403.0643.v1

Phytophthora are associated with several diseases of foundation forest trees, such as oak (*Quercus* spp.) [11] and beech (*Fagus sylvatica*) [12] in Europe, and jarrah (*Eucalyptus marginata*) in Australia [13,14]. *Phytophthora* diseases of these tree species threaten whole ecosystems and the consequences of losing many such trees is hard to predict [15]. In New Zealand (NZ), the foundation tree species *Agathis australis* (kauri) is under threat from dieback caused by *Phytophthora agathidicida* [16].

Phytophthora agathidicida invades the root system of kauri, disrupting the vascular tissue causing resin 'bleeding' around the root collar and lower trunk, yellowing of the leaves and crown decline often leading to death [17,18]. To date the origin of *P. agathidicida* is unknown; it has only been found in NZ but has characteristics, e.g., extremely low genetic diversity, of an introduced species [16]. Recent research on the molecular clock of *P. agathidicida* suggests it may have been introduced to NZ prior to 1945 and disease expression is a result of recent changes between the pathogen, host and environment [19].

Kauri is the longest lived and largest tree species in NZ and the only indigenous member of the ancient conifer family Araucariaceae in the country [20]. The natural distribution of kauri is limited to the northern North Island. Due to extensive past disturbance and milling, less than 1% (7,500 Ha) of old growth kauri forests present at the time of European settlement remains [21]. In addition, there are approximately 60,000 ha of regenerating stands across the upper North Island, much of which are now protected [22]. A significant stand of kauri forest (over 17,000 ha) occurs in Te Wao Nui o Tiriwa / Waitākere Ranges Regional Park (WRRP), situated to the west of the most populous city in NZ, Auckland. Kauri is a key flagship species for conservation and a valuable aspect of NZ tourism and recreation. Kauri are a sacred (taonga) species to Māori (indigenous) people and are revered for their importance culturally and for their role as a foundation species in their ecosystem [23,24].

The soils around kauri are typically acidic with limited fertility due to the buildup of deep litter layers, and excessive leaching (podsolisation) [21]. As a result, distinctive communities of plants [25] and microbes [26] are associated with kauri. Not only is the kauri ecosystem distinct, it is also highly diverse [27]. The loss of kauri from kauri dieback is likely to lead to the decline and/or loss of these distinctive and diverse ecosystems.

Several *Phytophthora* species have previously been detected from kauri forests using a traditional soil baiting method [28] and through baiting waterways [29]. In addition to *P. agathidicida*; *P. cinnamomi, P. pseudocryptogea, P.multivora, P. nicotianae, P. chlamydospora* and *P. kernoviae* have been isolated from kauri forest soils [13,17,18,30–32] and *P. gonapodyoides, P. chlamydospora, P. asparagi, P. kernoviae, P. amnicola* and an unknown clade six species called *P.* sp "Waitākere" have been previously detected in waterways of the Waitākere Ranges [29,32]. Of the species frequently isolated from kauri soils, *P. agathidicida* has been found to be significantly more pathogenic to kauri than other *Phytophthora* species during pathogenicity bioassays with kauri seedlings [33].

The use of environmental DNA (eDNA) and metabarcoding has been used more recently to survey for *Phytophthora* species from water and soil in natural ecosystems and urban or other human disturbed environments worldwide [34–37]. Using these methods, natural ecosystems consistently yield greater numbers of *Phytophthora* species (through metabarcoding) compared to non-native ecosystems [34,35,38]. As well, eDNA and metabarcoding methods typically detect more species than traditional baiting and plating methods [37–39]. This is most likely because they can detect unculturable species and can deal with low levels of inoculum [39]. In NZ kauri forests, eDNA and metabarcoding has been used to detect fungi and bacteria present in the soil around healthy and unhealthy kauri trees [40,41] but it has not yet been used to detect *Phytophthora* species. The current study is the first time eDNA and metabarcoding have been used to survey *Phytophthora* species in NZ forests.

The aim of this study was to investigate *Phytophthora* communities associated with healthy and symptomatic kauri in Te Wao Nui o Tiriwa / Waitākere Ranges, NZ, using both soil baiting and eDNA analysis and to compare both association of *Phytophthora* species with kauri dieback and detection rates between methods.

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2. Materials and Methods

2.1. Site Characteristics and Kauri Dieback History

Te Wao Nui o Tiriwa (the Great Forest of Tiriwa) / Waitākere Ranges is located on NZ's North Island and is one of the largest areas of native forest in the greater Auckland region and one of the largest areas of kauri forest in NZ. The study area was limited to the Waitākere Ranges Regional Park (WRRP; 36°53–37°03S, 174°27–174°34E) which covers most of Te Wao Nui o Tiriwa, consisting of more than 17,000 ha of parkland between metropolitan Auckland, the coast of the Tasman Sea to the west and the Manukau Harbour to the south (Figure 1). The terrain is hilly ranging from sea level to 474 m elevation [42]. Mean annual temperature ranges from 12.5 to 14.5°C. Total annual rainfall measured at a nearby station (Arataki, 7.5 km northeast of the study area, 190 m above sea level) is approximately 1,600 mm (1981– 2010) (Environmental Monitoring, Auckland Council GeoMaps, https://geoma pspub lic.auckl andco uncil.govt.nz/viewe r/index.html).

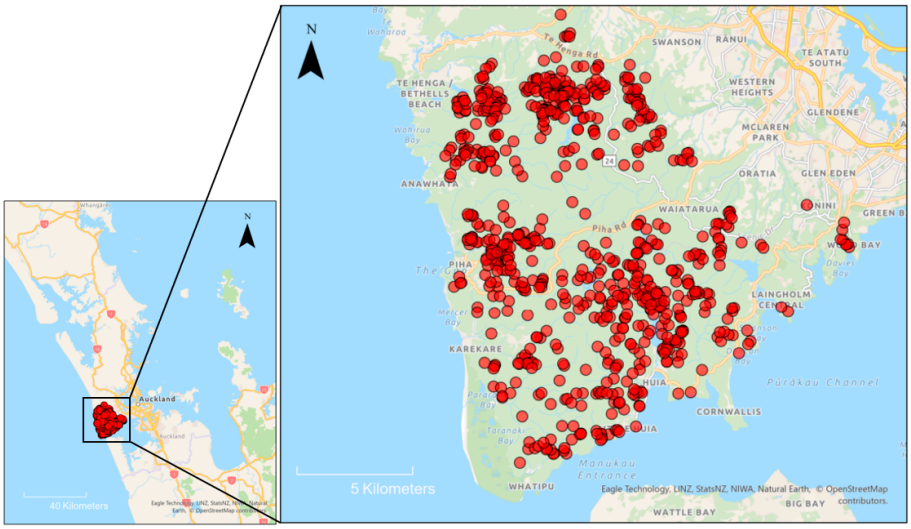

Figure 1. Location of the 767 soil samples (represented by red circles) collected in the Waitākere Ranges Regional Park survey for the current study. The smaller map on the left shows the location of the Waitākere Ranges Regional Park in relation to Auckland City on the upper North Island of New Zealand.

Phytophthora agathidicida was first detected on North Island in Te Wao Nui o Tiriwa / Waitākere Ranges in 2006; it was isolated from the margin of bleeding lesions on symptomatic trees [17]. Kauri health in association with *P. agathidicida* was first monitored there in 2010 and 2011 and showed a high prevalence of kauri dieback with about 7.9% of surveyed kauri affected. A follow-up survey conducted in 2017 raised concerns with respect to how far the disease and pathogen had spread [43]. In response to this discovery, a landscape scale rāhui (cultural restriction) was put in place over Te Wao Nui o Tiriwa / Waitākere Ranges in 2017 by the local indigenous people responsible for the land Te Kawerau ā Maki [43]. The rāhui was focussed on minimising the risk of spreading *P. agathidicida* until more information on the extent of the pathogen distribution was available and appropriate risk mitigation measures were in place. This placed a temporary ritual prohibition on the area and restricted access to the forest to separate people from things that are tapu (sacred or prohibited). The rāhui involved track closures and prevented public access to remote areas of the forest.

In response to the rāhui, Auckland Council, in partnership with Te Kawerau ā Maki implemented a further forest-wide survey in 2021 to determine the extent of kauri decline and the extent of *P. agathidicida* infestation throughout Waitākere Ranges Regional Park. Our study is part of this survey.

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2.2. Survey Design and Sampling

Full details of the surveillance design are presented by Froud, *et al.* [44]. In brief, mature kauri crowns were detected by remote sensing with 68,420 mature kauri greater than 15 m in height putatively identified. Of these, 2,140 were selected at random for field surveying with soil samples collected from 767 of these trees [44]. Soil samples were collected between March and July 2021. Trees were classified as symptomatic if they had a canopy dieback score > 3 (details of canopy scoring in Froud et al [44]) and/or the presence of a basal bleed [45].

Four soil sub-samples were taken at 90° intervals around each tree and at 1 – 2 m from the trunk starting either below the tree tag, or if the tree had a basal or lateral root resin 'bleed', below the most active 'bleed'. Soil was taken to a depth of 10 – 15 cm after brushing away the loose litter layer and contained a mixture of organic material, mineral soil, and kauri feeder roots. The four sub-samples were amalgamated into one sample per tree totalling approximately 650 – 750 g. The soil samples were double-bagged and stored in a dark place at temperatures from 10 – 25°C until dispatched to the laboratory. Samples were thoroughly mixed and homogenised by sieving (6 mm) in the lab. A 2 g tube was filled with a subset of each soil sample (containing feeder root fragments and rhizosphere soil) and stored at -20°C in the dark until was DNA extraction.

2.3. Soil Baiting

Separate aliquots of each soil sample were used to isolate *Phytophthora* species in a *P. agathidicida*-selective [28] and standard baiting assay [46], although results from both assays were combined for data analysis.

The procedure for the *P. agathidicida*-selective baiting assay was as follows [28]. Approximately 100 g including fine feeder roots and soil from each soil sample were air dried at 20°C for 3 – 4 days on a lab bench with a dehumidifier (Mitsubishi Electric MJ-E22VX, Tokyo, Japan) set to 50% running constantly. The samples were re-moistened carefully and incubated in diffuse natural light for 3 – 4 days at 20–22°C to stimulate sporangia production. The samples were then carefully flooded with distilled water to a depth of 3 – 5 cm above the soil surface. Soil disturbance and water turbulence were minimised by flooding slowly. Five Himalayan cedar (*Cedrus deodara*) needles were floated on the water surface and three freshly germinated (3-day-old), intact blue lupin (*Lupinus angustifolius*) radicles were suspended over the water surface using parafilm with the root tip submerged in the water.

The samples for the standard assay were not dried prior to flooding and were only baited with Himalayan cedar needles.

The baits were incubated at 20°C in light and monitored for lesions. After 2-3 days the baits were removed, surface sterilised in 50% ethanol followed by two rinses in distilled water and blotted dry on paper towels. The lupins were cut into 1 cm pieces and plated into *Phytophthora*-selective P_6ARPH media [47]. The cedar needles were plated whole, taking care to submerge the proximal end into the media. Plates were incubated in the dark at 18 – 20°C and monitored for *Phytophthora*-like growths daily for 5 days. Such growths were sub-cultured onto clarified 20% V8 agar for morphological characterisation. *P. cinnamomi* and *P. agathidicida* isolates were identified based on distinguishing morphological characteristics but any other *Phytophthora* isolates were submitted for DNA sequence analysis.

2.4. Sequencing of Cultures

All unknown isolates were identified by amplification and sequencing of the Internal Transcribed Spacer (ITS) and cytochrome c oxidase subunit 1 (*coxI*) gene regions. Mycelium was harvested from 3-day old cultures grown in clarified 20% V8 broth, rinsed three times in sterile deionised water and blotted dry on paper towels. The mycelium was placed in a 2 mL cryo tube and freeze dried for 24 hours. The cap was replaced, and the mycelium was stored at -20°C prior to DNA extraction. Genomic DNA was extracted using the Qiagen DNeasy Plant Pro Kit (Hilden, Germany) following the manufacturer's instructions. The ITS1 gene region was amplified by PCR using the

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following primers ITS 5 (5′-GGAAGTAAAAGTCGTAACAAGG) and ITS 4 (5′-TCCTCCGCTTATTGATATGC) [48] while the *coxI* gene region was amplified using COIF (5′-TCAWCWMGATGGCTTTTTTCAAC-3′) and COIR (5′-RRHWACKTGACTDATRATACCAAA-3′) [49].

PCR products were run in 1.5% agarose gel and quantified using the NanoPhotometer® NP80 Spectrophotometer (Implen, Munich, Germany) for quality and quantity before sending to University of Auckland DNA Sequencing Facility (Auckland Genomics), for PCR purification and DNA sequencing (AMPure magnetic beads and Sanger sequencing (1/4 Big Dye v3.1). Sequences were viewed using Geneious Prime v.2022.0.1 (Biomatters Ltd, Auckland, NZ). The sequences were trimmed and (MUSCLE) aligned using the features within the Geneious software. Sequences were aligned with a curated library of validated type and isotype isolates of all the currently described species of *Phytophthora* supplied by Professor Treena Burgess (Harry Butler Institute, Murdoch University, WA, Australia; Sarker et al., 2023).

2.5. Soil DNA Extraction and PCR Amplification

Genomic DNA was extracted from up to 250 mg of soil per tree sample using the DNeasy® PowerSoil® Pro Kit (Qiagen, Germany) following the manufacturer's instructions. The sample was crushed in a Qiagen Tissue LyserIII machine for 50 seconds at 30 cycles for step 6. Final elution's were performed in 100 µL of Tris (10 mM) buffer (buffer C6 of the kit). All DNA was stored at -20°C before amplicon generation.

For all samples, amplicon libraries for ITS gene region were created by applying a nested PCR using the primary Oomycete specific primers oom18S/ITS7 [50] in the first round, and the nested *Phytophthora*-specific primers 18ph2f/5.8S-1R [51]. In addition, 30 samples (selected based on the ITS1 gene sequencing results; Supplementary Materials Table S1) were amplified in a nested approach targeting the 40S ribosomal protein S10 (RPS10) gene region with primers PRV9-F and PRV9-R in the first round [52] and oomycete-specific primers RPS10-F and RPS10-R in the second round to resolve detections to species level where this is not possible with the ITS1 region alone [53]. In both cases the second round PCR primers had Illumina MiSeq (MS) adapter sequences attached to the 5′ end, as per standard protocols for the MiSeq platform (Illumina Demonstrated Protocols: Metagenomic Sequencing Library Preparation) [54].

The PCRs were performed in 25 µL volumes containing 12.5 µL of PCR buffer KAPA HiFi HotStart ReadyMix (KAPA Biosystems). For the ITS1 PCRs there was 8.5 µL of PCR grade water, 400 nM of each primer and 2 µL of genomic DNA (first round) or 1 µL of the PCR product (second round had 9.5 µL of PCR grade water instead of 8.5 µL). For the RPS10 PCRs there was 1 µM of each primer, 2.5 µL of genomic DNA or PCR product and 8 µL of PCR grade water. No-template negative PCR controls were included each time a PCR reaction was set up and carried forward to the second round in the same manner as for the experimental samples.

PCRs were carried out in a Mastercycler® X50s thermal cycler (Eppendorf, Hamburg, Germany) with the following steps for the primary ITS1 PCR: 3 min at 95°C for initial denaturation, followed by 35 amplification cycles of 98°C for 20 s, 60°C of annealing for 15 s, and 72°C for 60 s, a final extension cycle at 72°C for 7 min and holding at 10°C. For the nested ITS1 PCR the conditions were 94°C for 2 min, 25 cycles of 95°C for 20 s, 60°C for 25 s, and 72°C for 60 s, and 72°C for 7 min and holding at 10°C.

For the RPS10 primary PCR the conditions were 94°C for 2 min, 35 cycles of 94°C for 30 s, 59°C for 45 s, and 72°C for 60 s, and 72°C for 10 min and holding at 4°C. For the nested RPS10 PCR the cycling conditions were as follows; 94°C for 2 min, 30 cycles of 95°C for 20 s, 60°C for 25 s, and 72°C for 60 s, and 72°C for 7 min and holding at 4°C.

Amplicon library preparation was performed according to recommended protocols (Illumina Demonstrated Protocol: 16S Metagenomic Sequencing Library Preparation) [54]. After visualisation of 5 µL on 1.5% agarose gels, a duplicate PCR was run for all samples which produced an amplification product. The duplicate PCRs were combined and purified with the AMPure XP PCR purification system (Beckman Coulter Life Sciences) following the manufacturer's instructions (0.8x

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of AMPure XP beads were used per sample). The purified products were quantified using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA). Purified PCR amplicons, adjusted to 5 ng/μL for indexing. Indexing and sequencing was done at Auckland Genomics, University of Auckland, NZ on Illumina MiSeq using 600-cycle V3 chemistry (300 bp paired-end reads).

2.6. Controls for Illumina Sequencing of ITS1 Gene Region

Positive controls were developed using DNA extracted from the following *Phytophthora* isolates sourced from the The New Zealand Institute for Plant & Food Research Limited isolate collection: *P. cinnamomi* (H1454), *P. agathidicida* (H1453), *P. pseudocryptogea* (H1258) and *P. multivora* (H1467). All four species are known to occur in kauri forests and the isolates used were from the North Island of NZ. The DNA extraction, amplification and sequencing methods were the same as described above.

eDNA from five samples (5316, 5340, 5502, 5630, and 5597) which were negative for *Phytophthora* (based on the *Phytophthora* specific ITS PCR) were combined as background eDNA and spiked with DNA from the four *Phytophthora* species to create mock community control samples (ranging from 0.0001 to 0.1 ng/μl in various combinations). The combined background eDNA was also sequenced, as described above.

Additional negative controls were sequenced for four samples (5547, 5674, 5675, and 5678) which did not produce an amplification product from the *Phytophthora*-specific PCR of the soil DNA extractions.

The sequencing was performed over two runs. To check for differences between the runs, five samples from the first sequencing run (5084, 5085, 5170, 5185 and 5203) were included in the second sequencing run as controls. All steps were repeated for the second run (including DNA extraction).

2.7. qPCR Validation of Mock Communities for the ITS1 Gene Sequencing

An enriched multiplex real-time PCR for *P. agathidicida* and *P. cinnamomi* was developed to validate the mock community control samples and the presence or absence of *P. agathidicida* and *P. cinnamomi* in field samples. The primers used for *P. agathidicida* and *P. cinnamomi* for the multiplex enriched qPCR targeted the ITS1 gene and were within the region that the oomycete specific primers for the metabarcoding PCR amplified (Table 1). The *P. agathidicida* qPCR was highly specific and did not amplify other species, while *the P. cinnamomi* qPCR primers were specific to clade 7.

Table 1. Primers used for target species in the qPCR validation assay.

Primer or probe name	Target species	Sequence (5′ to 3′)	Notes*	Reference
ITS_PTA_F2	*P. agathidicida*	AACCAATAGTTGGGGGCGA		[55,56]
ITS_PTA_R3		CTCGCCATGATAGAGCTCGTC		[55]
ITS_PTA_probe2		AGCCAAAGCCAGCAGCCG	5′ FAM, 3' BHQ1	[55]
PCIN F6	*P. cinnamomi*	CGTGGCGGGCCCTATC		[57]
PCIN R2		AAAAGAGAGGCTACTAGCTCAGTTCCC		[57]
PCIN probe		TGGCGAGCGTTTGGGTCCCTCT	5′ HEX, 3' BHQ2	[57]

*Notes: Florescent dye label for the probes. .

The PCR products from the oomycete specific ITS PCR (oom18S/ITS7) for metabarcoding were used as the template for the enriched qPCR validation. The qPCRs were performed in 15 μL volumes, containing 7.5 μL of TaqMan® Environmental Master Mix 2.0 (Life Technologies), 2.92 μL of PCR

 doi:10.20944/preprints202403.0643.v1

grade water, 350 nM of each primer, 160 nM of each probe, and 2 µL of template (1:100,000 dilution of oomycete specific ITS PCR oom18s/ITS7). No-template negative PCR controls were included each time a PCR reaction was set up and carried forward to the second round in the same manner as for the samples. qPCRs were carried out in an Eco Real-time PCR instrument (Illumina, San Diego, California) with the following steps: 10 min at 95°C for initial denaturation, followed by 40 amplification cycles of 95°C for 15 s, and 60°C of annealing for 60 s. A no template negative control was always included in the reactions. The cycle threshold was set to 0.02.

To determine the minimum read count for a true positive in the metabarcoding, all samples with less than 500 reads for *P. agathidicida* and 23 samples with less than 180 reads for *P. cinnamomi* were run in the enriched qPCR.

In addition, to check the ability of the metabarcoding to give a quantitative measure of *Phytophthora* in samples, the spiked mock community DNA mixes and the oom PCR products for control samples 6000 to 6006 and 6011 (Table 1) were used as templates in a multiplex qPCR to quantify the amount of *P. cinnamomi* and *P. agathidicida* DNA in each sample.

2.8. Bioinformatics

Paired-end reads were imported into the DADA2 pipeline in R Studio [58] to remove the primers and inspect read quality. Reads were trimmed to a minimum length of 100 base pairs, dereplicated and merged. The quality of the DNA libraries before and after the trimming step were checked with FASTQC [59], and all output files were merged into a single report using MultiQC [60].

An amplicon sequence variants (ASVs) table was generated for the merged ITS reads and for the R2 reads of the RPS10 only (after filtering and trimming to 260 bp), potential chimeras were detected and removed by means of the DADA2 pipeline.

Any ASV with a total library less than 50 reads were removed. Any samples with less than 100 reads total were removed. Singletons were discarded but noted for any with >3000 reads. Any samples with less than 50 reads for a single ASV were removed (as determined by the qPCR validation).

The ITS1 and RPS10 ASV tables were compared to the NCBI database comparing to the Nucleotide collection (nr/nt) and the Whole-genome shotgun contigs (wgs) Oomycete (taxid: 4762) and *Phytophthora* (taxid: 4783) databases.

The ITS1 ASVs were trimmed to the ITS1 gene region to check against the curated database supplied by Professor Treena Burgess (Harry Butler Institute, Murdoch University, WA, Australia). Using Geneious Prime (Version 2022.0.1), the RPS10 ASV table was compared to the reference database downloaded from OomyceteDB (www.oomycetedb.org; accessed on 30/1/2024) [53]. The reference database consisted of 886 sequences of oomycetes.

Those ASV sequences showing <99% but more than 98% similarity to a *Phytophthora* species after blasting or in the reference database were submitted to a phylogenetic analysis to check their positions in the different *Phytophthora* taxonomic clades. A simple phylogenetic analysis was conducted using Geneious (Version 2022.0.1) tree builder. The phylogenetic analysis of phylotypes which were not a described species were included.

2.9. Data Analyses

All analyses were conducted using R version 4.2.3 [61].

To determine how sensitive and quantitative the ITS1 metabarcoding primers were, the number of reads of each species found in the 'mock' communities were compared to the DNA concentration of each species, using a negative binomial generalised linear model with function glm.nb (package MASS, version 7.3-60; [62]). The response variable was the number of reads, and model predictor was the DNA concentration. Model assumptions were verified by visually inspecting residuals for assumptions of normality and homoscedasticity [63].

Species co-occurrence was assessed using the cooccur function with a threshold of >1 in the R package cooccur version 1.3 [64]. Sites with the dominant invasive *Phytophthora* species, *P. cinnamomi,*

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P. agathidicida and *P. multivora* were visualized using a Venn diagram constructed with the eulerr package version 7.0.0 [65].

The impact of canopy dieback score was analysed with a negative binomial generalised linear model NBGLMs (logit link; package MASS [62]) and visualised with ggplot2 version 3.4.3 [66].

The distribution of the three most abundant species (*P. agathidicida, P. cinnamomi* and *P. multivora*) were mapped using the geographical information system (GIS) software ArcGIS Pro version 2.7.1.

3. Results

3.1. Characteristics of Sampled Trees

The trees had a minimum diameter at breast height (~1.4 m DBH) of 10 cm and were >15 m tall (n = 767) [44]. Samples were taken from across the whole of the Waitākere Ranges Regional Park (Figure 1).

3.2. Identification of Phytophthora Species by Soil Baiting

Four species of *Phytophthora* were isolated by soil baiting from the 767 samples (*P. cinnamomi, P. agathidicida, P. multivora*, and *P. pseudocryptogea*), and an additional three species were detected through metabarcoding of soil eDNA (*P. kernoviae*, a clade 1 species - likely *P. cactorum* or *P. aleatoria*, and an unknown *P. europaea*-like clade 7 species; Table 2). *Phytophthora kernoviae* is putatively native to NZ [67] and the specific origins of the other species are unknown.

Table 2. *Phytophthora* species (n = 7) and their clades detected by soil baiting and metabarcoding from *Agathis australis* (kauri) rhizosphere samples (n = 767) in the Waitākere Ranges.

***Phytophthora* species**	**Clade**	**Soil baiting**	**Metabarcoding (soil eDNA)**	**Total detections**	**Total detections (%)**
P. cinnamomi	7	404	231	455	59.3
P. agathidicida	5	79	44	84	11.0
P. multivora	2	63	6	68	8.9
P. sp (*P. europaea*-like)	7	0	20	20	2.6
P. cactorum/*P. aleatoria*	1	0	4	4	0.5
P. pseudocryptogea	8	1	2	2	0.3
P. kernoviae	10b	0	2	2	0.3

3.3. Sequencing Output and Performance of Control Reactions

After the quality control filtering and merging, the ITS1 metabarcoding runs yielded an average of 14,448 ± 513 reads per sample (± standard error; range: 4– 58959). After the quality control steps, the RPS10 reverse reads (trimmed to 260 bp) from 30 samples had an average of 8187.9 ± 1028.1 reads per sample (± standard error; range: 2208– 28672). The Q score of quality was higher than 34 for the trimmed ITS library and higher than 25 for the RPS10 trimmed reverse reads.

No *Phytophthora* species were detected within any of the negative control reactions. *Phytophthora cinnamomi, P. agathidicida* and *P. pseudocryptogea* were detected in 100% of the mock community control samples. *Phytophthora multivora* was only detected in two mock community samples in which *P. cinnamomi* was at 0.01 (sample ID 6005) and 0.001 (sample ID 6006) ng/µl DNA (Table 3).

The sequence reads for each species were not directly proportional to the amount of DNA present in the mock community samples (Figure 2). For example, sample 6000 was spiked with 0.1 ng/µl of DNA for each of the four species, however *P. cinnamomi* made up 53.3% of the reads in that sample and *P. multivora* was not detected (Table 3). Overall, for the mock communities there was a positive correlation between DNA concentration and the number of reads in the sample ($z = 4.665$, $P < 0.001$; R^2 from a simple linear regression = 0.5529).

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Table 3. Mock community samples created by spiking eDNA from five samples with known concentrations of DNA extracted from pure cultures of *Phytophthora cinnamomi* (isolate H1454), *P. agathidicida* (isolate H1453), *P. pseudocryptogea* (isolate H1258) and *P. multivora* (isolate H1467) and the proportion of sequence reads detected for each species in each sample. Isolate IDs in brackets refer to the Plant & Food Research *Phytophthora* isolate collection located at the Hawkes Bay Site, New Zealand.

		Reads (%)*			
Sample ID	**DNA (ng/µl)**	***P. cinnamomi* (H1454)**	***P. agathidicida* (H1453)**	***P. pseudocryptogea* (H1258)**	***P. multivora* (H1467)**
6000	0.1	53.3	16.0	27.9	0.0
6001	0.01	51.0	19.4	27.4	0.0
6002	0.001	55.9	15.3	26.8	0.7
6011	0.0001	60.7	2.8	33.4	0.0
6003	0.01	-	0.3	-	-
	0.1	58.3	-	38.2	0.0
6004	0.001	-	31.4	-	-
	0.1	16.5	-	48.7	1.1
6005	0.01	3.3	-	-	-
	0.1	-	37.5	57.6	1.0
6006	0.001	69.6	-	-	-
	0.1	-	19.2	7.0	0.0
6007	0.01	-	-	0.7	-
	0.1	71.0	22.8	-	0.3
6008	0.001	-	-	30.2	-
	0.1	52.0	17.5	-	0.0
6009	0.01	-	-	-	0.0
	0.1	53.2	17.1	29.7	-
6010	0.001	-	-	-	0.0
	0.1	62.2	9.1	26.6	-

*The reads (%) was calculated by summing the total number of reads per sample and then dividing that number by the total number of reads for each species.

The number of sequence reads in the mock communities were different between *P. cinnamomi* and *P. agathidicida* ($z = 4.202$, $P < 0.0001$; Figure 2).

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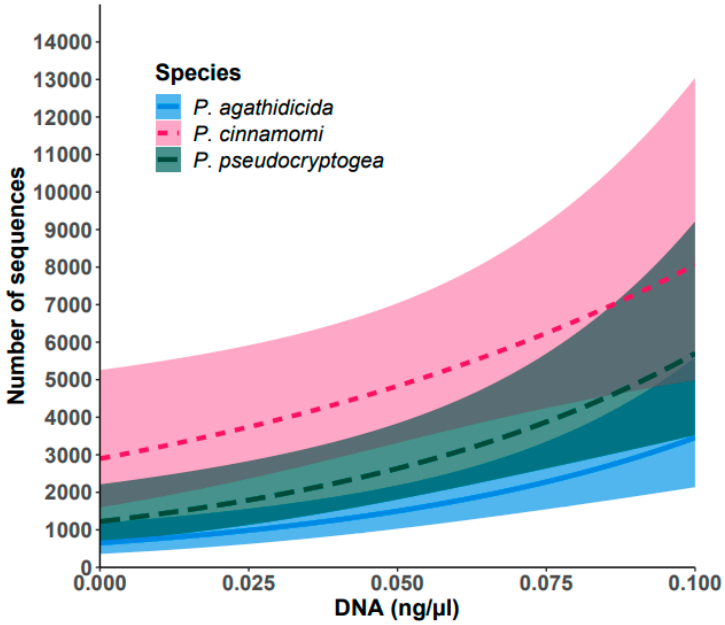

Figure 2. The number of sequences produced for *Phytophthora agathidicida* (blue with solid line) *P. cinnamomi* (pink with short dashes) and *P. pseudocryptogea* (green with long dashes) in the mock community samples with known DNA concentrations. Each line represents the best-fit line from the negative binomial generalised linear model (made using the R package MASS version 7.3-60) for each species and the surrounding shaded areas indicate 95% confidence intervals (CI). *Phytophthora multivora* omitted due to lack of sequence reads.

The spiked mock community DNA concentrations of *P. agathidicida* and *P. cinnamomi* were re-tested with qPCR which produced DNA ratios as expected for the DNA quantities loaded into each of the mock communities (Supplementary Materials Figure S1). The qPCR of the oom18s/ITS7 PCR products for the mock community samples in which the four species were spiked in equal concentrations (6000 to 6006 and 6011 from Table 3) provide evidence of sequencing bias either in the enrichment process of the nested PCRs or in the metabarcoding and hence are not a reliable quantitative measure of species abundance (Supplementary Materials Figure S1).

3.4. Identification of Phytophthora Phylotypes by NGS

From the 767 soil DNA extractions, 441 produced PCR products with the ITS primers indicating amplification of at least one *Phytophthora* target. The DADA2 workflow generated 816 ASV from which, after filtering and eliminating artefacts, seven *Phytophthora* species corresponding to six known species and one potentially new phylotype were detected (Table 2).

There were three ITS ASV's which had more than 3000 reads and were possibly a *Phytophthora* species but they were present in single samples (Appendix A Table A1).

3.4.1. Unknown Phytophthora Species in Clade 7

There was a potentially new species in 20 samples as detected with ITS metabarcoding (Table 2; Supplementary Materials Table S1) that was similar to clade 7 *Phytophthora* species (including *P. europaea, P. uliginosa and P. abietivora*) but distinctly different from the other clade 7 species (*P. cinnamomi*) detected in this study based on the ITS metabarcoding (Figure 3). The cropped sequence to the start of the ITS1 gene (200 bp) had a 100% query cover and 100% match to *P. uliginosa* isolate Ex-type IFB-ULI 1 (MG865597.1 NCBI reference) and 100% cover and 99.5% matches to *P. abietivora* isolate Ex-type (MK163944.1 NCBI Reference) and *P. europaea* (MG865488.1 NCBI Reference).

From the 30 samples selected for amplification with the RPS10 primers, all produced PCR products. The DADA2 workflow generated 176 ASVs after filtering. RPS10 sequencing confirmed the

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identity of a *P. europaea*-like species in 11 of the 20 samples which were positive with ITS (>99 reads) (Supplementary Materials Tables S1 and S2). The RPS10 sequencing confirmed the presence of *P. agathidicida, P. cinnamomi* and *P. pseudocryptogea* but read counts were very low or single samples were positive (Supplementary Materials Table S1 and S2). It did not confirm the presence of *P. cactorum*/*P. aleatoria* in the four samples which were positive with ITS (Supplementary Materials Table S1 and S2).

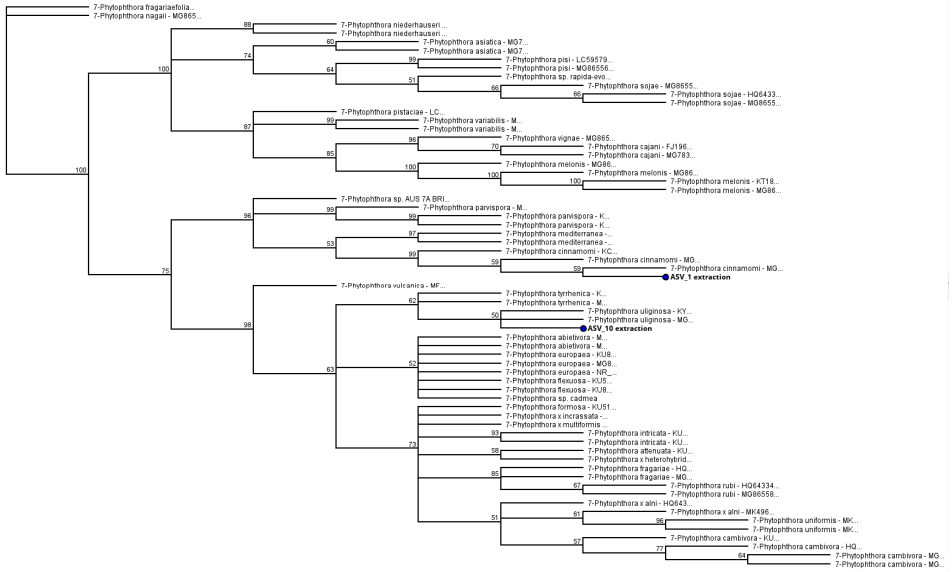

Figure 3. A phylogram based on ITS1 gene sequence data indicating the placement of the two clade 7 species detected in this study in relation to closely related taxa. *Phytophthora cinnamomi* represented by ASV1_extraction and *Phytophthora* sp. unknown represented by ASV10_extraction. The sequences were trimmed to the ITS1 gene region and compared with the curated ITS1 gene database supplied by Professor Treena Burgess (Harry Butler Institute, Murdoch University, WA, Australia). Numbers above the branch represent the bootstrap support based on parsimony analysis.

3.5. Comparison of Baiting to Metabarcoding

Of the seven phylotypes detected in this study, all were detected with metabarcoding and four were detected with baiting. Three of these phylotypes were detected in less than 0.5% of the samples tested using one or both of the detection methods.

Overall, 255 samples were negative for a *Phytophthora* using both methods and 268 samples were positive with one method only. With either method, 17 samples were positive for three species and one sample had four species present (including *P. agathidicida* and *P. cinnamomi* detected by sequencing and *P. multivora* and *P. pseudocryptogea* detected by baiting). Using both detection methods at least one *Phytophthora* species was detected in 506 samples. Of these, one sample had four species, 16 had three species, 87 had two species and 402 had 1 species detected.

Metabarcoding identified more species richness than baiting, but there were more positives with baiting compared to metabarcoding. The differences in counts for the three main species detected were not significantly different (ANOVA F = 0.44146, *P-value* = 0.5428).

The baiting and metabarcoding detections for *P. cinnamomi* and *P. agathidicida* were closely correlated well (Figure 4). There were 62 samples which were positive for *Phytophthora multivora* with baiting alone and five with sequencing alone; only one sample was positive with both methods (Figure 4).

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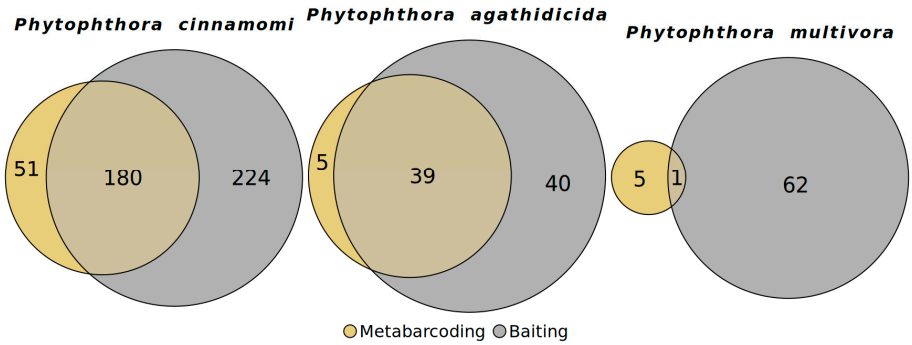

Figure 4. Venn Diagrams (created using the R package eulerr) showing the number of positive samples for *Phytophthora cinnamomi, P. agathidicida* and *P. multivora* using either baiting or ITS metabarcoding.

3.6. Phytophthora Community

The eDNA analysis added an additional five *P. agathidicida*, five *P. multivora* and 51 *P. cinnamomi* positive detections to the distribution map reported by Froud and colleagues [44] (Figures 4 and 5).

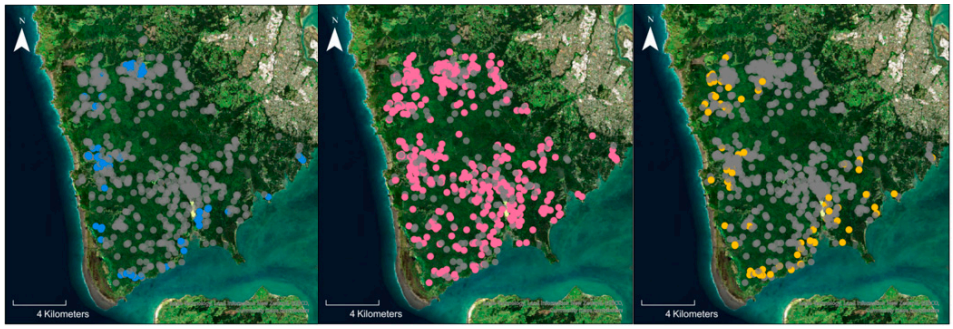

Figure 5. Distribution of the three key species *P. agathidicida* (blue), *P. cinnamomi* (pink) and *P. multivora* (yellow), across the Waitākere Ranges Regional Park, New Zealand. The grey circles indicate the position of samples negative for each relevant *Phytophthora* species. Created in ARC GIS Pro version 2.7.1. Scale bar is 4 km.

Overall, the distribution of these species does not differ substantially from that previously reported by Froud and colleagues [44], with *P. cinnamomi* distributed across much of the Waitākere Ranges while *P. agathidicida* is predominantly located in the peripheral areas with limited extension into the central area of the ranges (Figure 5). *Phytophthora multivora* has similarly had limited penetration into the centre of the park (Figure 5).

All three of the key species (*P. cinnamomi, P. agathidicida* and *P. multivora*) were detected from both asymptomatic and symptomatic trees either alone or cooccurring with either of the other two species (Figure 6). Of the 595 asymptomatic trees, no *Phytophthora* species was detected from 202 trees. From the 172 symptomatic trees, no *Phytophthora* species was detected from 60. There was one asymptomatic sample positive for *P. agathidicida* and *P. multivora* only (Figure 6).

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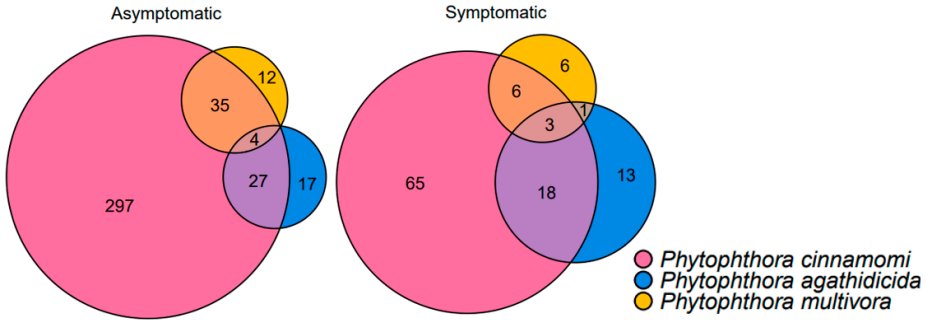

Figure 6. Cooccurrence of *Phytophthora cinnamomi* (pink), *P. agathidicida* (blue) and *P. multivora* (yellow) in the sampled kauri trees which were asymptomatic for kauri dieback (canopy score less than 3; n = 595) or symptomatic (canopy dieback score ≥3 and/or the presence of a basal bleed; n = 172). Positive detections with either baiting or ITS metabarcoding.

Phytophthora cinnamomi was more likely than by chance to occur together with *P. multivora* (*P*-value = 0.0305) and the unknown clade 7 *Phytophthora* species (*P*-value = 0.0429) as determined by the pairwise cooccurrence matrix (Supplementary Materials Figure S2).

All three species were found to have a significant relationship with increasing canopy dieback scores (i.e. declining tree health), however, this was strongest for *P. agathidicida* when plotted (all *P*-values from negative binomial generalised linear model <0.005; Figure 7).

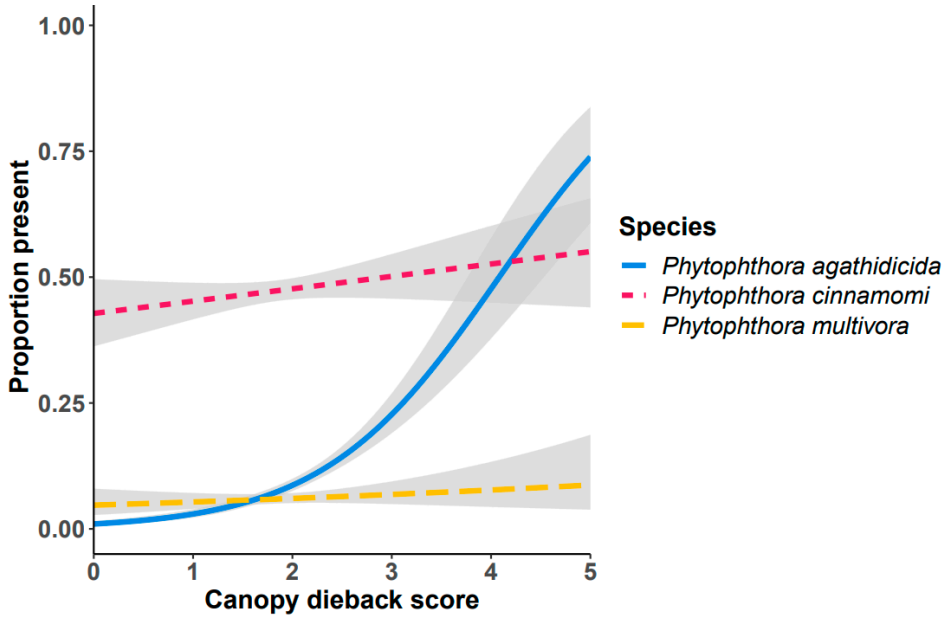

Figure 7. *Phytophthora agathidicida* (blue with solid line), *P. cinnamomi* (pink with short dashes), and *P. multivora* (yellow with long dashes) presence in kauri soil samples in association with canopy dieback scores (score of 0 = healthy tree, score of 5 = dead tree). Solid lines and surrounding grey areas indicate the fits and 95% confidence intervals (CI) from negative binomial generalised linear models (NBGLM) created in R with the package MASS.

4. Discussion

Despite the large number (n > 57) of *Phytophthora* species already present in NZ [32,68], the *Phytophthora* community (seven species total) detected around kauri in Te Wao Nui o Tiriwa / Waitākere Ranges was surprisingly few. During *Phytophthora* surveys of temperate forests internationally many more *Phytophthora* taxa are typically either associated with single trees or found within the same ecosystem. For example, 13 *Phytophthora* species were isolated from *Alnus glutinosa*

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(black alder) tissue, rhizosphere and water samples in Portugal [69], eight species were isolated by soil and nine by water baiting from *Quercus suber* in Italy (14 species total) [70], and in a study across Austria, Czech Republic and Slovakia 19 *Phytophthora* taxa were isolated from rivers and streams in *Alnus* forests [71].

Few conifer trees have been surveyed for *Phytophthora;* of those that have, *Austrocedrus chilensis* in Argentina had five *Phytophthora* species detected [72], asymptomatic and symptomatic *Picea abies* (Norway spruce) in Bulgaria had two *Phytophthora* species [73], and 14 *Phytophthora* species were detected in a *Juniperus communis* woodland in Scotland [74]. Of these, only Riddell, Dun, Elliot, Armstrong, Clark, Forster, Hedley and Green [74] utilised a metabarcoding approach to explore *Phytophthora* species richness beyond baiting in coniferous stands.

Metabarcoding consistently detects more species than soil baiting methods. For example, eDNA methods detected 20 *Phytophthora* species in *Quercus* species in Italy, compared to five species detected with baiting [5]. Similarly, 15 species were detected from *Castanea sativa* in Italy with eDNA compared to nine with baiting [39]. Our study followed the same pattern and both detection methods yielded positive detections that would have otherwise been missed by the other method. Therefore, eDNA is a useful tool to implement in future *Phytophthora* surveys of kauri forests.

The current study was a targeted survey in which only one known host species (*Agathis australis*) and one substrate (rhizosphere soil) was sampled, and this may be a primary reason for this comparatively small community. The samples were from randomly selected trees in the forest and thus included a broad range of symptomatic and healthy trees. Another contributing factor could be that NZ is a remote island nation with intense biosecurity practices implemented at the border and thus likely has fewer introductions of *Phytophthora* species than other countries more connected by trade, e.g. countries of the European Union and the United Kingdom.

Kauri are known to alter the soil pH and nutrient levels in the surrounding soil as the leaf litter layer accumulates and slowly decomposes [21]. These unique acidic conditions may impact the survival of *Phytophthora* species (both native and invasive) around kauri. In addition, since the discovery of kauri dieback in Te Wao Nui o Tiriwa / Waitākere Ranges, a rāhui (cultural exclusion) was implemented and tracks closed which would have limited the spread of soil movement around the forest, however, this recent action is not likely to have significantly impacted the diversity or distribution of species observed in this study with the evidence suggesting each of the pathogens observed have been established within the forest for many years.

Researchers are starting to give more consideration to native *Phytophthora* species, their distributions, and roles in their native environment [2]. Although native and other introduced *Phytophthora* species likely play an important role within the kauri ecosystem, it is not yet understood if any *Phytophthora* other than *P. agathidicida* play a role in contributing to kauri dieback and/or has a beneficial role in forest health. While *P. agathidicida* is the primary pathogen of kauri and was the most aggressive in *in vitro* inoculations [33], *P. cinnamomi* has long been associated with declining kauri [75,76] and may have a significant impact on forest health and regeneration through dampening off seedlings [31]. This study showed that *P. cinnamomi* was widely spread throughout Te Wao Nui o Tiriwa / Waitākere Ranges and suggests it has been present in the natural environments of NZ for a long time. It is possible that *P. cinnamomi* will have a greater impact with climate change [30].

Both *P. cinnamomi* and *P. multivora* are well adapted to dry environments and are significant pathogens globally [5,77–79]. Our study showed that *P. multivora* was more likely to occur alongside *P. cinnamomi* than by chance this could be due to the similarities in their mechanisms for dispersal, broad host range and tolerance of drier environments. While *P. cinnamomi* is well established as a pathogen in different forest ecosystems across the world, *P. multivora* is emerging as an important pathogen to holm oak (*Quercus ilex*) in Italy [5]. Both species pose a threat to kauri and potentially other plant species which are often uniquely associated with kauri under future climate change conditions. Understanding the roles *P. cinnamomi* and *P. multivora* play in kauri dieback is important as they are already present in areas where *P. agathidicida* is not (yet) and they were each isolated from symptomatic trees in the absence of *P. agathidicida*. Understanding if either *P. cinnamomi* or *P. multivora* are antagonistic to *P. agathidicida* infection or whether the presence of either predisposes a

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kauri tree to infection by *P. agathidicida* is important for understanding the latency of kauri dieback symptom expression. Current work is underway to investigate alternate hosts of *Phytophthora* species in kauri forests and will help to determine the roles of *P. cinnamomi* and *P. multivora* in these systems. Future research should investigate the epidemiology of both of these co-occurring species to determine if they are synergistic, antagonistic or have no effect on *P. agathidicida*.

The distribution of the three main species around the forest points to different invasion histories and establishment methods. *P. cinnamomi* has likely been present for the longest time with its spread and establishment facilitated by its broad host range [80]. *Phytophthora cinnamomi* was isolated from kauri prior to 1959 [76]. Given its extensive distribution, it is likely that the plant community has already been impacted severely with any species highly sensitive to *P. cinnamomi* already affected. *P. cinnamomi* is frequently isolated from asymptomatic kauri which would also help in its spread. In contrast, *P. agathidicida* and *P. multivora* appear to be invading from the periphery of the park and are likely newer incursions into the forest [19].

During a stream baiting survey in 2011 in which *P. cinnamomi* and *P. agathidicida* were not detected, *P. multivora* was detected in all five catchments sampled (Cascades A and B, Piha A and B and Nihotapu) [29]. In the current study, approximately 10% of the samples from Piha A and B catchments (as described in Randall [29]) were positive for *P. multivora*. However, in contrast all of the samples collected in the Cascades A (n = 35) and B (n = 10) catchments, were negative for *P. multivora*. The Nihotapu catchment represents a central area of the forest: only two of our samples fell within the catchment area marked in the Randall (2011) study and both were negative for *P. multivora*. Nevertheless, the positive detections during the stream baiting survey (Randall 2011) suggest *P. multivora* inoculum levels are relatively high and so positive trees may have been missed during the current survey.

Of the four other *Phytophthora* species detected in the survey, *P. kernoviae* and *P. pseudocryptogea* have previously been detected in kauri forests [17,18]. The pathogenicity of *P. kernoviae* to kauri has not been tested, though it is likely native to NZ and does not pose a threat to most native plants [67]. *Phytophthora pseudocryptogea* can infect the feeder roots of kauri and cause damage like *P. multivora* and *P. cinnamomi,* however none were able to kill kauri seedlings alone during glasshouse inoculations [33]. The potentially unknown clade 7 *P. europaea*-like *Phytophthora* species detected in the ITS metabarcoding was later confirmed with RPS10 sequencing as being similar to *P. europaea*. To confirm if this is a new species, isolates need to be cultured so their morphology can be compared to other species and their DNA extracted and sequenced. This was not achieved in this study and is a focus for ongoing work.

This study showed evidence through the qPCR of the mock communities that the enrichment process by nested PCR for metabarcoding makes them useful for a qualitative but not quantitative description of *Phytophthora* communities, supporting the results of Burgess, *et al.* [81]. Legeay, Husson, Cordier, Vacher, Marcais and Buée [50], the designers of the primers also concluded this based solely on the read counts in mock communities. In our study, the qPCR assay allowed for a read count cutoff decision that was less subjective than the usual method basing this on the sequencing output alone.

The metabarcoding primers used were poor at detecting *P. multivora* compared to baiting. *Phytophthora multivora* was detected from 63 samples by baiting, of which only one sample was positive with both baiting and metabarcoding. Though five additional samples that were positive only with metabarcoding that would have been missed if only baiting was used. Two ASVs were detected for *P. multivora* (ASV7 was present in the field samples and ASV229 was present in the mock communities) which differed by one base pair. These two 'types' of *P. multivora* were detected during the soil baiting in the current study and during a stream baiting survey in Te Wao Nui o Tiriwa / Waitākere Ranges [29].

It is unknown if the primers have lower affinity for *P. multivora,* in the 50 bp ahead of the primer binding sites for the oom18S primer the GC content is 40% so there should not be a primer binding issue. There may be a masking effect occurring due to the presence of the other species. In the studies which previously used the same ITS metabarcoding primers, *P. multivora* was not present in the

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environment [7] or mock communities [50]. It is quite possible that *P. multivora* has a lower inoculum content in the rhizosphere around kauri and the use of 250 mg of soil for the eDNA extraction limited the detection of *P. multivora* compared to the number of positives found by baiting 100 g soil. The ecological role of *P. multivora* including its invasion history within NZ forests is not well understood and warrants further research.

This study focused on the diversity of *Phytophthora* species found in the rhizosphere of symptomatic and asymptomatic kauri. It is likely that there are more *Phytophthora* species present in the wider forest associated with other plant species. The stream baiting survey by Randall [29] found four additional species to the current study including *P. aspargia*, *P. gonapodyoides* and *P. chlamydospora* and an unknown *P. sp* "Waitākere". A more extensive survey of alternative host *Phytophthora* diversity with respect to the plant species across the various substrates (water, soil, leaves, and roots) would be needed to uncover the full diversity of *Phytophthora* in Te Wao Nui o Tiriwa / Waitākere Ranges. The baiting assay is optimised for *P. agathidicida,* however by sampling seasonally and increasing the diversity of plants used for baits, a higher richness of *Phytophthora* species may have been detected [82].

The results of the current study supports previous surveys that showed using both metabarcoding and isolation techniques alongside each other yields greater information about *Phytophthora* species [9]. One key disadvantage of metabarcoding is the small amount of soil from which DNA is extracted;, this is likely why there were fewer positive detections across samples with the sequencing compared to the baiting in the current study. This may be improved with increased replication or extract from bulk soil samples but these can be cost prohibitive. The soil DNA extraction kit used only allows for high-throughput, standardised extractions based on small samples. Metabarcoding has the key advantage of being able to detect more species in a sample, especially those that are difficult to isolate (such as the unknown clade 7 species detected here).

Routine monitoring of environments like Te Wao Nui o Tiriwa / Waitākere Ranges will help inform predictive models and our understanding of the spread of *Phytophthora* inoculum through the environment. When there are enough data, it may also become possible to unravel the invasion histories of different species, paths of introduction and the latency period of kauri dieback disease progression (the time between when trees become infected, exhibit symptoms, and succumb to the disease). All of this will help to inform effective management of kauri dieback and help protect valuable areas, like the centre of Te Wao Nui o Tiriwa / Waitākere Ranges, from becoming infected.

5. Conclusions

Phytophthora agathidicida and *P. multivora* appear to have a limited distribution around the edge of Te Wao Nui o Tiriwa / Waitākere Ranges. In comparison, *P. cinnamomi* is widely distributed throughout the forest, suggesting an earlier invasion history or greater capacity for dispersal. All three species showed a significant relationship with decreasing canopy health of kauri but this relationship was vastly stronger for *P. agathidicida*, confirming it as the main driver of disease. Further analysis into spatial variation of *Phytophthora* species assemblages and historical points of disturbance (such as roads, logging sites and tracks) may help uncover the different invasion histories of the *Phytophthora* community in the Ranges. The use of metabarcoding alongside baiting complimented each other well and should be used in future surveys. This survey of Te Wao Nui o Tiriwa / Waitākere Ranges provides hope that large areas in the centre of the forest may still be protected from the devastating disease that is kauri dieback.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. **Figure S1:** Relationship between the DNA concentration (log femtograms) of the spiked mock community samples (for the ITS metabarcoding) and the cycle threshold (Cq) values in the multiplexed qPCR validation for *Phytophthora agathidicida* and *P. cinnamomi*. 'Mock DNA samples' shows the Cq values of the neat spiked eDNA samples as template and 'Oom PCR template' shows the Cq values when 1:100,000 dilutions of the oomycete specific PCR product (amplified with ITS primers oom18s/ITS7 for the primary metabarcoding PCR) was used as template in a qPCR.; **Figure S2.** Pairwise co-occurrence matrix for the *Phytophthora* species detected in the current study. Orange and blue tiles correspond to species pairs that were less or more likely to co-occur than predicted by a null model. Created in R with the package cooccur version

 doi:10.20944/preprints202403.0643.v1

1.3.; **Table S1:** Sequence read counts from the ITS metabarcoding for the 30 soil samples which were selected for amplification with the nested RPS10 metabarcoding approach.; **Table S1:** Sequence read counts from the RPS10 metabarcoding for the 30 soil samples which were selected for amplification with the nested RPS10 metabarcoding approach.

Author Contributions: Conceptualization, S.H, N.Wi and I.H.; methodology, S.H, I.H, and N.Wi.; validation, J.H and E.C.; formal analysis, S.H and P.S.; investigation, S.H, J.H, E.C, M.A, I.H and J.N.; resources, I.H, N.Wi, and N.Wa.; data curation, S.H.; writing—original draft preparation, S.H.; writing—review and editing, S.H, P.S, B.B, and N.W.; visualization, S.H.; supervision, N.Wi, N.Wa, B.B and P.S.; project administration, I.H and N.W.; funding acquisition, N.Wi and N.Wa. All authors have read and agreed to the published version of the manuscript.

Funding: Auckland Council funded the primary analysis of soils by baiting. Funding for soil curation and isolate sequencing was provided through the Ngā Rākau Taketake program of the New Zealand National Science Challenge. Funding for eDNA analysis was contributed from the George Mason Centre for the Natural Environment at the University of Auckland. This work was funded by the New Zealand Ministry of Business, Innovation and Employment (Ngā Rākau Taketake – Myrtle Rust and Kauri Dieback Research, C09X1817).

Data Availability Statement: The data will be made available at the following data repository https://data.bioheritage.nz/ with a reference DOI once the paper is accepted for publication.

Acknowledgments: The authors wish to acknowledge and thank Te Kawerau ā Maki whose rohe this research was carried out in and for their support of this research. The surveillance program was carried out by Auckland and the team from BioSense and we thank Yue-Chin Chew and Lee Hill and their teams for their tremendous efforts in implementing this substantial surveillance program. Preeti Panda is thanked for her advice during the bioinformatic analysis. Indigo Michael and Gavin Lear contributed to the soil DNA extraction efforts and kit costs.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Individual ASVs from the ITS sequencing which had more than 3000 reads. Sequences were trimmed to the start of the ITS1 gene and compared to the reference database from Treen Burgess in Geneious Prime v.2022.0.1. Full length sequences were compared to the Whole Genome Shotgun contigs (wgs) database in NCBI.

Sample	ASV	Reads	Database	Similarity	Query cover	Most similar species
5359	24	24937	ITS1 ref	100%	100%	*Phytophthora*_AUS_1A_KY110340
			wgs (whole seq)	93.36%	100%	*Phytophthora taxon totara*
5480	26	19767	ITS1 ref	99.50%	100%	*Phytophthora* sp. in clade 12A
			wgs (whole seq)	99.05%	100%	*P. tubulina*
5129	81	3105	ITS1 ref	100%	100%	*P. gregata/gibbosa/gonapodyides*
			wgs (whole seq)	98.84%	100%	*P. chlamydospora*

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