Phytophthora species assemblages in kauri forests:
Detected by isolating cultures and through metabarcoding

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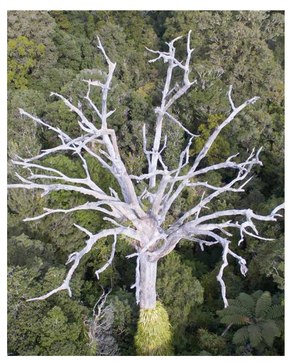
Introduction

- The endemic, foundation tree species *Agathis australis* (kauri) is under threat from *Phytophthora agathidicida* in New Zealand (Waipara et al., 2013).
- This root-rot pathogen is widespread in fragmented forest remnants and causes basal resin 'bleeds', canopy thinning and eventually tree death (Bradshaw et al., 2020).
- Several *Phytophthora* species have been isolated from the rhizosphere of declining kauri during previous routine surveys including *P. cinnamomi*, *P. multivora*, and *P. pseudocryptogea* (Waipara et al., 2013).
- We aimed to characterise the *Phytophthora* species assemblages from healthy and symptomatic kauri in the Waitākere Ranges Regional Park, Auckland, New Zealand.

Healthy kauri



Dead kauri

Methods

- Tree health was surveyed with rhizosphere samples collected from 767 of randomly selected kauri trees across 17,000 ha of the Waitākere Ranges Regional Park.
- *Phytophthora* cultures were isolated using soil baiting and identified morphologically and with DNA sequencing.
- Environmental DNA was extracted and ITS1 gene amplified with *Phytophthora* specific primers for high-throughput-sequencing metabarcoding (Legeay et al., 2019).

Results

- The *Phytophthora* community was relatively small (n = 7; Table 1).
- Three key invasive species were detected (*P. agathidicida, P. cinnamomi* and *P. multivora;* Fig 1).
- *P. agathidicida* was more likely to be detected in trees with **higher canopy scores** and closer to **historic timber mill sites** (Fig 2).
- *P. cinnamomi* and *P. multivora* were more likely to occur together than by chance alone (pairwise cooccurrence matrix, 5.3%).
- Interestingly, a potentially unknown *Phytophthora* species in clade 7 (similar to *P. 'cadmea'/tyrrhenica/uliginosa/europeae/abietivora*) was detected in 20 samples with metabarcoding. These samples will be retested with primers targeting different gene regions.

Figure 1. Distribution of the three main species *Phytophthora agathidicida* (pink), *P. multivora* (purple) and *P. cinnamomi* (khaki) across the Waitakere Ranges, NZ. Small dots were sampled and negative for the *Phytophthora* species in the relative frame.

Table 1. *Phytophthora* species (n = 7) detected by metabarcoding and soil baiting from *Agathis australis* rhizosphere samples in the Waitākere Ranges Regional Park, NZ.

Species	Clade	Metabarcoding	Baiting	Total detections	Total detections (%)	Status
P. cinnamomi	7	231	404	455	59.3	Introduced
P. agathidicida	5	44	79	84	11.0	Introduced
P. multivora	2	6	63	68	8.9	Introduced
P. sp (Clade 7)	7	20	0	20	2.6	Unknown
P. cactorum/aleatoria	1	4	0	4	0.5	Introduced
P. pseudocryptogea	8	2	1	2	0.3	Introduced
P. kernoviae	10b	2	0	2	0.3	Native?

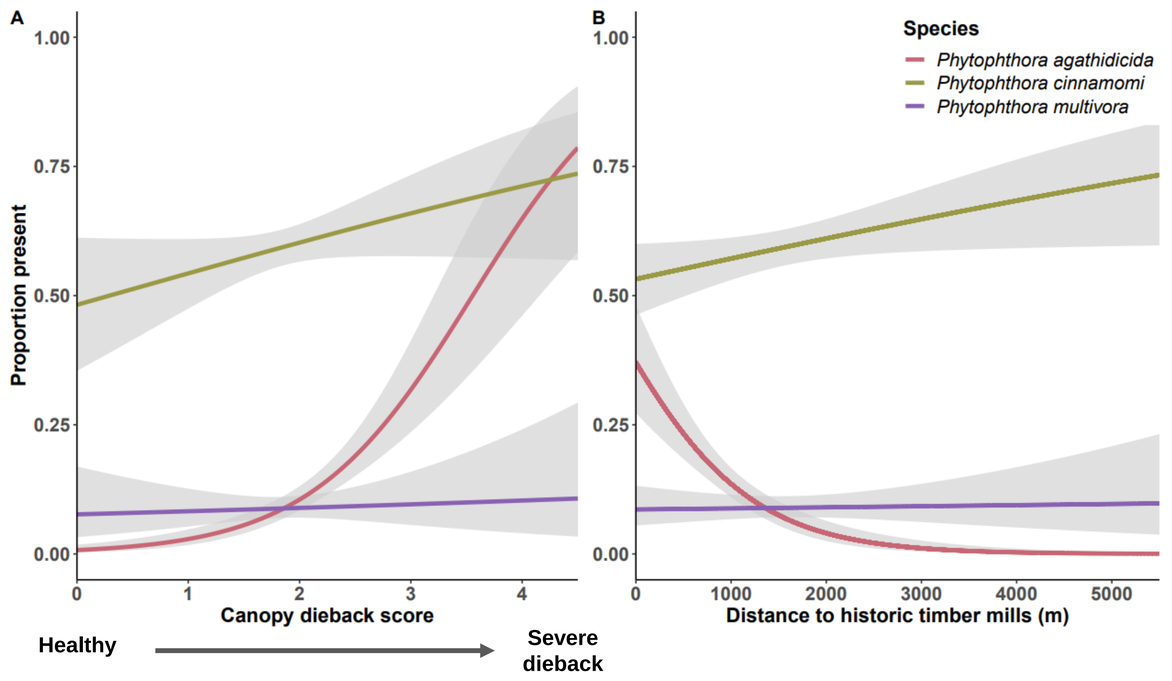


Figure 2. Proportion presence of *Phytophthora agathidicida* (pink), *P. cinnamomi* (khaki) and *P. multivora* (purple) detected through isolation with soil baiting and high-throughput-sequencing metabarcoding in relation to (**A**) canopy dieback scores and (**B**) distance to historic timber mill sites. Lines represent binomial generalised linear model fits and grey areas indicate the 95% confidence intervals.

Conclusions

- Surprisingly low diversity of *Phytophthora* species compared to surveys in temperate forests overseas.
- The two detection methods complimented each other well and were useful for a qualitative description of detected species.
- *Phytophthora agathidicida* was the main driver of disease.
- More research is needed to understand the role of *P. cinnamomi* and *P. multivora* in kauri dieback disease and to explore the relationship between *P. agathidicida* and historic logging sites.

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