2018 – 2019 Powell River Project Annual Research Report

Effects of Glyphosate Herbicide on *Phytophthora cinnamomi* and Mine Soil Microbial Communities

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Introduction:

The American Chestnut (*Castanea denata*) was once the dominant hardwood species within the forests of the Appalachians and an important resource for people and wildlife. In the early 1900s, a fungal blight (*Cryphonectria parasitica*) was introduced from imported 'Japanese Giant' nursery trees that caused topkill of American chestnuts (Tallamy 2007). Trees infected with *C. parasitica* die back, then continually resprout from the roots. Since 1983, the American Chestnut Foundation has been conducting a backcross breeding program to produce hybrid trees resistant to the blight, and more recently, various research institutions have been working to develop transgenic varieties of American chestnut which express genes that may give the trees resistance to the blight. Additional pathogens, such as the fungus-like oomycete which causes root rot (*Phytophthora cinnamomi*), have furthered threatened *C. dentata,* and research of transgenic American chestnuts has included the identification of genes that may provide resistance to *P. cinnamomi.*

Phytophthora cinnamomi is a water and soil-borne pathogen of global economic significance for its effects on agricultural and nursery plant production (Rossman and Palm 2006). It originated from Papua New Guinea and evidence suggests that it was introduced to the Americas on infected avocado rootstock (Zentmyer 1977, Linde et al. 1999, Hardham 2005). The pathogen can reproduce both sexually, forming oospores, and asexually, forming zoospores, and it is capable of spreading vegetatively as well (Weste 1983, Hardham 2005). Zoospores have flagella, enabling them to move through soil water, thus facilitating rapid infestation in water-logged soils; as a result, the pathogen is most active where soil is saturated (Duniway 1983, Weste 1983, Hardham 2005, Keen 2006). Soil texture and slope can affect the speed of spread

through a site, with faster spread through soils with fewer impediments and down steeper slopes (Weste 1983). *Phytophthora cinnamomi* is most active in soils where pH is slightly acid (6.0 to 6.5), but tolerates a fairly wide pH range of 4.0 to 8.0, and soils outside of this range appear to suppress *P. cinnamomi* (Zentmyer 1980, Weste 1983, Ko and Shiroma 1989).

Impacts of *P. cinnamomi* can be minimized through the use of phosphorous acid fungicides, and some evidence has suggested that changes in microbial community composition can facilitate or suppress *Phytophthora cinnamomi* infection (Pegg et al. 1988, Keen 2006). For example, bacteria from genera including *Pseudomonas* and *Chromobacterium*, and the fungal genus *Trichoderma* are believed to stimulate vegetative growth and/or sexual reproduction (Ribeiro 1983, Elliot 1983). Conversely, other studies observed that higher populations of bacteria and actinomycetes, particularly *Bacillus* sp. and *Pseudomonas* sp. have been associated with low *P. cinnamomi* activity as well as evidence of antagonism (Broadbent and Baker 1974, Malajczuk 1983, Mass and Kotze 1989, Duvenhage et al. 1990). Furthermore, higher organic matter content in soils appears to result in the suppression of *P. cinnamomi*, which has largely been attributed to increased populations and activity of antagonistic bacteria (Malajczuk 1983, Keen 2006). A thorough summary of antagonistic microbes in soils with low *P. cinnamomi* activity has been described in Keen (2006), suggesting that the presence of certain bacterial and fungal species can result in lower disease incidence.

Glyphosate (i.e., Roundup ®) is an herbicide commonly used in agriculture around the world, largely due to the increasing usage of genetically modified crops that have been developed with resistance to the herbicide (Haney et al. 2002, Newman et al. 2016). A number of studies have investigated the effects of glyphosate on microbial community composition and function, as well as the effects on abundances and gene expression within specific microbial populations, with varying results. For example, Gomez et al. found that total microbial biomass decreased in the week after glyphosate application (2009), while a number of other studies have found no changes in microbial biomass at field application rates (Wardle and Parkinson 1990, Liphadzi et al. 2005, Nguyen et al. 2016, Rose et al. 2016). In a greenhouse study, Newman et al. found that after exposure to glyphosate, relative abundance of Proteobacteria increased and relative abundance of Acidobacteria decreased (2016). A greenhouse experiment by Arango et al. studying the rhizosphere bacterial communities of glyphosate resistant soybeans observed an increase in *Gemmatimonadetes* sp. and a decrease in *Burkholderia* sp. in response to glyphosate application

(2014). Araújo et al. observed an increase in Actinomycetes one month after glyphosate application, and found that increased exposure to glyphosate more significantly affected soil microbes (2003). Araújo et al. (2003) and Wardle and Parkinson (1990) both observed an increase in the overall fungal populations after application of glyphosate, though neither studied investigated changes in fungal communities. In a culture study, Rosa et al. found that glyphosate acts as a fungicide against *Phytophthora capsici* (2010), and Meriles et al. found that glyphosate stimulated population growth of the related genus, *Pythium* in a long-term field experiment (2008). Overall, results suggest that field, greenhouse, and culture studies may all produce variable results, and that field and greenhouse experimental results may be significantly affected by myriad factors including site history, timescale, soil type, plant species, and sampling methods (Imfeld & Vuilleumier 2012). Given that some evidence has suggested that glyphosate may affect microbial communities, and therefore the ability of plants to resist pathogens, further research is critical.

Research Site History:

The two research sites we will be studying are located at the Kentland Research Farm in Blacksburg, VA (37°12'20.65"N 80°35'27.74"W) and a location on the Powell River Project (PRP) (36°59'9.75"N 82°42'5.47"W).

Soils at the Kentland Farm planting location are in the Unison and Braddock series, which are well-drained and characterized by a loamy texture with common cobbles. The Kentland site was formerly a peach orchard, converted to upland pasture, and was planted with a mix of transgenic and non-transgenic chestnuts in November 2013. Due to the site history of upland pasture, we have used a combination of mechanical weed control (mowing, string-trimming, hand-pulling weeds) and chemical control (backpack spray with 1.5% glyphosate herbicide, 2-3 times per growing season).

The PRP site was seeded after regrading in 2012 with a tree compatible seeding mix. At planting, the substrate was very rocky and comprised primarily of weather sandstones and shales. Fine materials collected from the site before planting ranged in pH from 4.83 to 5.83 (mean 5.19), and organic matter was overall fairly low ranging from 0.4 to 1.1% (mean 0.7 %). The

site was first planted in June 2013 with a mix of hybrid chestnuts, then planted with additional hybrid chestnuts as well as transgenic chestnuts in May 2014 and May 2015. The existing cover on this site is overall fairly sparse and non-aggressive, and so weed control has been limited to string-trimming and hand-pulling.

In June 2013, several chestnuts at both Kentland and the PRP showed symptoms that appeared to be due to *Phytophthora cinnamomi*. Three sets of tissue samples and soil samples from the associated rooting zones of these trees were collected from each site and sent to the Bartlett Tree Research Lab in Charlotte, NC for *P. cinnamomi* testing. All of the plant tissue and soil samples from the PRP tested positive for *P. cinnamomi*, whereas none of the samples tested positive for *P. cinnamomi* from the Kentland site. Since then, no evidence has been observed suggesting that any mortality at Kentland was due to *P. cinnamomi*. The purpose of this study was to investigate the effects of glyphosate on the presence and abundance of *P. cinnamomi* and the remaining microbial community.

Methods:

To understand the effects of glyphosate application on the presence of *Phytophthora cinnamomi* at our PRP research site and not our Kentland research site, we conducted two related experiments:

1. Community study: Microbes have been observed to facilitate or suppress *P. cinnamomi*, and glyphosate has been documented to alter microbial community composition. We investigated the possibility that glyphosate is directly or indirectly affecting the ability of *P. cinnamomi* to colonize our research site at Kentland Farm. Soil samples were collected from locations where trees had died at both Kentland and the PRP sites (at the PRP, these will be sites where trees died from *P. cinnamomi*), as well as locations where trees are still alive and healthy. We characterized the soil bacterial and fungal communities within these soils, and confirmed the presence of *P. cinnamomi*. Sampling locations were then treated with glyphosate, and resampled to repeat the community analyses as well as *P. cinnamomi* presence to determine if there were any changes.
2. Culture study: In order to directly investigate the effects of glyphosate on *P. cinnamomi*, we conducted a culture study in which glyphosate was applied to isolated cultures of *P. cinnamomi* to observe whether growth or survival were inhibited. Study included positive

and negative controls, and cultures were isolated from the *P. cinnamomi* strains present on the PRP site.

On October 30, 2018 we collected twenty-four approximately 500 g soil samples from our two research sites from sample locations that fell into one of two categories: locations where a tree has died (on the mine site, half of these were locations where the tree definitively died from *P. cinnamomi*), and locations where the planted tree was still in good health. A total of six samples were collected from each location category at each site (e.g. Kentland, live tree) (Table 1).

Table 1. Summary of all treatment groups from both sites. Each treatment group resulted in two soil samples: before and one week after glyphosate herbicide application.

Site	Tree #	A/D	Herbicide applied?
Kentland	223	A	N
Kentland	184	A	N
Kentland	174	A	N
Kentland	166	A	Y
Kentland	336	A	Y
Kentland	170	A	Y
Kentland	433	D	N
Kentland	290	D	N
Kentland	325	D	N
Kentland	311	D	Y
Kentland	444	D	Y
Kentland	181	D	Y
PRP	32	A	N
PRP	118	A	N
PRP	15	A	N
PRP	49	A	Y
PRP	141	A	Y
PRP	3	A	Y
PRP	140	D	N
PRP	131	D	N
PRP	150*	D	N
PRP	14*	D	Y
PRP	148*	D	Y
PRP	135	D	Y

*Confirmed mortality from *P. cinnamomi* infection.

Of each of the 24 soil samples, 50 g was collected and stored on ice in the field for pre-glyphosate application analysis of bacterial and fungal communities, and 200 g was placed in ziplocs to be kept moist for detection and culturing of *P. cinnamomi*. The remaining 250 g soil samples was dried, sieved, and analyzed for pH, basic chemistry, and organic matter content in the departmental soil testing lab. Frozen samples were stored in a -80 ºC freezer until DNA extraction and analysis. The unfrozen soil samples were stored in a cold room at approximately 10 ºC to maximize *P. cinnamomi* survival until the culture experiment was set up (Jeffers and Martin 1986). Following soil sample collection, we treated half of the sample locations in a 50 cm radius around trees with 50 mL of 1.5% glyphosate, applied evenly with a spray bottle (Table 1). Only half of the sample locations were treated with glyphosate to account for natural changes in bacterial/fungal communities and *P. cinnamomi* abundance. On November 7, 2018 we collected a second set of soil samples from the same 24 sample locations at both Kentland and the PRP. Again, 50 g of soil from each sample location were collected and stored on ice in the field for bacterial and fungal community analysis, and the remainder of samples were collected and kept moist for the quantification of *P. cinnamomi*.

Bacterial and fungal diversity and community structure were characterized by sequencing amplified regions of 16S rRNA (Caporaso *et al.* 2012) and ITS (O'Brien *et al*. 2005) genes, respectively. First, total DNA was extracted from each soil sample using the PowerSoil DNA extraction kit (MoBio, Carlsbad, CA). Amplification was performed in triplicate to smooth out PCR biases and samples were barcoded so that they could be cost-effectively pooled into two sequencing runs – one for bacteria (16S) and one for fungi (ITS). Sequencing was performed on the Illumina MiSeq platform at Duke University's core genomics facility. Sequences were quality filtered, aligned, clustered into operational taxonomic units (OTUs) at the 97% similarity cutoff, and classified using QIIME (Caporaso *et al.* 2010) and identified against the Greengenes database (DeSantis *et al.*, 2006). To detect the presence of *P. cinnamomi* within soil samples, we used PCR with the LPV3 primer to determine whether *P. cinnamomi* was present (Kong et al. 2003).

To quantify *P. cinnamomi*, we used a baiting technique using 4 mm discs of *Rhododendron sp.* leaves (Jeffers and Martin 1986). Each of the 48 soil samples from the two sampling dates was placed in a sterile centrifuge tube and flooded with sterilized ultrapure DI water. Discs of sterilized, healthy *Rhododendron sp.* leaves were placed into flooded soil samples on February

21, 2018 and incubated at 27 ºC for one week. Three leaf discs were selected at random from the flooded soils and placed in the center of plates prepared with PAR(PH)-V8 selective medium (Jeffers and Martin 1986, Table 2, Figure 1). Petri dishes were incubated in the dark at 27 ºC, and colony formation was recorded every 24 hours. *P. cinnamomi* presence was confirmed via microscopic identification (Erwin and Ribeiro 1996). The proportion of plates with resulting *P. cinnamomi* colonies from each sample was used as a proxy for P. *cinnamomi* relative abundance. The cultures produced on the selective medium were transferred to fresh plates with PAR(PH)-V8 selective medium to produce colonies for the culture experiment.

Table 2. Recipe for PAR(PH)-V8 selective medium (Jeffers and Martin 1986).

Ingredient	**Amount per 1.0 L**	**Notes**
Basial medium		
Clarified V8 concentrate*	50 mL	Promotes Phytophthora spore production
DI water	950 mL	
Difco Bacto agar	15 g	
Amendments		
Delvocid (50% pimaricin)	10 mg	Antifungal
Sodium ampicillin	250 mg	Antibacterial
Rifamycin-SV (sodium salt)	10 mg	Antibacterial
Terraclor (75% PCNB)	66.7 mg	Inhibits soilborne fungi
Hymexazol	50 mg	Inhibits most Pythium spp.

*Buffered V8 juice (1.0 g CaCO3/100 mL V8 juice clarified by centrifugation at 7000 rpm for 10 minutes

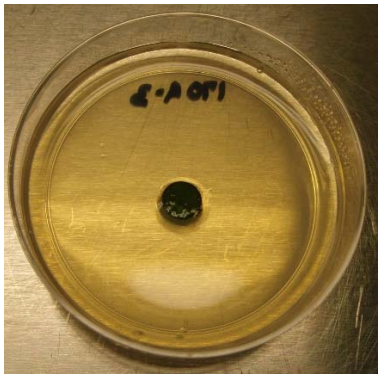

Figure 1. Setup of leaf-bait cultures. Dark circle is baited *Rhododendron sp.* leaf.

The best *Phytophthora cinnamomi* isolates with no observed contaminant species were transferred to fresh 60 mm petri dishes prepared with the PAR(PH)-V8 selective medium for the glyphosate dosing experiment. Selected plates were produced from five original leaf discs from three original soil samples. We produced 72 total plates for this study, with one 4 mm plug transferred to each plate. On March 1, 2019, each plate received one of 4 treatments: 1.5 % glyphosate, undiluted glyphosate concentrate, a negative control (ultrapure DI water), and a positive control (AgriFos fungicide). Each treatment was replicated on 18 plates. Each treatment was administered by soaking two 4 mm discs of Whatman #1 filter paper in the corresponding treatment and placing them on opposite sides of the transferred plug, approximately 1 cm away (Figure 2). Plates were covered and incubated in the dark at 27 ºC and colony growth was photographed and observed under the microscope every 24-72 hours through April 1, 2019. To quantify differences in *P. cinnamomi* growth on treatment discs, we ranked the presence of *P. cinnamomi* on each filter paper disc on a scale from 0 (none) to 4 (abundant) (Table 3). As other species colonized the plates over time, we also quantified the presence of other species on the discs using a similar ranking system from 0 (absent) to 3 (abundant).

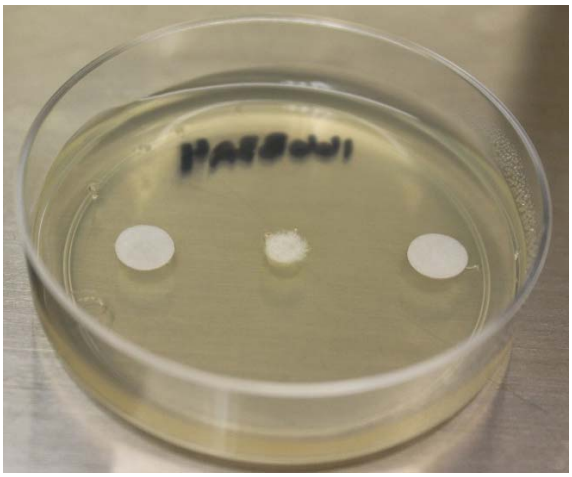

Figure 2. Setup of glyphosate dosing experiment. Center circle is *P. cinnamomi* plug with two treated filter paper discs on either side.

Table 3. Phytophthora cinnamomi ranks for glyphosate dosing experiment.

Rank	Observation
0	No P. cinnamomi on disc
1	Present (e.g. just at edge or a few chlamydospores present on disc)
2	Covering up to 50% of disc
3	Covering more than 50% of disc
4	Abundant, covering entire disc

Phytophthora cinnamomi abundance data from each of the three experiments was analyzed using analysis of variance (ANOVA) to assess differences based on site, field application of glyphosate, or sampling date. We also ran several correlation analyses to assess whether there were any differences in *P. cinnamomi* abundance related to soil chemistry. Changes in bacterial and fungal community structure were visualized using non-metric multidimensional scaling (NMDS) and statistically tested with permutational MANOVA (PERMANOVA) using Adonis in the R statistical package.

Results:

Microbial communities

Relative abundances of bacterial (16S) and fungal (ITS) OTUs were used to perform NMDS using Bray-Curtis dissimilarities to visualize differences in community composition among treatment groups (Anderson 2001, Oksanen, et al. 2008, R Core Team 2013). The analysis calculates dissimilarity between pairs of samples based on presence/absence and abundance of all species in the data matrix; two plots with the exact same species and abundances would have a dissimilarity of 0. The goal of this analysis is to graph each plot as a point in an ordination with x-number of axes, based on their dissimilarity, while aiming to maximize the 'goodness of fit' by minimizing 'stress'. Generally, the more axes, the lower the stress, but the more difficult it becomes to present and interpret the data. Ordinations show all the points in relation to one another, based on the calculated dissimilarities, to help visualize trends among groups. Points that are closer together are more similar than points that are further apart.

We were able to plot our bacterial community data on two axes with minimal stress (stress=0.09). Analysis of soil bacterial communities showed significant differences between the two study locations, which form two clusters on either side of the ordination (p=0.001) (Figure 3, Table 3) However, no significant differences in soil bacterial community structures were observed between glyphosate treatment groups (Figure 3, Table 4).

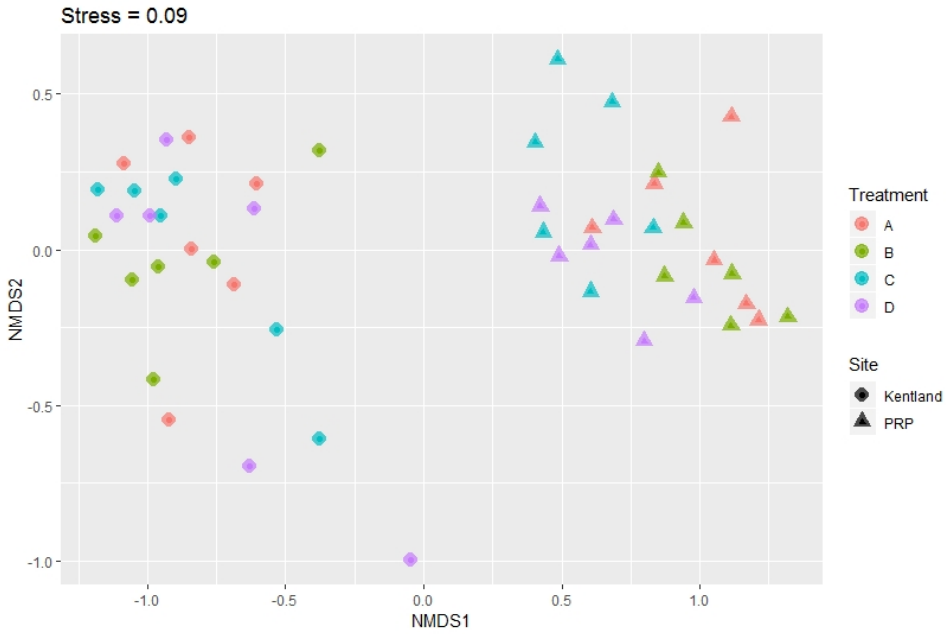

Figure 3. NMDS ordination of soil bacterial communities at the two study sites and among the four treatment groups: A) Glyphosate, pre-treatment, B) Glyphosate, 1 week post-treatment, C) Control, pre-treatment, D) Control, 1 week post-treatment.

Table 4. PERMANOVA statistical output for analysis of bacterial communities between sites and among glyphosate treatment groups.

	Df	**Sum of squares**	**Mean squares**	**F Model**	**R^2 probability**	**p-value**
Treatment	3	0.7074	0.2358	0.9043	0.04485	0.577
Site	1	3.9036	3.9036	14.9698	0.24746	0.001
Treatment x Site	3	0.7328	0.2443	0.9367	0.04645	0.554
Residuals	40	10.4305	0.2608		0.66124	
Total	47	15.7743			1	

Significant differences in soil bacterial community structures were also detected between samples collected at live trees and those collected at dead trees, although the grouping among live and dead trees is less distinct at the Kentland Farm site (p=0.035) (Figure 4, Table 5).

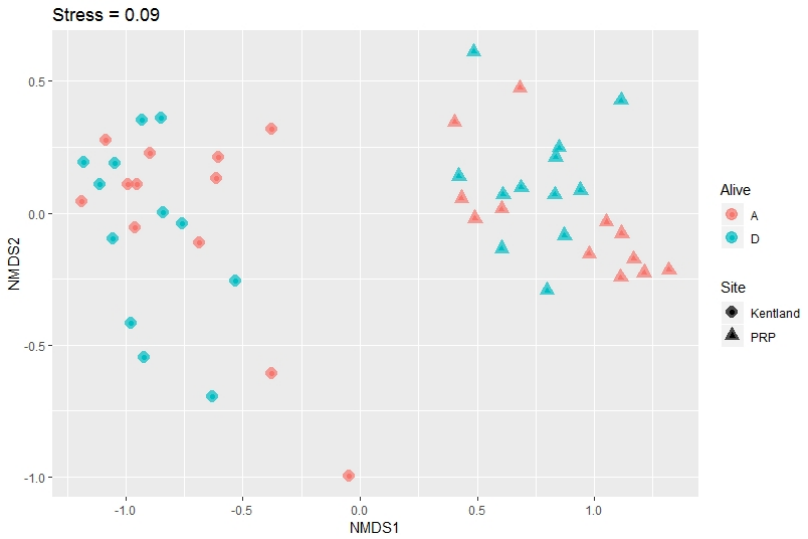

Figure 4. NMDS ordination of soil bacterial communities at the two study sites and trees that were alive or dead.

Table 5. PERMANOVA statistical output for analysis of bacterial communities between sites and between alive and dead trees.

	Df	Sum of squares	Mean squares	F Model	R^2 probability	p-value
Alive	1	0.4985	0.4985	2.0052	0.0316	0.035
Site	1	3.9036	3.9036	15.7027	0.24746	0.001
Alive x Site	1	0.4342	0.4342	1.7466	0.02752	0.049
Residuals	44	10.938	0.2486		0.69341	
Total	47	15.7743			1	

We were able to plot our fungal community data on two axes with minimal stress (stress=0.09). With the exception of two outliers, soil fungal communities at the two study sites

were distinct and showed little variability within each site (p=0.001) (Figure 5, Table 6). No clear differences in soil fungal community structures were detected between glyphosate treatment groups.

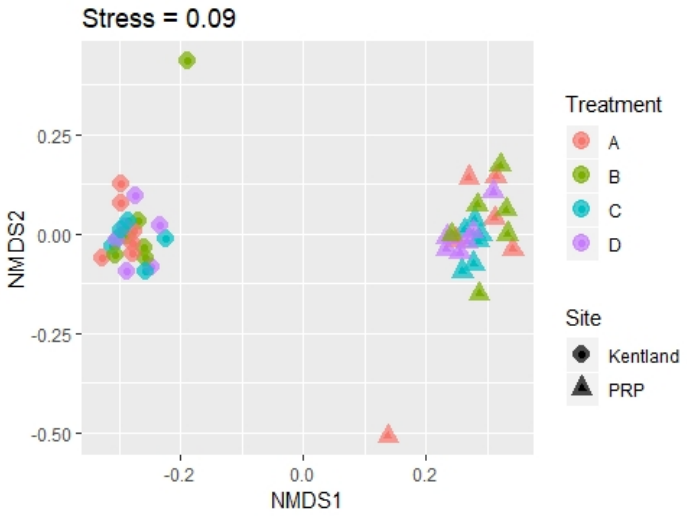

Figure 5. NMDS ordination of soil fungal communities at the two study sites and among the four treatment groups: A) Glyphosate, pre-treatment, B) Glyphosate, 1 week post-treatment, C) Control, pre-treatment, D) Control, 1 week post-treatment.

Table 6. PERMANOVA statistical output for analysis of soil fungal communities between sites and among glyphosate treatment groups.

	Df	Sum of squares	Mean squares	F Model	R^2 probability	p-value
Treatment	3	0.7074	0.2358	0.9043	0.04485	0.579
Site	1	3.9036	3.9036	14.9698	0.24746	0.001
Treatment x Site	3	0.7328	0.2443	0.9367	0.04645	0.554
Residuals	40	10.4305	0.2608		0.66124	
Total	47	15.7743			1	

Soil fungal community structures showed distinct differences between live and dead trees at each of the study sites, though these differences were slightly less distinct at the Powell River Project (PRP) site (p=0.036) (Figure 6, Table 7).

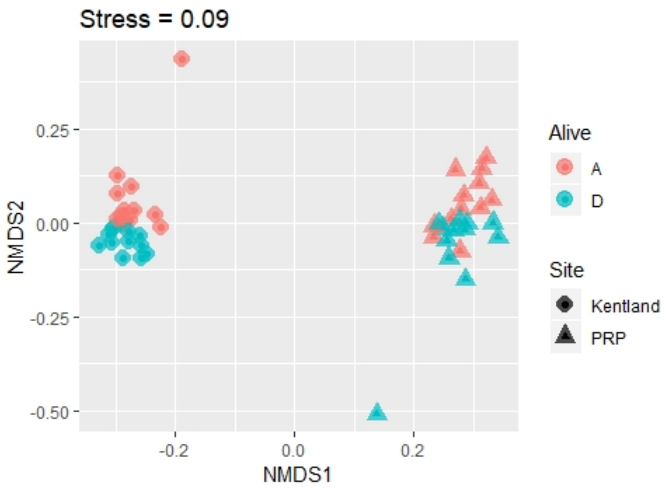

Figure 6. NMDS ordination of soil fungal communities at the two study sites and trees that were alive or dead.

Table 7. PERMANOVA statistical output for analysis of soil fungal communities between sites and between alive and dead trees.

	Df	Sum of squares	Mean squares	F Model	R^2 probability	p-value
Alive	1	0.4985	0.4985	2.0052	0.0316	0.036
Site	1	3.9036	3.9036	15.7027	0.24746	0.001
Alive x Site	1	0.4342	0.4342	1.7466	0.02752	0.046
Residuals	44	10.938	0.2486		0.69341	
Total	47	15.7743			1	

To understand which species were driving the observed microbial community differences, we looked at the average relative abundance of the 10 most abundant OTUs at each site within bacteria and fungi. As we would expect based on the NMDS ordinations, the most visible differences between bacterial communities were between the two study sites, rather than the glyphosate treatments or alive/dead trees (Figures 7 and 8). Several phyla were notably more

abundant at Kentland than the PRP: Proteobacteria, Firmicutes, Bacteriodetes, Actinobacteria, and Gemmatimonadetes. Acidobacteria were notably more abundant at the PRP than at Kentland.

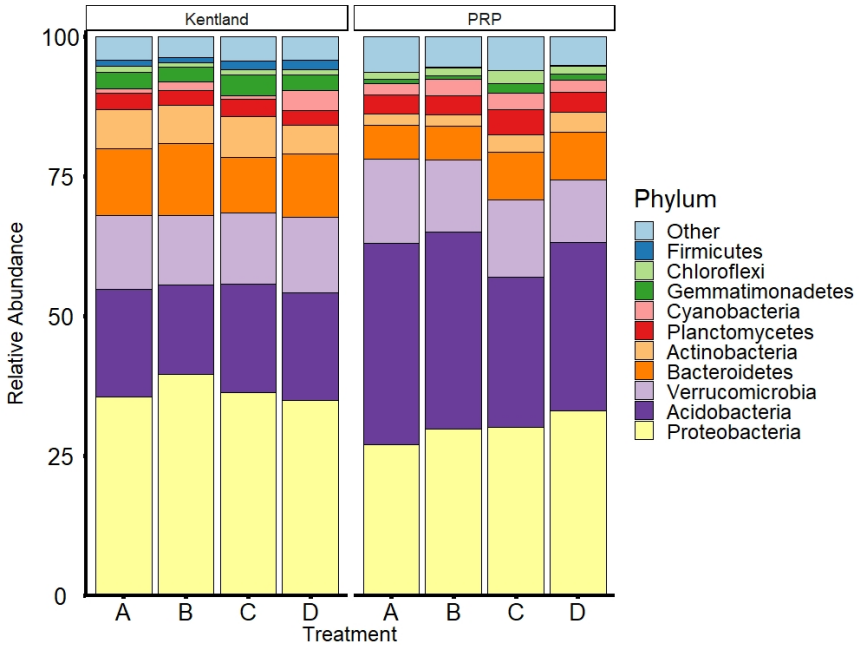

Figure 7. Average relative abundance of 10 most abundant bacterial phyla among glyphosate treatments at both sites. Treatments A/B represent the glyphosate application treatment before/after application, and treatments C/D represent the control treatments sampled before/after treatment application.

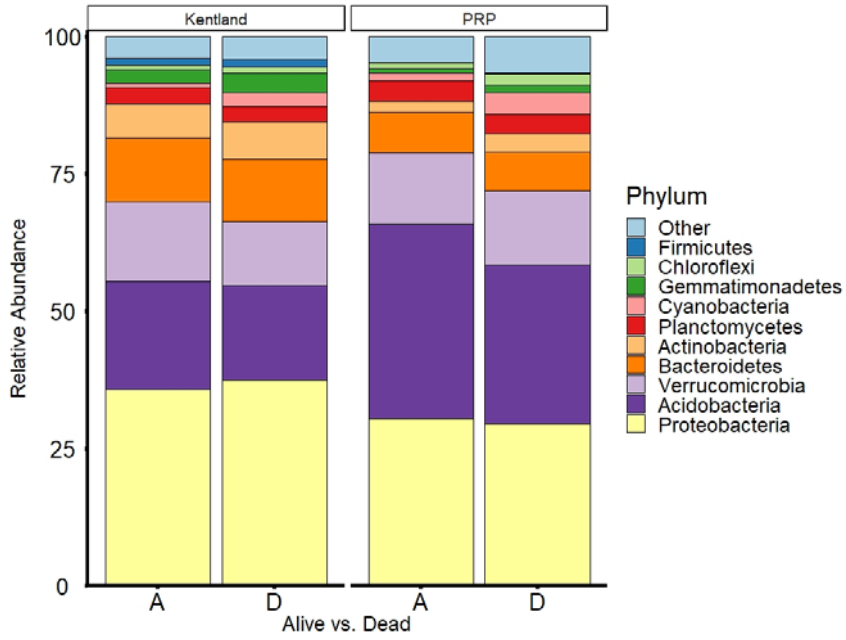

Figure 8. Average relative abundance of 10 most abundant bacterial phyla between alive and dead trees at both sites.

Within the fungal community, we observed clear differences between the two sites, as well as between live and dead trees (Figures 9 and 10). We had expected this database to include *Phytopthora* spp., including *P. cinnamomi*, but because they were not included, we were unable to directly quantify *P. cinnamomi* abundance in our soil samples. Soil samples from the PRP had greater relative abundance of the fungal classes Archareorhizomycetes and Leotiomycetes, as well as a greater mix of 'other' taxa. Soil samples from Kentland had greater abundance of several fungal classes: Sordariomycetes, Dothideomycetes, Mortierellomycetes, and Pezizomycetes. Soil samples from live trees appear to have greater relative abundance of Agariomycetes than dead trees. At Kentland, Sordariomycetes and Dothideomycetes appear to have greater relative abundance near dead trees, whereas these classes do not appear different at the PRP. At the PRP, dead trees have greater relative abundance of Archaerhizomycetes as well as other classes of fungi.

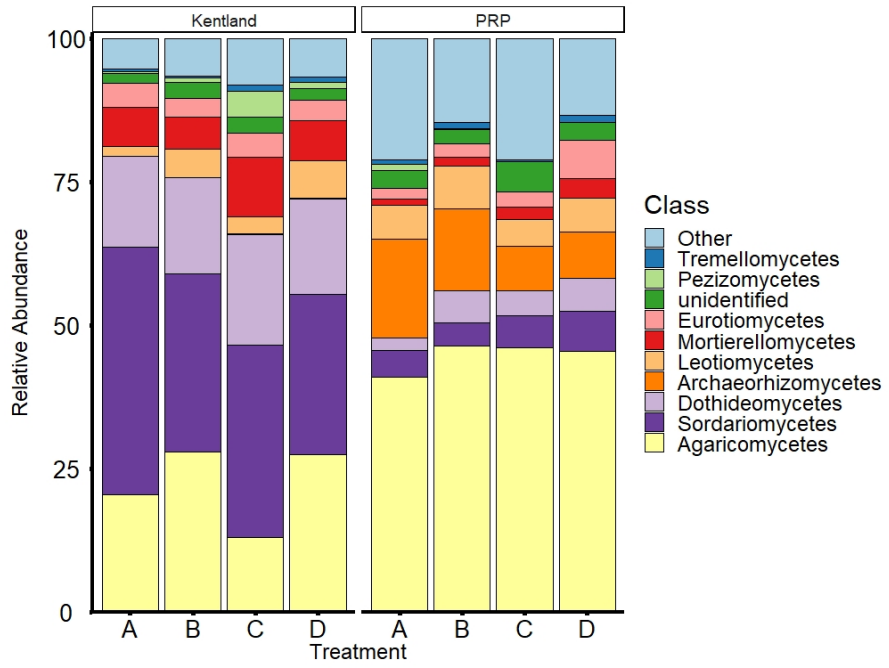

Figure 9. Average relative abundance of 10 most abundant fungal phyla among glyphosate treatments at both sites. Treatments A/B represent the glyphosate application treatment before/after application, and treatments C/D represent the control treatments sampled before/after treatment application.

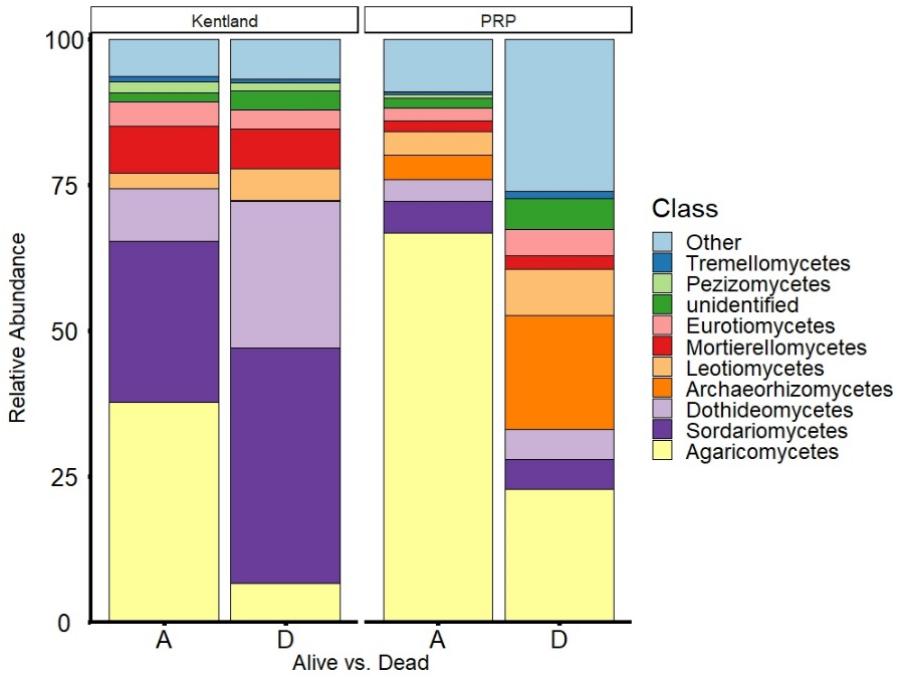

Figure 10. Average relative abundance of 10 most abundant fungal phyla between alive and dead trees at both sites.

We compared species richness and Shannon-Wiener diversity values of bacterial and fungal communities to see whether these metrics were associated with any of our treatment groups. Within the bacterial communities, we observed no differences in species richness or diversity among treatments, sites, or between live and dead trees (Figures 11 and 12).

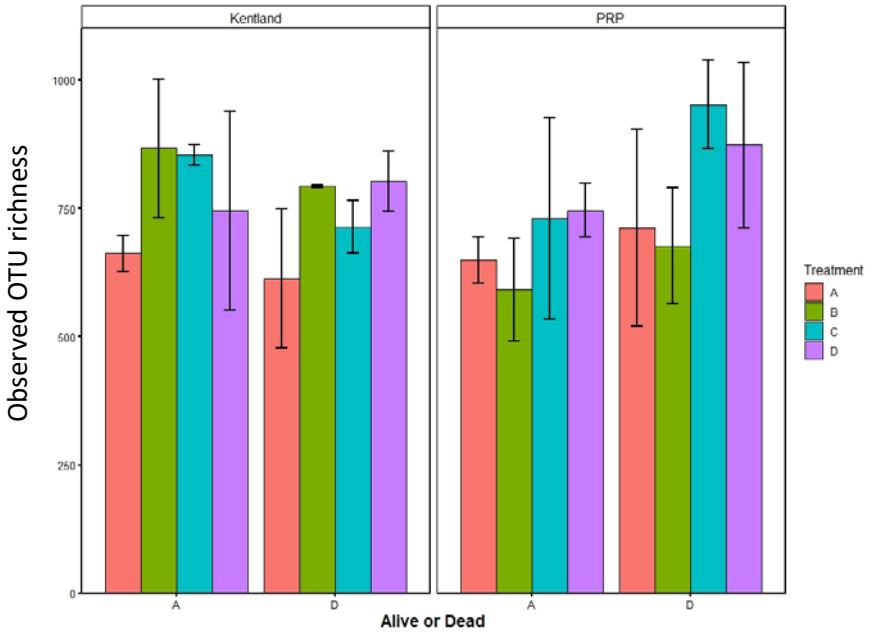

Figure 11. Bacterial community species richness among all treatment combinations at both sites. Treatments A/B represent the glyphosate application treatment before/after application, and treatments C/D represent the control treatments sampled before/after treatment application.

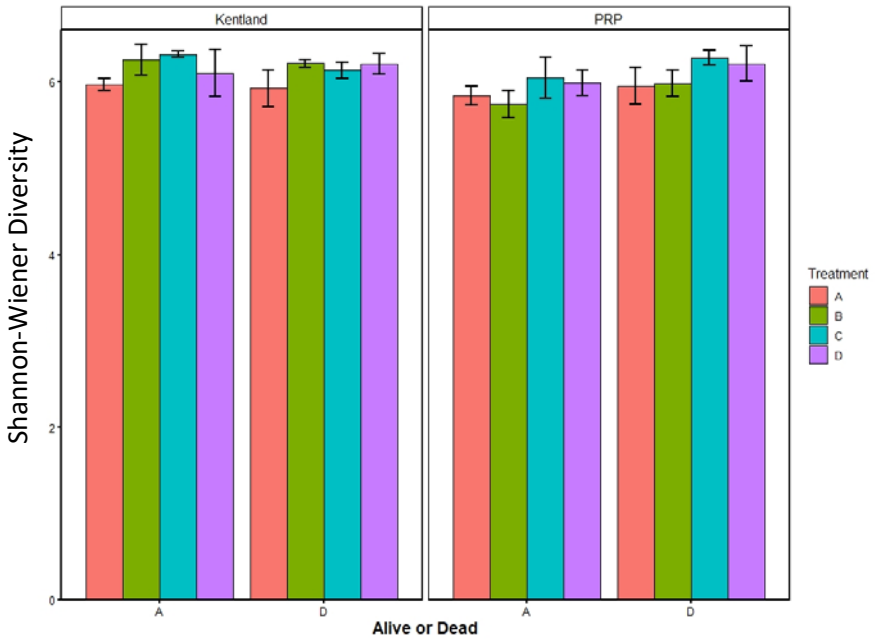

Figure 12. Bacterial community Shannon-Wiener diversity among all treatment combinations at both sites. Treatments A/B represent the glyphosate application treatment before/after application, and treatments C/D represent the control treatments sampled before/after treatment application.

Within the fungal communities, soil samples near live trees had greater OTU richness ($p=0.01$) and Shannon-Wiener diversity ($p=0.0106$) than samples collected near dead trees (Figures 13 and 14). In addition, we observed a slightly significant interaction for OTU richness at the $\alpha=0.10$ level between site and alive/dead trees, such that live trees had greater OTU richness at Kentland, but live and dead trees did not differ at the PRP ($p=0.0746$)

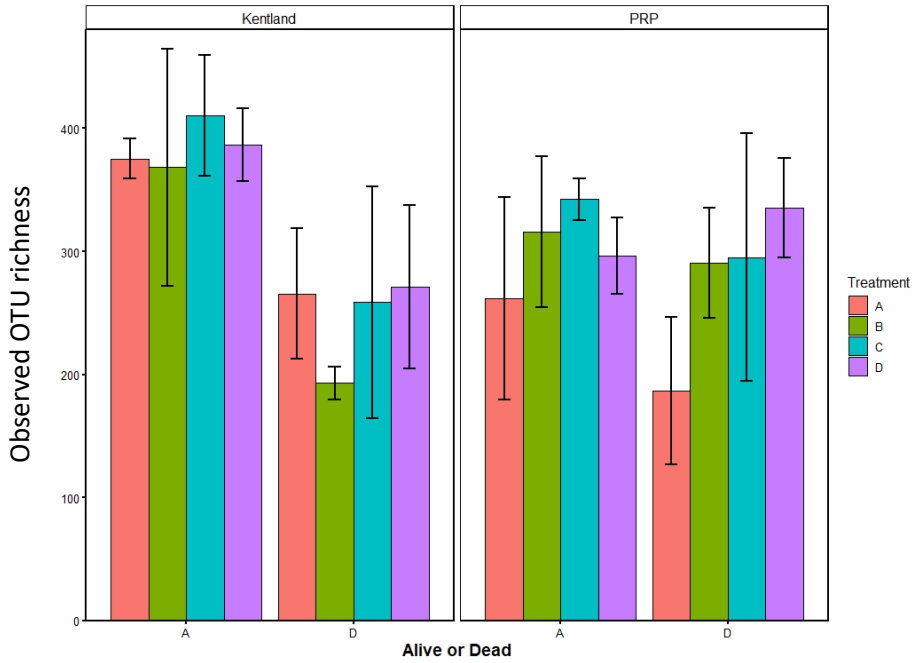

Figure 13. Fungal community species richness among all treatment combinations at both sites. Treatments A/B represent the glyphosate application treatment before/after application, and treatments C/D represent the control treatments sampled before/after treatment application.

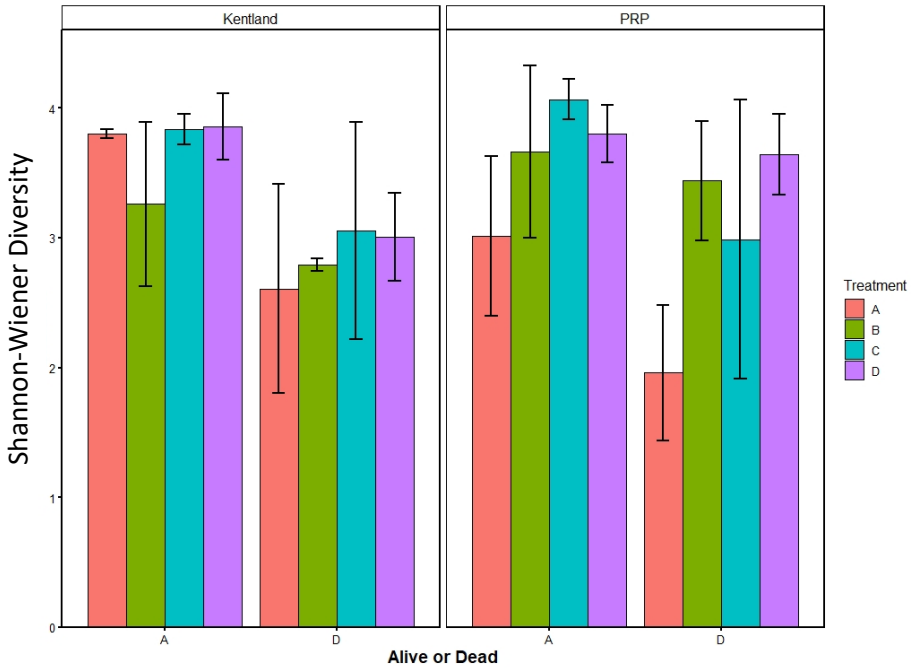

Figure 14. Fungal community Shannon-Wiener diversity among all treatment combinations at both sites. Treatments A/B represent the glyphosate application treatment before/after application, and treatments C/D represent the control treatments sampled before/after treatment application.

Phytophthora cinnamomi PCR

We had difficulty obtaining consistent results detecting *P. cinnamomi* using PCR with the LPV3 primer. Samples were initially run in January 2019, and run several times through October 2019 (Appendix 2, Tables 8a and 8b).

Table 8a. Summary results of PCR runs to detect *P. cinnamomi* in soils from the Kentland Farm study site.

Tree #	Sample ID	Alive	Site	Treatment (A/B=pre/post glyphosate +, C/D= pre/post control)	# PCR runs	Positive IDs
223	SK1	A	Kentland	C	6	0
223	SK13	A	Kentland	D	4	3
184	SK4	A	Kentland	C	4	1
184	SK16	A	Kentland	D	5	2
174	SK5	A	Kentland	C	6	6
174	SK17	A	Kentland	D	5	5
166	SK2	A	Kentland	A	2	0
166	SK14	A	Kentland	B	5	4
336	SK3	A	Kentland	A	3	2
336	SK15	A	Kentland	B	5	5
170	SK6	A	Kentland	A	1	0
170	SK18	A	Kentland	B	1	0
433	SK7	D	Kentland	C	3	0
433	SK19	D	Kentland	D	3	0
290	SK10	D	Kentland	C	6	6
290	SK22	D	Kentland	D	6	5
325	SK11	D	Kentland	C	2	0
325	SK23	D	Kentland	D	5	3
311	SK8	D	Kentland	A	3	0
311	SK20	D	Kentland	B	6	6
444	SK9	D	Kentland	A	4	2
444	SK21	D	Kentland	B	4	4
181	SK12	D	Kentland	A	1	0
181	SK24	D	Kentland	B	1	0

Table 8b. Summary results of PCR runs to detect *P. cinnamomi* in soils from the Powell River Project study site.

Tree #	Sample ID	Alive	Site	Treatment (A/B=pre/post glyphosate +, C/D= pre/post control)	# PCR runs	Positive PCR IDs
32	SK25	A	PRP	C	3	0
32	SK37	A	PRP	D	4	2
118	SK28	A	PRP	C	3	0
118	SK40	A	PRP	D	5	3
15	SK29	A	PRP	C	3	0
15	SK41	A	PRP	D	4	0
49	SK26	A	PRP	A	9	8
49	SK38	A	PRP	B	4	3
141	SK27	A	PRP	A	7	6
141	SK39	A	PRP	B	6	1
3	SK30	A	PRP	A	3	3
3	SK42	A	PRP	B	3	1
140	SK31	D	PRP	C	4	1
140	SK43	D	PRP	D	3	2
131	SK34	D	PRP	C	4	2
131	SK46	D	PRP	D	3	1
150	SK35	D	PRP	C	2	0
150	SK47	D	PRP	D	3	1
14	SK32	D	PRP	A	2	0
14	SK44	D	PRP	B	2	0
148	SK33	D	PRP	A	3	0
148	SK45	D	PRP	B	2	0
135	SK36	D	PRP	A	4	3
135	SK48	D	PRP	B	3	1

While several samples consistently showed negative results (e.g. trees 14, 15, and 148 from the Powell River Project and trees 170, 181, and 433 from the Kentland Farm site), others consistently showed positive results (e.g. tree 49 from the Powell River Project and trees 290, 336, and 174 from Kentland Farm). Several other samples showed clear positives in some runs and clear negatives in others, occasionally even when run on the same tray, as with SK27 on August 29, 2019. Interestingly, *P. cinnamomi* presence was confirmed in soil and tissue samples from trees 14 and 148 at the Powell River Project by the Bartlett Tree Research Lab in 2015,

though it was never detected using PCR in this study. We have not yet determined why we were observing these inconsistencies, but it is clear that while this method may be helpful in detecting *P. cinnamomi* in certain conditions, it is critical to replicate PCR runs, as well as use a secondary method of detection to confirm results.

Rhododendron leaf baiting

Several of the baited soil samples resulted in *P. cinnamomi* growth on the selective medium (Figure 15, Table 9). Presented data represent microscopic identification of cultures grown on plates and was not corrected based on PCR results, as there can be differences in detection through these methods (Dai et al. 2019). In fact, we never positively detected *P. cinnamomi* in any samples from trees 14 and 148 using PCR, but we did grow *P. cinnamomi* cultures from the *Rhododendron* sp. leaves baited in these samples and, as stated above, it was confirmed present by the Bartlett Tree Research Lab.

Although we had expected to only see *P. cinnamomi* in our soil samples from the Powell River Project, soil samples from Kentland Farm (58.3 ± 42.0) had a significantly higher percentage of plates that grew *P. cinnamomi* from baited leaf discs compared to the Powell River Project samples (36.1 ± 31.0) (p=0.041) (Table 10). There were no differences observed among glyphosate field treatment groups (p=0.219), between collection dates (p=0.219), or between live or dead trees (p=0.610) (Table 10).

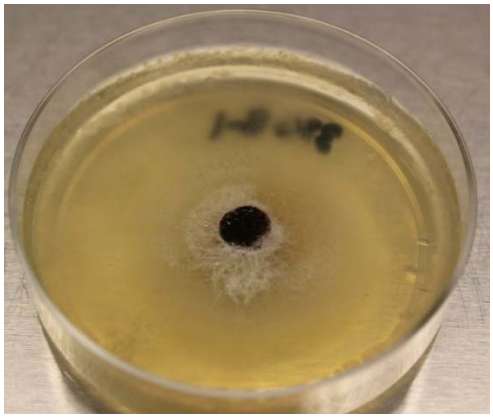

Figure 15. Growth of *Phytophthora cinnamomi* from *Rhododendron sp.* leaf disc baited in tree 336 soil sample collected on 7 November, 2018 (7 March, 2019, S. Klopf).

Table 9. Percent of plates that grew *P. cinnamomi* among all treatment groups before and after glyphosate application.

Site	Block	Tree #	Alive/Dead	Herbicide applied?	Before herbicide application	After herbicide application
					Plates with P. cinnamomi growth (%)	
Kentland	1	223	A	N	100	0
Kentland	3	184	A	N	66.7	100
Kentland	4	174	A	N	66.7	66.7
Kentland	1	166	A	Y	66.7	100
Kentland	2	336	A	Y	33.3	100
Kentland	5	170	A	Y	33.3	33.3
Kentland	1	433	D	N	100	100
Kentland	3	290	D	N	100	100
Kentland	4	325	D	N	0	33.3
Kentland	1	311	D	Y	100	100
Kentland	2	444	D	Y	0	0
Kentland	5	181	D	Y	0	0
PRP	1	32	A	N	0	0
PRP	2	118	A	N	66.7	33.3
PRP	3	15	A	N	66.7	66.7
PRP	1	49	A	Y	33.3	66.7
PRP	2	141	A	Y	33.3	0
PRP	4	3	A	Y	66.7	0
PRP	1	140	D	N	100	0
PRP	3	131	D	N	100	0
PRP	4	150	D	N	33.3	33.3
PRP	2	14	D	Y	33.3	33.3
PRP	2	148	D	Y	0	33.3
PRP	4	135	D	Y	33.3	33.3

Table 10. Mean percent of plates within main treatment groups.

Site	Plates with P. cinnamomi growth (%)	SD
Kentland	58.3 a	42.0
PRP	36.1 b	31.0
Alive/Dead		
Alive	50.0	34.1
Dead	44.4	42.4
Collection Date		
Pre-treatment	51.4	36.8
Post-treatment	43.1	39.9
Glyphosate treatment		
Glyphosate+, pre-treatment	36.1	30.0
Glyphosate+, post-treatment	41.7	40.5
Control, pre-treatment	66.7	37.6
Control, post-treatment	44.4	41.0

Soil chemistry

Basic soil chemistry was compared between the two study sites. Organic matter content, P, K, Ca, Mg, Zn, Mn, and B were all higher in soil samples from Kentland (Table 11). Soil pH was also higher at Kentland, but soils from both sites were acidic. The complete dataset is included in Appendix 1. Samples from the PRP study site had higher Fe concentrations.

Table 11. Basic soil chemistry from the Kentland Farm and Powell River Project study sites.

	Kentland	PRP	
	mg kg^{-1} ± SD		p-value
P	15 ± 9 a	7 ± 10 b	0.049
K	94 ± 18 a	75 ± 25 b	0.047
Ca	829 ± 217 a	225 ± 69 b	<0.001
Mg	182 ± 55 a	99 ± 19 b	<0.001
Zn	2.2 ± 0.7 a	1.3 ± 0.4 b	<0.001
Mn	19.4 ± 4.1 a	15.2 ± 3.8 b	0.017
Cu	0.4 ± 0.3	0.3 ± 0.1	0.166
Fe	8.7 ± 3.1 b	17.5 ± 3.0 a	<0.001
B	0.4 ± 0.2 a	0.1 ± 0.0 b	<0.001
	Mean ± SD		
pH	5.63 ± 0.40 a	5.24 ± 0.18 b	0.006
Organic matter (%)	4.5 ± 0.6 a	1.7 ± 0.3 b	<0.001

We ran a series of correlation analyses to determine whether any of these basic soil chemical properties were related to the frequency of *P. cinnamomi* detection in our leaf bait experiment. We found significant, albeit weak, positive correlations between the percent of plates that grew *P. cinnamomi* and P, Ca, Mg, Zn, and Cu concentrations in soil samples (Table 12). The strongest relationships were with P and Cu concentrations, both of which were positively correlated to the percent of plates that grew *P. cinnamomi*, however Cu concentrations did not differ between our two study sites (Figures 16 and 17).

Table 12. Summary of correlation analyses between the percent of plates that grew *P. cinnamom*i in the *Rhododendron* leaf baiting experiment and basic soil chemistry.

	Correlation coefficient	**p-value**
pH	0.123	0.404
P	0.367	0.0103
K	-0.104	0.484
Ca	0.286	0.0491
Mg	0.292	0.044
Zn	0.292	0.0444
Mn	-0.0264	0.859
Cu	0.368	0.0101
Fe	-0.1	0.498
B	0.17	0.249
Organic matter (%)	0.277	0.0563

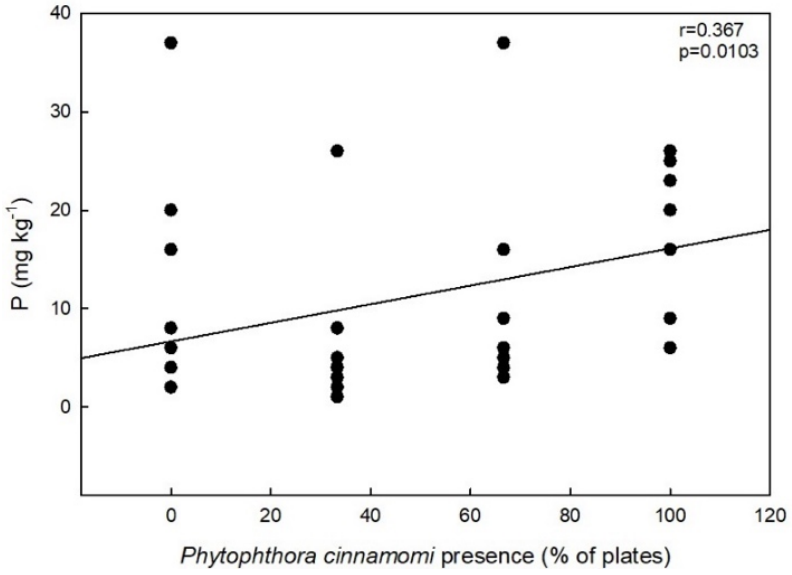

Figure 16. Correlation between soil P concentrations and the percent of plates that produced *P. cinnamomi* cultures.

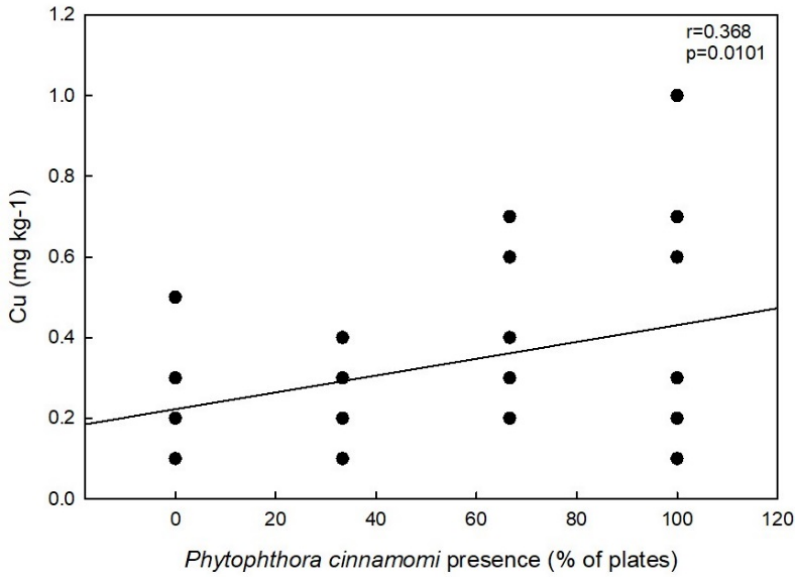

Figure 17. Correlation between soil Cu concentrations and the percent of plates that produced *P. cinnamomi* cultures.

Glyphosate dosing

The glyphosate dosing experiment was conducted using cultures that originated from the *Rhododendron* leaf baiting of soil samples from trees 336, 174, and 166. We observed and ranked differences in *P. cinnamomi* growth on filter paper discs treated with 1.5% glyphosate,

41% glyphosate, Agri-Fos fungicide, or deionized water. We observed no significant differences in growth of *P. cinnamomi* among treatment groups (p= 0.479) or among culture sources (p=0.182) (Tables 13 and 14).

Table 13. Mean ranked abundance of *P. cinnamomi* growth on filter paper discs (0 to 4 range) in the four treatment groups.

Treatment	Mean ranked abundance of *P. cinnamomi*	SD
Glyphosate (1.5 %)	3.2	1.2
Glyphosate (41%)	3.2	1.1
Agri-Fos fungicide control	3.2	1.1
DI control	2.7	1.1

Table 14. Mean ranked abundance of *P. cinnamomi* growth on filter paper discs (0 to 4 range). Different trees indicate different origins of grown and isolated cultures.

Tree #	Mean ranked abundance of *P. cinnamomi*	SD
166	3.5	0.9
174	3.2	1
336	2.8	1.2

Although we did not observe any differences in *P. cinnamomi* growth among treatment groups, we did observe differences in the ranked abundance of other species among treatment groups. Filter paper discs treated with 41% glyphosate had significantly lower abundance of other microbes compared to other treatment groups (p<0.001) (Figure 18). No other differences were detected.

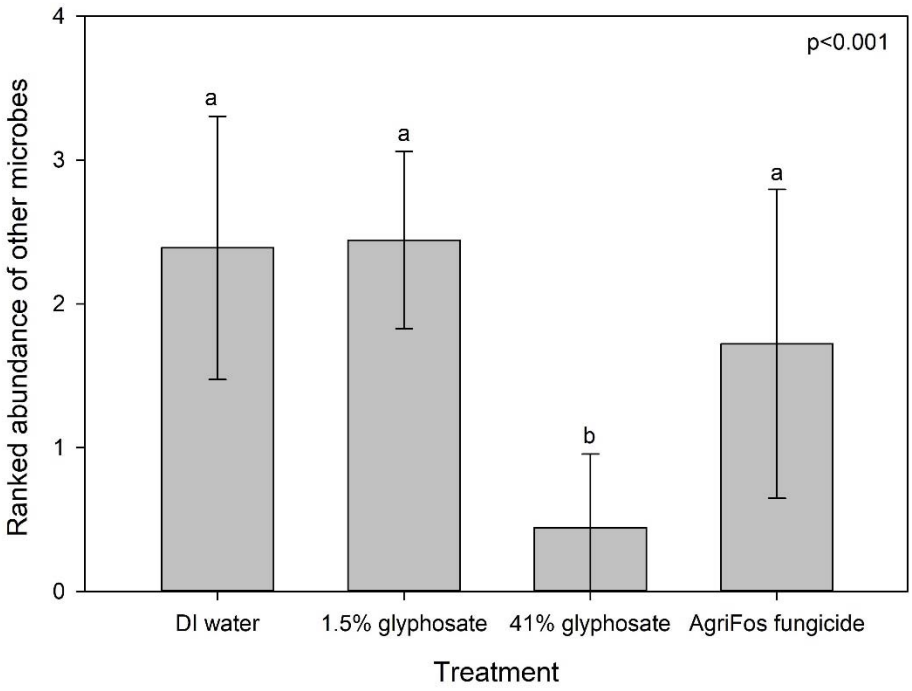

Figure 18. Ranked abundance of microbes other than *P. cinnamomi* on plates within four treatment groups in glyphosate dosing experiment.

Discussion

Although to date we have only detected *P. cinnamomi*-related mortality at the Powell River Project site and did not expect to find any *P. cinnamomi* from Kentland samples, our PCR analysis and soil baiting experiment both detected *P. cinnamomi* presence at multiple sampling locations at both sites. However, we also found some discrepancies in *P. cinnamomi* presence between analytical methods. Furthermore, *P. cinnamomi* appeared to be even more abundant at Kentland, despite the lack of evidence of tree mortality from this pathogen. We suggest that there may be some soil property (or properties) at Kentland that suppresses *P. cinnamomi* infection rates, a hypothesis that is well-established in the literature (Baker and Cook 1974, Malajczuk 1983, Schmitthenner and Canaday 1983, Weste 1983, Alabouvette et al. 1996, Keen 2006).

Soil chemistry differed between the two study sites, and it is possible that these differences resulted in greater colonization (as at the Kentland site) or greater pathogenicity (as at the Powell River Project site) of *P. cinnamomi*. While *P. cinnamomi* can survive in pH 4.0 to 8.0 soils, some evidence has suggested that soils between pH 5.5 and 7.0 may able to suppress *P.*

cinnamomi (Broadbent and Baker 1974). Soils at Kentland were within the range observed in suppressive soils (pH=5.63), while soils at the Powell River Project were more acidic (pH=5.24), which would support our observations of high *P. cinnamomi* abundance but lower pathogenicity at Kentland than the Powell River Project site. However, other studies have suggested that more acidic soils were more capable of suppressing *P. cinnamom*i than soils in which pH was closer to 6 (Ko and Shiroma 1989). Soil Ca has also been associated with *P. cinnamomi* disease incidence and severity, though data are conflicting. Soils at Kentland had notably higher Ca concentrations compared to the Powell River Project. Both Broadbent and Baker (1974) as well as Makajczuk (1979) observed that soils high in Ca were suppressive of *P. cinnamomi*. However, the addition of $CaCO_3$ to soil has shown to both decrease (Boughton et al. 1978) and increase (Halsall 1980) disease incidence and severity. While higher pH and Ca may play a role in the lower pathogenicity of *P. cinnamomi* at Kentland, the conflicting results from these studies suggest that other variables may be involved as well.

Increasing soil macronutrient concentrations have shown varying responses in *P. cinnamomi* disease incidence and severity. Increased P and K have been related to decreases in disease incidence and severity, although these studies conclude that soil P and K do not appear to be primary drivers in *P. cinnamomi* activity (Weste, 1983, Erwin and Ribeiro 1996). Some studies have shown decreases in disease incidence with the addition of inorganic N fertilizers (Zentmyer and Bingham 1956, Broadbent and Baker 1974), while others have observed either no change or increases in disease incidence (Marks et al. 1972). However, application of nitrogen via high-N organic amendments versus inorganic fertilizers has resulted in decreased disease incidence and severity in multiple studies (Tsao and Zentmyer 1979, Schmitthener and Canaday 1983). This relationship may be an indirect result of increasing soil organic matter, which has also resulted in the suppression of *P. cinnamomi* in several studies (Broadbent and Baker 1974, Malajczuk 1983, Weste, 1983, Downer et al. 1999, Keen 2006). We did not quantify soil N at either site, however, soil N was certainly higher at the Kentland Farm orchard site than the Powell River Project reclaimed mine soils. Our observations of higher soil organic matter, P, K, and likely also N at Kentland may help to explain the absence of *P. cinnamomi* disease incidence at this site. The relationship between organic matter and *P. cinnamomi* disease incidence and severity has also been linked to the increased abundance and diversity of microbes that suppress *P. cinnamomi* via antagonism, predation, or other mechanisms (Malajczuk 1983).

Overall, we observed clear differences within the soil bacterial and fungal communities. The most prominent observed differences were between the study sites, where we detected different relative abundance of several taxa, though no differences in richness or diversity. Given the observed differences between the sites in terms of organic matter content, pH, basic soil chemistry, climate, and site history, we would expect that soil microbial communities would differ. Within sites, we observed clear differences in soil fungal communities between alive and dead trees, and less distinct groups within the soil bacterial communities. This is expected, as many taxa of fungi and some bacteria are responsible for wood decomposition and would be more abundant at locations with dead trees (Zabel and Morrell 1992, Clausen 1996, Piskur et al. 2011, Fang et al. 2018). Several of the bacterial and fungal taxa that were more abundant at Kentland have been described as antagonistic to *P. cinnamomi* in numerous studies. Among bacteria, several genera within the phyla Proteobacteria, Firmicutes, and Actinobacteria have been associated with antagonism of *P. cinnamomi,* including *Chromobacterium sp.*, *Micromonospora sp.*, and *Streptomyces sp*. (Broadbent and Baker 1974, You et al. 1996). Among the fungi, the classes Sordariomycetes and Dothideomycetes both include numerous taxa that have been associated with *P. cinnamomi* antagonism. Within Sordariomycetes, *Trichoderma spp*., *Fusarium spp*., *Myrothecium roridum*, *Petriella spp*, and *Sporothrix sp*. are associated with P. *cinnamomi* antagonism (Malajuk 1979, Mass and Kotze 1989, Casale 1990, McLeod et al. 1995, Chambers and Scott 1995, Costa et al. 1996, Duvenhage and Kohne 1997, Costa et al. 2000, Downer et al. 2001). Within Dothideomycetes, the taxa *Alternaria spp*., *Aureobasidium spp*., *Cladosporium spp*., *Pullularia spp.*, *Ulocladium spp*., *Pleospora spp*., and *Epicoccum purpurascens* are all associated with *P. cinnamomi* antagonism. While we were unable to classify many of our OTUs to genus or species, the abundance of *P. cinnamomi* but lack of root rot disease or mortality at Kentland would suggest that there may be species from some, or all, of these taxa that may be suppressing the pathogen. In this study, we learned that the soil bacterial and fungal communities at Kentland and the Powell River Project were distinctly different and that there may be greater abundance of antagonistic taxa at Kentland. However, at the level of detail in our dataset, we cannot confirm with certainty that these taxa are present and responsible for *P. cinnamomi* suppression.

While we were unable to directly compare *P. cinnamomi* abundance to soil microbial communities, we were able to conclude that there were no changes in the soil microbial

communities related to glyphosate at our application rate. Therefore, because there were no changes to microbial communities resulting from glyphosate application, there could be no indirect effects to *P. cinnamomi* abundance related to potential community changes. Although we had expected to see a response to glyphosate, it is possible that our application rate, application frequency (e.g. once vs. multiple applications), or residence time in the field were too low to elicit a response in the microbial communities. Our observation of lower abundance of other microbe species on plates within the 41% glyphosate treatment compared to our 1.5% glyphosate treatment would support the hypothesis that our field application rates were too low to affect soil microbial communities. However, glyphosate is only applied at concentrated rates in targeted "cut-and-paint" applications to treat invasive shrubs such as *Elaeagnus umbellata,* and it is improbable that we would ever see impacts on microbes from these rates in the field.

Conclusions:

Overall, presence of *P. cinnamomi* at the two study sites did not appear to be in any way related to the application of glyphosate herbicide. We detected no differences in *P. cinnamomi* presence or abundance, nor did we detect any changes in the soil microbial community, that were associated with our glyphosate treatments in either the field or the laboratory. We sought to try and explain why *P. cinnamomi* was present at both sites, and in fact may be more abundant at the Kentland Farm site, but had only resulted in *Castanea dentata* mortality at the Powell River Project. We suggest that while *P. cinnamomi* has readily colonized both sites, several factors, including higher macronutrient concentrations, higher pH, higher Ca concentrations, and higher soil organic matter content may have created soil conditions that were suppressive to the pathogen at Kentland. In addition, there is evidence to suggest that antagonistic bacteria and fungi may be both present, and more abundant at Kentland than at the PRP, though we cannot definitively confirm this. Further study of these microbial communities and additional analyses to quantify *P. cinnamomi* on the two sites would help elucidate why root rot disease has not been detected at Kentland. This information would help provide guidance on possible site preparation methods, such as the addition of organic matter amendments, that may help provide proper conditions for the establishment of antagonistic bacteria and fungi.

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Appendix 1- Basic soil chemistry of samples collected at Kentland and the Powell River Project in October 2018.

Tree #	Alive	Site	Treatment (all pre-treatment) A=glyphosate+ C=control	Collection date	pH	P ppm	K ppm	Ca ppm	Mg ppm	Zn ppm	Mn ppm	Cu ppm	Fe ppm	B ppm	% OM
223	A	Kentland	C	10/30/2018	4.89	20	91	563	121	3.1	19	0.2	14.3	0.2	5.5
166	A	Kentland	A	10/30/2018	5.51	9	66	719	167	2	13	0.7	9.2	0.3	4.4
336	A	Kentland	A	10/30/2018	6.08	26	115	1064	250	1.6	15.7	0.2	6.7	0.6	4.5
184	A	Kentland	C	10/30/2018	6.03	16	85	894	195	1.3	18.9	0.6	7.4	0.4	3.8
174	A	Kentland	C	10/30/2018	5.48	6	87	693	149	1.8	25.4	0.6	9.8	0.2	4
170	A	Kentland	A	10/30/2018	5.52	4	68	764	132	1.5	25.5	0.1	7.8	0.4	4.5
433	D	Kentland	C	10/30/2018	5.11	25	100	608	148	2.6	15.9	0.1	12.7	0.2	4.4
311	D	Kentland	A	10/30/2018	5.35	26	91	686	142	3.2	18.6	1	12.1	0.3	4.1
444	D	Kentland	A	10/30/2018	5.79	16	101	810	216	1.8	16.1	0.5	6	0.4	4.1
290	D	Kentland	C	10/30/2018	6.21	23	94	1350	313	2.9	18.2	0.3	4.3	0.7	5.6
325	D	Kentland	C	10/30/2018	5.99	4	98	930	185	1.8	21.9	0.2	5.7	0.5	4.1
181	D	Kentland	A	10/30/2018	5.62	6	134	872	167	2.7	24	0.1	7.8	0.5	5.1
32	A	PRP	C	10/31/2018	5.48	4	82	276	120	2	14.1	0.2	12	0.1	2
49	A	PRP	A	10/31/2018	5.23	5	75	205	88	1.6	18.9	0.2	19	0.1	1.7
141	A	PRP	A	10/31/2018	4.99	2	71	135	61	0.8	16.1	0.2	16.3	0.1	1.6
118	A	PRP	C	10/31/2018	5.62	3	88	345	126	1.8	14.3	0.2	12.8	0.1	2.1
15	A	PRP	C	10/31/2018	5.25	4	66	244	111	0.7	10.3	0.4	18	0.1	1.3
3	A	PRP	A	10/31/2018	5.2	37	65	312	111	1.2	15.7	0.3	19.2	0.1	1.4
140	D	PRP	C	10/31/2018	5.07	6	62	195	99	1.6	24.6	0.2	20.7	0.1	2
14	D	PRP	A	10/31/2018	5.28	8	66	226	103	1.1	15.6	0.4	22	0.1	1.7
148	D	PRP	A	10/31/2018	5.19	8	144	205	88	1.1	15.1	0.3	15.2	0.1	1.6
131	D	PRP	C	10/31/2018	5.34	6	69	278	110	1.5	14.9	0.3	19.3	0.1	2.2
150	D	PRP	C	10/31/2018	5.15	2	77	157	96	0.9	12.9	0.2	17.2	0.1	1.8
135	D	PRP	A	10/31/2018	5.09	1	40	122	74	0.7	10.1	0.2	18.1	0.1	1.3

Appendix 2- Summary tables of *P. cinnamomi* detection from soil DNA extractions with PCR- 1 of 4

Tree #	Sample ID	Alive	Site	Treatment (A/B=pre/post glyphosate +, C/D= pre/post control)	1-21-19 to 1-23-19 (run at lower annealing T°)	8/27/2019	8/28/2019	8/29/2019
3	SK30	A	PRP	A	Bright band	Strong thin band	Bright band	DNR- no sample
3	SK42	A	PRP	B	Not detected (ND)	DNR	DNR	DNR
14	SK32	D	PRP	A	ND	DNR	DNR	DNR
14	SK44	D	PRP	B	ND	DNR	DNR	DNR
15	SK29	A	PRP	C	ND	DNR	DNR	DNR
15	SK41	A	PRP	D	ND	DNR	DNR	DNR
32	SK25	A	PRP	C	ND	DNR	DNR	DNR
32	SK37	A	PRP	D	ND	DNR	ND	Small band
49	SK26	A	PRP	A	3 lanes, small band	Small band, v. faint	2 lanes, small bands	Small band
49	SK38	A	PRP	B	ND	DNR	DNR	DNR
118	SK28	A	PRP	C	ND	DNR	DNR	DNR
118	SK40	A	PRP	D	Small band, v. faint	DNR	Small band, v. faint	ND
131	SK34	D	PRP	C	ND	DNR	DNR	DNR
131	SK46	D	PRP	D	ND	DNR	DNR	DNR
135	SK36	D	PRP	A	ND	DNR	Bright band	Small, bright band
135	SK48	D	PRP	B	ND	DNR	DNR	DNR
140	SK31	D	PRP	C	ND	DNR	DNR	DNR
140	SK43	D	PRP	D	ND	DNR	DNR	DNR
141	SK27	A	PRP	A	Bright band	Small indistinct band, v. faint	2 lanes, 1 w/ Bright band, 1 ND	Small band, v. faint
141	SK39	A	PRP	B	Small band, faint	ND	Small band	Small band, v. faint
148	SK33	D	PRP	A	ND	DNR	DNR	DNR
148	SK45	D	PRP	B	ND	DNR	DNR	DNR
150	SK35	D	PRP	C	ND	DNR	DNR	DNR
150	SK47	D	PRP	D	ND	DNR	DNR	DNR

Appendix 2- Summary tables of *P. cinnamomi* detection from soil DNA extractions with PCR- 2 of 4

Tree #	Sample ID	Alive	Site	Treatment (A/B=pre/post glyphosate +, C/D= pre/post control)	10/14/2019	10/15/2019 Run 1	10/15/2019 Run 2	10/16/2019
3	SK30	A	PRP	A	DNR	DNR	DNR	DNR
3	SK42	A	PRP	B	DNR	ND	DNR	Small band
14	SK32	D	PRP	A	DNR	ND	DNR	DNR
14	SK44	D	PRP	B	DNR	ND	DNR	DNR
15	SK29	A	PRP	C	DNR	ND	ND	DNR
15	SK41	A	PRP	D	DNR	ND	ND	ND
32	SK25	A	PRP	C	DNR	ND	DNR	ND
32	SK37	A	PRP	D	DNR	Small band, v. faint	DNR	DNR
49	SK26	A	PRP	A	DNR	ND	DNR	Bright band
49	SK38	A	PRP	B	DNR	Small band	Small band	Small band, v. faint
118	SK28	A	PRP	C	DNR	ND	ND	DNR
118	SK40	A	PRP	D	DNR	Small band, v. faint	DNR	ND
131	SK34	D	PRP	C	DNR	Small band	ND	Small band, faint
131	SK46	D	PRP	D	DNR	ND	DNR	Small band, v. faint
135	SK36	D	PRP	A	DNR	Small band, v. faint	DNR	DNR
135	SK48	D	PRP	B	DNR	Small band, v. faint	DNR	ND
140	SK31	D	PRP	C	DNR	Small band, v. faint	ND	ND
140	SK43	D	PRP	D	DNR	Small band, v. faint	DNR	Small band, v. faint
141	SK27	A	PRP	A	DNR	ND	DNR	Bright band
141	SK39	A	PRP	B	DNR	Small band, v. faint	DNR	Bright band
148	SK33	D	PRP	A	DNR	ND	DNR	ND
148	SK45	D	PRP	B	DNR	ND	DNR	DNR
150	SK35	D	PRP	C	DNR	ND	DNR	DNR
150	SK47	D	PRP	D	DNR	Small band, v. faint	DNR	ND

Appendix 2- Summary tables of *P. cinnamomi* detection from soil DNA extractions with PCR- 3 of 4

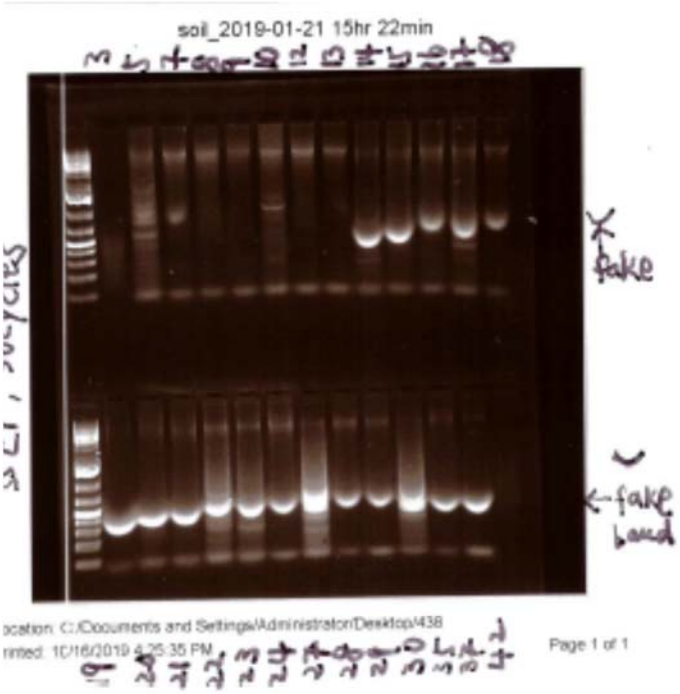
Tree #	Sample ID	Alive	Site	Treatment (A/B=pre/post glyphosate +, C/D= pre/post control)	1-21-19 to 1-23-19 (run at lower annealing T°)	8/27/2019	8/28/2019	8/29/2019
166	SK2	A	Kentland	A	ND	Did not run (DNR)	DNR	DNR
166	SK14	A	Kentland	B	Small band, faint	DNR	Small band	Small band
170	SK6	A	Kentland	A	ND	DNR	DNR	DNR
170	SK18	A	Kentland	B	ND	DNR	DNR	DNR
174	SK5	A	Kentland	C	Small band, v. faint	DNR	Small band	Small
174	SK17	A	Kentland	D	Small band	DNR	Bright band	Small band
181	SK12	D	Kentland	A	ND	DNR	DNR	DNR
181	SK24	D	Kentland	B	ND	DNR	DNR	DNR
184	SK4	A	Kentland	C	ND	DNR	DNR	DNR
184	SK16	A	Kentland	D	ND	DNR	Small band, v. faint	Small band, v. faint
223	SK1	A	Kentland	C	ND	DNR	DNR	DNR
223	SK13	A	Kentland	D	ND	DNR	Small band	Small band
290	SK10	D	Kentland	C	Small band, v. faint	DNR	Small band	Bright band
290	SK22	D	Kentland	D	Small band	ND	Small band	Small band
311	SK8	D	Kentland	A	ND	DNR	DNR	DNR
311	SK20	D	Kentland	B	Small band, v. faint	DNR	Small band, v. faint	Small band
325	SK11	D	Kentland	C	ND	DNR	DNR	DNR
325	SK23	D	Kentland	D	ND	DNR	Bright band	ND
336	SK3	A	Kentland	A	Small band, v. faint	DNR	Small band, v. faint	ND
336	SK15	A	Kentland	B	Small band, v. faint	DNR	Small band, v. faint	Small band
433	SK7	D	Kentland	C	ND	DNR	DNR	DNR
433	SK19	D	Kentland	D	ND	DNR	DNR	DNR
444	SK9	D	Kentland	A	Small band, v. faint	DNR	ND	ND
444	SK21	D	Kentland	B	Small band, v. faint	DNR	Small band, v. faint	Small band

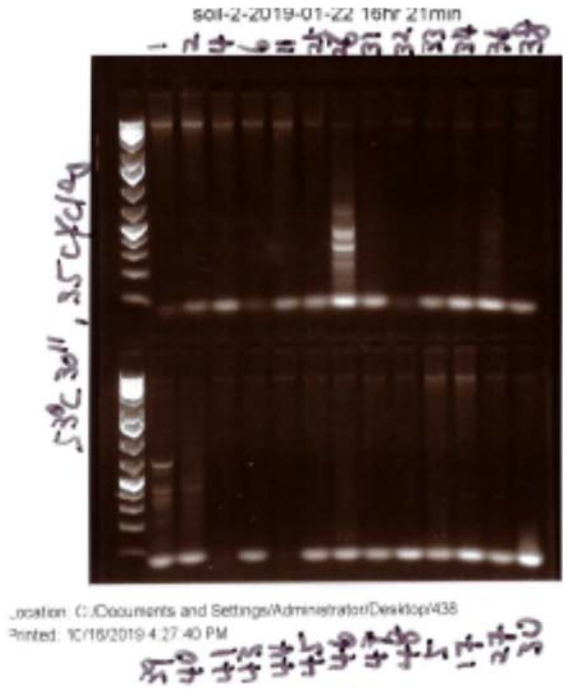
Appendix 2- Summary tables of *P. cinnamomi* detection from soil DNA extractions with PCR- 4 of 4

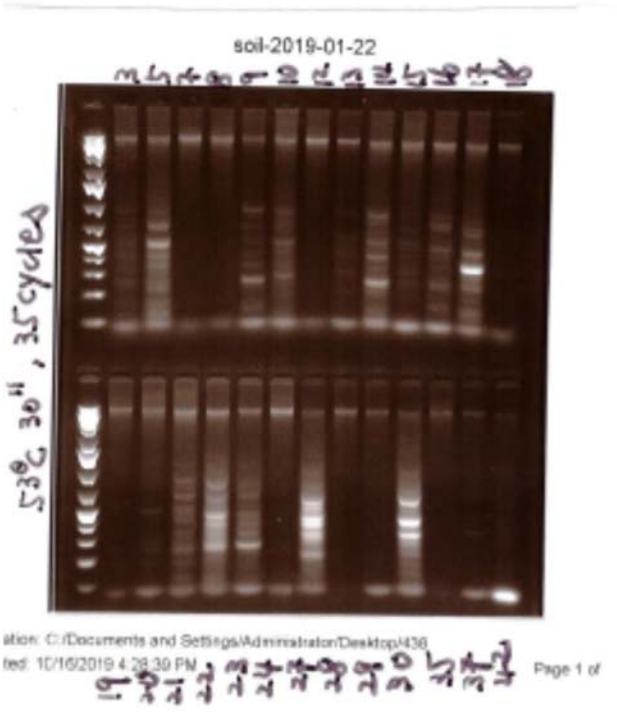
Tree #	Sample ID	Alive	Site	Treatment (A/B=pre/post glyphosate +, C/D= pre/post control)	10/14/2019	10/15/2019 Run 1	10/15/2019 Run 2	10/16/2019
166	SK2	A	Kentland	A	DNR	DNR	ND	DNR
166	SK14	A	Kentland	B	DNR	DNR	ND	Small band
170	SK6	A	Kentland	A	DNR	DNR	DNR	DNR
170	SK18	A	Kentland	B	DNR	DNR	DNR	DNR
174	SK5	A	Kentland	C	Small band, v. faint	DNR	Small band, v. faint	Small band, v. faint
174	SK17	A	Kentland	D	DNR	DNR	Small band, v. faint	Small band
181	SK12	D	Kentland	A	DNR	DNR	DNR	DNR
181	SK24	D	Kentland	B	DNR	DNR	DNR	DNR
184	SK4	A	Kentland	C	Small band	DNR	ND	ND
184	SK16	A	Kentland	D	DNR	DNR	ND	ND
223	SK1	A	Kentland	C	ND	DNR	ND	Three reps, ND
223	SK13	A	Kentland	D	DNR	DNR	DNR	Small band, v. faint
290	SK10	D	Kentland	C	Small band, v. faint	DNR	Small band	Small band, v. faint
290	SK22	D	Kentland	D	DNR	DNR	Small band, v. faint	Small band
311	SK8	D	Kentland	A	DNR	DNR	ND	ND
311	SK20	D	Kentland	B	Small band	DNR	Small band, v. faint	Small band, v. faint
325	SK11	D	Kentland	C	DNR	DNR	DNR	ND
325	SK23	D	Kentland	D	DNR	DNR	Small band, v. faint	Small band, v. faint
336	SK3	A	Kentland	A	DNR	DNR	DNR	DNR
336	SK15	A	Kentland	B	DNR	DNR	Small band, v. faint	Small band
433	SK7	D	Kentland	C	DNR	DNR	ND	ND
433	SK19	D	Kentland	D	DNR	DNR	ND	ND
444	SK9	D	Kentland	A	DNR	DNR	DNR	Small band, v. faint
444	SK21	D	Kentland	B	Small band	DNR	DNR	DNR

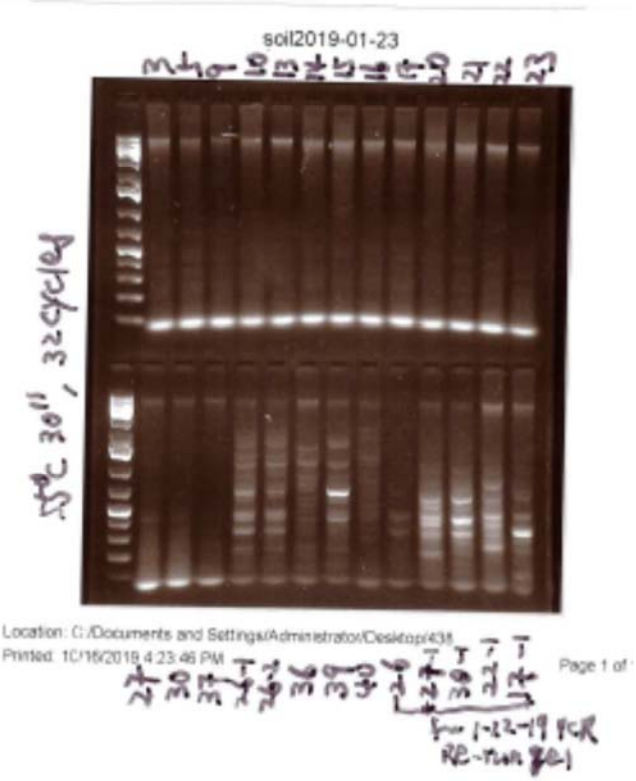
Appendix 3 – PCR with LPV3 primer gels Page 1 of 5

January 21, 2019 to January 23, 2019, various settings. No positive controls included. Annealing temperature likely too low for effective detection of *P. cinnamomi*.



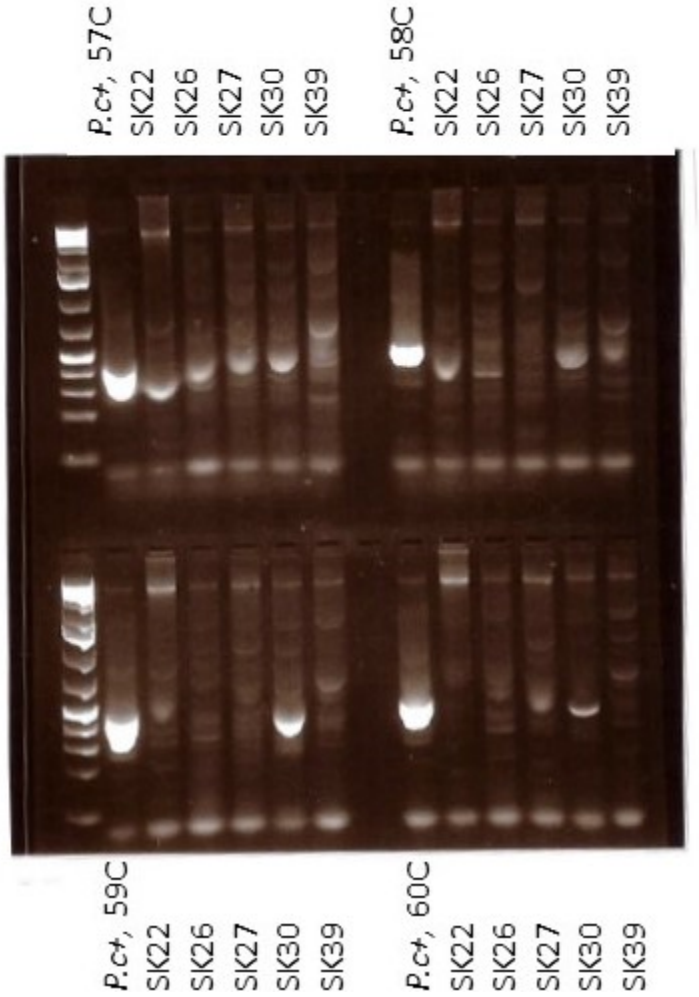




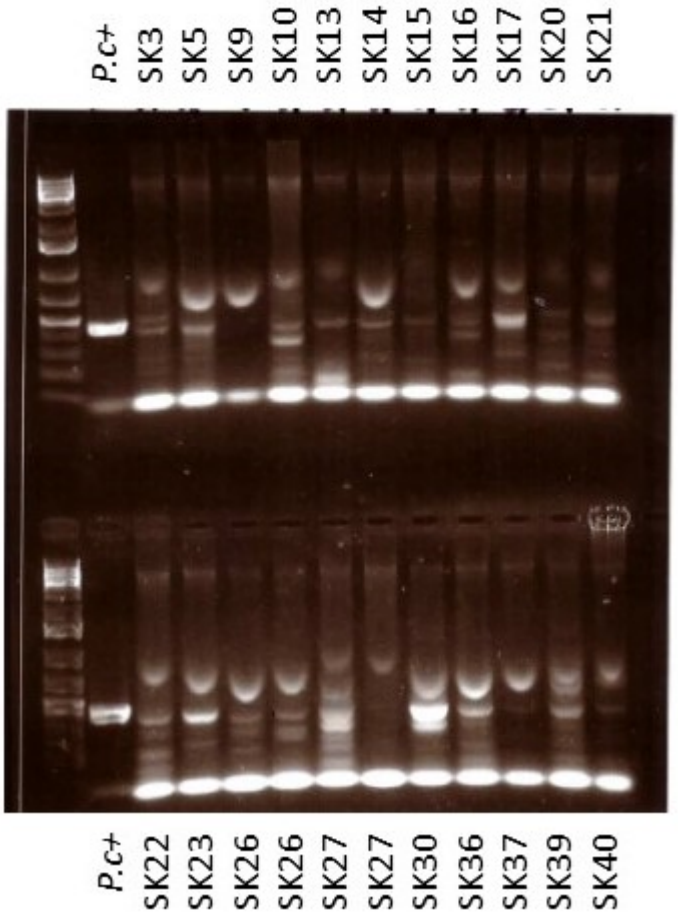


Appendix 3 – PCR with LPV3 primer gels Page 2 of 5

8-27-19,35", 40 cycles, sample load 15uL, Pc+ load 5uL
Upper L 57C, Upper R 58C, Lower L 59C, Lower R 60C

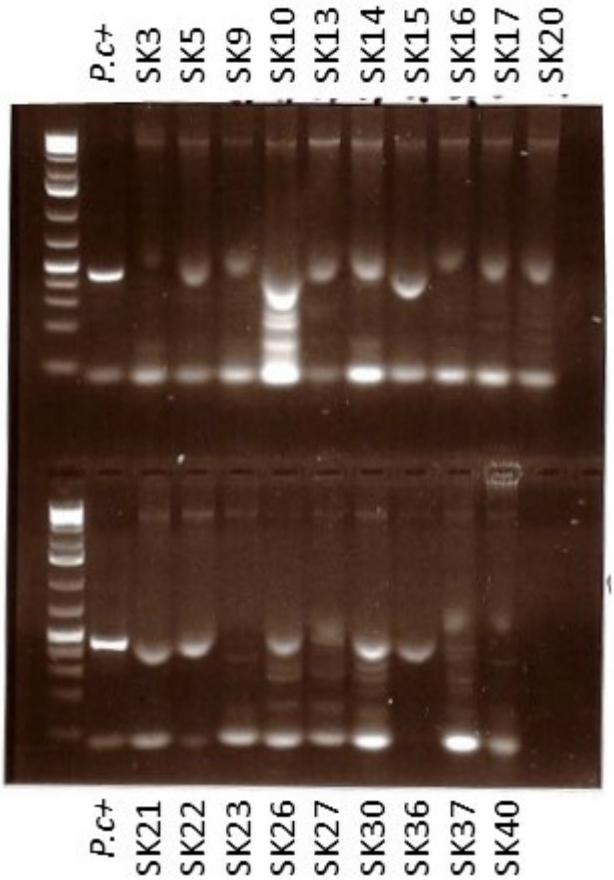


8-28-19, 60C, 35", 40 cycles, sample load 15uL, Pc+ load 5uL

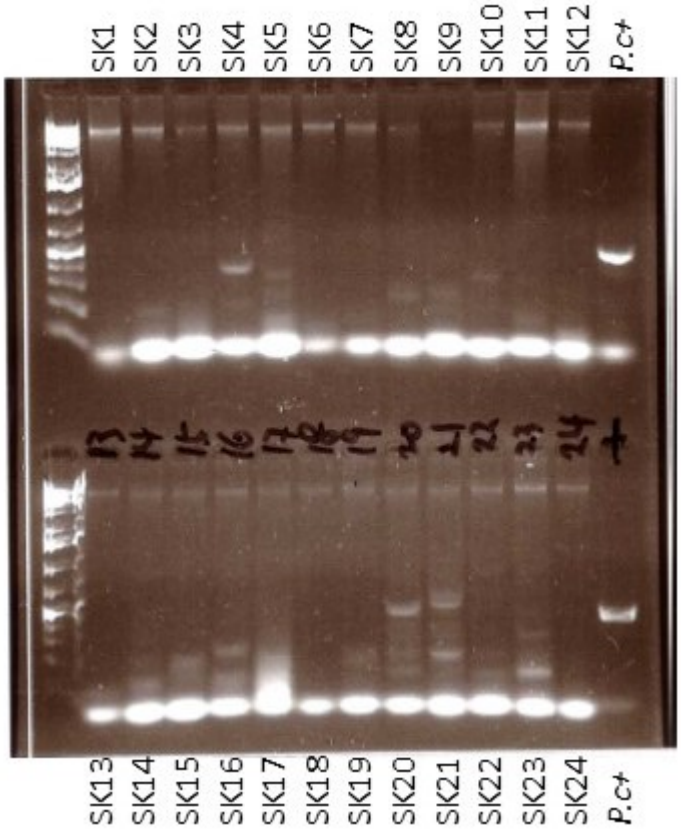


Appendix 3 – PCR with LPV3 primer gels Page 3 of 5

8-29-19, 60C, 35", 40 cycles, sample load 15uL, Pc+ load 5uL



10-14-19, 60C, 35", 45 cycles, sample load 12uL, Pc+ load 3uL



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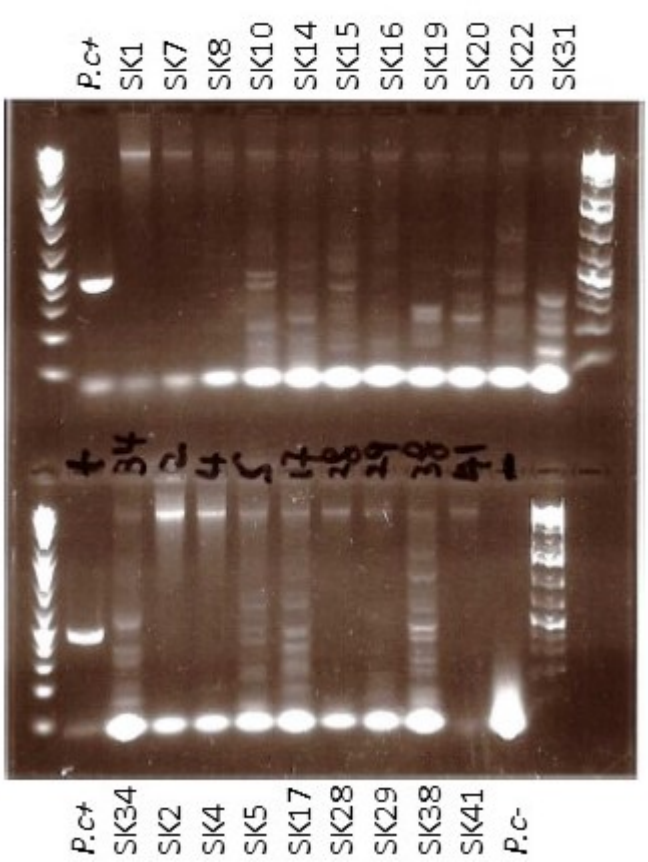
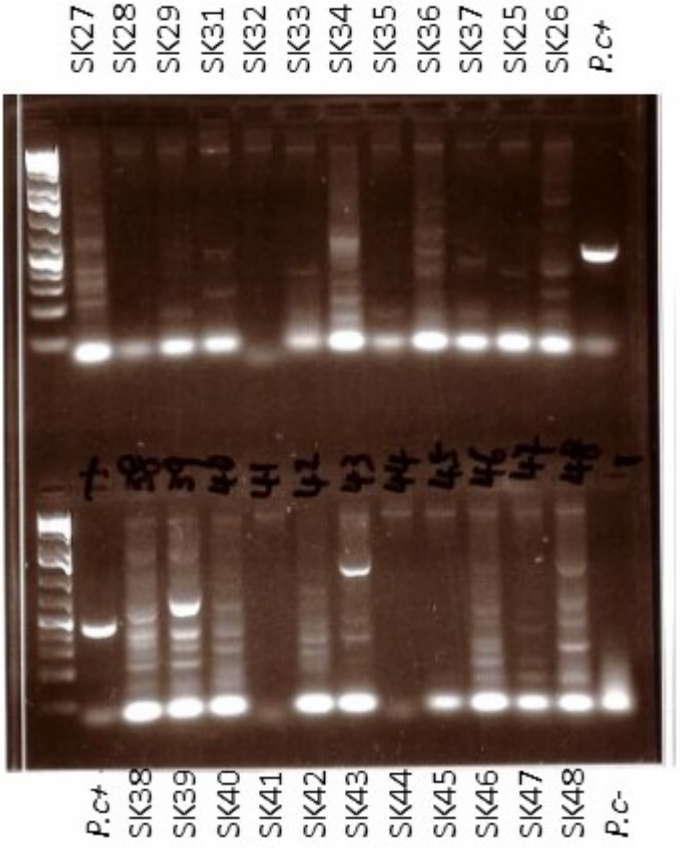
10-15-19, 60C, 35", 45 cycles, sample load 25uL, Pc+ load 5uL



10-15-19, 60C, 35", 45 cycles, sample load 25uL, Pc+ load 5uL



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10-16-19, 60C, 35", 45 cycles, sample load 25uL, Pc+ load 5uL