Persistence and degradation of Phytophthora cinnamomi DNA and RNA in different soil types

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Abstract
DNA and RNA detected in soil using molecular techniques may originate from a living or dead organism. It is therefore of interest to know how long the DNA and RNA from a decaying organism can persist in soil, and how environmental conditions such as soil temperature, moisture, and microbial populations impact on the survival time. This study determined the difference between the persistence of Phytophthora cinnamomi mRNA and DNA in different soil types. DNA and RNA were extracted from P. cinnamomi and 10 ng/250 mg of soil was applied to five different soil types that were either air-dried or maintained at 70% field capacity. The persistence of DNA at 20°C was tested after intervals of 0, 3, 7, 14, 90, 241, and 378 days, and for RNA at 0, 1, 3, and 7 days using qPCR and RT-qPCR techniques, respectively. Persistence was longer in dry than moist soil, P. cinnamomi DNA could be readily detected in dry soil conditions for up to 90 days and was found at extremely low levels at 241 and 378 days. RNA was detected only on day 1, except for dry river sand, and moist sandy loam in which it persisted for 3 days; it was not detected after seven days. These results confirm that RNA degrades very quickly, making it a valuable tool for determining the presence of viable Phytophthora in soil. In contrast, DNA can be remarkably stable in some environments, and positive results could be obtained even after the death of the organism for a year or more prior to the test. For diagnostics, the use of an RNA-based test avoids the possibility of such false positive results. In the context of the research project, this study is relevant to determining how long viable Phytophthora remains in soil after the eradication protocols have been instigated. In a broader context, the persistence of DNA is relevant to any study using environmental DNA for diagnostics or for metabarcoding when undertaking community ecology or microbiome studies. These results are relevant for studies using detection of P. cinnamomi nucleic acids in soils for purposes of diagnostics, ecological research, or projects on eradication.

Keywords
diagnostics, eDNA, humic acid, microbial DNase, qPCR assay, survival

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**1 | INTRODUCTION**

*Phytophthora cinnamomi* is a global, broad host range invasive soil-borne plant pathogen (Burgess et al., 2017). It is the cause of root and collar rot in many woody plant species resulting in a disease known as *Phytophthora* dieback (Colquhoun & Hardy, 2000). This disease can cause significant economic losses in production of avocado, pineapple, peach, chestnut, and macadamia (Hardham, 2005; Scott, Burgess, Hardy, & Lamour, 2013). For example, Coffey (1992) reported that in California, *P. cinnamomi* was affecting 60–70% of avocado trees causing losses in excess of US$40 million annually. The forests of the south-west of Western Australia are particularly vulnerable to this disease (Hardy, Barrett, & Shearer, 2001; Shearer, Crane, Barrett, & Cochrane, 2007), and for 40 years, there has been an extensive program to delineate areas of the forest infested or free of the disease in order to restrict its spread (Shearer et al., 2007). Alcoa of Australia conducts open-cut bauxite mining in this forest and spends approximately AUS$7 million annually on mitigating the spread and impact of this pathogen (Colquhoun & Hardy, 2000). For these reasons, there is a high demand by industry and land managers for accurate, consistent, and cost-effective diagnostic tools for the detection of *P. cinnamomi*.

The genus *Phytophthora* belongs to a group of microorganisms commonly referred to as water molds, but they are not fungi and are placed in the kingdom, Stramenopila (Beakes, Glockling, & Sekimoto, 2012). Taxonomic classification of *Phytophthora* has traditionally been based on morphological features (Waterhouse, 1963). These features can be ambiguous, variable, and require painstaking and time-consuming culture and microscopic observations. Also, because different species can share the same morphological features, they cannot be used alone for diagnostics. All species descriptions since 2000 have included a molecular phylogeny to support the species description (Blair, Coffey, Park, Geiser, & Kang, 2008; Brasier, 2009) and diagnostics is almost always based on sequencing of gene regions such as ITS and cox1.

There were 60 described *Phytophthora* species in 1996 (Erwin & Ribeiro, 1996), but since then more than 70 species have been described following the application of molecular tools (Yang, Tyler, & Hong, 2017), and the molecular phylogeny divides *Phytophthora* species into 11 clades (Yang et al., 2017). *Phytophthora* species have been shown to be prevalent in natural ecosystems as well as in horticulture.

In detection, baiting of a flooded soil sample is the usual technique to determine the presence or absence of *P. cinnamomi* in a soil. This method has limitations, and false negatives are common (Hüberli, Tommerup, & Hardy, 2000; McDougall, Hardy, & Hobb, 2002). Alternatively, *P. cinnamomi* can be detected directly from soil or plant material using species-specific primers. However, until recently the primers used in such studies have not been sufficiently specific for *P. cinnamomi*, giving rise to possible confusion of the results with other *Phytophthora* species (Kunadiya et al., 2017).

There is however an additional problem in using DNA-based molecular detection methods. DNA can persist in soil and host tissues after the death of the organism (Greaves & Wilson, 1970; Paget, Monrozier, & Simonet, 1992; Recorbet, Picard, Normand, & Simonet, 1993; Romanowski, Lorenz, & Wackernagel, 1993), and simply identifying the presence of the DNA does not distinguish between signals originated from viable cells or those released from dead cells (Scheu, Berghof, & Stahl, 1998). An alternative is to use an RNA-based approach, as RNA is only produced by living organisms (Sheridan, Masters, Shallcross, & Mackey, 1998). RNA can be used as a viability marker as it degrades rapidly in comparison with DNA (Chimento, Cacciola, & Garbelotto, 2012; Vettraino, Sukno, Vannini, & Garbelotto, 2010). Recently, primers have been developed that are not only specific to *P. cinnamomi*, but can also be used to detect RNA, (Kunadiya et al., 2019).

When an organism dies, the DNA and RNA can be released from the cells during lysis (Hebsgaard, Phillips, & Willerslev, 2005; Jakubovics, Shields, Rajarajan, & Burgess, 2013; Nicholls, 2005). Both DNA and RNA are degraded in soil by nuclease-producing microorganisms and the activity of these microorganisms will depend upon soil type, temperature, and moisture availability (Garbeva, Van Veen, & Van Elias, 2004; Greaves & Wilson, 1970; Novitsky, 1986; Paul, Jeffrey, David, DeFlaun, & Cazares, 1989). Early studies showed that pure nucleic acids in soil microcosms were quickly digested by nucleases from pro- or eukaryotic microorganisms with the release of inorganic phosphorus (Blum, Lorenz, & Wackernagel, 1997; Greaves & Wilson, 1970; Keown, O’Callaghan, & Greenfield, 2004). The persistence of DNA in surface soil (0-15cm) and sediment can range from a few days to several years depending on environmental conditions (Boessenkool et al., 2014; Haile et al., 2009; Nielsen, Ray, & Van Elias, 2004). Nucleic acids in the soil solution are more readily enzymatically degraded than those bound to sand or clay (Khanna & Stotzky, 1992; Lorenz & Wackernagel, 1987; Paget et al., 1992; Romanowski, Lorenz, & Wackernagel, 1991). When DNA molecules are adsorbed to soil particles, their properties change based on cations and water molecules adsorbed on the surface. Adsorption of nucleic acids to clay minerals is pH dependent (Khanna & Stotzky, 1992) with adsorption generally increasing when the pH is below 5, and decreasing as pH rises above 5 (Greaves & Wilson, 1969; Khanna & Stotzky, 1992).

Microorganisms are the primary decomposers in soils. The moisture present in soil determines microbial motility and activity, diffusion of nutrients and waste, and the activity of extracellular enzymes. Soil texture and structure control the availability of moisture between soil particles (Carter, Yellowlees, & Tibbett, 2010), and microbial activity depends on the matric potential of the soil (Linn & Doran, 1984). Microbial activity is highest when the matric potential is approximately ~0.01 megapascals (MPa), but declines rapidly as soil dries (Moldrup, Olesen, Rolston, & Yamaguchi, 1997; Papendick & Runkles, 1965). There are many factors influencing movement of organisms in soil, but larger organisms are more restricted than smaller ones. Bacteria are unable to cross air-filled pores on their own; however, studies have shown that some motile bacteria can move along fungal hyphae (Kohlmeier et al., 2005; Warmink, Nazir, Corten, & Van Elias, 2011; Warmink & Van Elias, 2009).
As mentioned above, a situation in which an accurate detection method for *P. cinnamomi* in soil would be of great value comes from the bauxite mines in the jarrah (*Eucalyptus marginata*) forest of Western Australia. The open-cut mining in both areas free or infested with *Phytophthora* requires the construction of haul roads preferably comprised of gravel free of the pathogen to ensure that it is not spread by vehicles during movement of the ore (Colquhoun & Hardy, 2000). Logistical constraints have made it necessary to make roads with materials taken from infested sites. When such haul roads are being rehabilitated, they are now left unused and free of plant growth for approximately 3 years. This “fallowing” is expected to eliminate the pathogen, but proof of this has to be shown both by baiting, and also using molecular diagnostics. Information was thus required on how long *P. cinnamomi* nucleic acids remain detectable in different soil types and could result in a positive detection using molecular methods, but actually be due the presence of remnant DNA, rather than the viable organism.

Therefore, the current work investigated the length of time *P. cinnamomi* DNA and RNA can survive in different soil types when dry or moist. The results of this study are particularly relevant for the assessment of success of various eradication programs.

2 | METHODS

2.1 | Soil collection, characterization, and inoculation of soil samples

Soil samples were collected from five different *P. cinnamomi*-free sites to a depth of 10 cm including a Jarrah Forest silty loam 32.157340°S 116.047446°E (Armadale, Darling Plateau); a sandy soil from a Jarrah Forest riparian zone 32.140710°S 116.047265°E (Armadale, Darling Plateau), swamp peaty sand 32.071502°S 115.834876°E (Chelodina Swamp, Murdoch, Swan Coastal Plain); Banksia Woodland sand 32.074433°S 115.834733E (Murdoch University Bushland Reserve, Swan Coastal Plain); and a river sand (Mundaring region in the Perth Hills). The five soils were sieved through a 2 mm screen to remove plant roots or leaves and debris before the soil analyses (for Ca, S, C, N, Mg, P, K, Fe, Al, Na, and Zn), and then air-dried for one week, weighed, and stored at room temperature for further analysis.

Analyses of the physical and chemical characteristics of the soils were all performed by CSBP (Soil and Plant Analysis Laboratory, Bibra Lake Western Australia 6163) using standard methods. DNA extractions and PCR were performed on the five soil samples to confirm their *P. cinnamomi*-free status using a nested PCR with cox2F and cox2RC4 primers, used in the first round, and the *P. cinnamomi*-specific qPCR assay PCINS in the second round (Kunadiya et al., 2019).

2.2 | Gravimetric soil water content

The gravimetric soil water content was determined for each soil type by the microwave drying method. Three replicates of 10 g air dried-soil were placed in Petri plates, weighed, then reweighed after microwaving at 2,450 MHz (Routledge & Sabey, 1976). The water content of the sample was calculated as the mass of water per mass of dried soil and is expressed as \( m = M_w/(M_s) \), where \( m \) is the water content on a dry weight soil, \( M_w \) is the mass of water contained in the sample, and \( M_s \) is the mass of the soil sample used.

2.3 | Field capacity of the soil

Samples of each of the five soil types were placed in small free-draining plastic pots (1 l), and enough water added to saturate the whole soil, then the pots were allowed to stand for 48 hr to be free drained gravitationally. After 48 hr, samples were taken from the center of the moist soil and the water content calculated using the above microwave drying method for 10 min. The water content was determined using \( Q_g = \text{mass of water/mass of microwaved soil dry weight} \times 100 \). The mass of water equals the mass of moist soil minus the mass of dry soil.

2.4 | Fluorescein diacetate hydrolytic activity assay

Fluorescein diacetate (FDA) hydrolysis was used to determine the total microbial activity present in soil samples (Adam & Duncan, 2001). All the reagents were prepared according to (Adam & Duncan, 2001).

The standards against which to compare the fluorescein released during soil assays were prepared using concentrations from 0 to 7 \( \mu \)g fluorescein which cover the normal range of FDA activity in soil (Adam & Duncan, 2001; Battin, 1997; Green, Stott, & Diack, 2006). The standards were measured at 490nm wavelength on the spectrophotometer (UV-VIS mini 1,240 spectrophotometer, Shimadzu, Europe). All soil samples were run in triplicate using controls without substrate, and the mean, standard deviation, and coefficient of variation were determined. The enzyme activity was reported as \( \mu \)g of product formed g\(^{-1}\) dry weight of soil per hour.

2.5 | Metabarcoding of prokaryotes in soil

Illumina sequencing was performed on Illumina MiSeq platform, utilizing Illumina's Nextera XT v2 Indices and paired-end sequencing chemistry, and Illumina paired-end reads were used to analyze microbial communities by targeting amplicons of the 16S rRNA gene. The purified DNA templates isolated from each of the five soils were amplified using universal prokaryotes primers 341F-CCTAYGGGRBGCASCAG and 806R-GGACTACNGGGTATCTAAT (Bates et al., 2011), 341F-806R targets the variable region V4 bacteria and archaeal 16S rRNA genes. The expected length of the amplification product was 300bp. Amplicon library generation, quantification, and Illumina sequencing run of extracted DNA were performed by the Australian Genome Research Facility (VIC, Australia).
Paired ends reads were assembled by aligning the forward and reverse reads using PEAR1 (version 0.9.5). Primers were identified and trimmed. Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8.4) USEARCH2.3 (version 8.0.1623) and UPARSE software. Using USEARCH tools, sequences were quality filtered, full length duplicate sequences were removed and sorted by abundance. Singletons or unique reads in the data set were discarded. Sequences were clustered followed by chimera filtering using the “rdp_gold” database as a reference. To obtain number of reads for each of the operational taxonomic units (OTUs), reads were mapped back to OTUs with a minimum identity of 97%. Using QIIME, taxonomy was assigned using Green genes database (DeSantis et al., 2006).

DNA and RNA extraction and amplification

The *P. cinnamomi* isolate MP94-48 from the Phytophthora Science and Management culture collection at Murdoch University was used to determine the persistence and viability of DNA and RNA in the five soil types. It was grown on half strength potato dextrose agar (Difco, Becton Dickson) at 20°C for 2 weeks in the dark. Genomic DNA was extracted from the mycelium using a ZR Fungal/Bacterial DNA Mini prep kit (Zymo Research), following the manufacturer’s instructions. The DNA concentrations were determined by a Qubit® 2.0 Fluorometer (Invitrogen, Life Technology). Extracted DNA was stored in DNA elution buffer at −20°C.

Total RNA was extracted from 50 mg of mycelium with the Power Plant RNA isolation kit (MO BIO Laboratories), according to the manufacturer’s instructions. Total RNA was further treated with the DNA-free ™DNase treatment kit (Ambion, Inc.) to remove any genomic DNA contamination, according to the manufacturer’s instructions. Total RNA concentration was determined by a Qubit® 2.0 Fluorometer (Invitrogen, Life Technology). Extracted RNA was stored at −80°C for further analysis. There were three replicates of each set of dry and wet soil types. The RNA experiment was repeated once.

Nested real-time PCR was performed using DNA and cDNA from extracted soil samples using cox2F and cox2RC4 primers (mitochondrial locus encoding subunit 2 of cytochrome c oxidase) used in the first round, and the *P. cinnamomi*-specific qPCR assay PCINS in the second round (Kunadiya et al., 2019).

### RESULTS

#### 3.1 Characterization of the soil physical and chemical properties

Clay content ranged from 1.93% to 22.25% and was highest in the silty loam soil. Silt content ranged from < 0.01 to 5.07% and was also highest in the silty loam. Coarse sand ranged from 55.09% to 95.33% and was highest in the river sand. Fine sand ranged from 2.74% to 29.02% and was much higher in sandy loam and silty loam compared to the other soils (Table 1). Organic carbon content ranged from 0.08% to 4.15% and was highest in the peaty soil (Table 1).

Exchangeable sodium content ranged from 0.01 to 0.47 meq/100 g (milli-equivalents per 100 gram of soil), potassium content from 0.01 to 0.22 meq/100 g, calcium content from 0.05 to 6.01 meq/100 g, iron content from 3.12 to 133.73 mg/kg, and all were highest in the silty loam. Magnesium ranged from 0.020 to 4.47 meq/100 g and was highest in the peaty soil. Aluminum content ranged from 0.058 to 0.581 meq/100 g and highest in the sandy loam (Table 1). Available potassium content ranged from 15 to 87 mg/kg, boron content ranged from 0.1 to 0.98 mg/kg, manganese content ranged from 0.16 to 7.56 mg/kg and all were highest in the silty loam. Zinc content ranged from 0.16 to 4.46 mg/kg, ammonium nitrogen content ranged from 1 to 32 mg/kg, sulfur content ranged from 0.9 to 29.8 mg/kg and all were highest in the peaty soil. Copper content ranged from 0.21 to 1.40 mg/kg and was highest in the sandy loam (Table 1).
Characterization of biological properties

Based on the FDA assay, there were no significant differences in microbial activity between the soil types (Table 2). The microbial composition, however, was very different between the soil types (Figure 1). Proteobacteria and Actinobacteria accounted for 36.8 and 29.9%, respectively, of the total reads and were evenly distributed between soil types. Acidobacteria, Chloroflexi, and Firmicutes

### TABLE 1 Physical and chemical properties of the five soil types (0–10 cm depth)

| Soil type     | Color       | Texture | Organic carbon | Gravel | Clay | Silt | Course sand | Fine sand | Sand |
|---------------|-------------|---------|----------------|--------|------|------|-------------|-----------|------|
| Sandy loam    | Brown–gray  | 1.0     | 1.28           | 0      | 8.0  | 5.0  | 57.95       | 29.02     | 86.97|
| Silty loam    | Gray        | 2.0     | 2.68           | 0      | 22.25| 5.07 | 55.09       | 17.59     | 72.68|
| Peaty soil    | Gray        | 1.0     | 4.15           | 0      | 5.99 | 3.02 | 85.52       | 5.46      | 90.98|
| Sand          | Gray        | 1.0     | 0.71           | 0      | 2.91 | 1.97 | 91.42       | 3.70      | 95.12|
| River sand    | Brown–white | 1.0     | 0.08           | 1.93   | <0.01| 95.33| 2.74        | 98.07     |      |

### TABLE 2 Gravimetric water contents, field capacity, and fluorescein diacetate activity of the five soils

| Soil type     | Gravimetric water content at field capacity (g) | Amount of water in 10g of soil at field capacity (g) | Amount of water in 10 g soil at 70% field capacity (g) | Fluorescein diacetate hydrolysis (mg/kg) |
|---------------|-----------------------------------------------|-----------------------------------------------------|------------------------------------------------------|----------------------------------------|
|               | Mean | SE   | Mean | SE   | Mean | SE | Mean | SE | Mean | SE |
| Sandy loam    | 0.63 | 0.013| 1.69 | 0.057| 1.187| 0.104| 0.0010|    |      |    |
| Silty loam    | 0.72 | 0.020| 3.00 | 0.004| 2.104| 0.122| 0.0008|    |      |    |
| Peaty soil    | 1.31 | 0.010| 2.95 | 0.007| 2.069| 0.111| 0.0002|    |      |    |
| Sand          | 0.34 | 0.001| 1.72 | 0.043| 1.207| 0.105| 0.0011|    |      |    |
| River sand    | 0.15 | 0.004| 2.06 | 0.002| 1.442| 0.109| 0.0003|    |      |    |
| P value       | <0.05| <0.05|      |      |      |      | <0.05|    |      |    |

3.2 Characterization of biological properties

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accounted for 12.7, 4.9, and 3.4% of total reads, respectively, and were under-represented in the sand. There was a high degree of variability in the relative proportion of all the remaining bacterial phyla with some being completely absent for some soil types (Figure 1).

3.3 | Persistence of DNA

Overall, the \emph{P. cinnamomi} DNA persisted longer in all of the dry soils, although there was a large variation between soil types (Figure 2). The half-life of DNA persistence was similar for dry peaty soil (250 days) and silty loam (200 days) and was higher than other soil types which were 60, 60, and 10 days for dry sandy loam, sand, and river sand, respectively (Figure 2). \emph{P. cinnamomi} DNA degradation was much higher in all moist soil types. The half-life of DNA persistence was 7, 5, 3, 3, and 2 days, respectively, for moist sandy loam, river sand, sand, silty loam, and peaty soil. DNA persisted up to 90 days in moist soil of all types, except for the sandy loam where it was negligible by day 14. In sandy loam, silty loam and peaty soil DNA persisted for up to 378 days or more in dry conditions, while in sand and river sand, it survived up to days 241 and then rapidly degraded and was not detectable on day 378 (Figure 2) (Table S1).

3.4 | Persistence of RNA

RNA was rapidly degraded in soil in both experiments and could not be detected at day 7 for any soils either moist or dry (Table 3). RNA degradation was so rapid in moist silty loam that it could not even be recovered when extraction occurred within ~ 30 min of the RNA being added to the soil. After 1 day, it could only be recovered from two of the moist soils, sandy loam, and river sand, and on day 3, in one experiment, it could still be detected in moist sandy loam (Table 3). RNA was detected on day 1 for all dry soils except sandy loam, and on day 3, it could only be detected in dry river sand.

4 | DISCUSSION

Naked \emph{P. cinnamomi} DNA can persist in soil for more than 378 days, and thus a positive detection using molecular methods (in absence of a living host) is not necessarily indicative of the presence of living \emph{P. cinnamomi}. In contrast, naked \emph{P. cinnamomi} RNA could be detected in soil for only 3 days or less after inoculation in moist and dry soil, making it an excellent indicator of viable \emph{P. cinnamomi}.

Both abiotic and biotic characteristics of soil are important in determining DNA and RNA longevity (Garbeva et al., 2004). In the

**FIGURE 1** Relative abundance of operational taxonomic unit (OUT’s) assigned to different prokaryote phyla across the five soil types. The percentage total number of reads for each phylum is given at the top of the column.
present study, conducted a temperature at 20°C, soil moisture was shown to be an important factor for nucleic acid longevity, as overall survival was much longer in dry rather than moist soil. Soil moisture allows microbes to function, and it can be assumed that the rapid degradation of DNA in moist soil is due to microbial activity. The considerable differences observed in DNA persistence between the different dry soil types may be linked to soil physical properties.

4.1 Biotic factors affecting DNA and RNA persistence

As in the current study, all other similar studies confirm microbial activity increases in moist soil and plays an important role in the degradation of DNA and RNA. England, Lee, and Trevors (1997) assessed *Pseudomonas aureofaciens* DNA over 44 weeks in autoclaved and nonautoclaved sandy loam, and after 30 weeks in nonautoclaved soil, *P. aureofaciens* was not detectable by viable plating, while it was detectable over 44 weeks in autoclaved soil. This increased activity in moist soil is due to two factors. Firstly, moist soils may support higher populations of microbes, and secondly, in moist conditions, more DNA may remain in solution rather than in a bound form on the soil surface, and DNA thereby becomes available for DNase degradation by microorganisms (Greaves & Wilson, 1970; Romanowski, Lorenz, Sayler, & Wackernagel, 1992; Romanowski et al., 1993). Microbial functions associated with soil were also observed to be significantly different between summer dry and winter moist conditions (Kuffner et al., 2012).

Many soil microbes produce the DNase enzyme, which degrades nucleic acids in the soil (Blum et al., 1997). Upon hydration of dry soil, an increase in the growth of prokaryotes was associated with increased DNase activity in the interstitial soil solution (Blum et al., 1997). Mostly bacteria use DNA fragments, nucleotides and nucleosides for their growth (Benedik & Strych, 1998; Finkel & Kolter, 2001), and also release nucleases into their surrounding environment for the primary purpose of scavenging for resources (Benedik & Strych, 1998). In the current study, there were only small differences between the soil types in the total microbial activity in moist soils. The same groups of microbes were present in similar abundance in all soil types, and the degradation rate in moist soil was similar for all soil types.

4.2 Influence of soil type on microbial degradation

Many studies have shown that nucleic acids can persist in soil for a significant amount of time, especially when the clay content is very high (Paget et al., 1992; Pietramellara et al., 2009; Widmer, Seidler,
When DNA is complexed with clay minerals, it provides a niche that protects DNA from DNase activity, and thus increases the time DNA persists in the soil (Demanèche, Jocteur-Monrozier, Quiquampoix, & Simonet, 2001; Khanna & Stotzky, 1992). In the present study, silty loam had the highest clay content which helps explain why DNA persisted for so long in this soil type. Not all clay minerals have equal DNA binding capacity; for example, montmorillonite adsorbs a higher amount of DNA than kaolinite (Khanna & Stotzky, 1992; Pietramellara et al., 2009). However, in general the capacity of clay to bind DNA is a hundred-fold greater than that of sand (Blum et al., 1997). Adsorption of nucleic acid to soil is also affected by other soil minerals and ions such as Na\(^{+}\), Mg\(^{2+}\), and Ca\(^{2+}\) which are responsible for DNA immobilization and higher DNA adsorption in clay soil (Paget et al., 1992). These elements were higher in the silty loam and peaty soil and were likely responsible for the extended DNA longevity in them. Mineral complexes with DNA also protect the nucleic acids by shielding certain bonds from chemicals (Ferris, 2005; Keil, Montluçon, Prahl, & Hedges, 1994), enzymatic hydrolysis (Lorenz & Wackernagel, 1994), or UV radiation (Scappini et al., 2004).

Degradation of DNA is faster in the soil solution than when the molecules are absorbed from the soil solution onto soil minerals or particles. Several studies have given similar results to those obtained here showing much faster loss of DNA from wet than dry soils. Extracellular DNA can be readily used by soil prokaryotes. A study found that when eDNA was consumed by bacteria in soil, a fraction of the eDNA can be resistant to decomposition, particularly when stabilized by soil minerals, and it could accumulate over time (Crecchio & Stotzky, 1998). These authors demonstrated that both chromosomal and plasmid DNA from various strains of *B. subtilis* were rapidly adsorbed and bound on clay minerals and sands; and when it was bound, the surface-active particles in soil and other natural habitats protected the DNA from degradation by DNase I.

### 4.3 Other abiotic factors affecting DNA and RNA persistence

Adsorption increases with increasing cations and decreasing pH (Greeaves & Wilson, 1970; Khanna & Stotzky, 1992; Paget et al., 1992; Romanowski et al., 1992). In the present study, the pH of all soils was above 5 when the H\(_2\)O method was used, while the pH of sandy loam and sand was below 5 when the CaCl\(_2\) method was used, and there was no clear relationship between pH and DNA persistence. Some divalent cations such as Mg\(^{2+}\) and Ca\(^{2+}\) concentrations are known to increase binding of DNA (Lorenz & Wackernagel, 1987; Greaves & Wilson, 1970; Khanna & Stotzky, 1992; Paget et al., 1992; Romanowski et al., 1992).

### Table 3 Persistence of Phytophthora cinnamomi RNA in five different soil types determined by nested qPCR. A 10ng sample of RNA was added to each tube on Day 0. The Day 0 extract was commence immediately after addition of the RNA. The extracted RNA was converted to cDNA prior to the qPCR assay and data are presented as the means of three replicates ± the standard error.

| Soil type     | Moisture | Experiment | Day 0       | Day 1       | Day 3       | Day 7       |
|---------------|----------|------------|-------------|-------------|-------------|-------------|
| Sandy loam    | Dry      | 1          | 2.00 ± 0.115| 0           | 0           | 0           |
|               |          | 2          | 1.90 ± 0.600| 0           | 0           | 0           |
| Silty loam    | Dry      | 1          | 0.60 ± 0.265| 0.015 ± 0.013| 0           | 0           |
|               |          | 2          | 1.27 ± 0.233| 0.160 ± 0.167| 0           | 0           |
| Peaty soil    | Dry      | 1          | 0.64 ± 0.319| 0.300 ± 0.300| 0           | 0           |
|               |          | 2          | 3.40 ± 0.436| 0           | 0           | 0           |
| Sand          | Dry      | 1          | 2.60 ± 0.208| 0.080 ± 0.061| 0           | 0           |
|               |          | 2          | 4.16 ± 0.186| 1.330 ± 0.418| 0           | 0           |
| River sand    | Dry      | 1          | 0.77 ± 0.715| 0.800 ± 0.153| 0.027 ± 0.022| 0           |
|               |          | 2          | 3.70 ± 0.100| 3.300 ± 0.265| 0.020 ± 0.012| 0           |
| Sandy loam    | Moist    | 1          | 1.93 ± 0.433| 0.019 ± 0.016| 0.007 ± 0.007| 0           |
|               |          | 2          | 0.93 ± 0.120| 0.027 ± 0.027| 0           | 0           |
| Silty loam    | Moist    | 1          | 0           | 0           | 0           | 0           |
|               |          | 2          | 0           | 0           | 0           | 0           |
| Peaty soil    | Moist    | 1          | 0.90 ± 0.493| 0           | 0           | 0           |
|               |          | 2          | 4.06 ± 0.167| 0           | 0           | 0           |
| Sand          | Moist    | 1          | 1.76 ± 0.463| 0           | 0           | 0           |
|               |          | 2          | 4.10 ± 0.321| 0           | 0           | 0           |
| River sand    | Moist    | 1          | 2.50 ± 0.153| 0.50 ± 0.153| 0           | 0           |
|               |          | 2          | 4.00 ± 0.153| 2.03 ± 0.273| 0           | 0           |
Paget et al., 1992; Romanowski et al., 1991). The concentration of these elements was higher in the silty loam and peaty soil than the other three soils, and it was in silty loam and peaty soils that DNA persisted for the longest time.

Other studies have shown that DNA can persist in the soil for up to 2–3 years when soils are rich in organic matter and clay particles, which absorb the nucleic acids and thus protect them against enzymatic degradation (Paget, Lebrun, Freyssinet, & Simonet, 1998; Widmer, Seidler, Donegan, & Reed, 1997). Soil organic matter is composed of a variety of organic compounds, among these humic acids are compounds which can be isolated from soil very easily, and which supply a solid surface for DNA adsorption (Crecchio & Stotzky, 1998; Saeki & Sakai, 2009). Organic matter is considered surface-active in soil and provides binding sites and protection for extracellular DNA. Extracellular DNA can bind to mineral particles and humic compounds, which protect the DNA and allow it to persist in sediments for short time periods (29 to 93 days) (Dell’Anno & Corinaldesi, 2004) or in some situations, even thousands of years (Haile et al., 2009; Hofreiter, Mead, Martin, & Poinar, 2003; Jorgensen et al., 2012). Pietramellara, Ascher, Ceccherini, Nannipieri, and Wenderoth (2007) reported that the presence of organic compounds in soil improved the adsorption of the DNA molecules on montmorillonite and kaolinite.

The contribution of soil organic matter to DNA adsorption will vary considerably among different soils, depending on soil particles, organic matter, pH, and clay content. The detailed mechanism(s) of DNA adsorption on organic matter remains unknown (Saeki, Ihyo, Sakai, & Kunito, 2011). Further investigations are required to understand the mechanisms of DNA adsorption to organic matter, and the role of soil microbial community it contains.

4.4 | DNA and RNA persistence

Survival of *P. cinnamomi* RNA was considerably shorter than for DNA, but again dry soils supported longer persistence than moist soil. However, in contrast to DNA, RNA survival was shortest on moist silty loam and degraded in a few hours, while in one of the two experiments, RNA survived longest (3 days) on dry river sand or moist sandy loam. The same soil properties affecting the longevity of DNA are true for RNA. A half-life of less than 3d for *DvSnf7* RNA from genetically modified maize in clay loam and loamy sand has also been reported by Fischer et al. (2017). While, Dubelman et al. (2014) using a range of agricultural soils found half-life of *DvSnf7* RNA ranged from about 14 to 30h, and Tab ata, Takada, Sato, Suzuki, and Suzuki (1993) demonstrated heterotrophic bacteria RNA degraded over 2 days in a freshwater environment. Chimento et al. (2012) showed mRNA was detected only for a week after cultures of *P. ramorum* were killed by rapid lyophilization, while its DNA was detected for 3 months after the same treatment. 235 Escherichia coli rRNA molecules adsorbed on clay minerals have been shown to persist for longer in the presence of a degrading agent (RNase-A), than free rRNA which is not bound or adsorbed to any clay particles on soil (Marco Franchi & Gallori, 2005). Franchi et al. (1999) reported adsorption of bacterial RNA on to clays was rapid and reached a maximum after 90 min of contact, and the adsorption was greater on montmorillonite than on kaolinite clay. In the current study, RNA was degraded very quickly and the time frame of testing (0, 1, 3, and 7 days) may have been too coarse to allow discrimination between rates of degradation in soils with different clay contents.

The survival times reported here for DNA and RNA are likely to be at the upper end of the range possible in the natural world as the soil samples were kept at a constant temperature of 20°C and either dry or at 70% field capacity. In nature, survival times are likely to be less if soil is alternately wet and dry, and there are fluctuations in daily and seasonal temperatures. The current experiment was carried out in the absence of living host plant roots and is relevant to some eradication protocols. However, when living plants are present, the microbial population will be very different in the rhizosphere than in bulk soil and this will influence survival time of nucleic acids in natural ecosystems (Uroz, Buée, Murat, Frey-Klett, & Martin, 2010).

4.5 | *Phytophthora cinnamomi* survival in the environment

When *P. cinnamomi* mycelium and spores die, the released DNA might persist in a soil for at least a year meaning that DNA analysis of soil gives an inflated estimation of the longevity of viable inoculum in the soil. The presence of RNA in the soil in contrast is highly likely to indicate the presence to predict the possibility of viable propagules in the soil. However resting structures such as oospores and chlamydospores may be dormant and not producing enough RNA to be within the detection limits of the various eRNA protocols (Kunadiya et al., 2019). Future experiments are necessary with these propagules in soil to determine if they produce sufficient RNA to be measured.

Several diagnostics tools have been developed that allow detection of *P. cinnamomi* from infested soil, or infected plant tissue or whole plant, but many of these techniques vary in specificity and sensitivity and are still not widely used (Tsao & Ocana, 1969; Zentmyer, Erwin, Bartnicki-Garcia, & Tsao, 1983). They include enzyme-linked immunosorbent assays (Comstock, 1992; Kokoskova & Janse, 2009), serological techniques (Malin, Roth, & Belden, 1983), and single-step lateral flow devices (Tomlinson, Dickinson, & Boonham, 2010). Antibody-based methods are fast, sensitive, and inexpensive, but it may show cross-reactivity with other pathogens resulting in erroneous in identifications (Franken, Zilverentant, Boonekamp, & Schots, 1992). Because of the limitation of conventional techniques, molecular diagnostics have been developed to detect and identify pathogens (Davidson, Wickland, Patterson, Falk, & Rizzo, 2005; Kliejunas, 2010). Dai et al. (2019) developed a loop-mediated isothermal amplification assay based on target gene *Pcinn* 100,006 that can detect 1ng *P. cinnamomi* DNA in a 25ul reaction.
MANUSCRIPT FOR REVIEW

Emeritus Professor Jen McComb reviewed the authors contributed to the manuscript and gave final approval.

The technique for the analysis of soil used here will be most applicable to soils free of large organic debris or living plant roots, such as those prepared for nursery or horticultural use. It is also possible that RNA detection might be a very useful tool after eradication protocols such as for the stockpiled topsoils and overburden soils required for use in the rehabilitation of bauxite mines. Baiting can be used in an initial analysis for the presence of living Phytophthora, but as baiting is known to give false negatives (Hüberli et al., 2000), RNA detection will provide more rigor to any other analyses to determine whether Phytophthora is still alive in the soil. The RNA analysis technique will also have application in testing of soils for the nursery trade and for quarantine purposes. The RNA analysis technique is faster than baiting. However, the effectiveness of such an assay is currently limited (as sample size is relatively small, 50 mg per plant or 250 mg of soil sample) and molecular assay reaction chemicals are expensive (Kunadiya et al., 2019). This contrasts with baiting of soil/ rhizosphere samples of much larger soil volumes (500 g or greater). Further work is needed to reduce the cost of each test before it could be used on a wide scale. Particularly important is for the kits and chemicals to be less costly to reduce the overall expense of an assay.

5 CONCLUSIONS

This experiment demonstrated the large disparity between the survival time in soil of DNA and RNA of P. cinnamomi. Survival of both nucleic acids is affected by soil type and moisture content, but the shorter persistence of RNA makes it a fairer tool for assessing the presence of viable P. cinnamomi. For all studies of the soil microbiome, it is important to remember that an assessment of the DNA content in the soil includes contributions from both living and dead organisms.

AUTHOR CONTRIBUTIONS

TB, GH, and WD supervised the project; MK, TB, GH, and WD contributed in the forming of ideas, experimental design, and editorial assistance; MK collected and analyzed the data; DW assisted with molecular laboratory work for the metabarcoding; all other laboratory work and manuscript prepared by MK. All authors contributed to the manuscript and gave final approval for publication. Emeritus Professor Jen McComb reviewed the manuscript.

6.1 Data Available Statement

All data generated during this study are included in this submitting manuscript (and in Table S1). We have provided the raw data in excel spreadsheets as Appendices S1–S3.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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