Midpeninsula Regional
Open Space District

R-21-63
Meeting 21-14
May 12, 2021

AGENDA ITEM 8

AGENDA ITEM

Oregon State University *Phytophthora* Research: Assessment of *Phytophthora* Soilborne Pathogens at Midpeninsula Regional Open Space District Restoration Sites

GENERAL MANAGER'S RECOMMENDATIONS

Receive Oregon State University's *Phytophthora* Research Presentation on the Assessment of *Phytophthora* Soilborne Pathogens at Restoration Sites. Provide feedback on recommendations and next steps. No formal Board action necessary.

SUMMARY

Invasive plant pathogens in the genus *Phytophthora* cause significant economic and ecological damage to horticultural and agricultural industries, and native wildlands. *Phytophthora* species can cause negative impacts across many different plant families in a variety of native habitats when introduced into the wildlands. These species have been introduced into California wildlands via infected native plant nursery stock and through other disturbances such as soil importation. Oregon State University's (OSU) Dr. Jennifer Parke and Dr. Ebba Peterson, have completed their research on *Phytophthora* in Midpeninsula Regional Open Space District (District) lands and will provide key findings and recommendations for future management actions to minimize the risk of impacts to the natural environment caused by *Phytophthora.*

Background

Soilborne *Phytophthoras* are a group of water molds that infect plants. There are over 150 described *Phytophthora* species, including the sudden oak death pathogen (*Phytophthora ramorum*; SOD). Although not known for certain, most experts believe that some types of *Phytophthoras* are native to California. They spread via spores in water, soil, or plant debris; some species are airborne.

In 2004, the District began to address SOD through staff trainings, use of Best Management Practices (BMPs), updating contract language, SOD Blitzes (coordinated with the University of California, Berkeley), and by supporting research on District lands and other locations. Since 2012, numerous species of soilborne *Phytophthoras* have been identified in native plant nurseries and revegetation areas of California. Some were inadvertently introduced into District preserves through use of infected nursery stock and other disturbances.

Starting in 2014, District staff ceased all native plant nursery stock installation in District Preserves for several years after learning about these plant pathogens and the destruction they are capable of causing at restoration sites and wildlands. District Natural Resources (NR) staff have

worked closely with native plant suppliers to ensure plants received for revegetation projects are grown utilizing phytosanitary BMPs and are tested for *Phytophthora* prior to being planted in District Preserves. Beginning in 2017, nursery plants grown with these BMPs that test negative have been installed at revegetation sites following the District's "Guidelines for Minimizing *Phytophthora* Contamination" BMPs (Attachment 2). These BMPs were compiled by NR staff specifically for planting projects to protect sensitive sites and prevent movement of *Phytophthoras* from known contaminated sites.

In 2016, spot sampling and testing completed by Phytosphere Research (a District Consultant) identified several soilborne *Phytophthora* species in several District Preserves, including the Skyline Ridge Tree Farm in Skyline Ridge Open Space Preserve (OSP), and the Mount Umunhum Summit and Bald Mountain parking lot in Sierra Azul OSP. To determine the presence and distribution of soilborne *Phytophthora* species at all District revegetation sites previously planted with nursery stock, NR staff released a Request for Proposals to twelve researchers and consultants to sample and test for *Phytophthora* species and determine what features may influence establishment and spread of these pathogens.

On June 28, 2017, the District's Board of Directors (Board) authorized an agreement with OSU to test District revegetation sites for soil diseases (R-17-85) using two methods: soil baiting[1] to isolate *Phytophthora* into culture, and the detection of *Phytophthora* DNA directly from the soil.

DISCUSSION

In December 2017 and 2018, OSU sampled and tested sites in 10 preserves. Sites were classified into one of four categories:

1) revegetation sites previously planted with nursery stock between 1993-2014,
2) recent revegetation sites previously planted with nursery stock between 2017-2018,
3) sites where future revegetation projects are planned, and
4) disturbed but non-remediated sites where no revegetation projects are planned.

The objectives of the OSU sampling, testing, analysis, and research were to:

1. Determine the presence and distribution of soilborne *Phytophthora* pathogens in representative revegetation sites on District preserves.
2. Identify conditions allowing for the designation of existing and future planting sites as either a high or low risk of *Phytophthora* introduction and establishment.
3. Provide recommendations for management of sites with *Phytophthora* contamination, and protective actions for uncontaminated sites.

Presence and Distribution of Soilborne Phytophthora Pathogens

Twenty (20) species of *Phytophthora* were cultured from District samples, including one provisionally new species *(P.* aff. *ilicis*[2]), one species recently discovered in California but not known to spread from restoration plantings (*P.* taxon *asparagi)*, and one species that had not been detected in North America previously (*P. boehmeriae)*. Some sites had a large number of taxa that were detected with DNA sequencing, despite having a low number of species detected with baiting. Importantly, this method found DNA of some pathogenic species of concern, which were *not* detected by baiting. Such DNA-only detections indicate either that the

[1] Baiting is a method which uses a growing medium, typically a pear, that *Phytophthora* present in the soil infect and grow on.
[2] Aff. (Latin: affinis) literally means "related to" and is used in scientific name for species that have not officially described in the scientific literature. In this case, the new species is closely related to *P. ilicis*.

Phytophthora sp. is present but could not be baited from these sites, or it may be remnant DNA from introductions of the pathogen that did not persist.

Risk of Phytophthora Introduction and Establishment
Several *Phytophthora* species were detected on District Preserves that are known to cause severe disease elsewhere and are considered high risk to native habitats. For many of the other *Phytophthora* species found, the amount of damage to native habitats is fairly unknown. Unlike SOD (a species that spreads via air, i.e., 'airborne'), the majority of soilborne *Phytophthoras* are not known to cause widespread plant death. However, many of these species, including many detected in OSU's sampling and analysis, are likely contributing to plant health decline associated with other environmental stressors. Some are likely native species thought to cause minor or sporadic disease, and other species can cause severe symptoms in nursery settings but are less likely to persist in native habitats. Given the different risks posed by each *Phytophthora*, OSU ranked each project area taking into account not only its total diversity, but also the potential for each detected species to cause harm. The risk ratings of each *Phytophthora* (low to very high risk of causing disease on District lands) were based on published literature and best professional judgement. These rankings can be used to create management recommendations for preventing the introduction of *Phytophthora* into District Preserves, containing *Phytophthora* species in heavily infested sites, and protecting less infected sites with a lower diversity of *Phytophthora* species.

Each *Phytophthora* was categorized into four risk classes (very high, high, moderate, or low) based on its ability to cause disease. Project areas with the highest infestation scores were at Pulgas Ridge OSP at the Blue Oak parking lot and forest site, the Mindego Gateway parking lot in Russian Ridge OSP, Skyline Ridge Tree Farm in Skyline Ridge OSP, and the La Honda Creek OSP site near the Event Center. Project areas with the lowest risk infestation scores were in El Corte de Madera OSP, Sierra Azul OSP, and Bear Creek Redwoods OSP. There are *Phytophthora* species present at the lower risk sites, however these may be less pathogenic and less diverse.

In general, OSU found that sites with hardwood, chaparral, and shrub vegetation had higher infestation scores, as did riparian areas. These sites tended to have a greater proportion of samples in which *Phytophthora* was detected, and greater diversity of high to very high-risk species. Rocky sites and those with limited forb cover had the lowest infestation scores, as did Douglas-fir or redwood dominant habitats when not adjacent to streams. These areas had lower species diversity; when higher-risk species were detected they were often limited to prior revegetation project sites. This could indicate that these sites have plant genera or other site factors that limit *Phytophthora* establishment.

Recommendations for Management
OSU developed three main methods (outlined below) to prevent further spread of *Phytophthoras* and reduce their impacts in District preserves and wildlands:

1. <u>Exclusion and Prevention</u>

Prevention of introducing *Phytophthora* species into District Preserves should be a top priority. Risk for introduction can be greatly reduced by:

- minimizing importation or movement of soils;
- using direct seed planting;

- installing native plant nursery stock that is grown utilizing phytosanitary BMPs and tested for *Phytophthoras*;
- cleaning and sanitizing boots, tools, equipment, and vehicles;
- managing trails to prevent spread of infested soils (including restricting human access to high-risk sites);
- controlling drainage and surface water; and
- planning and implementing considerations when staff, contractors, and volunteers move between sites or when they are working in known infested sites or sensitive sites.

Exclusion is especially important to protect less infested sites and sensitive sites that are known to host Special Status Species and is also important for heavily infested sites.

2. Eradication and Resistance

Eradication is costly, time consuming, extremely difficult, and has an unknown success rate once non-native *Phytophthoras* are introduced to a site. It requires the complete removal of *Phytophthora* and may involve heat treatment, removal of the host, or the application of fungicide, and also requires long term monitoring to ensure its success. Heat treatment of nursery stock potting media has been proven to be an effective BMP but heat treatment of soil in field settings via solarization and steaming is costly, time consuming, and often impractical. However, if conditions are right, solarization may be useful to eradicate *Phytophthora* in small areas or as a pre-planting treatment. Fungicide treatments should be used as a last resort.

Resistance breeding is a long-term method used for native tree species impacted by *Phytophthora* species like SOD. Efforts are underway at Bear Creek Redwoods OSP to experiment with potential SOD resistant trees through the collection and planting of acorns from trees that survive in the SOD infested preserve. The District should prioritize preservation of vulnerable plant populations located in areas with no or low infestations of *Phytophthora*.

3. Protection and Suppression

Protection and suppression methods focus on a range of strategies that can create a barrier between a pathogen and host or reduce impacts from pathogens once they are introduced. This may include management of surface waters and wet soils, or treatment with fungicides.

Applicable principles developed by OSU may be applied to District management decision-making:

- assessing and managing the risk of a given site around the goals of the restoration project;
- assessing and testing for *Phytophthora* at new revegetation sites;
- avoiding planting vulnerable species in severely *Phytophthora* infested areas;
- planting in a manner to reduce risk factors; managing soil moisture at planting sites;
- increasing spacing between host species that may be hosts; pre-emptive treatment with phosphite based compounds like Agri-Fos (currently on the List of Approved Pesticides within the District's Integrated Pest Management Program) that suppress *Phytophthora*; and
- completing periodic plant health assessments in heavily infested sites and sites that are fairly clean.

FISCAL IMPACT

OSU's *Phytophthora* Research Presentation has no immediate fiscal impact, however, the results and recommendations may have fiscal impacts to future District land management activities, including capital improvement, restoration, and maintenance projects.

BOARD AND COMMITTEE REVIEW

On February 10, 2016, District staff presented to the Board providing a 10-year update on the status of SOD (Board Report, minutes).

On June 28, 2017, the Board authorized the award of contract with OSU (Board Report, minutes).

On March 27, 2019, the Board authorized the General Manager to amend OSU's contract to add additional analysis for the research (Board Report, minutes).

CEQA COMPLIANCE

The *Phytophthora* research conducted by OSU was determined to be categorically exempt under Sections 15302 (Minor Alterations of Land) and 15306 (Information Collection) of the California Environmental Quality Act (CEQA) Guidelines at the June 28, 2017 board meeting.

NEXT STEPS

NR staff will develop more robust BMPs for District staff and contractors to minimize the potential for introducing or moving *Phytophthoras* throughout District Preserves, especially at sensitive sites with Special Status Species, and sites rated lower risk due to the type of habitat or lower detections of *Phytophthora* during OSU's study. NR Staff will start the development of remediation and implementation plans based on OSU's site-specific recommendations, as well as staff training, public outreach, and planning and implementation of prevention techniques. Preliminary internal meetings with NR staff and other District Departments in Fiscal Year 2021-22 will be conducted to determine the feasibility and fiscal impacts of implementing these strategies to minimize the potential for introducing or moving *Phytophthoras* throughout District Preserves.

Attachment(s)

1. Oregon State University Final Report: Assessment of *Phytophthora* Soilborne Pathogens at Restoration Sites in the Midpeninsula Regional Open Space District
2. Guidelines for Minimizing *Phytophthora* Contamination at Midpeninsula Regional Open Space District Preserves

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Dec. 31, 2020

Final Report

Assessment of *Phytophthora* soilborne pathogens at restoration sites in the Midpeninsula Regional Open Space District: minimizing the impacts of harmful pathogens to better attain restoration objectives

Agreement between the
Midpeninsula Regional Open Space District
and
Oregon State University

July 1, 2017 to December 31, 2020

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Executive Summary

Problem Statement & Approach

Plant pathogens in the genus *Phytophthora* are ecologically diverse, inhabiting water, soil, and plant roots, stems and canopies of many ecosystems worldwide. Many, such as *P. ramorum*, the causal agent of sudden oak death, are invasive and cause substantial harm to native plant communities; others reduce plant vigor and cause mortality in association with adverse climate events or other stressors. Introduced species often invade after historical disturbances involving the movement of soil, such as construction and road grading, however more recent evidence demonstrates that many *Phytophthoras* are associated with nursery-grown plants. Of major concern is the introduction of damaging species from native plant nurseries during restoration outplantings. Consequences of their establishment include failed plantings, reduced natural regeneration, and further spread of *Phytophthora* into vulnerable habitats.

To best guide *Phytophthora* management, we performed surveys to identify the distribution and diversity of *Phytophthora* species within the Midpeninsula Regional Open Space District (MROSD), focusing on prior restoration sites, future project areas, and the surrounding environment. To optimize our ability to detect *Phytophthora*, we used two different methods: soil baiting to isolate *Phytophthora* into culture, and detection of *Phytophthora* DNA directly from the soil, in a procedure called Illumina MiSeq high-throughput sequencing. A total of 38 past and future restoration areas across 10 preserves were sampled in December of 2017 and 2018. Project areas were ranked by their overall infestation level, which is based on the diversity of *Phytophthora* species detected there and their potential to cause disease. Rankings should be used to prioritize District lands for future management efforts.

Key findings

- **A high diversity of *Phytophthora* species was detected.** A total of 20 species were cultured, including one species not previously reported in North America (*P. boehmeriae*), one not known to have spread from restoration plantings (*P.* taxon *asparagi*), and one provisional new species (*P.* aff. *ilicis*).
- **The DNA-based method detected a greater diversity of *Phytophthora* than the soil baiting method.** Of the 34 *Phytophthora* taxa detected by Illumina, 19 were never baited. In many cases we likely detected DNA remnants in the soil (i.e. they are not viable), however many of these detections are of species which are difficult to bait.
- **Different *Phytophthora* species pose different levels of risk for disease development.** Some *Phytophthora* species are highly likely to cause disease on District lands, whereas other species represent a medium to low risk. For many taxa, the potential for damage is not known.
- ***Phytopththora* species that appear to be native are widely distributed; these are believed to represent a low risk to MROSD.**

- **High risk species include *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. crassamura*, *P. cryptogea*, *P. megasperma*, *P. multivora*, *P. rosacearum*, *P. syringae*, P. taxon *asparagi*, and *P. ramorum***. These were all baited; for most their distribution is greater as indicated by the DNA-analysis.
- **Many detections are strongly associated with nursery plants and disturbance.** 11 *Phytophthoras* were detected (by either method) only from nursery-grown plants or in heavily disturbed areas. However, many high risk species were also detected in unplanted areas, consistent with the complex disturbance history on MROSD lands.
- **Different vegetation types had different infestation levels, though this varied by preserve.** On average, areas dominanted by Douglas-fir or redwood (provided they were not adjacent to waterways) and areas with sparse vegetation were the least infested; riparian areas, hardwood forests, and chaparral/shrub communities were among the most infested. Despite having vegetation types associated with greater infestation, some preserves were relatively free of *Phytophthora*.

Key takeaways

- ***Phytophthora* has established and is impacting many plant communities located within MROSD preserves**. Some areas (notably Pulgas Ridge, Skyline Ridge, and Mindego Gateway) are heavily contaminated by *Phytophthora* and have symptoms characteristic of *Phytophthora* decline: stunting, chlorotic foliage, dying branches, and mortality. However,
- ***Phytophthora* species are not ubiquitous**. Many preserves, notably El Corte de Madera, Bear Creek Redwoods, and the higher elevation sites at Sierra Azul, were the least infested. When higher-risk species were detected they were mostly found in prior revegetation projects.
- **Nursery-grown plants are implicated in the introduction of a large diversity of species, including some of the highest risk species**. While not all introductions establish, some, such as *P. cactorum*, may be spreading into surrounding vegetation.

Management recommendations

- ***Phytophthora* management can and should be incorporated into existing IPM strategies to protect natural habitats for their educational, recreational, and ecological benefits.**
- **Areas with low infestation rankings should be a priority for treatment and protection**.
    - Protection principles minimize the risk of introduction, particularly of species not already at the site. This is achieved by minimizing importation or movement of soils; direct seed planting; installing native plant nursery stock grown utilizing phytosanitary BMPs; cleaning and sanitizing boots, tools, equipment, and vehicles; managing trails to prevent spread of infested soils; controlling drainage

and surface water; and coordinating and implementing phytosanitary considerations when staff, contractors, and volunteers move between sites.
    - Under some circumstances, heat treatment through solarization or steaming may be useful to eradicate *Phytophthora* in small areas or as a pre-planting treatment.

- **Heavily infested areas are best managed by containing the local spread of pathogenic species, and monitoring for their impacts**.
    - Even within heavily infested areas with a high diversity of aggressive species, some *Phytophthora* species may have a limited distribution. Sanitation measures within a site reduce the risk of further contamination.
    - Disease may be minimized by managing drainage and runoff at planting sites; increasing spacing between plant species that may be hosts when host-specific *Phytophthora* species are indicated at a site; and avoiding planting vulnerable species in severely *Phytophthora* infested areas.
    - Pre-emptive treatment with phosphite-based compounds like Agri-Fos (currently on the List of Approved Pesticides within the District's Integrated Pest Management Program) suppress *Phytophthora* and may be used to protect vulnerable populations close to infestation centers.

- **Assess for *Phytophthora* prior to planting at future revegetation sites.**
    - Greatest *Phytophthora* diversity was detected from *Alnus*, *Arbutus*, *Arctostaphylos*, *Frangula*, *Heteromeles*, *Lupinus*, *Mimulus*, *Notholithocarpus*, *Quercus* and *Rubus*. These plant genera (along with *Ceanothus*, which was not sampled here) should be targeted to assess future *Phytophthora* diversity. The presence of these genera may also indicate the area is conducive for *Phytophthora* establishment.
    - Areas in which we observed lower diversity should also be assessed. While these locations had fewer *Phytophthora*, aggressive species were detected at all preserves.

Future research to better guide Phytophthora *management*

- **Repeat baiting for high risk species detected only via the DNA-sequencing method.**
    - DNA-only detections indicate a species was introduced, however it does not indicate whether it is viable. Detections of greatest concern include *P. tentaculata*, *P. lateralis*, and *P. siskiyouensis*.

- **Monitor heavily infested sites (and less-infested sites for comparison) for the impacts of *Phytophthora* infection.**
    - Given the uncertainty about the extent to which many species may cause problems, monitoring of plants for symptoms of disease and decline will best guide the management of vegetation exposed to *Phytophthora.*
    - In particular, long-term monitoring will help determine if Phytophthoras are impacting natural regeneration of native plant populations or the success of revegetation efforts; monitoring may identify which plant species or individuals are particularily impacted by *Phytophthora*, and which are tolerant to disease.

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Abstract

Plant pathogenic *Phytophthora* species cause disease on agricultural crops, managed landscapes, and forests worldwide. Recent research indicates that *Phytophthora* species may have been introduced to wildlands as part of revegetation efforts. Surveys for *Phytophthora* have been limited by methodology to detect and identify *Phytophthora* species present in soil, and as a result, their distribution and impact on the health of wildland plant communities is only beginning to be understood. The purpose of this study was to survey restoration sites within the Midpeninsula Regional Open Space District (MROSD) for soilborne *Phytophthora* species with the goal of directing management to reduce future disease impacts. Objectives were to:

1. Determine the presence and distribution of soilborne *Phytophthora* pathogens in representative revegetation sites in MROSD preserves.
2. Identify conditions allowing for the designation of existing and future planting sites as either a high or low risk of *Phytophthora* introduction and establishment.
3. Provide recommendations for management of sites with *Phytophthora* contamination, and protective actions for sites not yet contaminated.

Ten MROSD preserves were selected, representing several different habitat types and disturbance histories. Sites were classified into one of four categories: revegetation sites previously planted with nursery stock, sites where future revegetation projects are planned, disturbed but nonremediated sites where no revegetation projects are planned, and relatively undisturbed control sites. Control sites were located adjacent to the revegetation, planned, or disturbed areas. Sites were sampled in December of 2017 (Year 1) and 2018 (Year 2). For each site, soil and roots were collected from the base of six plants belonging to three plant species. Plant tissue from plants with symptoms of *Phytophthora* disease (branch dieback, stem lesions, foliar symptoms) was also collected. GPS coordinates and plant health status were recorded for each sample and mapped with ArcGIS.

To maximize the likelihood of detecting the greatest diversity of *Phytophthora* species, two different methods were used. Baiting is a traditional method for detecting *Phytophthora* from soil that makes use of the attraction and colonization of susceptible plant tissue by live *Phytophthora*. Baits included unripe pears (Years 1 & 2) and oregano stem and leaf, rhododendron leaf, and *Quercus robur* acorn radicals (Year 2). Bait lesions were placed on a selective agar medium, and individual, single-species cultures growing from the bait were identified by Sanger sequencing of DNA extracted from hyphal growth on the agar plate. Our second method, Illumina MiSeq high-throughput sequencing, is a newer technique that involves extracting DNA directly from soil. Here, unique DNA sequences (operational taxonomic units, or OTUs) are matched to a reference database containing sequences of previously described species. Notably, this method can identify the DNA of all *Phytophthora* taxa present in the soil, even when they cannot be baited and are present in mixed populations. Details on the two methods, including their analysis and interpretation, are provided. In addition to soil samples, plant samples were cultured on selective media and isolates were identified with Sanger sequencing.

A total of 564 soil samples were collected from 72 sites. Of these, 22 sites (representing 252 samples) were sampled in both years. *Phytophthora* was baited from only 73 of 563 soil samples. Baiting success was nearly identical between the two years, with *Phytophthora* isolated from only 11.9% or 14.0% of the soils sampled in 2017 or 2018, respectively. Eighteen species

were recovered in total, of which *P. cactorum* and *P. cambivora* were the most common. Some species (e.g. *P. cactorum*) were baited both years, while others (e.g. *P. cambivora*, *P. megasperma*, *P. crassamura*) were only baited in one year, even from repeat samples. While species composition differed between revegetation and control sites, both site types supported a large diversity of species with 14 or 12 species baited, respectively. Both site types had up to 4 species baited per site. Two species, *P.* aff. *ilicis* and *P. nemorosa,* were only recovered from foliar lesions.

Of the 564 soil samples processed, 443 samples had DNA of sufficient quality for Illumina MiSeq sequencing. To reduce the likelihood of saying a particular OTU was present in error (false positives), we counted a given *Phytophthora* OTU as "present" for analysis purposes only when it comprised at least 0.095% of the total number of reads within a sample. At this threshold, we detected 34 distinct *Phytophthora* OTUs over both years. Of these, 11 OTUs represent clusters or complexes that cannot be identified to a single species. An additional 4 OTUs were detected at a minimum threshold of 0.01% and may also be present.

Compared to other genera, *Phytophthora* was uncommon, comprising only 1.28% of the total number of OTU reads within our samples. Despite the low relative abundance, *Phytophthora* was detected in nearly half of all soil samples. *Phytophthora* was present in all site classes, with no difference in the average number of *Phytophthora* OTUs detected in control, disturbed (combined disturbed and planned sites), or revegetation sites, with up to 9 *Phytophthora* OTUs detected at a single site. The *P. psychrophila* and the *P. cactorum*-cluster OTUs were the two most common *Phytophthora* taxa detected and were abundant in all site classes. The *P. nemorosa*-cluster and *P. syringae* OTUs were the next most commonly detected, followed by the *uliginosa*-cluster and the *quercina*-cluster which were detected almost exclusively in control sites.

Although *Phytophthora* was present in all site classes, the communities of OTUs differed between them. Compared to revegetation sites, controls sites contained a greater abundance of Clade 3 taxa. Clade 3 (the *nemorosa*-cluster and *psychrophila* OTUs) is comprised of species which may be native to western North America, as supported by the OTU data. Being native, these are thought to be less damaging to native flora and are considered less likely to cause substantial damage on MROSD lands. In contrast, revegetation and disturbed sites had a greater abundance of rare taxa; of the 13 OTUs with only 1 or 2 detections, 10 were detected exclusively at revegetation or disturbed (combined planned or disturbed) sites. Similarly, some *Phytophthora* species (*P. boehmeriae*, *P. crassamura*, *P. megasperma*, *P. nicotianae*, and *P. rosacearum*) were clearly associated with nursery outplantings or human disturbance as they were baited exclusively from revegetated or heavily disturbed areas.

One of the key findings is that the aggressive pathogen *P. cactorum* is widely distributed in all site classes at MROSD (as indicated via Illumina) and is especially active in revegetated sites (as indicated via baiting). *P. cactorum* has been associated with restoration nurseries in the Bay Area. It is likely that this pathogen was introduced some time ago with restoration plantings and dispersed to other areas. The amount of damage *P. cactorum* may cause to native plant communities is relatively unknown, as is the case for most of the other *Phytophthora* taxa we detected. In general, we expect the greater the diversity, particularly of more aggressive species, the greater the chances *Phytophthora* disease may manifest.

To summarize the overall infestation level of each site, we first categorized each species (baiting) or OTU (Illumina) into one of four risk classes based on their expected ability to cause

disease: very high, high, moderate, or low. Risk class is based on published literature, taking into account aggressiveness, host range, abundance, and prior history. Six *Phytophthora* species categorized as very high-risk (rating = 4) were baited from several preserves; Illumina detections of these taxa were similarly widespread. These very high-risk species include *P. cactorum, P. cambivora, P. cinnamomi, P. multivora, P. cryptogea*-complex, and *P. ramorum.* Five high risk species (rating = 3) were baited: *P.* taxon *asparagi, P. megasperma, P. crassamura, P. rosacearum,* and *P. syringae*. High risk OTUs corresponding to *P. siskiyouensis* and *P. tentaculata* were also detected with Illumina but were not recovered with baiting. Three medium risk (rating = 2) species, *P. boehmeriae, P. nicotianae,* and *P.* sp. *cadmea* were recovered; in addition, the medium risk *P. citrophthora*-cluster, *P. fallax, P. hedraiandra, P. hibernalis, P. lateralis,* and the *P. quercina*-cluster were detected. The remaining species recovered or detected were considered low-risk (rating = 1).

Risk ratings (1-4, representing low to very high-risk) for all detected *Phytophthora* were summed to calculate a soil infestation score for each site. Two scores were calculated: one for baiting and one for Illumina, which were summed for a final total score. Because there was little evidence revegetation or disturbed sites consistently contained a greater abundance of species or higher risk scores than their corresponding control sites, we summarized infestation for all species or OTUs detected within a project area.

Project areas (combined revegetated/disturbed site(s) and the corresponding control site) with the highest infestation scores were the Pulgas Ridge Blue Oak parking lot and the Pulgas Ridge forest site, Mindego Gateway, Skyline Ridge and La Honda Creek. These sites have evidence for heavy infestation using both detection methods, indicating numerous pathogenic *Phytophthora* species are present. Symptoms of Phytophthora dieback were also observed. Extreme caution should be used to prevent dispersal from these sites. A number of areas (Monte Bello, Purisima Redwoods, some Sierra Azul locations) had a high infestation score via Illumina, but had a relatively low score via baiting; these areas may have a history of failed introductions and/or we had a limited ability to bait some *Phytophthora* spp. from these sites.

Project areas with the lowest infestation ranking were in El Corte de Madera (3 project areas), Sierra Azul (3 project areas at the Mt. Umunhum Summit), and Bear Creek Redwoods (2 project areas). Despite a history of ornamental outplantings and disturbance, there was minimal to no evidence of dieback at these sites and *Phytophthora* isolates were largely restricted to outplanted nursery stock. These sites are not *Phytophthora*-free, however the species present may be less pathogenic and/or the community may be less diverse. Future efforts should focus on preventing new introductions into these sites.

Management of *Phytophthora* is aided by *a priori* information regarding a site's infestation status. To this end, we performed exploratory statistical analyses assessing many disturbance, habitat class, and topographical variables for their ability to predict if future sites should be classified as high or low risk of being infested. The odds of detecting a viable *Phytophthora* species via baiting differed significantly by habitat class (grossly defined as woodland, chaparral/shrub, rocky-forb, riparian, or upland-evergreen) and site type (control, disturbed & planned, or revegetated); areas with greater infestation scores were those in the riparian, woodland, and chaparral/shrub classes, especially if they had been revegetated with nursery plants. These three habitat classes were also associated with significantly larger infestation scores, indicating both a high diversity of species and presence of aggressive species. After inclusion of these variables, other factors which may influence the establishment of *Phytophthora* were relatively less important or had a negligible effect on our ability to predict

site infestation: plant health, elevation, slope, aspect, usage and distance to features associated with the spread of *Phytophthora*, and prior disturbance history. While still important to consider when assessing the current or potential infestation of an area; they are likely less predictive because of the complex history of the area and the broad distribution of *Phytophthora*, particularly the aggressive species.

Conclusion

Phytophthora species are widespread in MROSD. A total of 20 species were cultured, including a species not previously reported in N. America (*P. boehmeriae*), one not known to have spread from restoration plantings (*P.* taxon *asparagi*), and one provisional new species (*P.* aff. *ilicis*). Unfortunately, high-risk and very-high risk species were baited from both control and revegetated areas, in all preserves. Illumina MiSeq detected 38 distinct OTUs, providing evidence of even greater frequency, distribution, and diversity than detected by baiting.

The combination of two *Phytophthora* detection methods, baiting and Illumina MiSeq, resulted in a composite dataset that was more robust than if based on a single method alone. Baiting underestimates the diversity of species present, resulting in an incomplete picture of *Phytophthora* distribution. While Illumina is extremely sensitive and can detect the broadest range of *Phytophthora* taxa, notably of hard-to-bait species, it does not allow separation of some individual species belonging to clusters or complexes, cannot distinguish between live and dead propagules, and may in fact be too sensitive, resulting in false positives. Careful interpretation of both methods provides the most informative analysis of *Phytophthora* distribution currently possible.

Notably, both methods indicated a wide range of infestation severity among project areas and preserves. To the extent possible, tolerant hosts should be selected for highly contaminated sites, and sanitation measures should be implemented when moving between sites. This research demonstrates that some MROSD preserves have substantially less *Phytophthora* infestation than others, and these areas should be a priority for protection. *Phytophthora* diseases continue to emerge world-wide to the detriment of native vegetation. *Phytophthora* management can be incorporated into the broader goal of protecting natural habitats for their educational, recreational and ecological benefits.

This report contains the results from two years of sampling of MROSD preserves and includes the following deliverables:

- A full description of the sampling strategy and methods used.
- A detailed list of which *Phytophthora* spp. were recovered (via baiting) or detected (via Illumina) from each site, and from which hosts.
- Interpretation of results from baiting and the Illumina datasets.
- A brief overview of the published host range and historical impact of each *Phytophthora* species (if known).
- A ranking of the infestation level, taking into account *Phytophthora* diversity and aggressiveness of the species detected there, for each site and area.
- Management recommendations for the containment of *Phytophthora* as they pertain to MROSD revegetation sites.

Introduction & Project Objectives

Invasive phytopathogens in the genus *Phytophthora* (Phylum Oomycota; Order Peronosporales) cause significant economic and ecological damage to horticultural and agricultural industries and native wildlands. While case examples in Australia, South Africa and elsewhere (*P. cinnamomi*), and the western U.S. (*P. lateralis*) served as examples of this genus' capacity to cause widespread mortality, the global emergence of *P. ramorum* initiated interest in understanding the distribution of *Phytophthora* outside of agriculture. As the agent of sudden oak death (SOD), *P. ramorum* has caused the death of millions of tanoak, coast live oak and canyon live oak trees in California and Oregon since its establishment in the mid-1990s. More recently, widespread mortality has been reported in commercial Japanese larch plantations in the U.K. Surveys designed to delimit the extent of *P. ramorum* and other species have resulted in a surprising diversity of *Phytophthora* spp. in wildland ecosystems.

Prior to the emergence of SOD, ~ 50 species were described, mostly associated with food crops. In the past 25 years this list has expanded to include ~150 species, of which over 50 have been described since 2010. *Phytophthora* is divided into 12 phylogenetically distinct clades, although as new species are identified the number of clades continues to increase. Clades 6 and 9 are generally thought to be opportunistic aquatic species, only occasionally causing disease. However, some species from all clades are capable of causing disease under certain circumstances.

The diseases caused by this genus have similarly expanded globally. A single species is implicated as the causal agent in many diseases. In addition to *P. cinnamomi, P. lateralis*, and *P. ramorum,* these include: *P. austrocedrae*, causal agent of Mal del Ciprés of *Austrocedrus* in Patagonia and dieback of *Juniperus communis* in the U.K.; *P. pluvialis*, red needle cast of radiata pine (*Pinus radiata*) and Douglas-fir (*Pseudotsuga menziesii*) in New Zealand; *P. pinifolia*, Daño Foliar del Pino of radiata pine in Chile; and *Phytophthora* x *alni*, a hybrid taxon thought to have originated in tree nurseries, causal agent of *Alnus* decline in Europe. *Phytophthora* spp. have also been found in association with decline of hardwood and Mediterranean vegetation in numerous parts of world, instances in which a single species cannot be implicated.

What constitutes a native *Phytophthora* is a field of active debate, particularly because many *Phytophthora* spp. have been so widely distributed and native species are not thought to cause substantial disease in their native ranges. For example, *P. pluvialis* is widespread in western coastal U.S. forests, causing minor or sporadic defoliation of Douglas-fir. It has never been detected causing disease on radiata pine in this tree's native range, disease emerging first in commercial plantings in New Zealand where it was likely introduced. Similarly, *P. lateralis* and *P. ramorum* are thought to have been recovered in their native ranges causing minor disease on native vegetation. The lack of virulence cannot, by itself, be taken as an indication of origin, particularly because *Phytophthora* spp. have been transported globally much longer than we have been aware of the problems they cause. Disease of epidemic proportions occurs only when all three components of the disease triangle come together: an aggressive pathogen, a highly susceptible host, and a conducive environment for spread, establishment and disease development.

The horticultural nursery industry has been implicated in the inter- and intracontinental movement of pathogenic species. Human activities such as road and trail use have subsequently spread *Phytophthora* into surrounding environs. While less studied, restoration nurseries have also been a pathway by which non-native *Phytophthora* spp. have been introduced into

vulnerable habitats. Surveys indicate a high diversity of plant pathogenic *Phytophthora* spp. present in native (and many commercial production) plant nurseries, most of which are not apparent when plants are shipped and outplanted. The extent to which these species have been introduced, or were already established, in restoration sites is also not always readily apparent; the consequences, however, can result in failed future plantings, reduced natural regeneration, and further spread of *Phytophthora*. Better management of potentially infested sites requires a thorough assessment of which species are present, or, most importantly, the identification of areas with low abundance and minimal species diversity.

Just as the diversity of *Phytophthora* spp. and diseases have expanded, so has our appreciation of the diversity in disease etiologies, dispersal methods, and host ranges caused by this genus. Even then, the methods by which we have come to evaluate *Phytophthora* diversity have some limitations. By and large, surveys have focused on either direct isolation from symptomatic plant material or baiting from environmental substrates (e.g. rhizosphere or water samples). The former method is biased towards species capable of causing acute symptoms, and in some cases has proven to be difficult depending upon the host and season. The latter preferentially selects species which are biologically active at the time of baiting, may colonize the chosen bait (typically green pears, or oak and rhododendron leaves), and are capable of growing on selective media before being outgrown by competing genera. While baiting-based surveys have consistently indicated high species diversity, an overabundance of negative samples make landscape level analyses of *Phytophthora* distribution, impacts, and management options difficult to obtain. It also indicates that *Phytophthora* diversity is actually underestimated.

Within the past ten years a new technology, high-throughput sequencing (HTS) of environmental DNA (eDNA), has bypassed the need to obtain pure-culture isolates for diversity assessments. In this method, DNA is extracted directly from an environmental substrate, amplified, and identified by matching amplified sequences to those published in curated databases of known species. Multiple sequencing platforms exist (Illumina MiSeq, Illumina HiSeq, PacBio, MinION), all of which are capable of sequencing millions of DNA segments (amplicons) from multiple organisms in a single sample. By using primers more specific to Oomycetes, we can target the amplification of *Phytophthora* DNA in our samples, allowing for greater detection sensitivity than would be obtainable by baiting alone.

In this study, we performed surveys to identify the assemblage and distribution of *Phytophthora* species present within Midpeninsula Regional Open Space District (MROSD) preserves and restoration sites, with the goal of best directing current and future management of vulnerable areas. Using both baiting and Illumina MiSeq, we sought to accomplish the following objectives:

1. Determine the presence and distribution of soilborne *Phytophthora* pathogens in representative revegetation sites in MROSD preserves.
2. Identify conditions allowing for the designation of existing and future planting sites as either a high or low risk of *Phytophthora* introduction and establishment.
3. Provide recommendations for management of sites with *Phytophthora* contamination, and protective actions for sites not yet contaminated.

Methods

Site selection:

We collected soil and vegetation samples at past or future revegetation projects across 10 different preserves (Table 1). Sites were selected representing a range of habitat types and disturbances. Emphasis was placed on those deemed a high priority for MROSD, and on those conditions most likely to influence *Phytophthora* establishment and impact, including source nursery, host plant species installed, habitat type, and planting date. For each revegetation project (or set of adjacent projects) we also sampled an area with minimal disturbance to determine *Phytophthora* species diversity and the extent to which *Phytophthora* are already present in the general area.

Sites were classified as one of the following (Fig. 1):

1. Revegetation (reveg) sites: MROSD revegetation projects in which nursery-grown plants were outplanted prior to sampling.
2. Planned sites: locations of future MROSD revegetation projects.
3. Disturbed sites: locations with obvious disturbance or dieback. These were associated with a revegetation project but lacked remediation by MROSD.
4. Control sites: unplanted and minimally disturbed areas in close proximity to MROSD project areas, selected with the following criteria:
    - Adjacent to reveg or planned projects, or, when not adjacent, sharing a similar habitat type as the projects.
    - Containing plant species shared with reveg or planned site.
    - Upstream / uphill of reveg sites and major disturbances (when possible).

We visited and sampled sites in December of 2017 (year 1) and 2018 (year 2). Some sites had adjacent stream baits (Fig. 1), which are discussed separately (Appendix G). Twenty-two sites were visited both years to validate prior year's findings. For these samples in year two we added an analysis with the compound propidium monoazide (PMA) to help determine if DNA-only detections were the result of intact cells or are remnant, non-viable DNA (Appendix F).

Sample collection:

For each site we collected soil and roots from the base of six plants. We aimed to sample two individuals for three species per site; when a second individual could not be found or only two target plant species were present, we sampled additional individuals of the other target species (whereby the number of species sampled did not exceed 3). Two areas were sampled slightly differently: PR_E001 (Blue Oak Parking Lot at Pulgas Ridge) and the chinquapin sites (El Corte de Madera). Additional samples were collected at PR_E001 due to the severity of symptoms observed there. The chinquapin sites were sampled because of reports of dieback in the area; for each chinquapin site we collected 3 soil samples, which were combined and processed together, and there was no corresponding control site.

In addition to sampling any species or individuals in an area with decline symptoms, we targeted species that were both commonly outplanted by MROSD and were widespread in MROSD preserves. In each location, we first sampled plants in the revegetation or planned project areas. We then sampled the control area, preferring the same species as sampled in the adjacent project.

Approximately 1.5 L of the rhizosphere soil was collected from each plant, taken from a depth of 5-15 cm (Fig. 2). Soil was collected on the downhill (south side if on flat ground) side of the plant, which was placed in a plastic bag along with flagging labelled with the site ID, plant ID, and sample date. Soil was removed with a metal trowel, and the hole was then filled in with adjacent soil. The trowel was cleaned of large particulates and surface sterilized with isopropyl alcohol between samples.

For sites visited both years, we preferentially sampled the same individual plants, sampling adjacent to the prior year's hole. When the individual plant could not be found, we sampled the nearest plant of the same species, which was then given a new plant ID number.

In all cases we recorded the GPS coordinates of the sample, along with plant health status (healthy, dieback, fading, chlorotic, needle cast, canker, dead, or other). As samples were often close enough that low location accuracy would confound their relative position, we additionally hand-mapped the locations of all samples at a site relative to each other and local landmarks. GPS coordinates were cross-referenced to the maps and were adjusted in ArcGIS to match their locations when overlaid upon aerial imagery of the area.

While sampling soil, we collected leaves and cambium tissue from the area when foliar and canker symptoms were present. These were bagged and processed by site.

Soil processing (preparation):

We assessed *Phytophthora* spp. presence in soil two different ways: by baiting and isolation of *Phytophthora*, and by DNA extraction directly from 10 g of soil and sequencing on the Illumina MiSeq Platform (Fig. 3). Soil samples were stored in plastic bins for transport back to Corvallis, OR where they were stored in a walk-in cold room (temperature range 2.5 to 6°C; average 3.2°C) until baiting.

During our preparations for baiting we removed a sub-sample of soil for sequencing. The soil in each bag was thoroughly mixed. We then removed 9.0 to 10.0 g of soil using a metal scoop, sterilized in ethanol between samples, from multiple locations in the bag. This was placed in a 15 ml falcon tube labelled with the location ID, plant ID, and sampling date; we stored all tubes in -20°C before extraction.

Soil processing (baiting):

The remaining soil was slated for baiting (Fig. 3v-z). We completed baiting seven weeks after the soil was placed in the cold room. One liter of soil was removed from the sample bag and placed in a new 3.78 L plastic bag along with its identification information. Multiple bags were placed, side by side, in plastic tubs such that they supported each other from the side. We added 1 unripe, organic D'Anjou pear to each bag, then added 500 mL of deionized (DI) water to flood each bag, an amount sufficient to raise the water level to approximately 1-3 cm above the surface of the soil (Fig. 3v).

In 2018, all samples taken from locations also sampled in 2017 also received a window-screen pocket containing a 1-cm piece of oregano stem and leaf, half a rhododendron leaf, and a *Q. robur* acorn radical as additional baits, plus two Styrofoam packaging peanuts to keep the pocket at the water surface. Baits were left at room temperature (23°C) for five days, over which time we added additional DI-water as needed to keep baits partially submerged. Baits were then

removed, rinsed in a 10% bleach solution, rinsed in DI-water, dried, and placed in new sandwich-sized baggies for further incubation to allow for lesion development.

We checked the pear baits for lesions after one or two days, then periodically for an additional two weeks. For each individual lesion (up to four per pear; Fig. 3v) we removed a small segment at the lesion edge using a scalpel, blotted it dry, and placed it in PARPH selective media. These primary isolate plates were monitored for hyphal growth over a two week period; different morphotypes were sub-cultured onto new PAR plates to produce single-isolate cultures (Fig. 3w). As cultures became clean of contaminants they were then sub-cultured onto CMA with beta-sitosterol for longer-term storage and future identification with Sanger sequencing.

We plated all oregano and rhododendron baits regardless of symptoms. We preferentially plated two lesion margins per bait, however when lesions were not present we plated the stem and leaf (oregano) or the leaf-tip and cut edge (rhododendron). All material was surface sterilized in 10% bleach, then rinsed in DI-water before plating in PAR. As with the pear, we monitored all plates for hyphal growth and sub-cultured different morphotypes to produce single-isolate cultures.

Acorn radicals were targeting slow-growing clade 12 species that would otherwise be overgrown by faster growing species and genera. To obtain these, each radical was surface sterilized, rinsed in DI-water, and then placed in a 60 mm diameter petri plate containing a filter saturated with DI-water. Radicals were checked every other day for two weeks for the production of sporangia, which were removed and placed on PAR media with a sterile needle.

Controls for the baiting periods included three negative DI-water controls in which only the baits and DI-water were included in each baiting bag. Positive controls contained the baits, DI-water, and sporulating cultures of *P. cambivora* (both years), or *P. tentaculata* or *P. quercina* (both only in year 2).

Foliage & Bark processing:

All symptomatic plant material collected in the field was stored at 3.2°C for up to two weeks before processing at Oregon State University. For leaves, lesion segments were placed in PAR selective media to isolate *Phytophthora* pathogens. For cankers, the exposed edges of the cambium tissues were removed to reveal the inner lesion margins, which were then segmented and plated in PAR. Culture isolations and storage were completed as with cultures obtained from the baits.

Species identification via Sanger Sequencing:

We extracted DNA from pure-isolate cultures produced from the baiting and foliage plating for species identification via Sanger sequencing. DNA was extracted from a small segment of mycelium using the Extract-N-Amp Plant PCR kit (MilliporeSigma, St. Louis, MO). The hyphae were aseptically transferred to 15 µl of extraction solution in a 200 µl tube, incubated at 95°C for 10 minutes in a thermocycler, after which 15 µl of dilution solution was added to each tube. Extracts were stored at -20°C.

We first amplified the nuclear internal transcribed spacer (ITS) region using DC6 and ITS4 primers (White et al. 1990, Cooke et al. 2000). For each sample, 0.5 µl of template was added to a 13 µl PCR reaction volume containing MyTaq^{TM} Plant-PCR kit 2x master mix

(Bioline, London, UK), nuclease-free water, and 0.5 mM each of forward and reverse primers. In the first set of samples processed in year two we used 1x Phusion® High-Fidelity PCR Master Mix with HF Buffer (ThermoFisher Scientific, Waltham, MA) instead of MyTaq, but the same primer concentration. PCR was performed on a Biorad DNA Engine® Peltier Thermal Cycler with a 3 min initial temperature of 95°C, followed by 35 cycles of 15 sec denaturation at 95°C, 15 sec annealing at 55°C, and 45 sec extension at 72°C, with a final 5 min extension at 72°C. PCR product was visualized on agarose gels and cleaned with ExoSAP-IT (USB, Cleveland, Ohio, USA) before submitting for Sanger sequencing with the forward primer ITS1 (White et al. 1990) as the sequencing primer. Ten samples with poor sequences were submitted again with ITS4 as the sequencing primer. Sequencing was performed at the Center for Genome Research and Biocomputing Core Laboratory (CGRB) at Oregon State University using an ABI Prism 3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were identified by querying the Phytophthora-ID curated database (http://phytophthora-id.org/seq-id.html) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using BLAST.

For isolates in which the two databases were not in agreement or for which multiple species were identified as close matches, we amplified the mitochondrial cytochrome oxidase c subunit 1 (COX1) gene using FM55 and FM84 primers (Martin 2000, Martin and Tooley 2003). PCR and sequencing were performed as described above with PCR products submitted for sequencing with primers FM55 and FM84.

Species identification via Illumina MiSeq sequencing:

To identify DNA fragments matching those belonging to *Phytophthora* spp. directly from environmental samples, without the need to obtain single-species cultures, we extracted DNA directly from up to 10.0 g of soil and sequenced it on the Illumina MiSeq Platform (Fig. 3a-e). DNA was extracted using DNeasy PowerMax Soil Kit (Qiagen, Hilden, Germany). DNA extracts were stored at -20 °C before quantification, amplification and sequencing as described by Redekar et al. (2019).

Briefly, the DNA concentration was measured using a spectrophotometer (NanoDrop 1000, Termo Fischer Scientific, Inc., Wilmington, DE) then diluted to 25 ng/µl. We amplified the ITS1 region using ITS6 and ITS7 primers using reagent concentrations as described in Ricercar et al. (2019), and amplicons were submitted to the CGRB for amplicon cleanup, indexing, and sequencing. Sequences were retrieved from CGRB after reads were demultiplexed into individual samples. We used Cutadept (version 1.10) (Martin 2011) to process de-multiplexed sequence reads to achieve the following: (i) trim low quality reads, whereby bases with quality-scores below 25 were trimmed from the 5' and 3' ends; (ii) trim adapters upstream of the ITS6 sequence in read1 and the ITS7 sequence in read2; (iii) trim 32 base pairs from the 5' end of read1, which mapped to the 18S segment not present in our Oomycete databases; and (iv) remove reads fewer than 100-bp in length for reliable identification.

A standalone Nucleotide-Nucleotide BLAST+ (version 2.2.29) search was performed separately for the read1 and read2 data to obtain the best high-scoring segment (HSP) pair. We required an HSP contain at least 99% similarity and a minimum 100-bp alignment length with the query sequence for positive identification. Due to unreliable entries in public DNA repositories, we limited the searches to curated databases: Phytophthora-ID (http://phytophthora-id.org; Grünwald et al. 2011), Robidaeu et al. 2011, and IDphy (https://idtools.org/id/phytophthora/index.php, Abad et al. 2019). Read1 and read2 HSP results

were compared and further processed as follows: (i) when both reads were in agreement, the query was identified as a positive match for that taxon; (ii) when a BLAST hit was present in only one of the paired reads (i.e. read1 or read2), the results were eliminated; (iii) for BLAST hits in which each paired read resulted in non-matching taxa, we assigned a designation showing either were indicated (e.g. taxon1 / taxon2).

An operation taxonomic unit (OTU) was designated as a group of sequences assigned to a single taxon (or paired taxon when both reads were not in agreement). In many cases, species could not be distinguished over the sequenced region, in which case the OTU was assigned a complex or cluster designation representing multiple potential species. A complex is composed of closely related species with identical ITS1 sequences (which may be distinguished at other loci); a cluster is composed of related species with sequences identical between the ITS6 and ITS7 primers, but differ and may be distinguished at other loci within the ITS region. All OTUs which could be placed in the genus *Phytophthora* but lacked an ITS match at greater than 99% sequence similarity were grouped into a single OTU listed as "*Phytophthora*-unknown".

To reduce the inclusion of false-positives we first eliminated any taxa observed in less than 2 query reads (singletons) before merging the taxon counts for read1 and read2. We then required an OTU comprised of at least 0.01% of the total numbers of reads within a single sample for reporting (number of reads for that OTU in the sample / total number of reads in that sample, hereby referred as a within-sample relative abundance of 0.01%). A more stringent minimum threshold was required to be considered "detected" for analytical purposes, whereby an individual OTU must have a within-sample relative abundance greater than or equal to 0.095%. For a discussion on the utilities and interpretations of thresholds, see Appendix E.

Site infestation levels and analysis:

Soil infestation level is a measure of a site or area's *Phytophthora* infestation that reflects both the *number* of *Phytophthora* species or OTUs detected (alpha diversity), and the relative *risk* of each species or OTU. Soil infestation levels were determined to guide management recommendations for each project area.

To determine infestation levels for each project area, we first sorted *Phytophthora* species (baiting) or OTUs (Illumina) into one of four risk classes based on the expected impact they may have on MROSD lands: very high, high, moderate, or low. Risk class was based on aggressiveness, host range, abundance, and prior history as an invasive species as assessed from published literature (Appendix A). Each risk class was assigned a rating of 1-4, representing low to very high-risk.

We then calculated an overall infestation score for each project area, which includes all reveg/disturbed/planned sites and their corresponding control site (Table 1). This score was calculated by summing the individual risk ratings (1-4) of all the species detected there. For example, if we recovered *P. ramorum* (risk rating = 4), *P. crassamura* (risk rating = 3), and *P.* sp. 'cadmea' (risk rating = 2) from a project the area, the area was given a baiting-infestation score of 9 (=4+3+2). This analysis only takes into account the presence/absence of a species or OTU within an area, not the number of samples from which a given taxon was detected. Two different scores were assigned to each area: one for baiting, and one for Illumina. These were compared to assess the overall infestation level for that location. We calculated a final site-level ranking by summing the two scores.

To test the hypothesis that reveg or disturbed sites have greater infestation scores than their corresponding control site, we calculated infestation scores separately for each site. For this analysis, disturbed sites included both planned sites (PLND) and those that were disturbed but unremediated sites (DIST). We performed a paired t-test to compare the infestation scores between each reveg or disturbed site and their corresponding control site. A paired t-test was also performed to test for differences between reveg/control or disturbed/control pairings using other infestation statistics: the number of *Phytophthora* OTUs detected, and the proportion of samples from each site in which a *Phytophthora* was detected. We hypothesized reveg or disturbed sites would have greater *Phytophthora* diversity and *Phytophthora* would be detected in a greater proportion of samples in the reveg or disturbed site relative to their control.

Additional analyses investigating the impacts of plant community, disturbance history, topography, and other factors affecting the odds of successfully baiting *Phytophthora* or the infestation level of a site are covered in Appendix C.

Results

Site & Sample Summary:

A total of 564 soil samples were collected from 72 sites over the course of this project (Table 2, Table 3). Of these, 22 sites (representing a total of 252 samples) were sampled in both years (Table 1). We sampled soil from the base of fifty species in total (Table 4). Of these, *Heteromeles arbutifolia*, *Mimulus aurantiacus*, *Arbutus menziesii* and *Frangula californica* comprised 41.67% of the samples.

Soil baiting:

A total of 563 soil samples were baited (one sample of the 564 collected was sequenced with Illumina, however the baiting sample went missing). While *Pythium* species were common, *Phytophthora* was only recovered from 73 samples. Baiting success was nearly identical between the two years, having isolated *Phytophthora* from only 11.85% or 13.99% of the soils sampled in 2017 or 2018, respectively. While the species composition differed between reveg and control sites, a high diversity of species was detected from both sites (Table 5). Eighteen species were recovered in total, of which *P. cactorum* and *P. cambivora* were the most common (Table 5). Some species (e.g. *P. cactorum*) were baited both years, while others (e.g. *P. cambivora*, *P. megasperma*, *P. crassamura*) were only baited in one year, even from repeat samples (Table 5).

Plant genera in which the majority of samples were taken account for the majority of positives (Table 6). Greatest *Phytophthora* diversity was obtained from soil collected at the base of *Frangula*, *Arbutus*, and *Mimulus* with 6-7 species detected each. Relative to their lower sampling effort, *Alnus* and *Rubus* also supported elevated species diversity (Table 6). Some plants (e.g. *Eriophyllum*) were positive only in 1 site.

We collected soil and roots from a total of 120 plants both in 2017 and 2018; these plants had no known additional disturbance between years. Of these, the majority were negative both years; in only five plants was the same *Phytophthora* sp. recovered both years (Fig. 4). Twenty-four samples were found to contain *Phytophthora* in only in one year (Fig. 4). *Phytophthora cactorum* (4 plants across 3 sites) and *P. nicotianae* (1 plant only) were the only two species detected in repeat samples.

In 2018, 126 samples were baited with pear, rhododendron, oregano, and oak acorn radicals. *Phytophthora* was recovered from pear in the majority of positive samples (Table 7); for the majority of samples in which rhododendron or oregano detected a *Phytophthora* species, that species was also recovered from the pear bait. In only three samples was a *Phytophthora* species detected only by the non-pear baits, all from rhododendron. Species recovered in these samples include *P. cactorum* (two samples; one negative in 2017 and one in which *P. cambivora* was isolated from pear in 2017) and *P. nicotianae* (which was recovered from pear at this location in 2017). No *Phytophthora* samples were recovered exclusively from oregano.

The baits successfully detected the targeted species in our positive controls: *P. cambivora* was recovered from pear, rhododendron, and oregano; *P. quercina* was recovered from oak; and *P. tentaculata* was recovered from oregano. No *Phytophthora* species were recovered from negative controls baiting DI-water.

Foliage & Bark recovery:

We were able to isolate *Phytophthora* from 24 plant tissue samples, from either *Umbellularia californica* or *Notholithocarpus densiflorus*. *P. ramorum* accounted for nearly all of these; two additional isolates of *P. nemorosa* and *P.* aff. *ilicis* were recovered from a single location at La Honda Creek (Table 8). *Phytophthora* aff. *ilicis* likely represents a novel species, with an assembled sequence at the COX locus matching only 96.8% of base pairs to that of *P. ilicis* in published databases (the ITS sequences matched at greater than 99% for closely related species *P. nemorosa*, *P. ilicis* and *P. pseudosyringae*). No species were isolated in *Phytophthora*-selective media from the limited number of bark samples plated.

Illumina MiSeq Sequencing:

In total we sequenced DNA extracted from 443 soil samples on the Illumina platform. *Phytophthora* was uncommon, comprising only 1.28% of the total number of OTU reads (Fig. 5). The majority (76.62%) of OTU reads were unidentified (ITS sequences not present in our Oomycetes database and not identifiable to any Oomycota genus). When an Oomycete was identified, the majority of reads were attributable to the presence of *Pythium* spp. (22% of reads). Genera listed under "other" include *Achlya*, *Aphanomyces*, *Apodachlya*, *Brevilegnia*, *Peronospora*, *Pythiopsis* and *Saprolegnia*, which collectively comprised only 0.06% of total OTU reads.

When detected, the majority of *Phytophthora* OTUs generally comprised a small proportion of the total number of reads within a sample. The majority (254 OTU signatures of the 573 signatures recorded with a minimum 0.01% within-sample relative abundance) comprised only 0.01 to <0.095% of the total number of reads within their respective samples (Fig. 6); these OTUs fell below the minimum 0.095% within-sample relative abundance required to be considered "detected" for analytical purposes (but see Appendix E).

Thirty-eight distinct *Phytophthora* OTUs were identified over both years at or above 0.01% within-sample relative abundance; of these 34 OTUs were detected at the minimum 0.095% within-sample relative abundance (Table 9). Eleven OTUs represent either clusters or complexes, and cannot be identified to a single species (Table 10). Eighteen OTUs, however comprised greater than 1% of the total number of reads within their respective samples (Table

11). Some OTUs (*cactorum*-cluster, *quercina*-cluster, *uliginosa*-cluster, *psychrophila*, and *P.* sp. unknown) comprised greater than 10% of the total number of reads in some samples.

Despite comprising a small proportion of the amplified DNA in each sample, nearly half (208, or 46.95% of all soil samples) had at least one *Phytophthora* OTU detected at a within-sample relative abundance greater than 0.095% (Fig. 7). When *Phytophthora* was detected, most samples had only a single *Phytophthora* OTU present; two samples recovered from revegetation sites had 7 or 8 distinct *Phytophthora* OTUs detected (taken from reveg sites RV_RS_B001 and RV_RR_A006).

The *psychrophila* and the *cactorum*-cluster OTUs were the two most common *Phytophthora* taxa detected and were abundant in all site classes (Fig. 8, Table 9). The *P. nemorosa*-cluster and *P. syringae* OTUs were the next most commonly detected, followed by the *uliginosa*-cluster and the *quercina*-cluster which were detected almost exclusively in control sites (Fig. 8, Table 9). OTUs which were identified as belonging to *Phytophthora* but lacking a greater than 99% match to known species (*Phytophthora* sp. unknown) comprised the seventh most common OTU grouping.

Differences were observed in the community members between the different site classes (reveg, control, and disturbed). Of the 13 OTUs with only 1 or 2 detections, 10 were detected exclusively at revegetation or disturbed (planned or disturbed but unremediated) sites (Fig. 8). Clade 3 species dominated control and disturbed sites, with 42.3 or 47.6% of the total number of reads belonging to this Clade (Fig. 9). In comparison, at reveg sites clade 3 OTUs were the third most common after clade 8 and 1. Control sites additionally had greater representation of clade 7 and clade 12 taxa (Fig. 9).

The greatest number of *Phytophthora* OTUs were detected from *Arbutus*, *Mimulus*, *Frangula*, *Alnus*, *Quercus*, *Arctostaphylos*, and *Heteromeles*, from which at least 10 distinct *Phytophthora* OTUs were detected at a minimum 0.095% within-sample relative abundance (Fig. 10). There were many genera in which *Phytophthora* was detected via Illumina but we were unable to recover any species via soil baiting, notably *Lupinus* and *Notholithocarpus* (Fig. 10).

We sequenced soil DNA for 67 samples in which a *Phytophthora* species was recovered via baiting (from which we recovered 69 isolates). The associated OTU fell below the detection threshold of 0.095% for 51 (74%) of these isolates (Fig. 11). While below detection thresholds, the associated OTU comprised between 0.01 to 0.094% of reads within the sample for 14 (20%) of the isolates. Species for which the OTU was detected at ≥0.095% relative abundance (17 isolates) from the sample in which they were isolated include *P.* taxon *asparagi*, *P.* taxon *oaksoil*, *P. cactorum*, *P. pseudotsugae*, *P. cambivora*, *P. multivora*, *P. cryptogea*-complex, *P. pseudosyringae*, *P. nicotianae*, *P. syringae*, and *P.* sp. ‘cadmea’.

For paired samples in which soil was taken and processed from the same plant both years, repeat detections occurred for only a subset of OTUs. The *P. psychrophila*, *P. cactorum*-cluster, *P. quercina*-cluster, *P. uliginosa*-cluster, and *P. nicotianae* were the only OTUs detected in both years from at least 1 paired-sample at the minimum 0.095% within-sample abundance required to be considered “detected” (Table 12). At lower thresholds (minimum 0.01% within-sample relative abundance), we also were able to repeat detections of the *P. syringae*, *P. nemorosa*-cluster, *P. tentaculata*, and *P. siskiyouensis* OTUs in at least one paired-sample (Table 12). No other OTUs were repeated from the same sample between years.

Detection by plant health:

Across all site classes, healthy plants represented 67% of all samples baited and 71% of all samples sequenced. Many species were baited only from healthy plants (Fig. 12A). Only 3% of samples were taken from dead plants; of these only one was positive from which we recovered both *P. syringae* and *P. pseudosyringae*.

Detection of *Phytophthora* OTUs were more evenly divided across health classes, with most OTUs being detected at a mix of healthy and unhealthy plants (Fig. 12B). 12 OTUs were only detected from healthy plants (at the 0.095% within-sample relative abundance detection threshold); healthy plants comprised over 50% of the detections for nearly all other OTUs.

Site and area infestation scores:

Similar numbers of *Phytophthora* species were baited at control and reveg sites; slightly fewer species were detected at disturbed sites (Fig. 13). Using Illumina, *Phytophthora* was abundant across all site classes (Fig. 7, Fig. 13). Both control and reveg sites had as many as 4 species baited, or 9 *Phytophthora* OTUs detected at a single site.

Correlations between infestation measures– the number of *Phytophthora* OTUs detected, the proportion of samples with at least one OTU detection, and the cumulative infestation score by baiting or by Illumina – of a reveg or disturbed site and the corresponding control site were all weakly positive (correlation coefficient r ranging between 0.14 and 0.33; Fig. 14). Despite this, we found no evidence that control sites had less *Phytophthora* contamination than their corresponding reveg or disturbed/planned sites for any of these measures (at $\alpha = 0.05$) (Fig. 14).

Phytophthora taxa, as detected by baiting or by Illumina were ranked 1-4 based on their likely risk to plant health on MROSD lands. In total, we baited 6 very high-risk species (rating = 4), 5 high-risk species (rating = 3), 5 moderate risk species (rating = 2) and 4 low-risk species (Table 13, Appendix A). We identified 6 very high-risk OTUs (rating = 4), 6 high-risk OTUs (rating = 3), 9 moderate-risk OTUs (rating = 2), and 15 low-risk OTUs (rating = 1) via Illumina (Table 13, Appendix A).

Very high-risk species were baited in many areas; in many cases they were the only species detected (Fig. 15). Low-risk species (risk rating = 1) were baited only from sites with the greatest diversity. In contrast, low-risk taxa were more broadly distributed as detected by Illumina (Fig. 15). In general, higher diversity was associated with greater infestation scores for both baiting and Illumina.

Many sites within Pulgas Ridge, Mindego Gateway (Russian Ridge), La Honda, and Skyline Ridge were scored as high-risk using both detection methods (Fig. 16, Appendix B). Some project areas, such as the Monte Bello Grassland and the Annex Garden areas scored high via Illumina but low via baiting; the inverse was true of others, such as Mt. Umunhum Bald Mountain and the Rancho San Antonio Equestrian lot (Fig. 16). A number of locations were rated low risk via both baiting and Illumina (Fig. 16).

Discussion

Consistent with prior studies within the region (Bourret et al. 2018 and Sims et al. 2018) and with others within Mediterranean climates around the world, *Phytophthora* has proven to be an abundant and widespread genus. This study reveals that a large number of *Phytophthora* species inhabit wildlands of the Midpeninsula Region Open Space District. We were able to culture 20 species in total, including one provisionally new species (*P.* aff. *ilicis*), one only recently known to be in California and not thought to have spread from restoration plantings (*P.* taxon *asparagi*), and one not known to be present in North America (*P. boehmeriae*). Illumina MiSeq sequencing identified even greater abundance and diversity, particularly of hard to bait species. Combined, we now have a baseline of which areas across ten preserves have a high prevalence of biologically active pathogens and historical introductions. More importantly, we identified which areas have low species diversity and are a priority for preventing future introductions.

Prevalence of nursery-associated Phytophthora

We hypothesized with greater disturbance and the introduction of nursery-grown plants, reveg sites would have a greater number of *Phytophthora* taxa detected via Illumina if not also by baiting. Surprisingly, there was a high diversity of *Phytophthora* OTUs detected at both unplanted control sites and revegetation sites. This was apparent in both baiting (Table 5) and in Illumina (Fig. 12). We did, however detect important differences between which species are present at each of the different site classes.

Among nursery-associated taxa, some species were more widely distributed than others. Both abundant and widespread, *P. cactorum* was sequenced across all site classes but was baited almost exclusively from reveg sites. The abundance of *P. cactorum* in nursery-grown outplantings is consistent with results from surveys of restoration nurseries in the Bay Area, whereby this species was among the most common recovered (Rooney-Latham et al. 2019, Sims et al. 2018). The extent to which Illumina detections occurred in the control areas indicates this species may be more widely dispersed than indicated by baiting. The implications of this, however, depends upon the interpretation of DNA-only detections.

As utilized in this study, Illumina MiSeq detects DNA present in both intact cells (hyphae or spores in the case of *Phytophthora*) and eDNA persisting in the environment after these cells are no longer viable. Without the addition of chemicals which bind to this "free" DNA, we cannot differentiate between a pathogen which may cause disease from a remnant population (as investigated in Appendix F). In the absence of being able to culture the pathogen, some proxies may be used to indicate if the species is viable: a high within-sample relative abundance of a particular OTU and the ability to detect the OTU in multiple years may indicate the taxon is viable and reproducing (although see discussion in Appendix E).

A second confounding factor is the short read length of Illumina and the use of the ITS region, resulting in an inability to differentiate between some species resulting in species clusters or complexes. The *P. cactorum*-OTU is a cluster comprised not only of *P. cactorum*, but also *P. pseudotsugae* and *P. idaei* (Table 10). *P. pseudotsugae* was also recovered via baiting, albeit less frequently, and may account for some of the *P. cactorum*-OTU detections. Given the differential abundance of these two species and the hosts they are associated with, however, we strongly suspect the majority of *P. cactorum*-cluster OTU detections are attributable to *P. cactorum*.

It is highly likely *P. cactorum* has been present on MROSD lands for some time and is highly capable of dispersing from disturbed and outplanted areas. The within-sample relative abundance of the *P. cactorum*-OTU was significantly higher at planned and reveg sites than at control sites, consistent with this taxon spreading outwards from nursery outplantings and disturbances. This species was the most reliably baited both years. We were able to repeat the detection of the *P. cactorum*-cluster between years for samples from both reveg and control sites (Table 12).

Little is known about the impact of *P. cactorum* on California native plant populations, though many genera are hosts within the nurseries and it has been implicated with disease in toyon (*Heteromeles arbutifolia*) and Pacific madrone (*Arbutus menziesii*). Complicating matters, *P. cactorum* may actually be a complex of multiple species with preferential virulence on different hosts. Until pathogenicity tests are undertaken with MROSD isolates this species should be considered an emerging problem with potential for contributing to disease of native vegetation at MROSD preserves.

Many other species were clearly associated with nursery outplantings or disturbances, but were not as abundant. Six species were exclusively baited in reveg areas only: *P. boehmeriae*, *P. crassamura*, *P. megasperma*, *P. nicotianae*, *P. pseudosyringae*, and *P. rosacearum* (Table 5); nearly half the OTUs were detected predominantly if not exclusively within reveg or more heavily disturbed areas (e.g. *P. nicotianae*, *P. tentaculata*, the *P. megasperma*-cluster; Fig. 9). By and large, these detections were limited to a smaller geographic range than *P. cactorum* or *P. syringae*. Such patterns are consistent with human activities being a pathway by which new *Phytophthora* spp. are introduced into new areas; the low detection frequency could indicate these species are either recently introduced and/or are not as capable of surviving or efficient at dispersing into adjacent vegetation.

Phytophthora *in control areas and the prevalence of Clade 3*

Fewer taxa had strong associations with control areas. Three species were baited exclusively from control sites (*P.* sp. 'cadmea', *P.* taxon *asparagi* and *P.* taxon *oaksoil*; Table 5). Of these, *P.* taxon *oaksoil* was recovered from a streamside-site and thus may represent disease presence anywhere upstream. *P.* taxon *asparagi* was recovered from the control area at the Hoita Rd. planting site (Sierra Azul), however strong signatures of this taxon were found in the adjacent planned planting area; this species has also been recovered recently from a restoration outplanting by Bourret (2018). Only one baited species, *P.* sp. 'cadmea', was clearly associated with minimally disturbed areas. Similarly, only two *Phytophthora* OTUs, the *P. quercina*-cluster and *P. uliginosa*-cluster (of which *P.* sp. 'cadmea' is a member) were strongly associated with control areas.

The distribution of the *P. quercina*-cluster and *P. uliginosa*-cluster may be partly influenced by where control areas were located. Reveg projects were generally located in more recently cleared lands, for which it was difficult to find an undisturbed equivalent. As such, control areas were adjacent to or within more heavily forested areas containing a greater abundance of plant genera associated with these taxa. Regardless, no disease was observed in associated with the detection of these two OTUs, or with *P.* sp. 'cadmea'.

Because of co-evolution with its host, minor disease development is thought to be one requirement of a native pathogen. That alone cannot be a defining feature as many plants with

known nursery-*Phytophthora* infections had no to only minor amounts of disease in the field. Arguably, regional abundance and genetic diversity are additional requisites. Lacking cultures (for the *P. quercina*-cluster) or population analyses (for the *P. uliginosa*-cluster), we cannot say if these detections represent native or naturalized *Phytophthora*. Rather, the best candidates for being native species are illustrated by the abundance of clade 3 taxa.

Phytophthora clade 3 is comprised of five described species: *P. ilicis*, *P. nemorosa*, *P. pseudosyringae* and *P. pluvialis* (which collectively form the *P. nemorosa*-cluster), and *P. psychrophila* (as its own OTU). All five have been recovered from western U.S. forests, largely as a result of monitoring for *P. ramorum.* Unlike the aggressive and widespread mortality associated with *P. ramorum*, these species generally cause minor or sporadic disease on native plants in coastal forests but greater amounts of disease in other environments or hosts. To varying degrees, these species are the best candidates to be considered as native to MROSD preserves. Greatest evidence exists for native origins of *P. pluvialis*; clonality or rarity in the other species places their origins in greater doubt.

Our results support the native origins of many members of clade 3. The clade 3 OTUs were widespread and abundant, occurred with above average within-sample relative abundance, and were amongst the most repeatable of the OTUs indicating ample DNA reserves within soils. While not isolated frequently, we recovered a high diversity within the clade, having isolated *P. pseudosyringae, P. nemorosa* and a potentially new species *P.* aff. *ilicis*. More studies using a greater diversity of isolates from North America and non-North American origins are needed to verify this conclusion. Should these taxa be native, the common paradigm indicates they should not cause substantial disease on native hosts in their native environments. They may be, however, indicative of plant communities and environments conducive for the establishment of other *Phytophthora* spp.

Comparison between the two methods and implications for future sampling

Even with primers targeting this genus, *Phytophthora* is an incredibly minor component of the rhizosphere community (Fig. 5). Baiting is sensitive to a large number of species, though its limitations result in an underestimation of species diversity. Notable omissions from detection include *P. psychrophila*, *P. lateralis*, *P. tentaculata*, and species from the *P. quercina*-cluster. The lower frequency of detections similarly underestimate the likely movement of taxa such as *P. cactorum* from outplanted areas into surrounding environs.

Illumina MiSeq sequencing, on the other hand, is remarkably sensitive but too comes with its own drawbacks. In addition to our inability to discern between viable vs. inviable detections and the inability to differentiate between taxa within species complexes or clusters, being so sensitive the method is prone to false positives. This most notably occurred with the detection of *P. tropicalis*, which was determined to be a lab contaminant and was eliminated from the dataset (see discussion in Appendix E). The interpretation of when to call a species "detected" is similarly nuanced and must be made on a case-by-case basis (again, see Appendix E).

The within-sample relative abundance of *Phytophthora*-OTUs which were also baited was surprisingly low (Fig. 11). For only a 24% of the samples in which a *Phytophthora* was isolated was the corresponding OTU detected at above the 0.095% within-sample relative abundance threshold. Some species were better represented by baiting: *P. cambivora*, for

example, was the second most commonly baited species (despite only being baited in year 1), but the 14th most common OTU. Similarly, *P. ramorum* and *P. crassamura* were somewhat underrepresented in comparison to how frequently they were detected.

Importantly, neither method repeated detections for most *Phytophthora* taxa (Fig. 4, Table 12). The most reliably repeatable sample came from the Mindego Gateway in which *P. nicotianae* was detected both years via culture and Illumina. *P. cactorum* was the only other species baited from the same plant between years. Repeatability was somewhat better with Illumina, repeating detections of as many 9 *Phytophthora* OTUs including the cryptic *P. psychrophila* and *P. quercina*-cluster. Fortunately, for the suite of species we detected we found pears to be an adequate bait source. But to obtain best estimates of species diversity within an area, sampling over multiple years and seasons is warranted.

The plant genera in which we detected *Phytophthora* were consistent with prior reports in the area. Notably higher species diversity was found via both methods on *Frangula*, *Mimulus*, *Arbutus*, *Heteromeles*, *Alnus* and *Quercus* (Fig. 10). While only two *Phytophthora* species were baited from *Arctostaphylos,* we noted high levels of symptoms in this species as well as a 12 distinct *Phytophthora* OTUs in association. Other genera for which a high diversity of *Phytophthora* were detected by Illumina and not baiting include *Lupinus* and *Notholithocarpus*. These genera may host a larger number of *Phytophthora* spp. than would be represented via baiting.

In both methods we cannot discern whether the pathogen present is infecting that particular host or on neighboring plants. This may partly account for why the majority of the plants from which *Phytophthora* was detected were classified as healthy (Fig. 12A,B). *Phytophthora* is poorly recovered from dead or dying plants, as best demonstrated in the Blue Oak parking lot planting of Pulgas Ridge (PR_E001). Many plants in the reveg area displayed clear signs of acute *Phytophthora* dieback (Appendix B), though we more reliably recovered *Phytophthora* from healthy-looking plants at this site.

Pathogenic *Phytophthora* are poor saprophytes, and recovery is generally easier from active infections rather than dead or near-dead material. Above ground symptoms such as crown dieback or branch flagging typically appear late in the disease cycle. Substantial root loss may have already occurred by the time these symptoms are apparent, resulting in sampling at a time when inoculum reserves may be decreasing due to the loss of host tissues and invasion by secondary agents. The implications of this include the need to sample plants with mild to no symptoms to determine *Phytophthora* abundance.

Disease may be more apparent in the future where pathogenic species are present, even in apparently healthy plants or areas. Management or environmental changes may favor disease development. For generalist pathogens, some hosts may support infection and harbor populations without suffering substantial damage, all the while passing the pathogen on to more severely affected genera. Disease may also be present but progressing slowly in a phenomemon known as *Phytophthora* decline.

The role of Phytophthora *in decline*

Decline is a complex process characterized by reduced plant vigor and increased mortality, often in association with a changing climate events (e.g. prolonged drought, lower snow pack) and a suite of insects and pathogens. In contrast to acute diseases such as SOD which

are attributable to a single species, declines involve a larger assemblage of primary and secondary agents and may progress much slower. Each individual agent may contribute varying degrees to the disease development, and assemblies often differ in their community structure. As such, it is often difficult to determine the importance of any particular agent.

Soilborne *Phytophthora* spp. have been implicated in declines of Mediterranean and temperate forests worldwide. In the case of flora of Mediterranean climates, declines are often in association with root health and drought. Chronic and increasing water stress benefit *Phytophthora* a number of ways. For oaks, changes in carbon supply and allocation resulting from leaf shedding and increased root production may increase the vulnerability of fine roots to root-rot pathogens, including many *Phytophthora* spp.; resulting fine root necrosis thus amplifies the stress of the drought, which may cause plant death itself or aid invasion by secondary agents not normally able to cause substantial harm.

Very few of the *Phytophthora* spp. we isolated have been evaluated for their ability to cause disease on native Californian hosts. Many of them, however, are likely causing some degree of fine root necrosis in the field. Taking samples of the rhizosphere, we, by and large, are selecting for those species with either incidental soil populations (for example *P. ramorum*, which produces inoculum in tree canopies which falls into and remains viable in the soil) or those which cause root infection. A third class of *Phytophthora* cause collar and stem cankers, which were not targeted as part of this study (although many species cause both fine root necrosis and cankers). We baited many species implicated as contributing to decline in some regions: *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. crassamura*, *P. cryptogea*-complex, *P. megasperma*, *P. multivora*, *P. pseudosyringae*, *P. syringae*, and *P.* taxon *asparagi*. We also found indications of others via Illumina: *P. fallax*, *P. hydropathica*, *P. lateralis*, the *P. quercina*-cluster (containing *P. quercina* and other potentially less pathogenic species), and *P. tentaculata*.

The impact of all these species depends upon the physiology of the plant, the secondary agents present, and the edaphic factors which may impact disease development (e.g. soil texture and pH). In many cases, *Phytophthora* may be secondary and of less actionable concern. For example, in other studies *P. drechsleri* and *P. boehmeriae* (the later being baited from Pulgas Ridge) have been recovered from impacted forests but themselves are poorly associated with decline, at least in comparison to more pathogenic species. Others (*P. syringae*, *P. nicotianae*) are more common but are of disputed importance. Their presence, however, cannot be discounted. Instead, greater incidence and species diversity, regardless of their known pathogenicity, could indicate a greater likelihood of *Phytophthora* establishment and decline developing in the future.

Locations with high infestation rankings

To account for the different threats posed by each *Phytophthora* species we detected, we sorted species into risk categories to calculate site and location-level infestation rankings. Greatest rankings were calculated for those sites with a combination of greater diversity and prevalence of high-risk species.

Some sites were heavily infested (Appendix B), and, on average, control sites were no more or less infested than their corresponding reveg or disturbed sites. This speaks to the widespread distribution of many high-risk *Phytophthora* spp. in MROSD preserves. Outplanting of nursery stock has resulted in the presence of numerous taxa not present in control sites, but the

majority of these detections were rare and rated low-risk. In contrast, the majority of high-risk and very high-risk *Phytophthora* OTUs (Appendix A) were recovered via baiting, many of them occurring in all site classes (Table 5, Fig. 8).

Interestingly, a given species was rarely baited from both the reveg site and the corresponding control site. Likely, many of these species were introduced prior to the reveg project and are thus not restricted to where nursery-grown plants were introduced. Due to the limitations of baiting and the small sample size, a single detection should not necessarily indicate a limited distribution. Illumina performed better in this regard. Take, for example, the detection of *P.* taxon *asparagi* and *P. boehmeriae*, for which we obtained only single isolates. DNA signatures of these two species were present in other plants at the site and the immediate surroundings (and nowhere else). As such, a detection of those more widely dispersed taxa should be interpreted as that particular pathogen being present in the general area.

In comparing the infestation levels of sites using Illumina, we did find positive correlations between the diversity and infestation scores of reveg/disturbed sites and their corresponding control sites (Fig. 14). This was expected: factors contributing to *Phytophthora* introduction and establishment – environmental suitability, host composition, disturbance history – are largely shared between all the sites within an immediate area. Taken together, sampling of both reveg sites and the surrounding vegetation will give the best picture to overall *Phytophthora* diversity.

Assessed by project area, some locations were clearly more infested than others (Fig. 16). Sites such as the Pulgas Ridge Blue Oak parking lot (PR_E001) and the Pulgas Ridge forest site (PR_B006) had a number of high-risk detections and symptoms of *Phytophthora* dieback. These areas have many features consistent with increased risk for *Phytophthora* contamination: prior ornamental plantings, restoration plantings, road grading and construction, ample public access and proximity to residential areas, and an abundance of host species known to be affected by *Phytophthora*. For others, such as La Honda Creek, infestation was more incidental to their location: being streamside and downstream of human disturbances increases their subsequent *Phytophthora* exposure.

Other project areas were rated as having low-infestation levels via baiting, but high-infestation levels via Illumina (Fig. 16, yellow quadrant). This may be due to a number of reasons. In the case of the Annex Garden, *Phytophthora* spp. were likely brought in on plants which failed to thrive; we thus may be detected a high proportion of non-viable, remnant DNA. In other cases, notably the Monte Bello sites, we found a number of moderate-risk but un-baitable taxa (*P. uliginosa*-cluster and *P. quercina*-cluster), and a high diversity of low-risk taxa (*P. psychrophila*, *P. nemorosa*-cluster, and others) indicating *Phytophthora* suitability at that location. Lastly, since not all species were baited all years (e.g. *P. cambivora*) it is very likely we missed some species detections via baiting in sites only sampled one year.

Locations with low infestation rankings

There were a number of project areas with low rankings as calculated from both Illumina and baiting (Fig. 16, blue quadrant). Many of these were located in El Corte de Madera (3 project areas), Sierra Azul (3 project areas), and Bear Creek Redwoods (2 project areas) preserves. Given the history of some of these sites, this is surprising. The Hendrys and Alma College sites had ample evidence of ornamental outplantings and disturbance, as did some locations on Mt.

Umunhum. For the most part, however, observations at these locations are in agreement with our results: there was minimal to no evidence of dieback consistent with the presence of soilborne *Phytophthora* at these sites and *Phytophthora* isolates were largely restricted to outplanted nursery stock.

In contrast to more highly ranked areas these locations have fewer pathogenic species and the community is less diverse. These areas, however, are not *Phytophthora*-free. Pathogenic species of concern were detected at nearly all these locations (Appendix B). El Corte de Madera and Bear Creek Redwoods were both notably impacted by *P. ramorum*, which was better detected by leaf and twig plating. Importantly, relative to more heavily infested areas, these locations represent a high priority for preventing new introductions to maintain the low diversity currently present.

Factors affecting the designation of sites as high or low risk of Phytophthora *contamination*

Thus far, we've largely discussed the risk posed by a particular species (due to, for example, a species' host range or its impacts in other areas). Alternatively, we may also talk about the risk that a plant community is "receptive" and may become invaded by *Phytophthora.* While *Phytophthora* spp. were widespread as detected via baiting and Illumina, substantial variation exists between the overall infestation of individual areas (Fig. 15, Fig. 16). Two possibilities may explain this observation: either *Phytophthora* has yet to be introduced, or an area may be inherently less at risk for *Phytophthora* establishment and spread.

Many factors can influence the introduction and establishment of a given *Phytophthora* species, as well as their total diversity. Most notable among these are the plant community and disturbance history. Many plant taxa were host to a larger diversity of *Phytophthora* species (Fig. 10), and some disturbances, especially the movement of nursery plants and soil, are more strongly associated with the introduction of new species. Risk of contamination is also thought to be greater the closer a site is to introduction points associated with access (distance to the nearest trail, for example), or topographical variables which maintain greater moisture at the site (aspect, for example).

Management of *Phytophthora* is aided by *a priori* information regarding a site's infestation status. To this end, we performed an exploratory analysis to assess if multiple disturbances, vegetation type, and topographical variables can predict if future sites should be classified as highly contaminated, or relatively free of *Phytophthora* (explained in detail in Appendix C).

In general, areas at highest risk of hosting *Phytophthora* were those in the riparian, woodland, and chaparral/shrub vegetation types, especially if the site had been revegetated with nursery plants. These three habitats were also associated with significantly larger infestation scores. This conclusion is consistent with known *Phytophthora* ecology in this region: plant taxa supporting the greatest diversity of species are most often found within the woodland and chaparral/shrub vegetation types; streamside vegetation may be exposed to *Phytophthora* dispersing in waterways, and the lower temperatures and higher relative humidity typical of riparian areas may favor their establishment. In contrast, upland-evergreen and rocky-forb vegetation types had the lowest *Phytophthora* diversity; these areas may be at lower risk for *Phytophthora* establishment.

Still, the effect did vary between preserves. We observed relatively low baiting success and infestation scores at El Corte de Madera, Sierra Azul, and Bear Creek Redwoods, despite sampling in areas with vegetation classes strongly associated with *Phytophthora* contamination. We expored which other factors may have influenced this, however all were relatively less important or had a negligible effect on our ability to predict site-level contamination risk: plant health, elevation, slope, aspect, usage and distance to features associated with the spread of *Phytophthora*, and prior disturbance history. These factors are still important to consider when assessing the likelihood an area may be or may become infested by *Phytophthora*; that they are less predictive is largely because of the complex history of the area and the broad distribution of *Phytophthora*, particularly the aggressive species.

Conclusions: management recommendations and future questions

Phytophthora is a widespread and diverse group of pathogens, able to inhabit water, soils, and canopies of both agricultural crops and native wildlands. As a result of emerging, devastating diseases such as sudden oak death, we have a new appreciation for how pervasive many high-risk species have become, and how many new species have yet to be described. Survey efforts are now being aided by new technologies, such as Illumina MiSeq Sequencing, which allow for greater detection sensitivity than ever before.

In contrast to sudden oak death, the vast majority of these species are not causing overt or widespread plant death. Some are opportunists, generally restricted to waterways where their ecological importance is unknown. Some are native, causing only minor or sporadic disease. Some cause severe symptoms in nursery environments, but are less likely to persist in natural habitats. Most others, including many of the species detected in our surveys, are thought to contribute to declining plant health in conjunction with other stresses. Plant pathogens may actually contribute to plant community health by initiating succession and selecting for greater plant diversity. In the case of *Phytophthora*, however, virulent non-native species have been so widely distributed by the nursery industry and their impacts are so poorly studied, the consensus has been that most species will have a net detrimental effect.

Management options depend largely on the infestation level of the site, particularly when aggressive species are detected. All future revegetation sites should be assessed for *Phytophthora* prior to planting. Areas with limited distribution or low diversity of *Phytophthora* species should be a priority for treatment and protection. Protection principles should be implemented to minimize the risk of introduction, particularly of species not already at the site. This is achieved by minimizing importation or movement of soils; direct seed planting; installing native plant nursery stock grown utilizing phytosanitary BMPs; cleaning and sanitizing boots, tools, equipment, and vehicles; managing trails to prevent spread of infested soils; controlling drainage and surface water; and coordinating and implementing phytosanitary considerations when staff, contractors, and volunteers move between sites. Under some circumstances, heat treatment through solarization or steaming may be useful to eradicate *Phytophthora* in small areas or as a pre-planting treatment.

Heavily infested areas, those with a high diversity of pathogenic species, are best managed by containing local spread and monitoring their impacts. Even within areas with heavy contamination, though, some *Phytophthora* species may have a limited distribution and sanitation measures within a site reduce the risk of further contamination; disease may be minimized by managing soil drainage and runoff at planting sites, increasing spacing between

plant species that may be hosts when host-specific *Phytophthora* species are indicated at a site, and avoiding planting vulnerable species in severely infested areas. Pre-emptive treatment with phosphite-based compounds like Agri-Fos (currently on the List of Approved Pesticides within the District's Integrated Pest Management Program) suppress *Phytophthora* and may be used to protect vulnerable populations close to infestation centers. Additional management recommendations are discussed in Appendix D.

Knowing current infestation levels is the first step to better *Phytophthora* management. However, to best aid the management of *Phytophthora* on MROSD lands, we propose the following questions be addressed:

1. Do DNA-only detections indicate the presence of viable pathogens?
    - DNA-only detections indicate that a species was introduced, however it does not indicate whether it is viable. Species of concern include *P. tentaculata*, *P. lateralis*, and *P. siskiyouensis*. *P. cactorum* was also widely detected via Illumina in control areas, although it was almost exclusively baited from revegetation areas. Further baiting of these detections will increase confidence as to whether high risk species are present, and if they are active in the surrounding vegetation. It is important to acknowledge, however, the limitations of baiting to detect the full diversity of *Phytophthora* species.

2. How are Phytophthora affecting management objectives?
    - Many plants from which we isolated *Phytophthora* were classified as healthy, even in areas where multiple aggressive species were recovered. The long-term outcome of these infections is not always clear, particularly since each *Phytophthora* species may affect various host genera differently and very little is known about most host:pathogen combinations. Comparing plant health outcomes in heavily infested and less infested areas will accomplish the following:
        1. Discern if infested plant communities develop disease and decline;
        2. Determine if *Phytophthora* contamination is affecting the success or failure of revegetation projects;
        3. Determine if *Phytophthora* contamination is affecting natural regeneration in native plant communities;
        4. Identify plant species or individuals which are tolerant to disease by a particular *Phytophthora* species; and
        5. Assist phylogenetic research indicating which *Phytophthora* species are native to MROSD lands.

Phytophthora must be incorporated into the broader goal of protecting natural habitats for their educational, recreational and ecological benefits. Management of *Phytophthora* diseases is best obtained through preventing the introduction of new species and genotypes, as the outcome of novel host:pathogen combinations cannot be predicted. To whatever extent is possible, tolerant hosts should be selected for highly contaminated sites, and sanitary measures should be implemented when moving between sites. Importantly, some MROSD preserves have substantially less *Phytophthora* infestation than others, and these areas should be a priority for protection.

Figures & Tables

Table 1. Site information, including location, project and site IDs, and year(s) sampled.

Preserve	Project	Project ID(s)[a]	Status[b]	Reveg ID[c]	Site ID[d]	2017	2018
Purisima Creek	Purisima Redwoods	RV_PC_A001	planned	PC_A001	PLND_PC_A001	x	x
		control	control	PC_A001	CON_PC_A001	x	
La Honda	La Honda Creek	RV_LH_F001, F002, & F003	reveg	LH_F001	RV_LH_F001		x
		control	control	LH_F001	CON_LH_F001		x
Pulgas Ridge	Blue Oak Parking Lot	RV_PR_E001	reveg	PR_E001	RV_PR_E001	x	x
		control	control	PR_E001	CON_PR_E001	x	x
	Pulgas Forest	RV_PR_B001 & B003	reveg	PR_B006	RV_PR_B006	x	x
		control	control	PR_B006	CON_PR_B006	x	x
	Pulgas Summit C-series	RV_PR_C002	reveg	PR_C002	RV_PR_C002	x	x
		RV_PR_C003	reveg	PR_C003	RV_PR_C003	x	x
		control (prior PR_D001)	control	PR_C003	CON_PR_C003	x	x
	Pulgas Summit A-series	RV_PR_A004	reveg	PR_A004	RV_PR_A004		x
		control	control	PR_A004	CON_PR_A004		x

[a] Project IDs as designated by MROSD, sampled collectively as a single "site." Occasionally project IDs were not assigned prior to sampling in which case they were assigned by OSU; some projects changed IDs in which case IDs used in prior documents are indicated in parentheses along with relevant notes related to the site.

[b] Site status upon sampling as designated by OSU: "reveg" sites are those in which nursery-grown plants were introduced to the site, and samples were taken from outplanted stock when they could be identified; samples from "planned" sites were taken from the area designated to be planted; "disturbed" sites are those in which a disturbance is apparent, however remediation is not planned; "control" sites are sites adjacent to planned and reveg sites containing a reduced level of disturbance. For analysis purposes, planned and disturbed sites are combined into a single site status designation "disturbed".

[c] Reveg IDs as designated by OSU, designating an identification code for each set of related sites (e.g. reveg site(s) and the paired control site)

[d] Site IDs as designated by OSU, indicating the reveg ID and the planting status. Each site is typically comprised of 6 soil samples per year.

Table 1 cont.

Preserve	Project	Project ID(s)[a]	Status[b]	Reveg ID[c]	Site ID[d]	2017	2018
Skyline Ridge	Skyline Ridge	RV_SR_A001	reveg	SR_A001	RV_SR_A001	x	x
		RV_SR_A002	reveg	SR_A002	RV_SR_A002	x	x
		RV_SR_A003	reveg	SR_A003	RV_SR_A003	x	x
		control	control	SR_A001	CON_SR_A001	x	x
		Tree Farm	disturbed	TreeFarm	DIST_TreeFarm	x	
	Big Dipper	RV_SR_B001	reveg	SR_B001	RV_SR_B001	x	
		RV_SR_B002	reveg	SR_B002	RV_SR_B002	x	
		control	control	SR_B001	CON_SR_B001	x	
Monte Bello	Bridge Planting	RV_MB_A001	planned	MB_A001bridge	PLND_MB_A001bridge	x	
		control	control	MB_A001bridge	CON_MB_A001bridge	x	
	Grassland Planting	RV_MB_A001	planned	MB_A001grass	PLND_MB_A001grass	x	
		control	control	MB_A001grass	CON_MB_A001grass	x	x
El Corte De Madera	El Corte de Madera Bridge	CM_C003	reveg	CM_C003	RV_CM_C003	x	
		control	control	CM_C003	CON_CM_C003	x	
	El Corte de Madera Parking Lot	CM_A003, A001	reveg	CM_A003	RV_CM_A003	x	
		control	control	CM_A003	CON_CM_A003	x	
	King Mt manzanita	CM_D001	planned	CM_D001	PLND_CM_D001		x
		control	control	CM_D001	CON_CM_D001		x
	Chinquapin	chinquapin	disturbed	CHIN1	DIST_CHIN1		x
		chinquapin	disturbed	CHIN2	DIST_CHIN2		x

Table 1 cont.

Preserve	Project	Project ID(s)[a]	Status[b]	Reveg ID[c]	Site ID[d]	2017	2018
Russian Ridge	Mindego Gateway	RV_RR_A001	reveg	RR_A001	RV_RR_A001	x	x
		RV_RR_A005 & RR_A006	reveg	RR_A006	RV_RR_A006	x	x
		control	control	RR_A001	CON_RR_A001	x	x
Rancho San Antonio	Annex Garden	RV_RS_B001	reveg	RS_B001	RV_RS_B001	x	
		control	control	RS_B001	CON_RS_B001	x	
	Rhus Ridge	RV_RS_A001	reveg	RS_A001	RV_RS_A001	x	
		control	control	RS_A001	CON_RS_A001	x	
	Equestrian Lot	RV_RS_C001, C002, & C003	reveg	RS_C001	RV_RS_C001		x
		control	control	RS_C001	CON_RS_C001		x
	RSA Field Office	RV_RS_D001, D002, D003, & D004	reveg	RS_D001	RV_RS_D001		x
		control	control	RS_D001	CON_RS_D001		x
Bear Creek Redwoods	Alma College	RV_BCR_A003	planned	BCR_A003	PLND_BCR_A003		x
		control	control	BCR_A003	CON_BCR_A003		x
	Webb Creek Bridge	RV_BCR_A004	reveg	BCR_A004	RV_BCR_A004		x
		control	control	BCR_A004	CON_BCR_A004		x
	Bear Creek Christmas Tree Farm	RV_BCR_A001&A002	planned	BCR_A001	PLND_BCR_A001		x
		control	control	BCR_A001	CON_BCR_A001		x

Table 1 cont.

Preserve	Project	Project ID(s)[a]	Status[b]	Reveg ID[c]	Site ID[d]	2017	2018
Sierra Azul	Mt. Umunhum Woods Trail	RV_SA_G001 & G002	reveg	SA_G001	RV_SA_G001	x	
		control	control	SA_G001	CON_SA_G001	x	
	Mt. Umunhum Bald Mountain	RV_SA_A005, A002, & SA_A006	reveg	SA_A008	RV_SA_A008	x	
		control (prior SA_B006)	control	SA_A008	CON_SA_A008	x	
	Mt Umunhum Hoita Road	RV_SA_H001	planned	SA_H001	PLND_SA_H001		x
		control	control	SA_H001	CON_SA_H001		x
	Flagpole	Flagpole (2017 planting)	reveg	Flagpole	RV_Flagpole	x	x
		control	control	Flagpole	CON_Flagpole	x	x
		Flagpole Lupin	disturbed	Flagpole	DIST_Flagpole	x	x
		RV_SA_F014 (2018 planting)	reveg	SA_F014	RV_SA_F014		x
	Mt. Umunhum weather shelter & stairway	RV_SA_F005 (weather shelter)	reveg	SA_F005	RV_SA_F005		x
		RV_SA_F011, F012 (stairway)	reveg	SA_F012	RV_SA_F012		x
		Teds	disturbed	teds	DIST_teds	x	x
		control	control	SA-F005	CON_SA-F005		x
	Mt. Umunhum summit plantings	SA_F001 (experimental)	reveg	SA_F001	RV_SA_F001	x	
		SA_F004 & F006 (cube; prior summit)	reveg	SA_I001	RV_SA_I001	x	x
		SA_F002 (new planting past cube)	reveg	SA_F002	RV_SA_F002		x
		control	control	SA_I001	CON_SA_I001	x	x
		SA_F013 (ceremonial circle; prior SA_G003)	reveg	SA_F013	RV_SA_F013		x
		control	control	SA_F013	CON_SA_F013		x
	Hendrys	RV_SA_L001, L002, L003 (prior Hendrys)	planned	SA_L001	PLND_SA_L001	x	
		control (prior Hendrys)	control	SA_L001	CON_SA_L001	x	

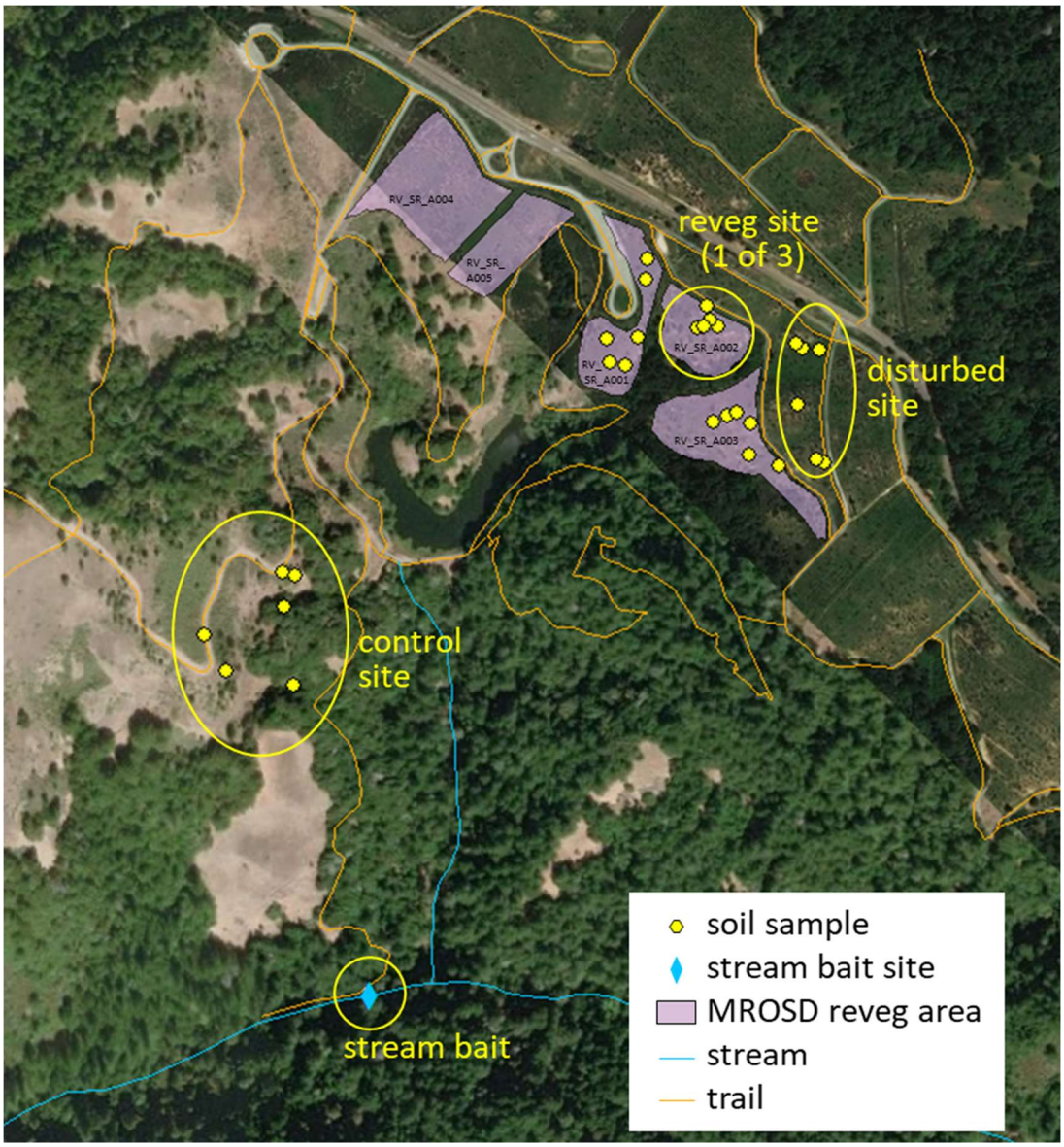


Fig. 1. Example of site classes, illustrated at Skyline Ridge Open Space Preserve. Pictured are reveg sites RV_SR_A001, RV_SR_A002 and RV_SR_A003, which had been outplanted in 2008, 2009 and 2010, respectively. These projects were to remediate a former Christmas Tree Farm, still in operation just upslope of the reveg projects and sampled as the disturbed site (designated as DIST_TreeFarm). The closest, minimally disturbed control area sharing species present in the reveg projects was located along the Sunny Jim Trail (designated as CON_SR_A001). At this location we also baited Lambert Creek, draining Horseshoe Lake. An additional site class category, planned sites, consisting of locations of future MROSD revegetation projects, is not represented in this figure.

Fig. 2. Sampling soil from the base of toyon with dieback symptoms characteristic of *Phytophthora* infection. RV_PR_E001 (Blue Oak Parking Lot, Pulgas Ridge).

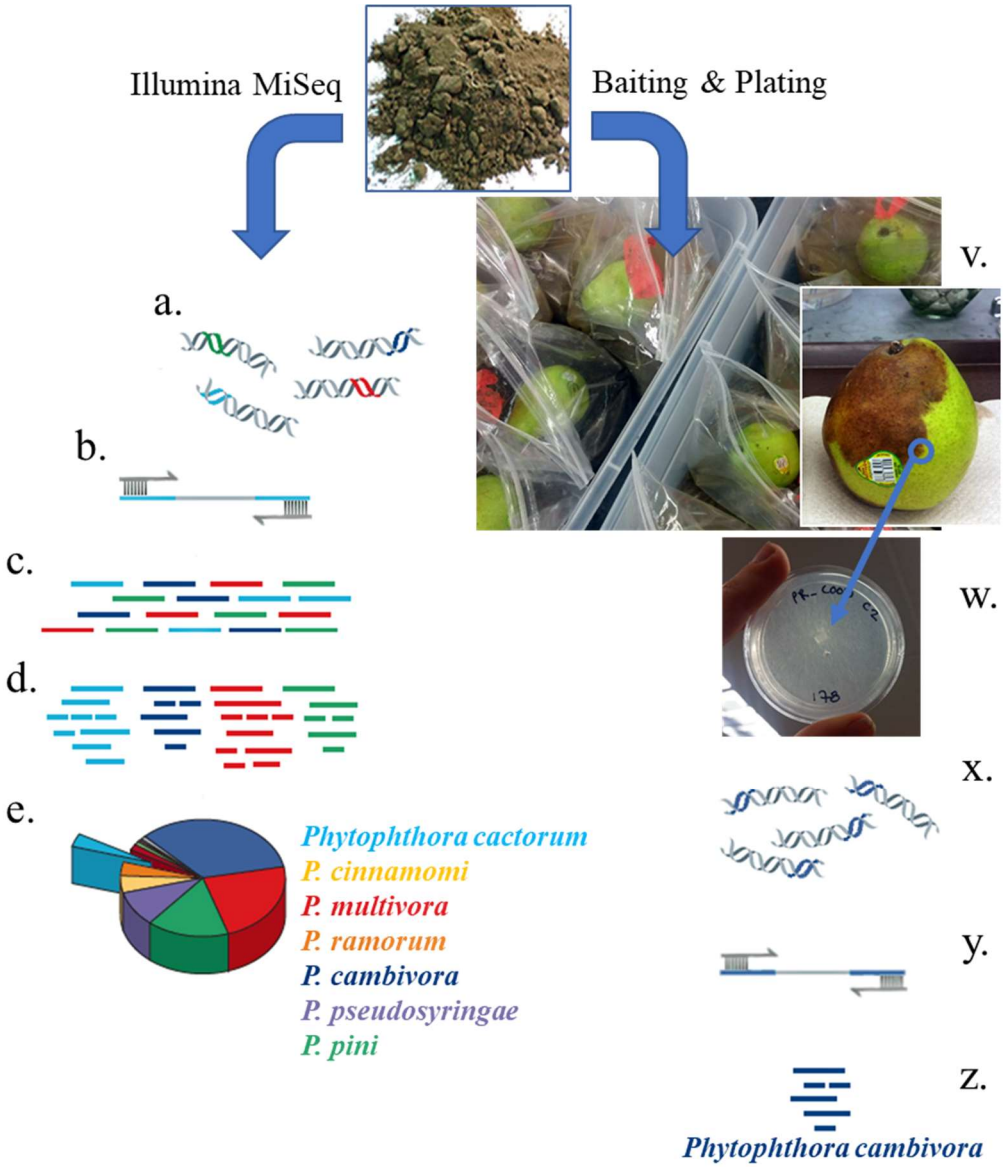


Fig. 3. Diagram showing how soil samples taken from potentially infested sites have been processed via the two mechanisms: Illumina MiSeq (left) and baiting and plating with identification via Sanger sequencing (right).

Illumina MiSeq methodology: DNA from multiple species was extracted directly from the soil (a), and copies were made with PCR (b, c). All DNA fragments (amplicons) are sequenced with Illumina high-throughput sequencing and sorted into similar groups (operational taxonomic units, or OTUs; d). These are then compared to a reference database of known species for identification (e).

Baiting and plating methodology: Soil was flooded and a pear bait was placed in the water (v). Lesions on the bait were then plated on agar media selecting for *Phytophthora* (w). DNA was extracted from the colony (x), copied with PCR (y), and sequenced (Sanger sequencing). This sequence was then compared to a reference database of known species for identification (z).

Figure adapted from Lasken, R.S. and McLean, J.S. Nature Reviews: Genetics 15:577-584.

Table 2. Summary of the number of sites sampled by year and status designation.

site status	no. sampled only in 2017	no. sampled only in 2018	no. sampled in both years	total no. sampled
reveg	9	10	11	30
control	10	11	8	29
disturbed	1	2	2	5
planned	3	4	1	8
Grand Total	23	27	22	72

Table 3. Summary of the number of soil samples collected and processed by year and site status. Samples collected in 2018 include those taken from new sites (sampled only in 2018) and repeat sites (sampled both years).

site status	no. sampled in 2017	no. sampled in 2018	total no. sampled
reveg	119	128	247
control	111	117	228
disturbed	16	18	34
planned	25	30	55
Grand Total	271	293	564

Table 4. Soil was collected from the following species, listed in order of frequency. The rank of the species indicates the relative frequency from which the taxon was sampled from each site-type, 1 being the most commonly sampled (ns = not sampled).

Species	No. samples	% of total samples	Ranking within each site type			
			control	reveg	disturbed	planned
Heteromeles arbutifolia	66	11.7%	2	1	ns	7
Mimulus aurantiacus	64	11.3%	1	4	ns	13
Arbutus menziesii	63	11.2%	3	3	3	1
Frangula californica	42	7.4%	6	2	ns	ns
Lupinus albifrons	30	5.3%	8	13	1	ns
Arctostaphylos crustacea	29	5.1%	4	6	ns	6
Notholithocarpus densiflorus	17	3.0%	9	14	ns	8
Rubus ursinus	17	3.0%	5	ns	ns	5
Quercus agrifolia	16	2.8%	7	ns	ns	3
Alnus rhombifolia	15	2.7%	10	10	ns	9
Penstemon heterophyllus	13	2.3%	ns	5	ns	ns
Quercus durata	12	2.1%	11	19	6	ns
Phacelia imbricata	11	2.0%	15	9	ns	ns
Alnus rubra	8	1.4%	14	18	ns	ns
Arctostaphylos andersonii	8	1.4%	12	29	ns	ns
Artemisia californica	8	1.4%	32	15	ns	ns
Monardella villosa ssp. *villosa*	8	1.4%	ns	7	ns	ns
Rosa californica	8	1.4%	18	ns	ns	2
Vaccinium ovatum	8	1.4%	ns	8	ns	ns
Quercus lobata	7	1.2%	17	21	ns	ns
Ribes californicum	7	1.2%	26	16	ns	ns
Chrysolepis chrysophylla	6	1.1%	ns	ns	2	ns
Cornus sericea	6	1.1%	13	ns	ns	ns
Eriophyllum confertifolium	6	1.1%	ns	20	ns	15
Erogonum saxatile	6	1.1%	ns	11	ns	ns
Rubus parviflorus	6	1.1%	23	ns	ns	4
Umbellularia californica	6	1.1%	ns	12	ns	ns
Pseudotsuga menziesii	5	0.9%	ns	28	5	ns
Quercus chrysolepis	5	0.9%	16	ns	ns	16
Ribes malvaceum	5	0.9%	ns	17	ns	ns
Sequoia sempervirens	5	0.9%	30	23	ns	ns
Baccharis pilularis	4	0.7%	31	32	ns	ns

Table 4 cont.

Species	No. samples	% of total samples	Ranking within each site type			
			control	reveg	disturbed	planned
bare soil[a]	4	0.7%	22	24	ns	ns
Carex ampifolia	4	0.7%	29	30	ns	ns
Rosa gymnocarpa	4	0.7%	28	ns	ns	14
Salvia mellifera	4	0.7%	20	ns	ns	10
Tellima grandiflora	4	0.7%	21	ns	ns	11
Pinus contorta	3	0.5%	ns	22	ns	ns
Quercus wislizeni	3	0.5%	ns	ns	4	ns
Ribes sanguineum	3	0.5%	27	ns	ns	17
Arctostaphylos regismontana	2	0.4%	24	ns	ns	ns
Buxus sp.	2	0.4%	ns	27	ns	ns
Corylus cornuta	2	0.4%	19	ns	ns	ns
Holodiscus discolor	2	0.4%	ns	26	ns	ns
Lepechinia calycina	2	0.4%	ns	31	ns	ns
Oxalis oregana	2	0.4%	ns	ns	ns	12
Pinus sabiniana	2	0.4%	ns	25	ns	ns
Sambucus racemosa	2	0.4%	25	ns	ns	ns
Dendromecon rigida	1	0.2%	ns	33	ns	ns
Rosa sp.	1	0.2%	ns	ns	ns	18
Grand Total	564	100%				

[a] Bare soil was sampled at two sites, CON_SA_I001 and PLND_CM_D001, where no plants were present in the immediate area of interest.

Table 5. Number of samples in which a particular *Phytophthora* sp. was recovered by baiting, organized by site status and year.

Phytophthora sp. baited	No. of samples in which sp. was recovered by site status[a]				No. samples in which sp. was recovered by year		Total
	control	disturbed	planned	reveg	2017	2018	
P. boehmeriae				1		1	2
P. cactorum	1			13	7	7	14
P. cambivora	7		1	5	13		13
P. cinnamomi	1			2	2	1	3
P. crassamura				7		7	7
P. cryptogea-complex[b]	3	1			2	2	4
P. lacustris	1			1		2	2
P. megasperma				2		2	2
P. multivora	1			1	1	1	2
P. nicotianae				2	1	1	2
P. pseudosyringae				1		1	1
P. pseudotsugae	1			1		2	2
P. ramorum	4		1	3	5	3	8
P. rosacearum				1		1	1
P. sp. 'cadmea'	3					3	3
P. syringae	3		1		1	3	4
P. syringae, P. pseudosyringae[c]				1		1	1
P. taxon *asparagi*	1					1	1
P. taxon *oaksoil*	2					2	2
Negative	199	33	52	206	238	252	
Total no. of samples baited	227	34	55	247	270[d]	293	
Proportion positive samples	0.12	0.03	0.05	0.17	0.12	0.14	
No. species detected	12	1	3	14	8	17	

[a] "reveg" sites are those in which nursery-grown plants were introduced to the site, and samples were taken from outplanted stock when they could be identified; samples from "planned" sites were taken from the area designated to be planted; "disturbed" sites are those in which a disturbance is apparent, however remediation is not planned; "control" sites are sites adjacent to planned and reveg sites containing a reduced level of disturbance.

[b] May be *P. cryptogea* s.s., *P. pseudocryptogea*, *P. erythroseptica*, or *P.* sp. *kelmania*. Phylogenetic relatinoships between these species are still being determined.

[c] Both *P. syringae* and *P. pseudosyringae* were recovered from the same sample.

[d] Note: the total number of samples baited is one less than the total number of samples collected in 2017 as one sample was missing upon bait assessment; however, DNA extract from this soil sample was submitted for Illumina MiSeq sequencing, hence its inclusion in the study.

Table 6. Recovery of *Phytophthora* species by associated genera. At least one *Phytophthora* was baited from 73 of the 563 soil samples collected and baited over 2017 and 2018. Data includes recovery results from samples collected both years. Note, as these *Phytophthora* species were baited from soils and not from lesioned tissue, the species detected was only found in association with the plant and may not be causing disease on that host. We were unable to bait *Phytophthora* from all genera not listed in the table.

Genus	total no. samples	no. positive samples	% positive	no. species	species recovered
Frangula	42	11	26.2%	7	*P. cactorum*, *P. cambivora, P. crassamura, P. multivora, P. ramorum, P. syringae, P.* taxon *asparagi*
Mimulus	64	9	14.1%	6	*P. boehmeriae, P. cinnamomi, P. cryptogea*-complex*, P. multivora, P. ramorum, P. syringae*
Arbutus	62	10	16.1%	6	*P. cactorum, P. cambivora, P. cryptogea*-complex*, P. megasperma, P. nicotianae, P. pseudosyringae*
Heteromeles	66	13	19.7%	4	*P. cactorum*, *P. cambivora*, *P. megasperma*, *P.* sp. 'cadmea'
Alnus	23	5	21.7%	4	*P. lacustris, P. ramorum, P. syringae, P.* taxon *oaksoil*
Quercus	43	5	11.6%	4	*P. cambivora, P. cinnamomi, P. crassamura, P.* sp. 'cadmea'
Rubus	23	3	13.0%	3	*P. ramorum, P. syringae, P.* taxon *oaksoil*
Arctostaphylos	39	4	10.3%	2	*P. cambivora, P. cinnamomi*
Eriophyllum	6	3	50.0%	2	*P. crassamura, P. rosacearum*
Rosa	13	3	23.1%	2	*P. pseudotsugae, P. ramorum*
Monardella	8	2	25.0%	1	*P. crassamura*
Oxalis	2	1	50.0%	1	*P. crassamura*
Sambucus	2	1	50.0%	1	*P. cactorum*
Carex	4	1	25.0%	1	*P. ramorum*
Tellima	4	1	25.0%	1	*P. ramorum*
Penstemon	13	1	7.7%	1	*P. pseudotsugae*

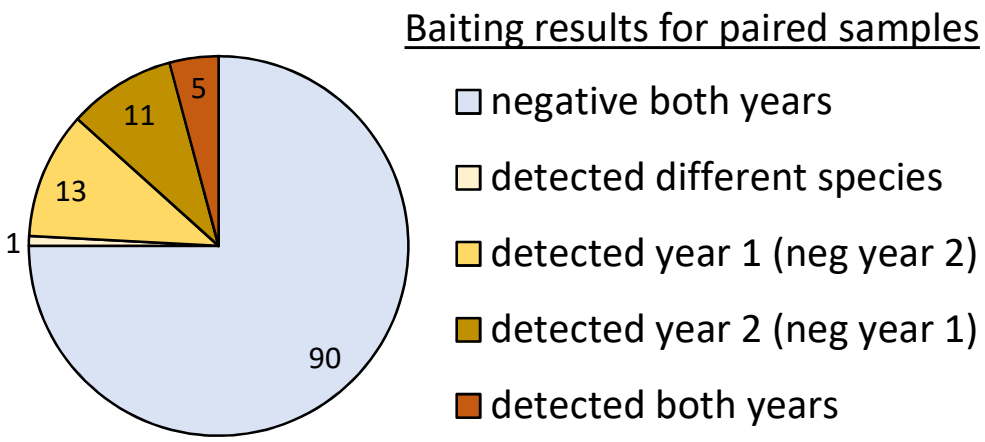


Fig. 4. Pie chart showing our ability to recover *Phytophthora* for all paired-samples collected in both 2017 and 2018. Soils from the base of a total of 120 plants were collected and baited in both years (representing a total of 240 samples). Of these 90 plants were negative both years; in only 5 plants was the same *Phytophthora* spp. detected both years: 4 plants from which *P. cactorum* was recovered and 1 plant from which *P. nicotianae* was recovered. In one sample taken from the Pulgas Ridge Blue Oak parking lot we baited *P. cambivora* one year, and *P.* sp. 'cadmea' the second year.

Table 7. Number of soil samples in which a given *Phytophthora* species was recovered, by bait type (pear, rhododendron, and oregano). Data is only presented for samples in which all bait types were tested. No species were recovered exclusively from oregano.

Phytophthora sp.	pear	rhod.	pear & rhod.	pear, rhod. & oregano
P. boehmeriae	1			
P. cactorum	3	2	2	
P. cinnamomi				1
P. crassamura	7			
P. cryptogea-complex	2			
P. lacustris	2			
P. megasperma	2			
P. multivora	1			
P. nicotianae		1		
P. pseudosyringae	2			
P. pseudotsugae	2			
P. ramorum	3			
P. rosacearum	1			
P. sp. 'cadmea'	3			
P. syringae	3			
P. taxon *asparagi*	1			
P. taxon *oaksoil*	2			
Total	35	3	2	1

Table 8. Recovery of *Phytophthora* spp. from foliage samples.

Site	No. samples with species detected			Total no. samples
	P. aff. *ilicis*	*P. nemorosa*	*P. ramorum*	
BCR_A001			2	2
BCR_A003			4	4
BCR_A004			5	5
LH_F001	1	1		2
PR_E001			1	1
RR_A001			1	1
SA_I001			1	1
SA_L001			1	1
SR_A001			3	3
SR_B001			1	1
SA_Teds			3	3
Total	1	1	22	24

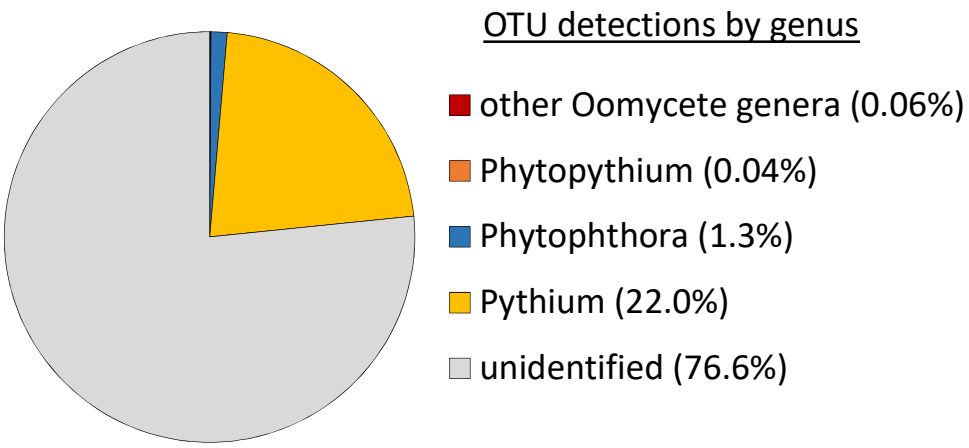


Fig. 5. Frequency of OTUs identified via Illumina MiSeq, separated by genera. Proportion of the total reads comprised by each group is indicated in parentheses. ITS6 and ITS7 primers are selective for Oomycetes (of which *Phytophthora* is one genus), however they do amplify non-Oomycete taxa. Unidentified reads are those which amplified using ITS6 and ITS7 primers, but did not match any known Oomycete genus.

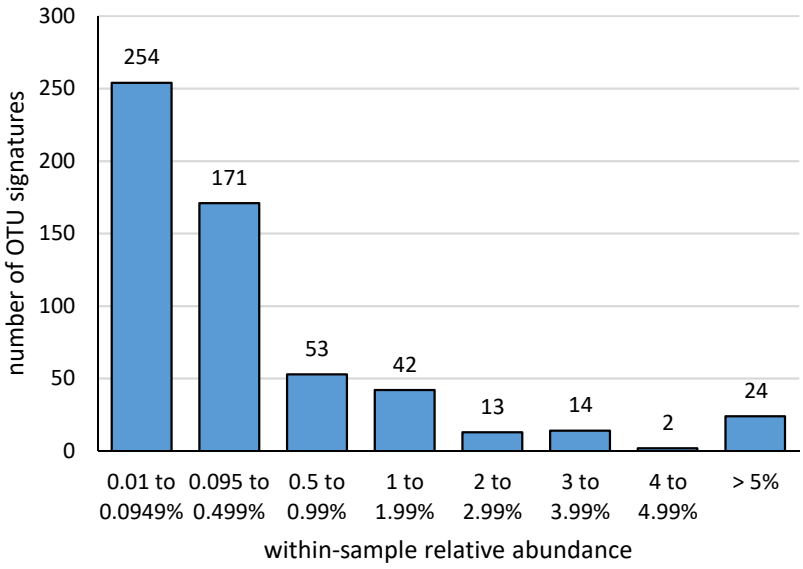


Fig. 6. Number of OTU signatures recorded via Illumina MiSeq sequencing, binned by the within-sample relative abundance for each particular OTU. In the majority of cases the OTU comprised only between 0.01% and 0.0949% of the total number of reads within their respective samples; in 24 detections a particular OTU comprised at least 5% of the total number of reads within their sample.

Table 9. *Phytophthora* OTUs detected from soil samples collected both years and processed with Illumina MiSeq sequencing, organized by frequency of detection. A complex is composed of closely related species with identical ITS1 sequences; a cluster is composed of related species with sequences identical only between the ITS6 and ITS7 primers. For species members in OTU clades or clusters, please see Table 10. OTUs with a "/" occur when the forward and backwards reads contradicted and either OTU in the pairing could be indicated. When applicable, the cultured species recovered via baiting is listed.

OTU designation	Clade[a]	No. of sites in which OTU was detected[b]	No. of samples in which OTU was detected[b]	Species recovered via baiting
P. psychrophila	3	27	72	
P. cactorum-cluster	1	28	52	*P. cactorum*, *P. pseudotsugae*
P. syringae	8	17	22	*P. syringae*
P. nemorosa-cluster	3	16	22	*P. pseudosyringae*, *P.* aff. *ilicis*[c], *P. nemorosa*[c]
P. uliginosa-cluster	7	11	21	*P.* sp. *'cadmea'*
P. quercina-cluster	12	8	19	
P. sp. unknown[d]	n/a	16	16	
P. citricola-complex	2	9	13	*P. multivora*
P. cryptogea-complex	8	11	11	*P. cryptogea*-complex
P. chlamydospora	6	8	8	
P. lateralis	8	7	7	
P. megasperma-cluster	6	6	6	*P. megasperma*, *P. crassamura*
P. cambivora-complex	7	4	5	*P. cambivora*
P. irrigata	9	4	5	
P. citrophthora-cluster	2	4	4	
P. ramorum	8	3	4	*P. ramorum*
P. cinnamomi	7	3	3	*P. cinnamomi*
P. formosa	7	3	3	
P. hydropathica	9	3	3	
P. asparagi	6	2	3	*P.* taxon *asparagi*
P. bilorbang-cluster	6	2	3	*P.* taxon *oaksoil*
P. clandestina	1	2	2	
P. fallax	9	2	2	
P. primulae	8	2	2	
P. tentaculata	1	2	2	

Table 9 cont.

OTU designation	Clade[a]	No. of sites in which OTU was detected[b]	No. of samples in which OTU was detected[b]	Species recovered via baiting
P. nicotianae	1	1	2	*P. nicotianae*
P. rosacearum	6	1	2	*P. rosacearum*
P. brassicae	8	1	1	
P. cambivora-complex / *formosa*	7	1	1	
P. hedraiandra	1	1	1	
P. porri	8	1	1	
P. riparia-cluster	6	1	1	*P. lacustris*
P. siskiyouensis	2	1	1	
P. virginiana	9	1	1	
P. boehmeriae[e]	10	0	0	*P. boehmeriae*
P. drechsleri[e]	8	0	0	
P. hibernalis[e]	8	0	0	
P. macilentosa[e]	9	0	0	

[a] As designated by Yang et al. 2017 and Jung et al. 2017

[b] To be considered detected, the OTU must have been present at ≥0.095% within-sample relative abundance.

[c] Cultured only from foliage. All other detections were from either soil, or foliage and soil.

[d] *P.* sp. unknown indicates a *Phytophthora* was detected, however it could not be identified to species (<99% sequence similarity to our reference database).

[e] A signature of the OTU was detected at >0.01% within-sample relative abundance, however the OTU did not comprise a minimum of 0.095% of the total number of reads in any samples.

Table 10. *Phytophthora* OTUs for which multiple species cannot be distinguished solely by sequencing the ITS region using ITS6 and ITS7 primers. Each OTU designation may indicate the presence of one or more of the member species.

Clade	OTU designation[a]	Member species
1	*cactorum*-cluster	*P. cactorum, P. idaei, P. pseudotsugae*
2	*citrophthora*-cluster	*P. citrophthora*, *P. terminalis*, *P. occultans*, *P. botryosa*, *P. himasilva*
2	*citricola*-complex	*P. citricola*, *P. citricola* E, *P. citricola* III, *P. citricola sensu stricto*, *P. pini*, *P. acerina*, *P. plurivora*, *P. pachypleura*, *P. multivora*, *P. caryae*, *P. capensis*, *P.* taxon *emzansi*
3	*nemorosa*-cluster	*P. nemorosa*, *P. ilicis*, *P. pseudosyringae, P. pluvialis*
6	*megasperma*-cluster	*P. megasperma*, *P. gonapodyides, P. crassamura*
6	*riparia*-cluster	*P. riparia*, *P. lacustris*
6	*bilorbang*-cluster	*P.* taxon *oaksoil*, *P. bilorbang*
7	*cambivora*-complex	*P. alni*, *P. alni* subsp. *alni*, *P. alni* subsp. *uniformis*, *P. alni* subsp. *multiformis*, *P. cambivora*
7	*uliginosa*-cluster	*P. europaea, P. uliginosa*, P. sp. 'cadmea'*, P. flexuos*
8	*cryptogea*-complex	*P. cryptogea, P. erythroseptica (= P. himalayensis), P.* sp. *kelmania, P. pseudocryptogea*
12	*quercina*-cluster	*P. quercina*, *P.* sp. "*ohioensis*", *P. versiformis*

[a] A complex is a group of phylogenetically related species that share an identical full-length ITS1 sequence; a cluster is a group of phylogenetically related species that have an ITS1 sequence identical only between ITS6 and ITS7 priming sites.

Table 11. Number of OTU detections which occurred at within-sample relative abundances greater than 1% or 5%. All OTUs not listed in this table comprised <1% of the total number of reads in all samples. For species members in OTU clades or clusters, please see Table 10. *P.* sp. unknown indicates a *Phytophthora* was detected, however it could not be identified to species (<99% sequence similarity to our reference database).

	Number of samples for which the OTU occurred with within-sample relative abundance…	
OTU	>1%	>5%
P. psychrophila	27	6
P. cactorum-cluster	23	9
P. quercina-cluster	9	4
P. sp. unknown	3	1
P. uliginosa-cluster	6	1
P. chlamydospora	2	1
P. syringae	4	1
P. citricola-complex	2	1
P. cryptogea-complex	4	0
P. cinnamomi	3	0
P. hydropathica	2	0
P. nicotianae	2	0
P. taxon *asparagi*	1	0
P. lateralis	1	0
P. nemorosa-cluster	3	0
P. riparia	1	0
P. bilorbang-cluster	1	0
P. ramorum	1	0
TOTAL	95	24

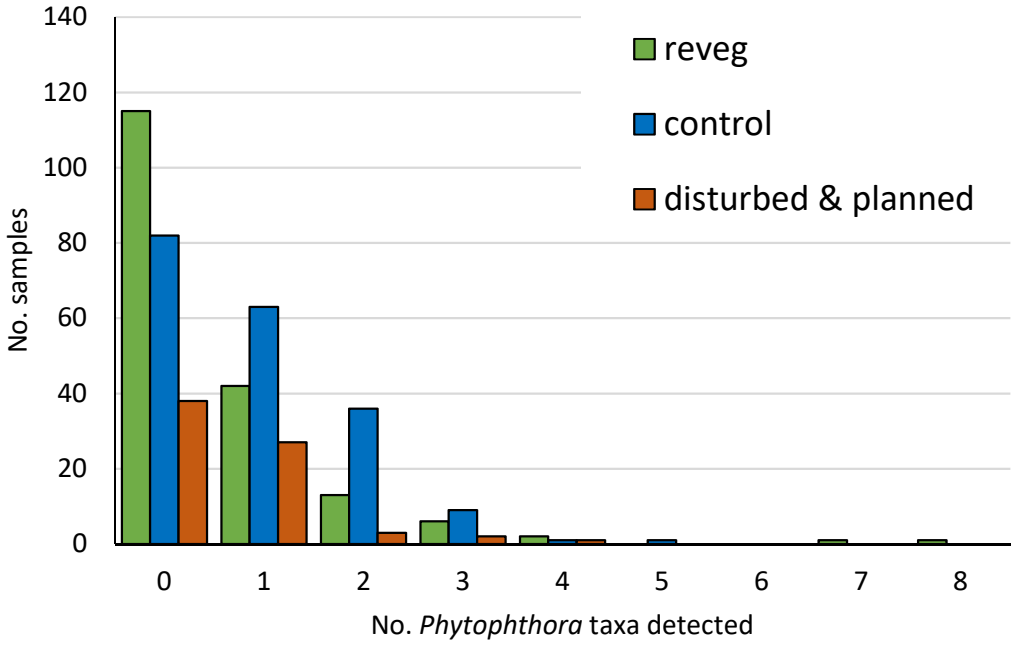


Fig. 7. Frequency chart indicating the number of OTUs detected in each 10g soil sample, separated by site status: revegetation, control, and combined disturbed and planned sites. Some samples from reveg sites had as many 7 or 8 OTUs present.

- Revegetation sites: 36.1% of samples had at least 1 *Phytophthora* OTU detected (at minimum 0.095% within-sample relative abundance)
- Control sites: 57.3% samples had at least 1 *Phytophthora* OTU detected
- Disturbed & planned sites: 46.5% samples had at least 1 *Phytophthora* OTU detected

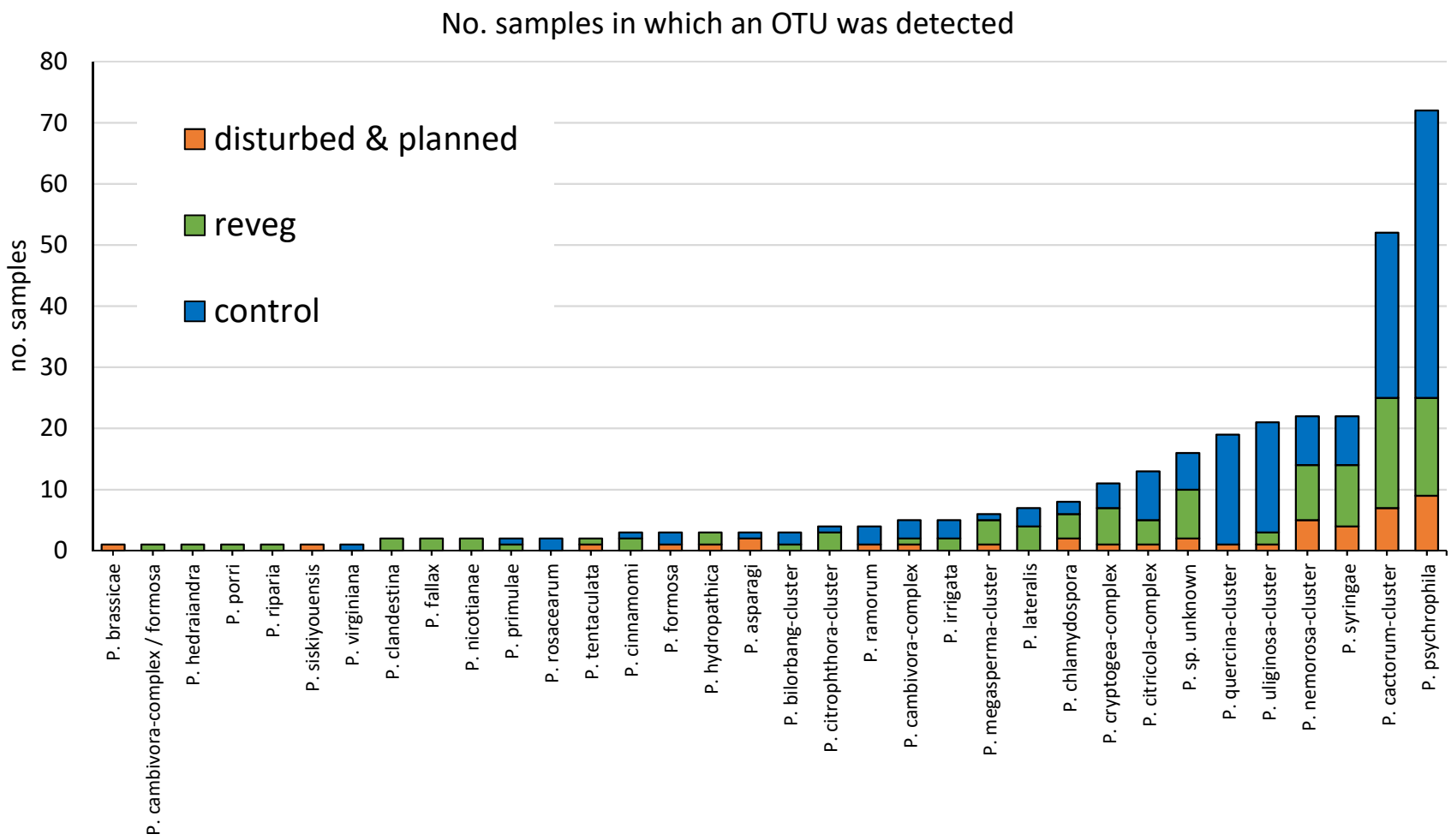


Fig. 8. Number of samples in which each *Phytophthora* OTU was detected via Illumina MiSeq sequencing. To be considered detected an OTU must have comprised a minimum of 0.095% of the number of reads within a sample. We sequenced a total number of 443 samples from planted restoration areas (reveg), adjacent, non-planted areas (control), or planned projects and disturbed areas. See Table 10 for the species composing *Phytophthora* complexes or clusters. When separated by a slash, forward and backwards reads of the DNA fragment being analyzed in that sample indicated either OTU may be present. *P.* sp. unknown indicates a *Phytophthora* was detected, however it could not be identified to species (<99% sequence similarity to our reference database).

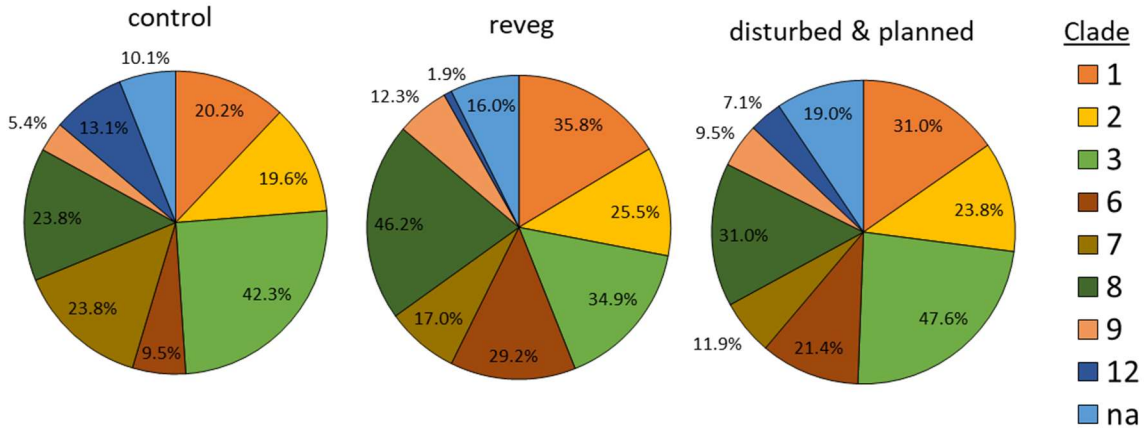
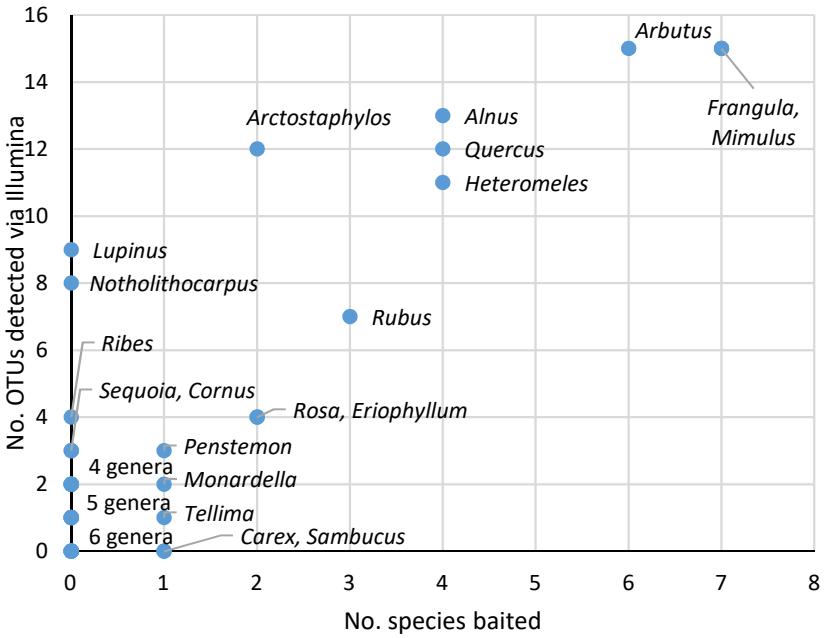


Fig. 9. Distribution of the number of detections organized by clade and site class: control, reveg, or disturbed (combined disturbed and planned sites). For example, 42.3% of all *Phytophthora* detections from control sites were OTUs within Clade 3. Detections from OTU designation *P.* sp. unknown are included as "na".



Plant genera for which 0 species were baited:

No. OTUs detected	Plant genera
0	*Artemisia, Dendromecon, Lepechinia, Pinus, Pseudotsuga, Umbellularia*
1	*Baccharis, Buxus, Chrysolepis, Erogonum, Holodiscus*
2	*Corylus, Phacelia, Salvia, Vaccinium*

Fig. 10. Comparison between the number of *Phytophthora* species detected by baiting and by Illumina, separated by associated plant genus.

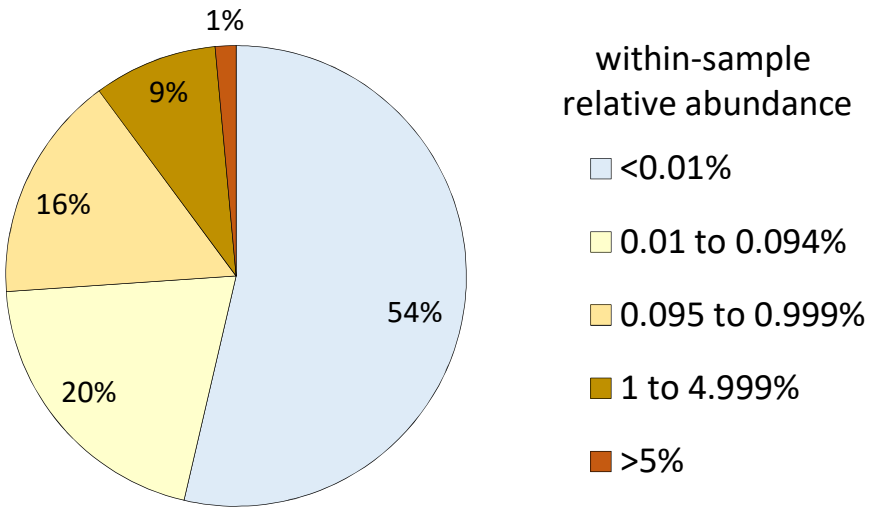


Fig. 11. Relative within-sample OTU abundance for those OTUs in which a *Phytophthora* species was isolated. For example, in 20% samples in which a species was isolated, the associated OTU comprised between 0.01 and <0.095% of the number of reads within the sample (within-sample relative abundance 0.01 and <0.095%). In 54% of samples the taxa was considered undetected at any threshold.

Table 12. Summary of our ability to detect a particular *Phytophthora*-OTU in samples taken from the base of same plant in both years. The number of paired samples in which the OTU was never detected, detected only 1 year, or detected both years are presented for two detection thresholds, ≥0.095% within-sample relative abundance (used as a general detection threshold for analysis) and ≥0.01% within-sample relative abundance (an indication the OTU may be present, albeit at low amounts). All OTUs not listed were either never detected in repeat-samples, or were detected a single year from each paired sample.

OTU	≥0.01% within-sample relative abundance			≥0.095% within-sample relative abundance			sites of repeat detections[a]
	never detected	detected one year	detected both years	never detected	detected one year	detected both years	
P. psychrophila	28	16	14	33	15	10	CON_MB_A001Grass, CON_PR_B006, CON_PR_E001, CON_RR_A001, CON_SR_A001, RV_PR_E001
P. cactorum-cluster	43	7	8	45	7	6	CON_PR_C003, CON_PR_E001, PLND_PC_A001, RV_PR_E001, RV_SR_A001, (RV_PR_B006), (RV_RR_A001)
P. quercina-cluster	47	2	9	47	6	5	CON_MB_A001Grass, CON_PR_C003, CON_PR_E001, CON_RR_A001, (CON_SR_A001)
P. uliginosa-cluster	48	6	4	50	6	2	CON_PR_B006, (CON_PR_E001)
P. nicotianae	57	0	1	57	0	1	RV_RR_A001
P. syringae	52	4	2	55	3	0	(PLND_PC_A001), (RV_RR_A006)
P. nemorosa-cluster	50	7	1	52	6	0	(CON_PR_C003)
P. tentaculata	56	1	1	57	1	0	(PLND_PC_A001)
P. siskiyouensis	57	0	1	57	1	0	(PLND_PC_A001)

[a] sites in parentheses are those for which an OTU signature comprised only 0.01% and <0.095% of total number of reads (below the detection threshold of ≥0.095%) in at least 1 year of the paired sample. When not in parentheses, the OTU was present in sample(s) at greater than ≥0.095% within-sample relative abundance both years.

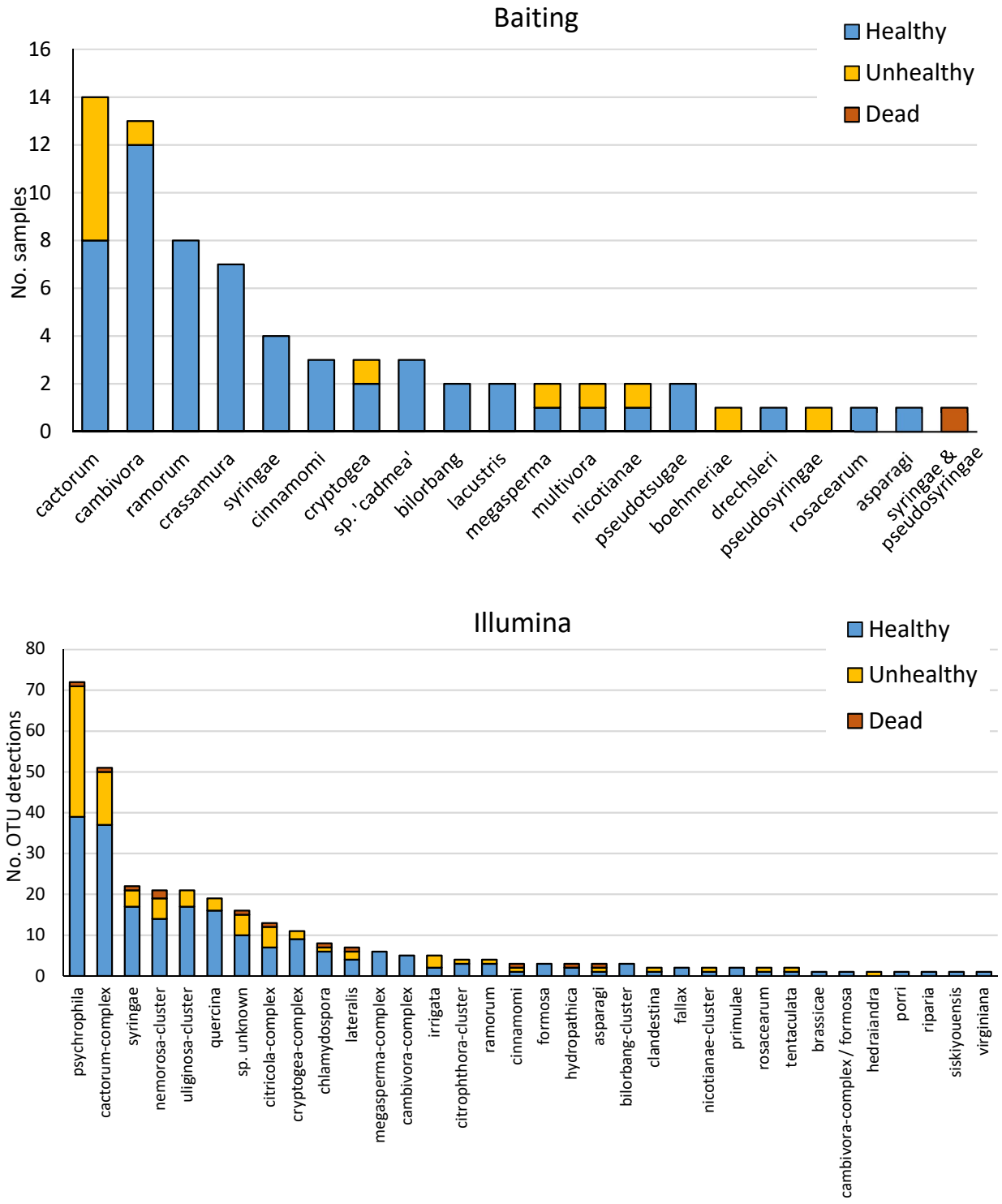


Fig. 12. Health status of the plants from which samples were taken and *Phytophthora* was detected via baiting or via Illumina.

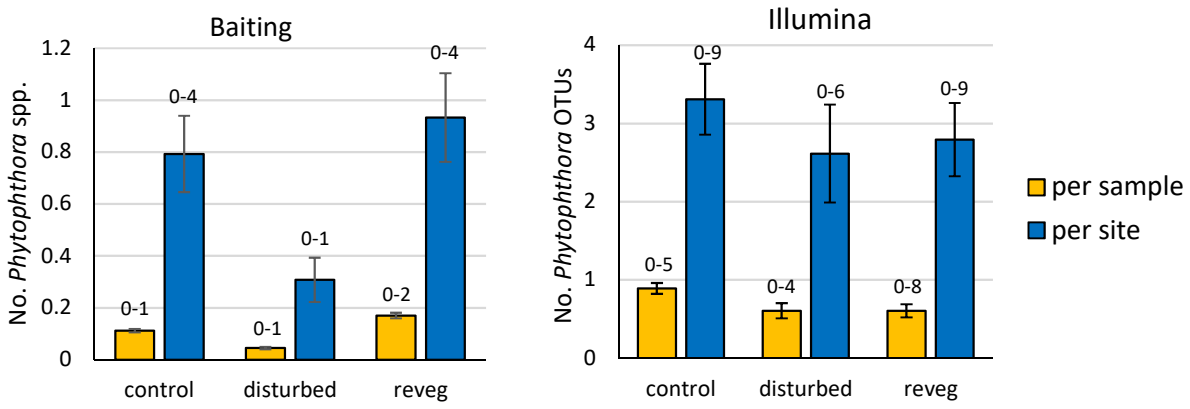


Fig. 13. Average (± s.e.) number of *Phytophthora* spp. recovered (baiting) or OTUs detected (Illumina, minimum of 0.095% within-sample relative abundance) per sample (yellow) or per site (blue), divided by site status: revegetation, control, and disturbed (combined planned and disturbed sites). Above each bar is the range (minimum – maximum) in the number of species or OTUs detected in each sample or site.

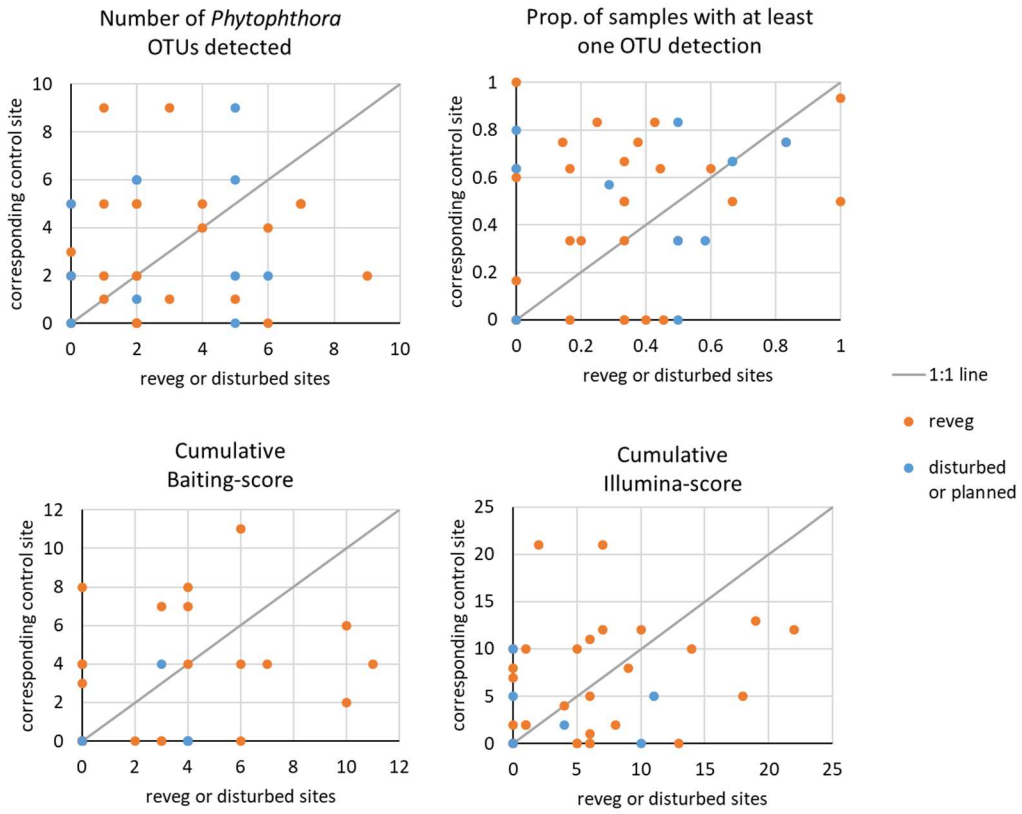


Fig. 14. Comparison in infestation statistics between reveg (orange) or disturbed sites (combined disturbed and planned sites; blue) and their corresponding control areas. All correlations were weakly positive, but non-significant. When the point falls below the 1:1 line, the reveg / disturbed site has greater OTU diversity, abundance, or infestation scores than the corresponding control site.

Table 13. Risk ratings and locations of the *Phytophthora* OTUs detected via Illumina, as well as the associated baited species.

Illumina			Baiting & Leaf Plating		
OTU[a]	risk rating[b]	preserves detected[c,d]	species	risk rating[b]	preserves detected[d]
cactorum-cluster	4	BCR, ECdM, LH, MB, PR, PC, RSA, RR, SA, SR	*P. cactorum*	4	PR, RSA, RR, SA, SR
			P. pseudotsugae	2	BCR, SA
cambivora-complex	4	MB, RR, SR, (PR, RSA, SA)	*P. cambivora*	4	MB, PR, RR, SA, SR
cinnamomi	4	PR, RSA, RR, (SA)	*P. cinnamomi*	4	PR, SA
citricola-complex	4	BCR, ECdM, MB, PR, PC, RSA, SA, (RR, SR)	*P. multivora*	4	PR, PC
cryptogea-complex	4	LH, PR, RR, SA, (BCR, PC, RSA, SR)	*P. cryptogea*-complex[e]	4	PR, SA
ramorum	4	MB, SA, (LH)	*P. ramorum*	4	BCR, ECdM, LH, MB, SA, SR
asparagi	3	SA	*P.* taxon *asparagi*	3	SA
megasperma-complex	3	LH, SA, (PR, RSA, SR)	*P. megasperma*	3	SR
			P. crassamura	3	BCR, RSA, SA
rosacearum	3	SA	*P. rosacearum*	3	SA
siskiyouensis	3	PC, (LH)	not detected via baiting		
syringae	3	BCR, ECdM, LH, MB, PR, PC, RSA, RR, SA, (SR)	*P. syringae*	3	LH, PC, RSA, SA
tentaculata	3	PC, SR	not detected via baiting		
boehmeriae	2	(PR)	*P. boehmeriae*	2	PR
citrophthora-cluster	2	MB, PR, SA, (RSA, RR)	not detected via baiting		
fallax	2	PR, SA	not detected via baiting		
hedraiandra	2	SR, (ECdM)	not detected via baiting		
hibernalis	2	(BCR)	not detected via baiting		
lateralis	2	MB, PR, RSA, RR, SA, (PC, SR)	not detected via baiting		
nicotianae	2	RR	*P. nicotianae*	2	RR
quercina-cluster	2	ECdM, MB, PR, RR, SR, (SA)	not detected via baiting		
uliginosa-cluster	2	BCR, PR, RSA, SA, SR, (MB)	*P.* sp. *cadmea*	2	BCR, PR
bilorbang-cluster	1	LH	*P.* taxon *oaksoil*	1	LH
brassicae	1	SA, (RSA)	not detected via baiting		
chlamydospora	1	MB, PR, RSA, SA, SR, (LH, PC, RR)	not detected via baiting		
clandestine	1	SA, SR, (RR)	not detected via baiting		
drechsleri	1	(RR)	not detected via baiting		
formosa	1	MB, PR, SR	not detected via baiting		

Table 13 cont.

Illumina			Baiting & Leaf Plating		
OTU[a]	rink rating[b]	preserves detected[c,d]	species	risk rating[b]	preserves detected[d]
hydropathica	1	RSA, SA, SR, (MB, PR)	not detected via baiting		
irrigata	1	MB, PR, RR, SA	not detected via baiting		
macilentosa	1	(SR)	not detected via baiting		
nemorosa-cluster	1	BCR, ECdM, LH, MB, PR, PC, RSA, RR, SA, (SR)	*P. nemorosa*[f]	1	LH
			P. aff. *ilicis*[f]	1	LH
			P. pseudosyringae	2	LH, PR
porri	1	SA	not detected via baiting		
primulae	1	SA, (RSA)	not detected via baiting		
psychrophila	1	BCR, ECdM, MB, PR, RSA, RR, SA, SR, (LH, PC)	not detected via baiting		
riparia-cluster	1	LH, (SR)	*P. lacustris*	1	LH
virginiana	1	MB, (BCR)	not detected via baiting		

[a] A complex is a group of phylogenetically related species that share an identical full-length ITS1 sequence; a cluster is a group of phylogenetically related species that have an ITS1 sequence identical only between ITS6 and ITS7 priming sites.

[b] Risk class, where 1=low risk, 2=moderate risk, 3= high risk, and 4= very high risk. Risk class is based on the species aggressiveness, host range, abundance, and prior history as an invasive species (Appendix A). For OTU complexes or clusters comprised of species with different risk rating, the OTU was assigned a rating best fitting the overall risk of the group based on known ecology of the member species and their abundance.

[c] Detection based on minimum 0.095% within-sample relative abundance. OTUs where the detected occurred at only 0.01 to <0.095% are indicated in parentheses.

[d] BCR = Bear Creek Redwoods, ECdM = El Corte de Madera Creek, LH = La Honda Creek, MB = Monte Bello, PR = Pulgas Ridge, PC = Purisima Creek Redwoods, RSA = Rancho San Antonio, RR = Russian Ridge, SA = Sierra Azul, SR = Skyline Ridge.

[e] May be *P. cryptogea* s.s., *P. pseudocryptogea*, *P. erythroseptica*, or *P.* sp. kelmania. Phylogenetic relationships between these species are still being determined.

[f] Recovered via leaf plating only.

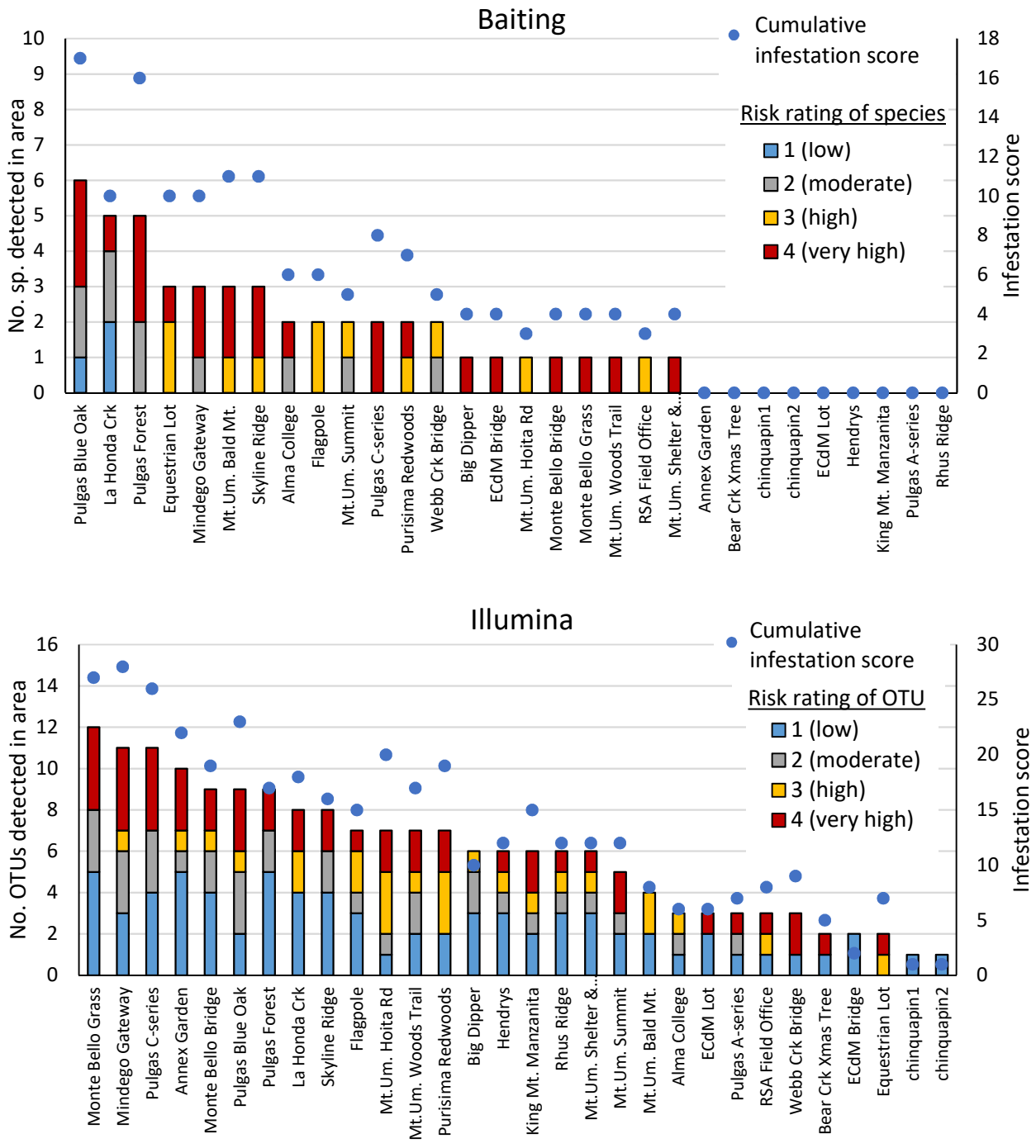
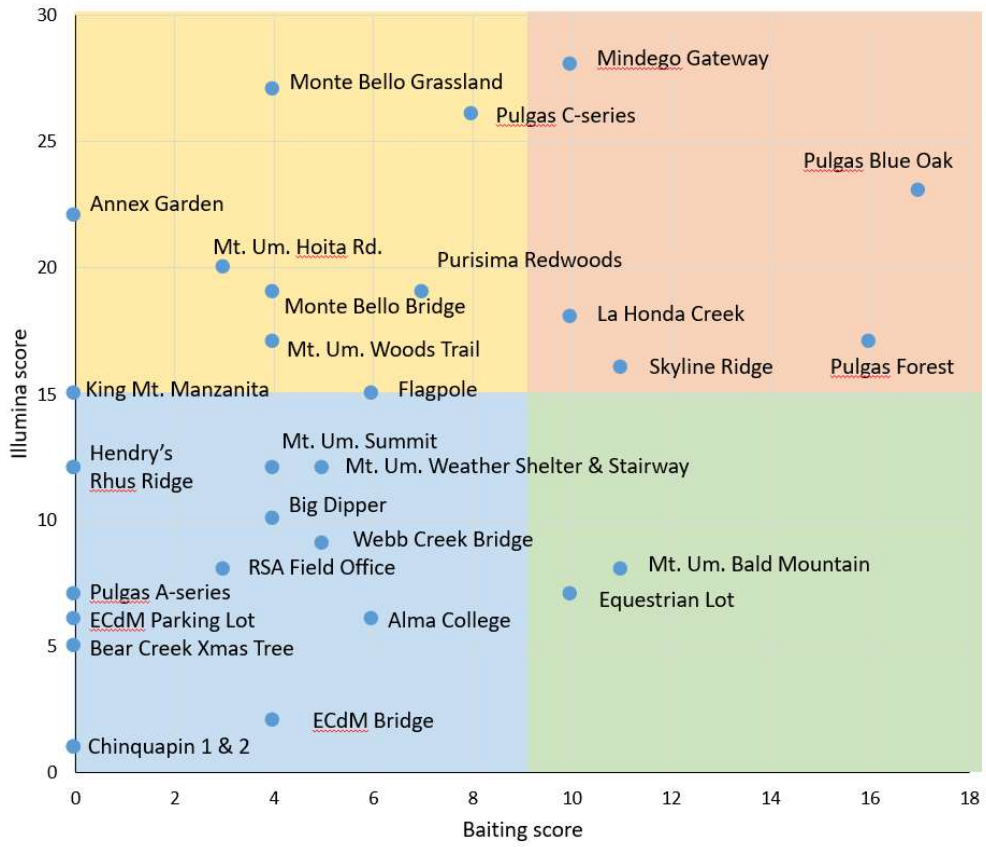


Fig. 15. Number of *Phytophthora* species or OTUs detected by baiting or Illumina, respectively, and cumulative infestation score for the area. Detections (presence/absence) are reported collectively for all RV, PLND, or DIST site(s) and their corresponding CON site located within a single project area (Table 1). Species or OTUs are color coded by their risk rating, based on their aggressiveness, host range, abundance, and prior history as an invasive species (Table 13, Appendix A). Infestation score was calculated by summing the risk rating (1-4) for each of the taxa detected.



Project Area	Baiting score	Illumina score	Final ranking
Pulgas Blue Oak	17	23	40
Mindego Gateway	10	28	38
Pulgas C-series	8	26	34
Pulgas Forest	16	17	33
Monte Bello Grass	4	27	31
La Honda Crk	10	18	28
Skyline Ridge	11	16	27
Purisima Redwoods	7	19	26
Mt.Um. Hoita Rd	3	20	23
Monte Bello Bridge	4	19	23
Annex Garden	0	22	22
Mt.Um. Woods Trail	4	17	21
Flagpole	6	15	21
Mt.Um. Bald Mt.	11	8	19
Equestrian Lot	10	7	17
Mt.Um. Summit	5	12	17
Mt.Um. Weather Shelter & Stairway	4	12	16
King Mt. Manzanita	0	15	15
Big Dipper	4	10	14
Webb Crk Bridge	5	9	14
Hendrys	0	12	12
Rhus Ridge	0	12	12
Alma College	6	6	12
RSA Field Office	3	8	11
Pulgas A-series	0	7	7
ECdM Lot	0	6	6
ECdM Bridge	4	2	6
Bear Crk Xmas Tree	0	5	5
Chinquapin1	0	1	1
Chinquapin2	0	1	1

Fig. 16. Comparison between the cumulative infestation scores as measured by baiting (x-axis) and Illumina (y-axis) for all project areas (combined RV, PLND, or DIST site(s) and their corresponding CON site). Scores were calculated by summing the risk ratings (1-4) for each *Phytophthora* species or OTU detected via baiting or Illumina within the project area. The site ranking is the infestation score combined with the baiting score.

Quadrants are color coded by general infestation level:

- Upper right (red): **Highest**, high scores via baiting and Illumina. These sites have evidence of heavy infestation from both detection methods, indicating numerous pathogenic *Phytophthora* species are present. Extreme caution should be used when moving from these sites.
- Upper left (yellow): **Moderate**, low score as detected by baiting, but high score as detected by Illumina. There may be a history of failed introductions and/or we had poor baiting success due to the limitations of baiting (e.g. a high proportion of un-baitable species or the species at the site were not baited the year the site was sampled).
- Lower right (green): **Moderate**, high score as detected by baiting, but low score as detected by Illumina. Pathogenic species were detected via baiting, but less so by Illumina. Community diversity is generally lower than highly infested sites.
- Lower left (blue): **Lowest**, lower scores via baiting and Illumina. Sites are not *Phytophthora*-free, however the species present may be less pathogenic and/or the community may be less diverse. Priority should be on reducing new introductions.

Select References & Resources

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Sims, L.L., and Garbelotto, M. 2021. *Phytophthora* species repeatedly introduced in Northern California through restoration projects can spread into adjacent sites. Biol. Invasions. DOI:10.1007/s10530-021-02496-6

Sims, L., Tjosvold, S., Chambers, D., Garbelotto, M. 2018. Control of *Phytophthora* species in plant stock for habitat restoration through best management practices. Plant Pathology Doi: 10.1111/ppa.12933.

Websites:

CDFA Plant Pathogen ratings:
http://blogs.cdfa.ca.gov/Section3162/

- ✓ California Department of Food and Agriculture website of risk ratings for numerous pests and pathogens threatening Californian agriculture and wildlands, including many *Phytophthora* spp.

California Oak Mortality Task Force & *Phytophthoras* in Native Habitats Work Group:
https://www.suddenoakdeath.org/
https://www.suddenoakdeath.org/welcome-to-calphytos-org-phytophthoras-in-native-habitats/

- ✓ Focuses on sudden oak death (*P. ramorum*) and other issues specific to restoration nurseries in California, including best management practices and a periodically updated newsletter.

Forest Phytophthoras of the World:
http://forestphytophthoras.org/

- ✓ Profiles of numerous *Phytophthora* sp. threatening wildlands worldwide. Also includes summary on *Phytophthora* basics.

USDA-ARS Fungal Databases, U.S. National Fungus Collections:
https://nt.ars-grin.gov/fungaldatabases/

- ✓ National database summarizing the location and plant host for which *Phytophthora* species have been recovered, as reported in academic literature.

Appendix A: *Phytophthora* species detected and a brief description of diseases they cause

Phytophthora spp. detected by soil baiting or leaf planting

Following is a summary table and review of the diseases and major host groups affected by each species encountered in the soil and vegetation surveys. The impact each species may have on native plant communities must be considered *in-situ*. Unfortunately, few species have been widely studied in native plant communities, particularly grassland or chaparral communities. Many agricultural species (e.g. *P. nicotianae* and *P. syringae*) have been detected at low frequencies in prior forest-soil surveys, although their persistence and impact on the native ecology is unclear. Others (e.g. *P. cambivora*) are responsible for the death of mature forest trees, but are not always associated with widespread mortality such as that caused by *P. ramorum* or *P. cinnamomi*. It is suspected some root-infecting species subsist on fine roots, ordinarily having only minor impacts on community health until environmental stress initiates substantial disease. Given the likelihood pathogenic species will encounter novel hosts, changing environments, and other biological stressors, future disease may develop. As a result, their threat status at MROSD is not immediately identifiable.

Nevertheless, the widespread distribution of some these species indicates past movement and potential establishment of disease-causing *Phytophthora* in MROSD preserves. The capacity of *Phytophthora*, as a genus, to establish within these wildlands should be taken to indicate pathogenic species could also survive. Caution to prevent new introductions and the spread of infested soils and plant material between sites is recommended.

Detailed reviews are provided for those species which were recovered via baiting. Included in the header for each are the clade and associated OTU corresponding to the *Phytophthora*, as well as the location and genera from which the species was recovered.

Lastly, we have summarized the results from other surveys of *Phytophthora* diversity in other restoration sites within the region and restoration nurseries within California. Some species, notably *P. quercetorum* and *P. niederhauserii*, where not detected by us but have been detected by others. These are not discussed below, but summaries of the risk associated with these species can be found on the CDFA Pest Raiting Proposal and Final ratings website (https://blogs.cdfa.ca.gov/Section3162).

Appendix A. Table 1. Summary table for *Phytophthora* spp. recovered by baiting from soil or direct leaf isolations.

Phytophthora species	Preserves detected	Host range	No. plant families affected[a]	No. plant genera affected[a]	Virulence on native hosts	Disease severity in other natural ecosystems & other relevant notes
Predicted risk to MROSD: Very high (rating = 4). Documented invasive species, known to cause widespread mortality or decline on some hosts, including California natives. Widespread throughout the region.						
P. cactorum	Pulgas Ridge, Rancho San Antonio, Russian Ridge, Sierra Azul, Skyline Ridge	very wide	91	255	aggressive on some hosts, contributor to decline	Severe, though may be a stronger contributor to decline than able to cause acute disease on most hosts.
P. cambivora	Monte Bello, Pulgas Ridge, Russian Ridge, Sierra Azul, Skyline Ridge	wide	28	59	aggressive on some hosts, contributor to decline	Severe, especially on *Castanea*, *Fagus, Chrysolepis;* multiple woody plants affected, especially in conjunction with other species.
P. cinnamomi	Pulgas Ridge, Sierra Azul	very wide	104	321	aggressive on many hosts, contributor to decline	Severe in many parts of the world. Entire plant communities may be affected. Future impacts for MROSD are unknown.
P. cryptogea-complex	Pulgas Ridge, Sierra Azul	very wide	83	236	aggressive on some hosts, contributor to decline	Commonly found in association with declining vegetation, many CA native plant genera are hosts
P. multivora	Pulgas Ridge, Purisima Creek Redwoods	wide	28	44	aggressive on some hosts, contributor to decline	Commonly found in association with declining vegetation, many CA native plant genera are hosts
P. ramorum	Bear Creek Redwoods, El Corte de Madera Creek, La Honda Creek, Monte Bello, Sierra Azul, Skyline Ridge	wide	26	56	aggressive on some hosts	Rapid and widespread mortality on *Quercus*, *Notholithocarpus,* and *Larix*; blight on multiple other native hosts

Predicted risk to MROSD: High (rating = 3). Species known to cause disease on California natives and/or vegetation within Mediterranean climates. Distribution may be limited and/or species may be newly described and impacts and host range are not certain.

P. taxon *asparagi*	Sierra Azul	narrow, but growing	4	4	moderate, but relatively new	Moderate, documented causing disease on Mediterranean vegetation.
P. crassamura	Bear Creek Redwoods, Rancho San Antonio, Sierra Azul	moderate, but growing	15	19	moderate, but relatively new	Moderate, documented causing disease on many California natives
P. megasperma	Skyline Ridge	wide	43	94	moderate	Moderate, documented causing disease on California natives. The species complex is currently being delineated into different species including *P. crassamura* and *P. rosacearum*.
P. rosacearum	Sierra Azul	narrow, but growing	5	6	moderate, but relatively new	Risk thought to be equivalent to *P. megasperma*, to which it is closely related.
P. syringae	La Honda Creek, Purisima Creek Redwoods, Rancho San Antonio, Sierra Azul	wide	32	60	potential contributor to decline	Associated with some declines, however role is not entirely clear. Found on some native CA genera in nurseries.

Predicted risk to MROSD: Moderate (rating = 2). Species thought to have limited impact on most hosts or very limited host range; disease may occur only in certain circumstances not present on MROSD lands. Range is either limited on MROSD preserves, or widespread but without causing apparent disease. May be low risk, however data may be limited.

P. sp. 'cadmea'	Bear Creek Redwoods, Pulgas Ridge	unknown	unk	unk	unknown, no disease observed in areas where recovered	Recently identified species found only in the region, but not observed causing disease where recovered. Some closely related species contribute to oak decline in Europe.
P. boehmeriae	Pulgas Ridge	moderate	12	16	moderate	First report in U.S., only forest disease reported is on *Acacia*.

P. nicotianae	Russian Ridge	very wide	110	376	moderate	Only forest disease reported is on *Acacia*, but may cause disease on Arbutus and other Ericaceae.
P. pseudotsugae	Bear Creek Redwoods, Sierra Azul	narrow, but growing	1	1	moderate	Only known to be pathogen of Douglas-fir seedlings, but the host list is growing. Closely related to *P. cactorum*.
P. pseudosyringae	La Honda Creek, Pulgas Ridge	moderate	12	20	moderate	Presumptive native, where it causes disease similar to *P. nemorosa,* however it has been observed causing greater root disease on hardwoods in Europe.

Predicted risk to MROSD: Low (rating = 1). Presumptive native species with only minor disease observed, or stream-associated species considered to be saprophytic in native environments.						
P. aff. *ilicis*	La Honda Creek	narrow	unk	unk	likely low	Minor, closely related species *P. ilicis* is limited to *Ilex* and *Quercus*. Observed causing symptoms similar to *P. nemorosa* on California bay laurel.
P. taxon *oaksoil / bilorbang*	La Honda Creek	narrow	4	4	low	Minor, stream associated
P. lacustris	La Honda Creek	moderate	6	8	low	Minor, stream associated
P. nemorosa	La Honda Creek	moderate	6	11	low	Minor, only reported in Western North America causing minor blight or sporadic tree mortality

[a] Retrieved from Farr, D.F., and Rossman, A.Y. 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved October 2, 2020; https://nt.ars-grin.gov/fungaldatabases/

Appendix A. Table 2. Summary table for all *Phytophthora* spp. recovered via baiting or direct plating from other studies in restoration sites within the region (Bourett 2018, Frankel et al. 2020, and Sims and Garbelotto 2021), and restoration nurseries within California (Rooney-Latham et al. 2019, Sims et al. 2018).

	Detected (county of detection is listed if indicated in manuscript)							
	surveys of other restoration sites & streams			surveys of CA native-plant nurseries		MROSD preserves		
Phytophthora sp. (excluding hybrids)	Bourett 2018[a]	Frankel et al. 2020[b]	Sims & Garbelotto 2021[a]	Rooney-Latham[c] et al. 2019	Sims et al. 2018[d]	This study	associated OTU detected via Illumina (unbaited taxa only)[e]	no. studies with culture detections
P. acerina	Santa Clara						citricola-complex	1
P. aff. *cactorum / c.f. cactorum*		x		x				2
P. aff. *ilicis*[f]						x		1
P. aff. *lacustris*		x						1
P. aff. *niederhauserii*					x			1
P. aff. *syringae*	Humboltd						syringae	1
P. amnicola		x				x[g]		2
P. boehmeriae[f]						x		1
P. borealis		x						1
P. cactorum	Humboldt, Del Norte, Santa Clara	x			x	x		4
P. cambivora		x		x		x		3
P. chalmydospora	Del Norte, Humboldt, Mendocino, Monterey, San Luis Obispo, Interior, Santa Clara	x				x[g]		3
P. cinnamomi		x		x		x		3
P. citricola - pini complex / aff. *citricola*	San Luis Obispo, Santa Clara	x		x	x		citricola-complex	4
P. crassamura	Santa Clara	x	San Mateo		x	x		5

P. cryptogea-complex (incld. taxon 'kelmani' & *P. pseudocryptogea)*	Mendocino, San Luis Obispo, Santa Clara	x	Marin, San Francisco	x	x	x		6
P. erythroseptica		x					cryptogea-complex	1
P. europaea		x					uliginosa-cluster	1
P. gonapodyides	Del Norte, Humboldt, Mendocino, Monterey, San Luis Obispo, Interior	x				x[g]		3
P. hedraiandra	Santa Clara			x	x		hedraiandra	3
P. hibernalis	Del Norte						hibernalis	1
P. humicola	Santa Clara							1
P. hydropathica	Santa Clara						hydropathica	1
P. inundata	Santa Clara		Marin					2
P. lacustris	Humboldt, Mendocino, Monterey, San Luis Obispo, Interior, Santa Clara	x				x		3
P. megasperma	Santa Clara	x	Marin, San Mateo			x		4
P. multivora	Monterey, San Luis Obispo, Santa Clara		San Francisco, San Mateo	x	x	x		5
P. nemorosa	Del Norte, Humboldt, Mendocino, Monterey					x		2
P. nicotianae	Santa Clara			x		x		3
P. niederhauserii				x				1
P. occultans	Santa Clara				x		citrophthora-cluster	2
P. palmivora	Santa Clara							1
P. parsiana - complex	Santa Clara							1
P. parvispora				x				1
P. plurivora		x		x			citricola-complex	2

P. pluvialis	Humboldt, Del Norte, Mendocino						nemorosa-cluster	1
P. pseudosyringae	Del Norte, Humboldt, Mendocino, Monterey, Santa Clara					x		2
P. pseudotsugae	Humboldt					x		2
P. psychrophila	Santa Clara						psychrophila	1
P. quercetorum	Santa Clara	x						2
P. quercina	Santa Clara						quercina-cluster	1
P. ramorum	Humboldt, Mendocino, Monterey, Santa Clara					x		2
P. riparia	Del Norte, Humbotld, Monterey, San Benito, San Luis Obpiso, Santa Clara, Interior Counties	x				x[g]		3
P. rosacearum	Santa Clara					x		2
P. siskiyouensis	Humboltd						siskiyouensis	1
P. sp. aureomontensis	San Luis Obispo							1
P. sp. *'cadmea'*	Santa Clara					x		2
P. sp. NJB-2015	Mendocino							1
P. sp. xWS	Santa Clara							1
P. syringae	Del Norte, Humboldt, Mendocino, Monterey, San Luis Obispo, Santa Clara					x		2
P. taxon 'agrifolia'		x						1
P. taxon *'*asparagi'	Santa Clara					x		2
P. taxon 'casuarina'	Santa Clara							1
P. taxon 'juncus'	Santa Clara							1
P. taxon 'mugwort'	Santa Clara							1
P. taxon 'oaksoil' / *P. bilorbang*	Monterey, Santa Clara	x				x		3

P. taxon 'raspberry'	Santa Clara		Marin		x			3
P. taxon 'walnut'	Santa Clara							1
P. taxon 'xguadalupesoil'	Santa Clara							1
P. tentaculata		x		x			tentaculata	2
P. thermophila	Del Norte, Monterey, Santa Clara	x			x	x[g]		4
no. taxa reported:	45	23	6	12	10	25		
no. taxa unique to study:	19	5	0	2	1	2		

[a] County of detection is indicated in manuscript and is listed here. Detections were in restoration sites (Santa Clara County only) or via stream sampling (all other counties).

[b] County of detection not indicated; counties surveyed: Alameda, San Mateo, Santa Clara.

[c] County of detection not indicated; counties surveyed: Butte, Contra Costa, Monterey, Orange, Placer, Sacramento, San Diego, San Francisco, San Mateo, Santa Clara, Santa Cruz. Only culture positive detections are included.

[d] County of detection not indicated; no counties are listed in manuscript.

[e] Non-isolated taxa only; Undescribed taxa (e.g. taxon 'mugwort') may not be represented in the database used for OTU identification.

[f] Unique to MROSD lands.

[g] Detected in stream surveys, but not from soil or vegetation samples.

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Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.

Frankel, et al. 2020. *Phytophthora* introductions in restoration areas: responding to protect California native flora from human-assisted pathogen spread. Forests. DOI:10.3390/f11121291

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Rooney-Latham, S., et al. 2019. *Phytophthora* species are common on nursery stock frown for restoration and revegetation purposes in California. Plant Disease 103:448-455

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Review of *Phytophthora* spp. detected by soil baiting or leaf plating (in alphabetical order)

Phytophthora aff. *ilicis* (clade 3; *nemorosa*-cluster OTU)

LH_F001; *Umbellularia* (foliage only)

We recovered a single isolate from lesioned California bay laurel leaves collected from the LH_F001 area, likely representing a novel species *P.* aff. *ilicis*. The ITS sequences for this isolate matched at 99% for closely related species *P. nemorosa*, *P. ilicis* and *P. pseudosyringae*, requiring sequencing of the COX region for positive identified. An assembled sequence at the COX locus matched only 96.8% of base pairs to that of *P. ilicis* in published databases, indicating this may be a novel species. Additional analyses and morphological comparisons to known species are underway to determine if this is the case.

Being undescribed, a risk assessment for this taxon cannot be completed with certainty. *P. ilicis* was first recovered on ornamental holly in the western U.S. in the 1950s (Erwin and Ribeiro 1996); this species has rarely been recovered in western forests, and never from a native host (Hansen et al. 2017). It is currently known to infect only *Ilex*. However, being closely related to *P. nemorosa* and causing similar disease symptoms on bay laurel, we presume *P.* aff. *ilicis* would behave similarly and there is no immediate cause for concern.

Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.
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Phytophthora taxon *asparagi* (clade 6; *asparagi* OTU)

SA_H001; *Frangula*

P. taxon *asparagi* was both recovered and detected via Illumina in the Hoita Road planting site of Sierra Azul. The species was cultured from the control area in a single sample (within sample relative abundance of 0.92% from the positive sample), and detected from two samples in the planned project area (at 0.24 and 2.32% within-sample relative abundance). The *asparagi*-OTU was not detected in any other samples at any other location.

This taxon has yet to be validated in publication, however the first record of this species was published as causing a disease of cultivated asparagus in Michigan (Grank et al. 2012). *Phytophthora asparagi* was the most common *Phytophthora* species recovered in association with declining Mediterranean vegetation in Sardinia, causing dieback and mortality of native *Asparagi albus*, *Juniperus phoenicae* and *Pistacia lentiscus* (Scanu et al. 2015). Additional reports of this species occur in the Agavaceae in Australia and Italy (Cacciola et al. 2006, Cunnington et al. 2005). It has only been recently recovered in California by Bourett (2018), from a restoration planting of *Baccharis salicifolia*.

Significant dieback was observed in *Arctostaphylos* at the planting site. While there are no reports of *P.* taxon *asparagi* causing infection of members of the Ericaceae (or Rhamnaceae, the isolate coming from soil collected at the base of *Frangula*), the relatively new recognition of this species causing disease in similar habitats and its recovery at the site are cause for concern. Likely the full host-range of this species is not yet described and infection of both *Frangula* and *Arctostaphylos* should be investigated.

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.
Cunnington JH, Alwis Sde, Pascoe IG, Symes P (2005) The 'asparagus' *Phytophthora* infecting members of the Agavaceae at the Royal Botanic Gardens, Melbourne. Australas Plant Path 34: 413–414.
Cacciola SO, Pane A, Raudino F, Chimento A, Scibetta S, Davino S, et al. (2006) Bud and heart rot of fox tail agave (*Agave attenuata*) caused by *Phytophthora asparagi*. J Plant Pathol 88 (3S): S34–S34.
Granke LL, Saude C, Windstam ST, Webster BJ, and Hausbeck MK. 2012. *Phytophthora asparagi* Saude & Hausbeck, sp. nov. Persoonia 28: 146–147.
Scanu, B., Linaldeddu, B.T., Deirdda, A., Jung, T. 2015. Diversity of *Phytophthora* Species from Declining Mediterranean Maquis Vegetation, including Two New Species, *Phytophthora crassamura* and *P. ornamentata* sp. nov. PLoS ONE 10(12): 15)e0143234. doi:10.1371/journal.pone.0143234

Phytophthora boehmeriae (clade 10; *boehmeriae* OTU)

PR_B006; *Mimulus*

P. boehmeriae was detected via Illumina at the forest site at Pulgas Ridge in 2017, where it was successfully isolated in 2018. Current ITS sequencing identifies this isolate as *P. boehmeriae*, though this would be the first record of this species occurring in the United States. As such, further verification is being conducted for confirmation. The *P. boehmeraie*-OTU was not detected from any other samples in any location. As such, we strongly suspect this species was introduced as part of prior plantings or human activities evident in the location.

This species was first recovered from *Boehmeria* (Urticaceae) in Taiwan. The most notable forest disease caused by *P. boehmeriae* is a gummosis (gumming in association with necrotic lesions along the truck) of black wattle (*Acacia mearnsii*), often in association with *P. nicotianae* and other *Phytophthora* spp. in Brazil and South Africa (Roux and Wingfield 1997, Santos et al. 2006; http://forestphytophthoras.org/species/boehmeriae/disease). Additional hosts include: Deodar cedar (*Cedrus deodora*), *Eucalyptus* spp., *Pinus* spp., *Citrus* spp., *Malus sylvestris* (European crab apple), and *Gossypium* (cotton)(Farr and Rossman 2020).

The risk this species poses is unknown. The plant from which this species was isolated (*Mimulus*) was listed as having dieback, as was an additional plant (*Heteromeles*) in which the *P. boehmeriae*-OTU was detected. We also observed significant dieback in *Arctostaphylos* in the immediate area. However, multiple other *Phytophthora* species were recovered at the PR_B006 location, so we cannot ascribe symptoms to *P. boehmeriae* alone.

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Phytophthora cactorum (clade 1; *cactorum*-cluster OTU)

PR_B006; *Heteromeles*
RR_A001; *Frangula*, *Arbutus*
RR_A006; *Frangula*
RS_C001; *Heteromeles*
SR_A001; *Frangula*
SR_A002; *Arbutus*, *Heteromeles*, *Sambucus*

Phytophthora cactorum has a very wide host range, infecting over 250 plant genera (Farr and Rossman 2020) and causing a wide range of disease symptoms including damping-off, root rot, collar and crown rot, fruit rot, stem canker, leaf blight, and wilts, depending upon the host species (Erwin and Ribeiro 1996; http://forestphytophthoras.org/species/cactorum/disease). This species has a wide distribution throughout California, and is a known pathogen of many California native plant genera including *Arbutus*, *Alnus*, *Arctostaphylos*, *Baccharis*, *Calocedrus*, *Ceanothus*, *Cedrus*, *Corylus*, *Mimulus*, *Frangula*, *Fremontia*, *Juglans*, *Lupinus*, *Pinus*, *Pseudotsuga*, *Quercus*, *Ribes*, *Rosa*, and *Salix*, among others (Farr and Rossman 2020). This species is often associated with poor establishment resulting from moving infested nursery material into the field, potentially in conjunction with other species, however it has been associated with bleeding cankers on mature forest trees (Hudler 2013).

P. cactorum was by far the most abundant and widespread *Phytophthora* spp. of concern detected on MROSD lands. It was recovered from 14 samples (taken from 10 plants, in 4 sample locations in 4 preserves). Notably, 13 of the 14 culture-positive samples were outplanted nursery plants at reveg sites. The exception to this was the detection of *P. cactorum* from a single *Heteromeles* sampled as part of the control to RV_RS_C001 (Equestrian lot planting in Rancho San Antonio). The *P. cactorum*-OTU was detected via Illumina at ≥0.095% within-sample relative abundance in 52 samples (taken from 46 plants, representing 21 sample locations in 10 preserves) from three site-types (control, planned, and revegetation sites).

P. cactorum was the species most likely to be baited from the same plant both years (Fig. 4). After the *P. psychrophila*-OTU, the *P. cactorum*-cluster OTU was the most reliably detected OTU between years (Table 12). Repeat detections also occurred at all site-types.

The abundance of *P. cactorum* in nursery-grown outplantings is consistent with results from surveys of restoration nursery in the Bay Area, whereby this species was amongst the most common detected (Rooney-Latham et al. 2019, Sims et al. 2018). *P. cactorum* has also been recovered on MROSD lands by Phytosphere in prior surveys at Bald Mountain (RV_SA_A008) and Skyline Ridge (RV_SR_A001, RV_SR_A002, RV_SR_A003), and in restoration outplantings in adjacent counties (Bourret 2018). Our findings are in agreement with Sims et al. (2018) that this species is common on *Heteromeles* and occurs on *Frangula*.

Sims et al. (2018) proposed *P. cactorum* is recently introduced into western U.S. wildlands and has not yet spread substantially away from planted areas. While our baiting results are in agreement, strong Illumina signatures of the *P. cactorum*-OTU suggest this species may be more widely dispersed. The *P. cactorum*-OTU, however, contains *P. pseudotsugae* and *P. idaei* in addition to *P. cactorum*. *P. idaei* was not recovered in our study and is reported only in the U.K.; *P. pseudotsugae* was, however, recovered and is distributed throughout the west coast. Unfortunately, we cannot distinguish if Illumina detections in control areas may be attributed to *P. cactorum* or *P. pseudotsugae*; however, *P. pseudotsugae* is thought only to infect Douglas-fir

and a number of detections from control areas occurred in association with genera better known as hosts to *P. cactorum* (*Heteromeles*, *Arbutus*, *Rosa*; though see discussion under *P. pseudotsugae*). Culturing results also indicate the majority of these detections may be attributable to *P. cactorum*.

Unfortunately, pathogenicity tests have not been performed for the majority of potential host associations. Prior studies have indicated virulence of a particular isolate depends upon the host from which the isolate was recovered (Bhat et al. 2006), hence it is difficult to make broad conclusions about the pathogenicity of this species across multiple genera. Its role in contributing to decline of native vegetation is generally regarded as unclear (e.g. Vettraino et al. 2001), although growing evidence supports its role in many hardwood declines (e.g. Jung 2009). Further pathogenicity tests are needed. Given the wide distribution and risk, *P. cactorum* should be considered an emerging problem with potential for contributing to decline of native vegetation at MROSD preserves.

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<u>*Phytophthora* sp. ‘cadmea’ (clade 7; *uliginosa*-cluster OTU)</u>

PR_B006; *Heteromeles*
PR_E001; *Heteromeles*
BCR_A003; *Quercus*

This species was first recovered by and provisionally described by T. Bourett as part of studies into *Phytophthora* diversity in restoration outplantings in Santa Clara County (Bourret 2018). No formal species description or pathogenicity tests have yet been performed. Closely related species *P. uliginosa* and *P. europaea* are known oak root pathogens in Europe, the later being one species implicated in oak decline (Jung et al. 2002). *P.* sp. ‘cadmea’, however, was widespread in forested areas in association with *Quercus* and *Notholithocarpus* and was not causing any apparent symptoms.

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Phytophthora cambivora (clade 7; *cambivora*-complex OTU)

MB_A001Grass; *Quercus*
PR_B006; *Arctostaphylos*, *Heteromeles*
PR_C003; *Arctostaphylos*, *Heteromeles*
PR_E001; *Heteromeles*
RR_A001; *Heteromeles*
SA_G001; *Frangula*, *Heteromeles*
SR_A001; *Arbutus*, *Quercus*

Phytophthora cambivora (currently *P.* x *cambivora* indicating its status as an interspecific hybrid; Jung et al. 2016) is known to cause ink disease (root rot and stem canker), especially on chestnut (*Castanea*), beech (*Fagus*) and chinquapin (*Chrysolepis*), though other deciduous tree genera (*Quercus*, *Fagus*, *Juglans*) are also affected (Saavedra, et al. 2007, Vannini and Vettraino 2011, Vettraino et al. 2001, 2005; http://forestphytophthoras.org/species/cambivora/disease). It is widely distribution in California, Oregon and eastern forests, where, with the exception of *Chrysolepis* and *Castanea*, it is generally not associated with widespread mortality in the absence of other species (Balci et al. 2007, Reeser et al. 2011). As an undisputed contributor to *Phytophthora* decline, however, this aggressive pathogen should be considered high risk.

P. cambivora was recovered from Monte Bello by Phytosphere in June 2015, September 2016, and June 2017, then again by us in December 2017. Its recovery from *Arctostaphylos* in both native plant nurseries (Rooney-Latham et al. 2019) and native, symptomatic populations (unpublished Phytosphere report, in association with *P. cinnamomi*) gives cause for concern on this host. The other genera from which we recovered this species is consistent with prior reports, and all should be considered at risk for disease development when infected by *P. cambivora*.

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Phytophthora cinnamomi (clade 7; *cinnamomi* OTU)

PR_B006; *Arctostaphylos*
SA_A008; *Quercus*
PR_E001; *Mimulus*

Phytophthora cinnamomi is considered one of the most destructive and widespread species globally. This species affects over 300 plant genera in forest, agricultural and horticultural systems (Farr and Rossman 2020; http://forestphytophthoras.org/species/cinnamomi). Infecting the roots, symptoms generally include dieback, root rot and canker (Robin et al. 2012). Pathogen impacts may be extreme. In the jarrah forests of western Australia, approximately 40% of the plant species present are susceptible, and spread of this pathogen has resulted in decline of entire plant communities (Shearer et al. 2004, Weste and Marks 1987). In the Mediterranean and SE U.S. this species is associated with ink disease of *Castanea*, often in conjunction with *P. cambivora* (Crandall et al. 1945, Sharpe 2017, Vettraino et al. 2005). In the SE U.S. *P. cinnamomi* causes littleleaf disease of short leaf pine (Campbell 1953, Campbell and Coyle 2016). It is furthermore considered to be a strong contributor to oak decline in *Quercus* populations in Mediterranean Europe (Brasier 1996, Jung et al. 2018, Vettraino et al. 2002), and more recently in the Eastern U.S. (Balci et al. 2007, McConnell and Balci 2014, Reed et al. 2019). *P. cinnamomi* also causes disease in numerous species of native Ericaceae and Proteaceae in the western cape province of South Africa (von Broembsen and Kruger 1985).

Despite this species known impact in areas with Mediterranean climates and being present in California since at least 1942 (Wager 1942; though it was likely introduced much earlier), with few exceptions native California flora populations have remained relatively unaffected. While this species may not always cause significant disease when present (Sena et al. 2018), it is considered an emerging problem in California in conjunction with changing climate regimes.

The first reports of an infestation of native vegetation by *P. cinnamomi* occurred in 2003 when it was recovered from declining *Arctostaphylos myrtifolia* and *A. viscida* in the Sierra foothills (Swiecki et al. 2003). Nearly all plants at the sites were affected (Swiecki et al. 2003). Infection of *Quercus* in native oak forests was not recorded until an investigation of root and collar rot of *Q. agrifolia* in San Diego County in 2008 (Garbelotto et al. 2008).

P. cinnamomi has been repeatedly introduced and is established throughout the San Francisco Bay Area. Indeed, scattered but verified reports of native plant mortality are occurring throughout the region (Swiecki and Bernhardt 2017, Swiecki et al. 2011). The future implications of these findings are unknown. It has been proposed that relative to other species *P. cinnamomi* is less tolerant to unfavorable environmental conditions, and that cool winters have limited the impact of *P. cinnamomi* if not its distribution (Balci et al. 2007, Roth and Kuhlman 1966, Vettraino et al. 2005). However, changing climates may create different host-pathogen dynamics whereby plant species which currently tolerate infection may show greater or lesser symptom expression in the future. Impacts are expected to become more apparent with greater degrees of summer drought stress predicted with changing climates (Brasier 1996, Corcobado et al. 2014, McConnell and Balci 2014).

Current modeling anticipates relatively minor changes in climatic suitability in the San Francisco Bay Area (Burgess et al. 2017). While the precise impact on any given species cannot be predicted, given this species current capacity to persist at low levels and the abundance of

susceptible hosts within MROSD preserves, extreme caution should be undertaken when *P. cinnamomi* is detected.

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Sena, K., Crocker, E., Vincelli, P., and Barton, C. 2018. *Phytophthora cinnamomi* as a driver of forest change: implications for conservation and management. Forest Ecology and Management 409:799-807.

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Phytophthora crassamura (clade 6; *megasperma*-cluster OTU)

Please see the discussion under *P. megasperma*.

Phytophthora cryptogea-complex (*P. cryptogea* s.s., *P. pseudocryptogea*, and *P.* sp. *kalmania;* clade 8; *cryptogea*-complex OTU)

PR_C003; *Mimulus*
SA_Teds; *Arbutus*

P. cryptogea is yet another cosmopolitan *Phytophthora* spp. with a wide host range (>200 genera, Farr and Rossman 2020), impacting forest, horticultural and agriculture systems. Recent genetic analysis has shown *P. cryptogea* sensu lato to be a complex of multiple distinct species including *P. cryptogea* sensu stricto, *P. pseudocryptogea*, and *P.* sp. *kelmania* (Safaiefarahani et al. 2015). Virulence of these species has shown to vary by species and host (Delshad et al. 2020). Diseases caused by this group include a range of root, stem and leaf symptoms. In forest trees and woody plants it predominately causes a root rot and stem canker.

Little is known about how this species complex, by itself, may affect native vegetation beyond its pathogenicity on Douglas-fir and radiata pine seedlings (Bumbieris 1976, Pratt et al. 1976) and its recovery on numerous native California plant genera in nursery settings (Rooney-Latham et al. 2019, Yakabe et al. 2009). Associated genera include *Arbutus*, *Arctostaphylos*, *Artemisia, Eriogonum, Eriophyllum, Heteromeles, Mimulus, Penstemon*, *Planatus*, *Quercus, Ribes*, and *Salvia*, though others are likely affected (Rooney-Latham et al. 2019, Koike et al. 1997, Yakabe et al. 2009). As a common component of the *Phytophthora* assemblage recovered from declining native vegetation (Aghighi et al. 2016, Pérez-Sierra et al. 2013, Vettraino et al. 2002, others), this species should be considered pathogenic and of concern.

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Phytophthora lacustris (clade 6; *riparia*-cluster OTU)

LH_F001; *Alnus*

P. lacustris is a newly described species previously referred to as *P.* taxon 'Salixsoil'. First identified in 1972, it has been frequently baited from riparian habitats and streams worldwide. Morphologically and ecologically similar to *P. gonapodyides*, a formal species recognition based on genetic differentiation and environmental tolerances was completed by Nechwatal et al. in 2013.

This species has been isolated from *Alnus, Prunus,* and *Salix*. Pathogenicity tests indicate it to cause fine root damage to seedlings under flooding conditions (Nechwatal et al. 2013). It was a weak pathogen relative to more aggressive species, likely causing disease opportunistically and persisting as a saprotroph as is the case for many members of clade 6 (Nechwatal et al. 2013).

Nechwatal, J., Bakonyi, J., Cacciola, S.O., Cooke, D.E.L., Jung, T., Nagy, Z.A., Vannini, A., Vettraino, A.M., and Brasier, C.M. 2013. Plant Pathology 62:355-369

Phytophthora megasperma & *P. crassamura* (clade 6; *megasperma*-cluster OTU)

P. crassamura
BCR_A004; *Oxalis*
Flagpole; *Eriophyllum*
RS_C001; *Frangula*, *Quercus*
SA_F014; *Eriophyllum*, *Monardella*
SA_I001; *Monardella*

P. megasperma
SR_A001; *Heteromeles*
SR_A002; *Arbutus*

P. megasperma constitutes a complicated group of pathogens, some of which are designated as new species *P. sojea*, *P. medicaginis*, and *P. trifolii* (all infecting Fabaceae; Hansen and Maxwell 1991), *P. rosacearum* and *P. sansomeana* (Hansen et al. 2008), and P. crassamura (Scanu et al. 2015). Of these, *P. crassamura* was detected at high frequency by Sims et al. (2018) in native plant nurseries and *P. rosacearum* was frequently detected by Bourret et al. (2018). Because the former species is so recently described, many of the *P. megasperma* records may, in fact, reference diseases caused by *P. crassamura*. As such, both are discussed here.

P. megasperma sensu lato has wide host range and broad distribution. Dissimilar to other aquatic members of clade 6, *P. megasperma* is often associated with disease (Erwin and Riberio 1996). Either *P. megasperma* (and/or *P. crassamura* have been recovered or baited by Sims et al. (2019) and Bourret (2018) from a broad range of native Californian hosts including those in the genera: *Alnus*, *Artemisia*, *Eriophyllum*, *Heteromeles*, *Mimulus*, *Pinus*, *Planatus*, *Fragula*, *Rosa*, *Salix*, and *Quercus*. Pathogenicity of *P. crassamura* has been confirmed on *Planatus* (Sims et al. 2019) and *Juniperus phoenicea* and *Pistacia lentiscus* (Scanu et al. 2015, which showed similar

disease severity as the ex-type of *P. megasperma*). Both *P. megasperma* and *P. crassamura* have been isolated causing stem cankers and dieback on native vegetation in California outplantings (Sims et al. 2019). Given the broad host range of *P. megasperma* and the emerging disease properties of *P. crassamura*, expanding pathogen:host combinations are likely to arise in the future.

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<u>*Phytophthora multivora* (clade 2; *citricola*-complex OTU)</u>

PC_A001; *Mimulus*
PR_E001; *Frangula*

Previously part of the *P. citricola*-complex, *P. multivora* was first described as a distinct species causing a decline in eucalyptus in Western Australia (Scott et al. 2009). Recovered during nursery surveys assessing for *P. ramorum* infection in 2013 (Scheck 2020), *P. multivora* has been frequently encountered in surveys of nurseries and restoration outplantings throughout CA (Bourret 2018, Rooney-Latham et al. 2019, Scheck 2020). It is currently considered widespread and an aggressive pathogen in Mediterranean environments, causing fine root decay and stem canker (Jung et al. 2018).

This species has been baited from CA native plant nurseries from *Baccharis*, *Arctostaphylus*, *Ceanothus*, and *Frangula* (Rooney-Latham et al. 2019); Bourret (2018) also recovered this species via baiting of soils collected from *Acer*, *Artemsia*, *Frangula*, and *Sambucus.* Its host range, over 50 sp. in 44 genera (Farr and Rossman 2020) may be underestimated due to prior disease attribution to *P. citricola* sensu lato.

The *citricola*-complex OTU was among the more abundant OTUs detected in our sampling and was detected in all site classes and in both samples from which *P. multivora* was isolated. Other species of this complex (*P. pini* and *P. plurivora*) were recovered by Rooney-Latham et al. (2019) and may be contributing to some of the detections, though they were not detected as part of our surveys. All members of the complex should be considered pathogenic and capable of establishing on MROSD preserves.

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.
Farr, D.F., and Rossman, A.Y. 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved August 28, 2020, from https://nt.ars-grin.gov/fungaldatabases/
Jung, T., Pérez-Sierra, A., Durán, A., Horta Jung, M., Balci, Y., Scanu, B. 2018. Canker and decline diseases caused by soil- and airborne *Phytophthora* species in forests and woodlands. Persoonia 40:182-220.
Rooney-Latham, S., Blomquist, C.L., Hosta, K.L., Gou, Y.Y., Woods, P.W. 2019. *Phytophthora* species are common on nursery stock grown for restoration and revegetation purposes in California. Plant Disease 103:448-455

Scott, P.M., Burgess, T.I., Barber, P.A., Shearer, B.L., Stukely, M.J.C., Hardy, G.E. St.J., and Jung, T. 2009. *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis,* and other plant species in Western Australia. Persoonia 22: 1–13.

Scheck, H.J. 2020. California pest rating proposal for *Phytophthora multivora* P.M. scott & T. Jung, 2009. Accessed from https://blogs.cdfa.ca.gov/Section3162/?p=7048 <August 29, 2020>

Phytophthora nemorosa (clade 3; *nemorosa*-cluster OTU)

LH_F001; *Umbellularia* (foliage only)

Phytophthora nemorosa has a limited distribution being known only within western coastal forests, coinciding with the distribution of *P. ramorum*. Like *P. ramorum*, this species causes a foliar blight on native hosts, notably on *Umbellularia*, and stems cankers on *Notholithocarpus* and some *Quercus* (Reeser et al. 2008, Wickland et al. 2008). Unlike *P. ramorum*, however, this species has a more limited host range and is not known to cause substantial damage to native populations, only sporadically causing mortality (Hansen et al. 2017). Its recovery coincided with surveys for *P. ramorum*, which causes similar symptoms on these hosts. Relative to other species, it is also rarely recovered from nurseries (Yakabe et al. 2009).

While this species is highly clonal (typically indicative of a bottleneck resulting from an introduction from elsewhere; Linzer et al. 2009), it is considered by many to be endemic to western U.S. forests (Bourret et al. 2020). Recovery of this species poses minimal cause for concern.

Bourret, T.B., Aram, K., Edelenbos, C., Fajardo, S.N., Lozano, E., Mehl, H.K., Rizzo, D.M. 2020. Intraspecific diversity of Californian Clade 3 *Phytophthora* isolates. In Frankel, S.J., and Alexander, J.M. tech. cords. 2019. Proceedings of the seventh sudden oak death science and management symposium: healthy plants in a world with *Phytophthora*. Gen. Tech. Rep. PSW-GTR-268. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific SW Research Station. 121 p.

Hansen, E.M., Reeser, P.W., Sutton, W. 2017. Ecology and pathology of *Phytophthora* ITS clade 3 species in forests in western Oregon, USA, Mycologia, 109:1, 100-114.

Linzer, R.E., Rizzo, D.M., Cacciola, S.A., Garbelotto, M. 2009. AFLPs detect low genetic diversity for *Phytophthora nemorosa* and *P. pseudosyringae* in the U.S. and Europe. Mycological Research 113:298-307.

Reeser, P.W. , Sutton, W. , and Hansen, E.M. 2008. Phytophthora Species Causing Tanoak Stem Cankers in Southwestern Oregon. Pl. Dis. 92: 1252.

Wickland, A.C., Jensen, C.E., and Rizzo, D.M. 2008. Geographic distribution, disease symptoms and pathogenicity of Phytophthora nemorosa and Phytophthora pseudosyringae in California, USA. Forest Pathol. 38: 288-298. (42755)

Yakabe, L.E., Blomquist, C.L., Thomas, S.L., and MacDonald, J.D. 2009. Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. Pl. Dis. 93: 883-890.

Phytophthora nicotianae (clade 1; *nicotianae* OTU)

RR_A001; *Arbutus*

Phytophthora nicotianae is found worldwide infection 255 plant genera in 90 familes (Erwin and Ribeiro 1996, http://forestphytophthoras.org/species/nicotianae). It affects a variety of horticultural and agricultural crops worldwide where it can cause substantial damage (Panabières et al. 2016). Despite the abundance and host range, this pathogen is not associated with substantial disease in California wildlands. The most notable forest disease caused by *P. nicotianae* is a gummosis (gumming in association with necrotic lesions along the truck) of black wattle (*Acacia mearnsii*), often in association with *P. boehmeriae* and other *Phytophthora* spp. in Brazil and South Africa (Roux and Wingfield 1997, Santos et al. 2006).

This species was recovered from an outplanted madrone at Mindego Gateway (Russian Ridge) in both years. This species was also detected exclusively from this sample via Illumina both years. The plant in question had only minor dieback of the lower branches in year 1, with no symptoms and ample healthy growth observed in year 2. No record exists for the infection of *P. nicotianae* from *Arbutus menziesii*, however *A. unedo* is a reported host in Spain (Moralejo et al. 2009) as are other genera in the Ericaceae (*Arctostaphylos*, *Pieris*, *Rhododendron*; Farr and Rossman 2020).

The limited distribution and prevalence of this species in native plant nurseries (Rooney-Latham et al. 2019) strongly supports its introduction via nursery stock, although we have limited evidence it has spread from the site and is causing substantial disease. It was similarly rarely detected by Bourret (2018) in surveys of Santa Clara County restoration sites. While not as apparently common in U.S. outplantings, greater spread has been observed in European surveys (Jung et al. 2016) and this pathogen has demonstrated a capacity to persist between years.

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.

Farr, D.F., and Rossman, A.Y. 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved August 28, 2020, from https://nt.ars-grin.gov/fungaldatabases/

Jung, T., Orlikowski, L., Henricot, B., Abad-Campos, P., Aday, A.G., Aguín Casal, O., et al. 2016. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases. Forest Pathology 46:134–163.

Moralejo, E., Perez-Sierra, A.M., Alvarez, L.A., Belbahri, L., Lefort, F., and Descals, E. 2009. Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. Pl. Pathol. 58:100-110.

Panabières, F., Ali, G.S., Allagui, M.B., Dalio, R.J.D., Gudmestad, N.C., Kuhn, M-L., Guha Roy, S., Schena, L., Zampounis, A. 2016. *Phytophthora nicotianae* diseases worldwide: a new knowledge of a long-recognized pathogen. Phytopathologia Mediterranea 55:20-40.

Rooney-Latham, S., Blomquist, C.L., Hosta, K.L., Gou, Y.Y., Woods, P.W. 2019. *Phytophthora* species are common on nursery stock grown for restoration and revegetation purposes in California. Plant Disease 103:448-455

Roux J, Wingfield MJ. 1997. Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. Forest Ecology and Management. 99(3):327-336.

Santos, A.F.D., Luz, E.D.M.N., and de Souza, J.T. 2006. First report of *Phytophthora boehmeriae* on black wattle in Brazil. Plant Pathology 55(6):813.

Phytophthora taxon *oaksoil* (clade 6; *bilorbang*-cluster OTU)

LH_F001; *Alnus*, *Rubus*

This taxa may be accurately identified as either *P.* taxon oaksoil or *P. bilorbang,* and both records are used in the literature. *P. bilorbang* was first described as a new species contributing to stream-side decline of invasive *Rubus* in Western Australia (Aghighi et al. 2012, Aghighi et al. 2016). Alternative references with the same ITS sequence match to that of *P.* taxon *oaksoil* which was a commonly recovered from surveys of western U.S. streamways (Reeser et al. 2011; Sims et al. 2015). The two taxa are differentiated only by its ability to produce sexual spores (*P.* taxon Oaksoil being self-sterile; *P. bilorbang* able to produce sexual spores in the absence of an opposite mating type). Morphological analysis indicates the isolate we recovered are self-sterile, hence we prefer the designation *P.* taxon *oaksoil.*

The streamside location of LH_F001 is consistent with the known ecology of *P. bilorbang* & *P.* taxon *oaksoil*. It may be a contributing factor to disease when other species or abiotic conditions conducive to disease are present (Ahighi et al. 2016; Scanu et al. 2015). Like many members of Clade 6, however, this species is not thought to be significantly damaging to native ecosystems in the western U.S.

Aghighi, S., Burgess, T.I., Scott, J.K., Calver, M., Hardy, G.E. St.J. 2016. Isolation and pathogenicity of *Phytophthora* species from declining *Rubus anglocandicans*. Plant Pathology 65:451-461.

Aghighi, S., St. J. Hardy, G.E., Scott, J.K., and Burgess, T.I. 2012. *Phytophthora bilorbang* sp. nov., a new species associated with the decline of *Rubus anglocandicans* (European blackberry) in Western Australia. Eur. J. Plant Pathol. 133:841-855.

Reeser, P.W., Sutton, W. Hansen, E.M., Remigi, P., and Adams, G.C. 2011. *Phytophthora* species in forest streams in Oregon and Alaska. Mycologia 103(1):22-35.

Scanu, B., Linaldeddu, B.T., Deirdda, A., Jung, T. 2015. Diversity of *Phytophthora* Species from Declining Mediterranean Maquis Vegetation, including Two New Species, *Phytophthora crassamura* and *P. ornamentata* sp. nov. PLoS ONE 10(12): e0143234. doi:10.1371/journal.pone.0143234

Sims, L.L., Sutton, W., Reeser, P., and Hansen, E.M. 2015. The *Phytophthora* species assemblage and diversity in riparian alder ecosystems of western Oregon, USA. Mycologia 107(5):889-902.

Phytophthora pseudosyringae (clade 3; *nemorosa*-cluster OTU)

PR_E001; *Arbutus*
LH_F001; *Rubus*

Phytophthora pseudosyringae is a clade 3 species closely related to suspected natives *P. nemorosa*, *P. psychrophila*, and *P. pluvialis*. This species has been frequently isolated during surveys for *P. ramorum* in western forests (Wickland et al. 2008). It has also been detected in eastern U.S. streams, and in Europe and Chile (Hansen et al. 2017, Jung et al. 2003, Fajardo et al. 2017). As with *P. nemorosa*, the clonal population structure of this species suggests it has been introduced into western North America (Linzer 2009); however more recent analyses indicate Europe and Chile populations likely originated from North America (Bourett et al. 2020).

While the origin of *P. pseudosyringae* remains under debate, this species is not known to cause widespread disease in Californian forests. Greater disease is reported in Europe where it has been collected in association with *Alnus*, *Aesculus*, *Castanea*, *Fagus*, *Nothofagus*, *Quercus*, and *Vaccinium* (Hansen et al. 2017, Jung et al. 2003). In west coast forests, it causes similar diseases on similar host ranges as *P. nemorosa*: leaf blight on *Umbellularia* and occasional stem

canker and mortality on *Notholithocarpus* and *Quercus* (Reeser et al. 2008, Wickland et al. 2008). Dissimilar to *P. nemorosa* is it more often found infecting roots of mature trees, especially beech and chestnut (Jung et al. 2003). Detection of this species poses no immediate concern, however its status as a potential root and canker pathogen may implicate it in contributing to decline of trees under stressful conditions.

Bourret, T.B., Aram, K., Edelenbos, C., Fajardo, S.N., Lozano, E., Mehl, H.K., Rizzo, D.M. 2020. Intraspecific diversity of Californian Clade 3 *Phytophthora* isolates. In Frankel, S.J., and Alexander, J.M. tech. cords. 2019. Proceedings of the seventh sudden oak death science and management symposium: healthy plants in a world with *Phytophthora*. Gen. Tech. Rep. PSW-GTR-268. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific SW Research Station. 121 p.

Fajardo, S.N., Valenzuela, S., Dos Santos, A.F., Gonzalez, M.P., Sanfuentes, E.A. 2017. *Phytophthora pseudosyringae* associated with the mortality of *Nothofacus obliqua* in a pure stand in central-southern Chile. Forest Pathology 47(6):e12361

Jung, T., Nechwatal, J., Cooke, D.E.L., Hartmann, G., Blaschke, M., Osswald, W.F., Duncan, J.M., Delatour, C. 2003. *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous trees species in Europe. Mycological Research 107:772–789.

Hansen, E.M., Reeser, P.W., Sutton, W. 2017. Ecology and pathology of *Phytophthora* ITS clade 3 species in forests in western Oregon, USA, Mycologia, 109:1, 100-114.

Linzer, R.E., Rizzo, D.M., Cacciola, S.A., Garbelotto, M. 2009. AFLPs detect low genetic diversity for *Phytophthora nemorosa* and *P. pseudosyringae* in the U.S. and Europe. Mycological Research 113:298-307.

Reeser, P.W. , Sutton, W. , and Hansen, E.M. 2008. Phytophthora Species Causing Tanoak Stem Cankers in Southwestern Oregon. Pl. Dis. 92: 1252.

Wickland, A.C., Jensen, C.E., and Rizzo, D.M. 2008. Geographic distribution, disease symptoms and pathogenicity of Phytophthora nemorosa and Phytophthora pseudosyringae in California, USA. Forest Pathol. 38: 288-298. (42755)

<u>*Phytophthora pseudotsugae* (clade 1; *cactorum*-cluster OTU)</u>

BCR_A004; *Rosa*
SA_F002; *Penstemon*

P. pseudotsugae is known exclusively as a pathogen of Douglas-fir (*Pseudotsugae menziesii*) in the western United States (Hamm and Hansen 1983, Farr and Rossman 2020). Disease was first reported in Douglas-fir tree nurseries, where it causes root rot of seedlings (Hamm and Hansen 1983). Its impacts on mature Douglas-fir are unknown. A presumptive isolate of this species was recovered from *Austrocedrus chilensis* during investigations into the causal agent(s) of Mal del Ciprés in Argentina (Rajchenberg et al. 1998), however the culture was lost before verification and the species was not recovered in subsequent surveys (Greslebin et al. 2005).

Douglas-fir was not present at either location this isolate was recovered. Both isolates were sequenced at both the COX and ITS region for confirmation of identify. While somewhat ambiguous with *P. cactorum*, assembled sequences best matched records for *P. pseudotsugae* as submitted by Bourret 2018. Species identification was confirmed for these isolates, as indicate by its inability to grow in agar media supplemented in malachite green, and low abundance and greater size of oospores.

Most likely, *P. pseudotsugae* has a larger host rage. BCR_A004, with a culture positive from a control site, was dominated by coast redwood *Sequoia sempervirens*. The culture positive samples from SA_F002 (planted 2018) was recovered from nursery-grown penstemon, though contamination could have originated in the nursery from other sources. Compared to *P. cactorum*, however, there is less basis to expect *P. pseudotsugae* will cause substantial disease on MROSD lands.

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.
Hamm, P.B., Hansen, E.M. 1983. *Phytophthora pseudotsugae*, a new species causing root rot of Douglas-fir. Can. J. Bot. 61:2626-2631.
Farr, D.F., and Rossman, A.Y. 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved August 28, 2020, from https://nt.ars-grin.gov/fungaldatabases/
Greslebin, A.G., Hansen, E.M., Winton, L.M., Rajchenberg, M. 2005. *Phytophthora* species from declining *Austrocedrus chilensis* forests in Patagonia, Argentina, Mycologia, 97:218-228
Rajchenberg, M., Barroetaveña, C., Cwielong, P.P., Rossini, M., Cabral, D., Sıvori, A. 1998. Preliminar survey of Fungi associated with the decline of ciprés in Patagonia. In: Delatour C, Guillaumin JJ, Lung-Escarmant B, Marc¸ais B, eds. Proceedings of the 9th International Conference on Root and Butt-Rots (Carcans, France), 1-7 Sept. 1997. Paris: INRA. P 235-244.

Phytophthora ramorum (clade 8; *ramorum* OTU)

BCR_A001; *Notholithocarpus* (foliage only)
BCR_A003; *Notholithocarpus* (foliage only), *Rosa*
BCR_A004; *Notholithocarpus* and *Umbellularia* (foliage only)
CM_C003; *Tellima*, *Carex*
LH_F001; *Alnus*, *Rubus*
MB_A001Bridge; *Rosa*
PR_E001; *Umbellularia* (foliage only)
RR_A001; *Notholithocarpus* (foliage only)
SA_A008; *Frangula*
SA_I001; *Umbellularia* (foliage only)
SA_L001; *Umbellularia* (foliage only)
SR_A001; *Umbellularia* (foliage only)
SR_B001; *Mimulus*, *Umbellularia* (foliage only)
Teds; *Umbellularia* (foliage only)

P. ramorum is generalist, invasive pathogen that emerged in the mid-1990's as causing sudden oak death (SOD) of *Notholithocarpus densiflorus* (tanoak) and *Quercus* spp. in the western U.S., and ramorum leaf and stem blight in Europe (Frankel 2008, http://forestphytophthoras.org/species/ramorum). This species has since been widely distributed by western U.S. nurseries, and has also emerged causing mortality of plantation Japanese larch in the U.K. (sudden larch death, or SLD)(Brasier and Webber 2010, Grünwald et al. 2012).

Different symptoms are observed on different hosts. Infecting over 50 genera (Farr and Rossman 2020), disease on most is limited to minor leaf blights and occasional stem dieback that does not kill the host (Rizzo and Garbelotto 2003). It is from these tissues that sporangia (the spore responsible for dispersal and spread) and chlamydospores (a survival spore) are produced. Susceptibility varies significantly by host, with *Umbellularia californica* being the host driving the epidemic in Californian forests (Davidson et al. 2008).

P. ramorum threatens tanoak as a codominant overstory species throughout its range in California and Oregon (Cobb et al. 2012). An estimated 30-45 million trees had been killed by 2014, and this pathogen continues to expand its range northward. *P. ramorum* is established on MROSD preserves. While it can be recovered from soils and streams, the majority of spread is

thought to be aerial via blowing wind and rain. As such, containment efforts are ineffective at eliminating pathogen spread from infested areas so long as infected hosts are present.

It remains vital to continue to prevent the re-introduction of *P. ramorum* into MROSD lands. Forest and nursery populations are clonal, and four distinct lineages are recognized (Grünwald et al. 2012, Van Poucke et al. 2012). Only one of these, the NA1 lineage, is established in Californian forests though other lineages are detected in west coast nurseries (Grünwald et al. 2012). Care should be taken to prevent new introductions from nurseries and residential outplantings, as the potential impacts of the other lineages on California native flora are as of yet not well assessed. Two relatively recent introductions – the emergence of the EU2 lineage on larch in the U.K., a decade after the initial emergence of SOD, and the introduction of the more aggressive EU1 lineage into Oregon forests – have had profoundly negative impacts on our ability to control *P. ramorum*. These incidents serve as examples of the importance of managing *P. ramorum* (and other *Phytophthora* spp.) in nurseries.

Brasier C., and Webber J. 2010. Sudden larch death. Nature 466:824-825.
Cobb, R.C., Filipe, J.A.N., Meentemeyer, R.K., Gilligan, C.A., and Rizzo, D.M. 2012. Ecosystem transformation by emerging infectious disease: loss of large tanoak fromk California forests. Journal of Ecology 100:712-722.
Davidson J.M., H.A. Patterson, and D.M. Rizzo. 2008. Sources of inoculum for *Phytophthora ramorum* in a redwood forest. Phytopathology 98:860-866.
Farr, D.F., and Rossman, A.Y. 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved August 28, 2020, from https://nt.ars-grin.gov/fungaldatabases/
Frankel, S.J. 2008. Sudden oak death and *Phytophthora ramorum* in the USA: a management challenge. Australasian Plant Pathology 37:19-25.
Grünwald, N.J., M. Garbelotto, E.M. Goss, K. Heungens, and S. Prospero. 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends in Microbiology 20:131-138
Rizzo, D.M., and M. Garbelotto. 2003. Sudden oak death: endangering California and Oregon forest ecosystems. Front. Ecol. Environ. 1:197–204.
Van Poucke K., S. Franceschini, J.F. Webber, A. Vercauteren, J.A. Turner, A.R. McCracken, K. Heungens, and C.M. Brasier. 2012. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum*: EU2. Fungal Biology 116(11):1178-1191.

Phytophthora rosacearum (clade 6; *rosacearum* OTU)

SA_F014; *Eriophyllum*

Phytophthora rosacearum was described as a species distinct from *P. megasperma* in 2009, causing infections of rosaceous fruit trees (Hansen et al. 2009). Limited work has been presented on this species, specifically, but reports from the *megasperma*-complex may be attributable to this taxa. *P. rosacearum* was commonly found by Bourett (2018) in many non-Rosaceae associations, including *Artemisia*, *Salix*, *Baccharis*, *Populus*, *Planatus*, and *Sambucus*. The risk associated with the species should be taken as similar to that of *P. megasperma* until more is known about its pathogenicity on native vegetation.

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.
Hansen, E.M., Wilcox, W.F., Reeser, P.W., Sutton, W. 2009. *Phytophthora rosacearum* and *P. sansomeana*, new species segregated from the *Phytophthora megasperma* "complex". Mycologia 101(1):129-135.

Phytophthora syringae (clade 8; *syringae* OTU)

LH_F001; *Rubus*
PC_A001; *Alnus*
RS_C001; *Mimulus*
RS_D001; *Mimulus*
SA_A008; *Frangula*

P. syringae is a widespread pathogen of ornamental and agricultural plants, particularly in the family Rosaceae (Erwin and Ribeiro 1996). It is known for causing foliar infections of ornamentals, and has been a common species detected in many commercial nurseries (Knaus et al. 2015, Parke et al. 2014, Yakabe et al. 2009). It may also cause shoot dieback, canker, and root disease in some woody hosts (Erwin and Ribeiro 1996). Relevant host genera from which *P. syringae* has been isolated include *Arbutus*, *Arctostaphylos*, *Ceanothus*, *Frangula*, and *Viburnum*.

This species was widespread in our surveys as detected via baiting and in Illumina. It was recovered from all site classes. The precise impacts of this species in native vegetation are unknown. In some areas, *P. syringae* has been recovered in declining forest stands in association with other *Phytophthora* spp. (Jung 2009, Balci and Halmschlager 2003, Greslebin et al. 2005, Scanu et al. 2015), but are not always thought to be involved with decline (e.g. Greslebin and Hansen 2010). With the exception of tests using *Arbutus unedo* in Spain (Moralejo et al. 2009), we found pathogenicity tests have not been performed with genera native to coastal California.

Balci, Y., and Halmschlager, E. 2003. Incidence of *Phytophthora* species in oak forests in Austria and their possible involvement with oak decline. Forest Pathology 33:157-174.d

Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.

Greslebin, A.G., Hansen, E, M. 2010. Pathogenicity of *Phytophthora austrocedrae* on *Austrocedrus chilensis* and its relation with mal del cipres in Patagonia. Plant Pathology 59:604-612.

Greslebin, A.G., Hansen, E, M., Winton, L.M., and Rajchenberg, M. 2005. *Phytophthora* species from declining *Austrocedrus chilensis* forests in Patagonia, Argentina. Mycologia 97(1):218-228.

Jung, T. 2009. Beech decline in central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. Forest Pathology 39:73-94.

Moralejo, E., Perez-Sierra, A.M., Alvarez, L.A., Belbahri, L., Lefort, F., and Descals, E. 2009. Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. Pl. Pathol. 58:100-110.

Knaus, B.J., Fieland, V.J., Graham, K.A., Grünwald, N.J. 2015. Diversity of foliar *Phytophthora* species on *Rhododendron* in Oregon nurseries. Plant Disease 99:1326-1332.

Parke, J.L., Knaus, B.J., Fieland, V.J. Lewis, C., and Grünwald, N.J. 2014. *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. Phytopathology 104:1052-1062.

Scanu, B., Linaldeddu, B.T., Deirdda, A., Jung, T. 2015. Diversity of *Phytophthora* Species from Declining Mediterranean Maquis Vegetation, including Two New Species, *Phytophthora crassamura* and *P. ornamentata* sp. nov. PLoS ONE 10(12): 15)e0143234. doi:10.1371/journal.pone.0143234

Yakabe, L.E., Blomquist, C.L., Thomas, S.L., and MacDonald, J.D. 2009. Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. Pl. Dis. 93: 883-890.

Phytophthora-OTUs detected by Illumina

Provided is summary table of the taxa detected via Illumina MiSeq Sequencing. These detections indicate many alternatives regarding the presence of a particular species: the taxa may be abundant but was un-baitable with our methods; it may have been introduced but failed to establish, in which case we are detecting remnant DNA; and/or the taxa may be a recent introduction or a rare component of the rhizosphere community, in which case it was missed by baiting. Because the reads in Illumina are so short, it is also possible that the species detected is not the particular *Phytophthora* species listed. Rather, it may be a closely related but undescribed species not present in our database.

For those taxa in which we only found OTU detections (i.e. no corresponding species as baited), we also included a brief description of the associated species. Because species determinations are problematic with OTUs, these detections are discussed by clade. Unless noted, all species distributions, diseases and hosts are credited to Farr and Rossman (2020) of the USDA-ARS U.S. National Fungus Collection database and Abad et al. (2019) of the online *Phytophthora* database IDphy.

Abad, Z.G., Burgess T., Bienapfl J.C., Redford A.J., Coffey M., and Knight L. 2019. IDphy: Molecular and morphological identification of Phytophthora based on the types. USDA APHIS PPQ S&T Beltsville Lab, USDA APHIS PPQ S&T ITP, Centre for Phytophthora Science and Management, and World Phytophthora Collection. https://idtools.org/id/phytophthora/index.php <accessed September 1, 2020>

Farr, D.F., and Rossman, A.Y. 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved September 1, 2020, from https://nt.ars-grin.gov/fungaldatabases/

Appendix. A. Table. 3. Summary table for *Phytophthora* OTUs detected via Illumina MiSeq Sequencing.

Phytophthora OTU	Associated species[a]	isolated	Preserves detected[b]	Disease severity in other natural ecosystems & other relevant notes
Predicted risk to MROSD: Very high (rating = 4). The OTU or member(s) of the OTU are documented invasive species, known to cause widespread mortality or decline on some hosts, including California natives. Widespread throughout the region.				
cactorum-cluster	*P. cactorum*	x	BCR, ECdM, LH, MB, PR, PC, RSA, RR, SA, SR	Severe, though may be a stronger contributor to decline than able to cause acute disease on most hosts. Most detections are likely *P. cactorum*; *P. pseudotsugae* is, relatively, of less concern however host range is growing.
	P. pseudotsugae	x		
cambivora-complex	*P. cambivora*	x	MB, RR, SR, (PR, RSA, SA)	*P. cambivora* causes severe root disease, especially on *Castanea*, *Fagus*, *Chrysolepis*; multiple woody plants affected, especially in conjunction with other species. *P. alni* sensu lato causes root disease on alder; the most aggressive subspecies (*P. alni* subsp. *alni*) has not been detected in North America.
	P. alni sensu lato			
cinnamomi	*P. cinnamomi*	x	PR, RSA, RR, (SA)	Severe in many parts of the world. Entire plant communities may be affected. Future impacts for MROSD are unknown.
citricola-complex	*P. citricola* sensu stricto		BCR, ECdM, MB, PR, PC, RSA, SA, (RR, SR)	*P. multivora c*ommonly found in association with declining vegetation, many CA native plant genera are hosts. Closely related species of the *citricola*-complex are found within regional nurseries (*P. pini* and *P. plurivora*) or cause decline in Mediterranean tree hosts (*P. acerina*).
	P. multivora	x		
	P. pini			
	P. acerina			
	P. plurivora			
cryptogea-complex	*P. cryptogea* s.s.	x	LH, PR, RR, SA, (BCR, PC, RSA, SR)	Members of the complex are known hosts to many CA native genera, and are commonly recovered from declining vegetation.
	P. pseudocryptogea			
	P. sp. *kelmania*			
ramorum	*P. ramorum*	x	MB, SA, (LH)	Rapid and widespread mortality on *Quercus*, *Notholithocarpus,* and *Larix*; blight on multiple other native hosts.

Predicted risk to MROSD: High (rating = 3). The OTU or member(s) of the OTU are known to cause disease on California natives and/or vegetation within Mediterranean climates. Distribution may be limited and/or species may be newly described and impacts and host range are not certain.				
asparagi	*P.* taxon *asparagi*	x	SA	Moderate, documented causing disease on Mediterranean vegetation.
megasperma-complex	*P. megasperma*	x	LH, SA, (PR, RSA, SR)	Moderate, both species documented causing disease on California natives. The *megasperma*-complex is currently being delineated into different species including *P. crassamura* and *P. rosacearum*.
	P. crassamura	x		
rosacearum	*P. rosacearum*	x	SA	Risk thought to be equivalent to *P. megasperma*, to which it is closely related.
siskiyouensis	*P. siskiyouensis*		PC, (LH)	Associated with alder root disease.
syringae	*P. syringae*	x	BCR, ECdM, LH, MB, PR, PC, RSA, RR, SA, (SR)	Associated with some declines, however role is not entirely clear. Found on some native CA genera in nurseries.
tentaculata	*P. tentaculata*		PC, SR	Emerging pathogen, with many known CA native hosts and documented ability to cause outplanting failure within the region.

Predicted risk to MROSD: Moderate (rating = 2). The OTU or member(s) of the OTU are thought to have limited impact on most hosts or very limited host range; disease may occur only in certain circumstances not present on MROSD lands. Either restricted range on MROSD preserves, or widespread but without causing apparent disease. May be low risk, however data may be limited and it may be closely related to more pathogenic species. If more pathogenic species are indicated within the cluster, there is no basis to expect it is present.				
boehmeriae	*P. boehmeriae*	x	(PR)	First report in U.S., only forest disease reported is on *Acacia*.
citrophthora-cluster	*P. citrophthora* sensu lato		MB, PR, SA, (RSA, RR)	*P. citrophthora* sensu lato found in ornamental nurseries & infects some CA native genera; *P. occultans* has caused outplanting failures in some relevant genera in Oregon.
	P. occultans			
fallax	*P. fallax*		PR, SA	Causes crown dieback of Eucalyptus; not yet reported in North America.
hedraiandra	*P. hedraiandra*		SR, (ECdM)	Within nurseries on host genera used in restorations.

hibernalis	*P. hibernalis*		(BCR)	Within nurseries on host genera used in restorations.
lateralis	*P. lateralis*		MB, PR, RSA, RR, SA, (PC, SR)	Causes severe root disease and rapid mortality of *Chamaecyparis lawsoniana*; infects other genera of the Cupressaceae.
nicotianae	*P. nicotianae*	x	RR	Only forest disease reported is on *Acacia*, but may cause disease on *Arbutus* and other *Ericaceae*.
quercina-cluster	*P. quercina*		ECdM, MB, PR, RR, SR, (SA)	Associated with oak decline and detected in outplanted *Q. lobata* in neighboring county. Longer sequence reads indicate the detected OTU represents a closely related species.
	P. sp. *'ohioensis'*			
uliginosa-cluster	*P. uliginosa*		BCR, PR, RSA, SA, SR, (MB)	*P.* sp. *cadmea* is a recently identified species found only in the region, but not observed causing disease where recovered. The other two, especially *P. europaea*, contribute to oak decline in Europe.
	P. europaea			
	P. sp. *cadmea*	x		

Predicted risk to MROSD: Low (rating = 1). OTUs corresponding to presumptive native species with only minor disease observed, or stream-associated species considered to be saprophytic in native environments. OTU may only be reported on agricultural crops, and/or associated within ornamental nurseries.

bilorbang-cluster	*P. bilorbang*		LH	Minor, stream associated.
	P. taxon *oaksoil*	x		
brassicae	*P. brassicae*		SA, (RSA)	Associated with disease on Brassicaceae.
chlamydospora	*P. chlamydospora*	x	MB, PR, RSA, SA, SR, (LH, PC, RR)	Minor, stream associated.
clandestine	*P. clandestine*		SA, SR, (RR)	Not detected in North America, known diseases only on clover and alfalfa.
drechsleri	*P. drechsleri*		(RR)	Minor, better associated with food crops. Has been confused with *P. cryptogea*.
formosa	*P. formosa*		MB, PR, SR	Not detected in North America, not thought to be aggressive.
hydropathica	*P. hydropathica*		RSA, SA, SR, (MB, PR)	Causes damping off and root disease, especially in Ericaceae but not known to be in North America.
irrigata	*P. irrigata*		MB, PR, RR, SA	Minor, stream associated.
macilentosa	*P. macilentosa*		(SR)	Minor, stream associated.

nemorosa-cluster	*P. nemorosa*	x	BCR, ECdM, LH, MB, PR, PC, RSA, RR, SA, (SR)	Minor, only reported in western North America causing minor blight or sporadic tree mortality. Presumptive native taxa to North America.
	P. ilicis			
	P. aff. *ilicis*	x		
	P. pseudosyringae	x		
	P. pluvialis			
porri	*P. porri*		SA	Associated with disease on *Allium* spp.
primulae	*P. primulae*		SA, (RSA)	Not yet detected in North America, reported only on *Primula.*
psychrophila	*P. psychrophila*		BCR, ECdM, MB, PR, RSA, RR, SA, SR, (LH, PC)	May be contributor to decline of oak and beech in Europe, but presumptive native to North America where it does not cause concernable disease.
riparia-cluster	*P. lacustris*	x	LH, (SR)	Minor, stream associated.
	P. riparia			
virginiana	*P. virginiana*		MB, (BCR)	Minor, stream associated.

[a] Only those species which are of concern and/or are potentially in the area are included.

[b] OTUs where the detected occurred at only 0.01 to <0.095% are indicated in parentheses. BCR = Bear Creek Redwoods, ECdM = El Corte de Madera Creek, LH = La Honda Creek, MB = Monte Bello, PR = Pulgas Ridge, PC = Purisima Creek Redwoods, RSA = Rancho San Antonio, RR = Russian Ridge, SA = Sierra Azul, SR = Skyline Ridge.

Review of *Phytophthora*-OTUs detected only by Illumina (organized by clade)

Clade 1

OTUs detected:

P. clandestine
P. hedraiandra
P. tentaculata

Two of the taxa detected in Clade 1, *P. tentaculata* and *P. hedraiandra*, are known to occur in California. *P. clandestine* is found only in Australia where it is a major root pathogen of *Trifolium* (clover) and *Medicago* (alfalfa). The species name '*clandestine*' is derived from the difficulty encountered in detecting and culturing this species. The *P. clandestine*-OTU was detected in two samples, only from reveg sites.

P. hedraiandra is found predominantly in Europe, however it has been detected in Minnesota nurseries from *Viburnum* and *Rhododendron* (Schwingle et al. 2006, 2007) and California restoration nurseries from *Arctostaphylos* (in response to nursery surveys for *P. tentaculata*, see below) and *Ceanothus* (Chitambar 2015, Rooney-Latham et al. 2019, Sims et al. 2018). It is predominantly reported as a foliar and shoot pathogen, however *P. hedraiandra* may infect roots of *Fagus* (Hejna et al. 2014). The *P. hedraiandra*-OTU was detected only from 1 sample from a revegetation area.

P. tentaculata was first detected in the U.S. at a 2012 restoration outplanting of *Mimulus aurantiacus* in Monterey County (http://forestphytophthoras.org/species/tentaculata). The outbreak was traced back to the nursery supplying the planting stock, however the ultimate source of this pathogen into the U.S. was never identified (Rooney-Latham and Blomquist 2014). This species causes a crown, root, and stem rot of nursery plants in Europe and China, and is listed by the USDA as a pathogen of concern. Genera associated with *P. tentaculata* include *Apium, Aucklandia, Chicorium, Chrysanthemum, Delphinium, Gerbera, Lavandula, Santolina, Origanum*, and *Verbena*. Rooney-Latham et al. (2019) have found this species in association with *Artemisia*, *Ceanothus*, *Mimulus*, *Monardella* and *Salvia*. *Frangula* and *Heteromeles* are also susceptible (Chitambar 2016).

P. tentaculata has now been recovered in nurseries in Placer, Butte, Santa Cruz, San Mateo, Orange, and Santa Clara Counties, and from restoration outplantings in Alameda, Monterey, and Santa Clara Counties (Chitambar 2016). Currently all detections of *P. tentaculata* have only occurred in nurseries, or in outplanted, nursery-grown plants (Chitambar 2016). Similar to other surveys of restoration sites in the SF Bay Area by Bourret 2018 and Sims et al. 2018, we never recovered *P. tentaculata* in culture; however, the *tentaculata*-OTU was detected at Purisima Creek Redwoods (an unplanted site) and the Big Dipper planting site of Skyline Ridge. Given the repeatability of the Purisima Creek detection, we consider this detection to be accurate. It is possible the presence of the *tentaculata*-OTU at this site is the result of a failed introduction.

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.
Chitambar, J. 2015. California pest rating for *Phytophthora hedraiandra* de Cock & Man in't Veld. Accessed from https://blogs.cdfa.ca.gov/Section3162/?p=829 <September 1, 2020>
Chitambar, J. 2016. California pest rating for *Phytophthora tentaculata* Krober & Marwitz 1993. Accessed from https://blogs.cdfa.ca.gov/Section3162/?p=1590 <September 1, 2020>

Rooney-Latham, S., and Blomquist, C.L. 2014. First report of root and stem rot caused by *Phytophthora tentaculata* on *Mimulus aurantiacus* in North America. Pl. Dis. 98(7): 996.
Rooney-Latham, S., Blomquist, C.L., Hosta, K.L., Gou, Y.Y., Woods, P.W. 2019. *Phytophthora* species are common on nursery stock grown for restoration and revegetation purposes in California. Plant Disease 103:448-455
Schwingle, B. W., Smith, J. A., and Blanchette, R. A. 2007. *Phytophthora* species associated with diseased woody ornamentals in Minnesota nurseries. Plant Dis. 91:97-102.
Schwingle, B. W., Smith, J. A., Blanchette, R. A., Gould, S., Blanchette, B. L., Pokorny, J., and Cohen, S. D. 2006. First report of dieback and leaf lesions on *Rhododendron* sp. caused by *Phytophthora hedraiandra* in the United States. Plant Dis. 90:109.
Sims, L., Tjosvold, S., Chambers, D., Garbelotto, M. 2018. Control of *Phytophthora* species in plant stock for habitat restoration through best management practices. Plant Pathology Doi: 10.1111/ppa.12933.

Clade 2

OTUs detected:

- *P. citrophthora*-cluster (*P. citrophthora*, *P. terminalis*, *P. occultans*, *P. botryosa*, and *P. himasilva*)
- *P. siskiyouensis*

The *citrophthora*-cluster is a group of poorly defined taxa, members of which are currently being differentiated into separate species. *P. citrophthora* sensu lato is a widespread generalist pathogen, infecting many plant parts of over 120 genera. It is best known as a pathogen of citrus. While not found in prior surveys of native plants nurseries and outplantings (Sims et al. 2015, Bourret 2018, Rooney-Latham et al. 2019, here), *P. citrophthora* is commonly found in ornamental nurseries (Parke et al. 2014, Yakabe et al. 2009). Associated genera of concern includes *Arbutus*, *Arctostaphylos*, and *Eucalyptus*.

Of the other described species in the cluster, *P. botryosa* and *P. himasilva* are known only to occur only in Asia. *P. terminalis* and *P. occultans* have caused significant damage to European nurseries, where they were first recovered (Man in 't Veld et al. 2015). *P. terminalis* is not yet reported in North America. *P. occultans* has been found on *Ceanothus*, *Buxus*, *Gaultheria*, *Mohonia*, and *Rhododendron* in an Oregon nursery; these recoveries occurred during an investigation into the cause of a failed restoration planting of *Ceanothus* spp. (Reeser et al. 2015). More recently *P. occultans* has been recovered from *Acer* in California native plant nurseries (Sims et al. 2018). *P. occultans* should be considered a risk due to its occurrence in nurseries and history affecting restoration success.

P. siskiyouensis was first recovered during surveys delineating the spread of *P. ramorum* in SW Oregon, where it was recovered from *Umbellularia* and *Notholithocarpus* (Reeser et al. 2008, http://forestphytophthoras.org/species/siskiyouensis). It has since been found in California, Oregon, and the U.K. infecting *Alnus* (Perez-Sierra et al. 2015, Rooney-Latham et al. 2009, Sims et al. 2015). While largely reported from tree nurseries, *P. siskiyouensis* has been recovered from mature Italian alder (*Alnus cordata*) in San Mateo County (Rooney-Latham et al. 2009). The risk status for this species is generally unknown, however it appears to only cause substantial disease in native and horticultural alder (Sims et al. 2015).

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.
Man in 't Veld, W.A., Rosendahl, K.C.H.M., van Rijswick, P.C.J., Meffert, J.P., Westenberg, M., van de Vossenberg, B.T.L.H., Denton, G. & van Kuik, F. A.J. 2015. *Phytophthora terminalis* sp. nov. and *Phytophthora occultans* sp. nov., two invasive pathogens of ornamental plants in Europe. Mycologia 107:54-65

Parke, J.L., Knaus, B.J., Fieland, V.J. Lewis, C., and Grünwald, N.J. 2014. *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. Phytopathology 104:1052-1062.
Perez-Sierra, A., Kalantarzadeh, M., Sancisi-Frey, S., and Brasier, C.M. 2015. *Phytophthora siskiyouensis* causing stem lesions and cankers on *Alnus incana*. New Disease Reports 31: 17. (47976)
Reeser, P.W., Hansen, E.M., and Sutton, W. 2008. *Phytophthora siskiyouensis*, a new species from soil, water, myrtlewood (*Umbellularia californica*), and tanoak (*Lithocarpus densiflorus*) in southwestern Oregon. Mycologia 99: 639–643.
Reeser, P.W., Sutton, W. , Hansen, E.M., Goheen, E.M., Fieland, V.J., and Grunwald, N.J. 2015. First report of *Phytophthora occultans* causing root and collar rot on *Ceanothus*, boxwood, rhododendron, and other hots in horticultural nurseries in Oregon, USA. Pl. Dis. 99: 1282. (48115)
Rooney-Latham, S., Blomquist, C.L., Hosta, K.L., Gou, Y.Y., Woods, P.W. 2019. *Phytophthora* species are common on nursery stock grown for restoration and revegetation purposes in California. Plant Disease 103:448-455
Rooney-Latham, S., Blomquist, C.L., Pastalka, T., and Costello, L. 2009. Collar rot on italian alder trees in California caused by *Phytophthora siskiyouensis*. Pl. Health Progr. Online: doi:10.1094/PHP-2009-0413-01-RS. (43175)
Sims, L., Tjosvold, S., Chambers, D., Garbelotto, M. 2018. Control of *Phytophthora* species in plant stock for habitat restoration through best management practices. Plant Pathology Doi: 10.1111/ppa.12933.
Sims, L.L., Sutton, W., Reeser, P., and Hansen, E.M. 2015. The *Phytophthora* species assemblage and diversity in riparian alder ecosystems of western Oregon, USA. Mycologia 107(5): 889-902. (48201)
Yakabe, L.E., Blomquist, C.L., Thomas, S.L., and MacDonald, J.D. 2009. Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. Pl. Dis. 93: 883-890.

Clade 3

OTUs detected:

P. psychrophila

P. psychrophila is a clade 3 species closely related to suspected natives *P. nemorosa*, *P. pseudosyringae*, and *P. pluvialis*. This species was first recovered from rhizosphere samples in declining oak and beech in Europe; the importance of this species in decline remains in question (June et al. 2002, Pérez-Sierra et al. 2013). In Oregon and California, it has been recovered primarily in traps capturing rainwater as part of surveys for *P. ramorum*, with occasional recovery from fine roots of tanoak (Bourret 2018, Hansen et al. 2017).

Relative to *P. nemorosa* and *P. pseudosyringae*, the recovery of this species is rare from western forests (Bourret 2018, Hansen et al. 2017). Similarly, we never baited *P. psychrophila* from the soils, however it is by the far the most abundant and widely dispersed OTU detected in both years (Fig. 9). This OTU was detected at ≥0.095% within-sample relative abundance from 27 sites on 8 preserves, from 72 samples (Table 10). In a large number samples, moreover, it was present comprising >1% of the total number of reads within the sample (Table 9), was the most reliably detected between years (Table 12); the majority of repeat-detections occurred from samples collected at control sites. Such metrics indicate *P. psychrophila* has likely been present and biologically active with MROSD preserves for some time, consistent with a hypothesis this species is native to coastal Californian forests.

Bourret, T.B., Aram, K., Edelenbos, C., Fajardo, S.N., Lozano, E., Mehl, H.K., Rizzo, D.M. 2020. Intraspecific diversity of Californian Clade 3 *Phytophthora* isolates. In Frankel, S.J., and Alexander, J.M. tech. cords. 2019. Proceedings of the seventh sudden oak death science and management symposium: healthy plants in a world with *Phytophthora*. Gen. Tech. Rep. PSW-GTR-268. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific SW Research Station. 121 p.
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Jung T, Hansen EM, Winton L, Oswald W, Delatour C. 2002. Three new species of *Phytophthora* from European oak forests. Mycological Research 106(4):397–411
Pérez-Sierra A, López-García C, León M, García-Jiménez J, Abad-Campos P, Jung T. 2013. Previously unrecorded low-temperature *Phytophthora* species associated with *Quercus* decline in a Mediterranean forest in eastern Spain. Forest Pathology 43(4):331–339

Clade 6

OTUs detected:

P. chlamydospora

P. chlamydospora, formally known as *P.* taxon Pgchalmydo, is a recently described clade 6 species genetically and ecologically similar to other species in this clade (http://forestphytophthoras.org/species/chlamydospora). This species is often recovered from waterways, irrigation water, and associated riparian soils (as other clade 6 species), and is distributed throughout temperature forests of North and South America, Europa, Asia, South Africa, and Australia.

As many other clade 6 species it is considered to be an opportunistic pathogen. It is occasionally recovered from cankers on stems and roots of mature trees in forest, nursery and horticultural settings (Hansen et al. 2018), or from leaf and stem lesions in nurseries (e.g. Yakabe et al. 2009). However, in the majority of cases disease seems to develop under circumstances where the environment is controlled in such a manner it is conducive for disease development; in many cases where it is locally abundant there is limited to no evidence of disease (Hansen et al. 2015, 2018). It may be a contributor, along with *P. siskiyouensis* and *P. gonapodyides* to alder disease in western riparian forests (Sims et al. 2015).

Hansen, E., Reeser, P., and Sutton, W., 2018. Phytophthora *chlamydospora*. Forest Phytophthoras 8(1). doi: 10.5399/osu/fp.8.1.4566

Hansen EM, Reeser P, Sutton W, and Brasier CM. 2015. Redesignation of *Phytophthora* taxon Pgchlamydo as *Phytophthora chlamydospora sp. nov.* North American Fungi 10 (2): 1–14.

Sims, L.L., Sutton, W., Reeser, P., and Hansen, E.M. 2015. The *Phytophthora* species assemblage and diversity in riparian alder ecosystems of western Oregon, USA. Mycologia 107(5):889-902.

Yakabe, L.E., Blomquist, C.L., Thomas, S.L., and MacDonald, J.D. 2009. Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. Pl. Dis. 93: 883-890.

Clade 7

OTUs detected:

P. formosa

P. formosa is reported only in Taiwan, being recovered from streams and from the rhizosphere collected beneath *Araucaria* (Araucariaceae) and *Quercus glanulifera* (Jung et al. 2017a,c). It was weakly pathogenic in inoculations of *Castenea sativa* and *Fagus sylvatica* (Jung et al. 2017c).

Species in clade 7-subclade a may be widespread and cyptic (Jung et al. 2017b). Surveys in SE Asia have recently detected an abundance of new species in this group, many of which are not associated with disease in their recovered habitat and whose risk to other areas remain unassessed (Jung et al. 2017a). Many members of clade 7a are aggressive plant pathogens (e.g. *P.* x*cambivora*, *P. cinnamomi*, *P.* x*alni*) and contributed to decline (e.g. *P. uliginosa*). The detection of the *P. formosa*-OTU (which could be *P. formosa* or closely related species) could indicate a pathogenic species may be present, however until the detection can be verified it is difficult to assess the risk of this rarely detected OTU. All three detections occurred at unplanted sites (Fig. 8).

Jung, T., Change, T.T., Bajonyi, J., Seress, D., Pérez-Sierra, A., Yang, X., Hong, C., Scanu, B., Fu, C.H., Hsueh, K.L., Maia, C., Abad-Campos, P., Léon, M., and Jung, M.H. 2017. Diversity of *Phytophthora* species in natural ecosystems of Taiwan and association with disease symptoms. Plant Pathology 66:194-211.

Jung T, Jung MH, Cacciola SO, Cech T, Bakonyi J, Seress D, et al. 2017b. Multiple new cryptic pathogenic *Phytophthora* species from Fagaceae forests in Austria, Italy and Portugal. IMA Fungus. 2017; doi:10.5598/imafungus.2017.08.02.02

Jung T, Jung MH, Scanu B, Seress D, Kovács GM, Maia C, Pérez-Sierra A, et al. 2017. Six new *Phytophthora* species from ITS Clade 7a including two sexually functional heterothallic hybrid species detected in natural ecosystems in Taiwan. Persoonia 38: 100–135.

Clade 8

OTUs detected:

- *P. brassicae*
- *P. drechsleri*
- *P. hibernalis*
- *P. primulae*
- *P. porri*
- *P. lateralis*

Of the OTUs detected in this clade, two *P. hibernalis* and *P. lateralis* are known to be present in California and pose some risk to MROSD lands. *P. porri* and *P. brassicae* are two closely related and often confused species, with a limited distribution in North America. *P. porri* is associated with disease on *Allium* spp.; *P. brassicae* is associated with disease on Brassicaceae. Similarly, *P. primulae* has not yet been detected in North America, reported only on *Primula* in Europe and New Zealand. These taxa were all only detected rarely (1 or 2 detections); mostly from reveg or planned/disturbed sites.

P. hibernalis has been recovered from nursery *Camellia*, *Photinia*, *Pieris*, *Rhododendron*, and *Xylosma*, though pathogenicity could only be verified on *Rhododendron* and *Pieris* (Yakabe et al. 2009). It is associated with diseases of citrus, and some woody ornamental plants. While being more common in California nurseries, this OTU fell below the detection threshold of 0.095% within-sample relative abundance (Table 10). It was detected from a single sample at the Bear Creek Alma College site comprising 0.02% of the reads within that sample.

P. lateralis is sister species to *P. ramorum* and is a known pathogen of concern in many parts of the world (http://forestphytophthoras.org/species/lateralis). *P. lateralis* affects the family Cupressaceae, notably causing root rot of Port-Orford cedar (*Chamaecyparis lawsoniana*) in endemic populations of northern California and southwest Oregon, and ornamental plantings in Europe. This species may also infect *Taxus brevifolia* (Pacific yew; Taxaceae). This species is not known to be present in coastal CA forests outside the range of Port-Orford cedar, however it has a wider geographical range in nurseries. Peterson (et al. 2020) recently recovered *P. lateralis* causing substantial root decay of *Juniperus* and *Microbiotica* (Cupressaceae) in Oregon nurseries.

The *P. lateralis*-OTU was fairly abundant, being detected in 7 samples from reveg and control sites; unless members of the Cupressaceae are present there is no immediate cause for concern. Even then, infection of non-*Chamaecyparis* genera in the forest (including *Cupressus* and *Hesperocyparis*) may be limited to when more susceptible Port-Orford cedars are present.

Lastly, *P. drechsleri* is a cosmopolitan, generalist species infecting hosts in over 100 genera in 45 families, causing root rot as well as damping-off disease of seedlings (Abad et al.

2019, Erwin and Ribeiro 1996). This species is predominantly known as a pathogen of food crops (Erwin and Ribeiro 1996). Reports of this species being recovered in native vegetation are limited, though this species has often been confused with *P. cryptogea* (Erwin and Ribeiro 1996). In pathogenicity studies, *P. drechsleri* was reported to be less virulent than *P. cryptogea* sensu lato on Douglas-fir seedlings (Pratt et al. 1976). While being genetically close to *P. erythroseptica* and the *P. cryptogea*-complex (Safaiefarahani et al. 2015), *P. drechsleri* is the only member of its associated OTU in our database. The *P. drechsleri*-OTU was detected in only one sample at 0.02% within-sample relative abundance from an outplanted plant at Russian Ridge. While likely being introduced, it should not be considered widespread or of major concern.

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Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN.

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Safaiefarahani, B., Mostowfizadeh-Ghalamfarsa, R., Hardy, G.E. St.J., and Burgess, T.I. 2015. Re-evaluation of the *Phytophthora cryptogea* species complex and the description of a new species, Phytophthora *pseudocryptogea* sp. nov. Mycol Progress 14: 1–12.

Clade 9

OTUs detected:

P. fallax
P. hydropathica
P. irrigata
P. macilentosa
P. virginiana

Of the Clade 9 species detected via Illumina, none are known to occur in California. *P. hydropathica, P. irrigata, P. macilentosa*, and *P. virginiana* are reported in the Eastern U.S. predominantly being recovered from irrigation water (Hong et al. 2008, Yang and Hong 2013, Yang et al. 2014). Two species are associated with disease: *P. fallax* causes a crown dieback of *Eucalyptus* in New Zealand and Australia (Dick et al. 2006); *P. hydropathica* causes damping off and root rot in a diversity of hosts, particularly *Rhododendron* and *Kalmia* (Ericaceae)(Hong et al. 2010).

No Clade 9 species were detected in culture. This may be because the optimal temperatures for growth for most of these species approaches 30°C, while the baiting was performed at more moderate temperatures. However, we were able to bait thermo-tolerant, aquatic species of clade 6 (*P.* taxon *oaksoil, P. lacustris*). It is hypothesized thermotolerance is an adaptation allowing for better survival in stream-side decaying vegetation, this clade being a common component to stream communities and leaf debris (as is clade 6). While less is known about this clade than other more aggressive species, the members of this clade should generally be regarded as opportunistic saprotrophs in natural landscapes.

Dick, M.A., Dobbie, K., Cooke, D.E.L., and Brasier, C.M. 2006. *Phytophthora captiosa* sp. nov. and *P. fallax* sp. nov. causing crown dieback of *Eucalyptus* in New Zealand. Mycol. Res. 110: 393–404

Hong C, Gallegly ME, Richardson PA, Kong P, and Moorman GW. 2008. *Phytophthora irrigata*, a new species isolated from irrigation reservoirs and rivers in eastern United States of America. FEMS Microbiol Lett 285: 203–211.

Hong C, Gallegly ME, Richardson PA, Kong P, and Moorman GW, Lea-Cox, J.D., Ross, D.S. 2010. *Phytophthora hydropathica*, a new pathogen identified from irrigation water, *Rhododendron catawbiense* and *Kalmia latifolia.* Plant Pathology 59:913-921.

Yang X, Copes WE, and Hong C. 2014. Two novel species representing a new clade and cluster of *Phytophthora*. Fungal Biology 118: 72–82.

Yang, X., and Hong, C. 2013. *Phytophthora virginiana* sp. nov., a high-temperature tolerant species from irrigation water in Virginia. Mycotaxon 126:167-176.

Clade 12

OTUs detected:

P. quercina-cluster (*P. quercina*, *P.* sp "ohioensis", *P. versiformis*)

Of the members of the *P. quercina*-cluster, only *P. quercina* is thought to be present within the region, being first detected in the United States in 2016 from an outplanted *Quercus lobata* seedling in neighboring Santa Clara County (Bourret 2018). As a potential cause of oak decline of some oak species in central Europe (Jung et al. 1999), this species is of concern to oak restoration in the United States.

The *P. quercina*-cluster was abundant (>5% within-sample relative abundance in some samples) and widespread (27 samples across 7 preserves), though we obtained no isolate matching this taxon. We also failed to amplify longer reads of *P. quercina* via qPCR using *P. quercina*-specific primers from samples containing strong *P. quercina*-cluster detections. Longer sequence reads (1,034 bp in length) provided by the MinION sequencer indicates the OTU matching the *P. quercina*-cluster is neither *P. quercina, P.* sp. "ohioensis", or *P. versiformis*; rather, the OTU is likely an unidentified clade 12 species.

The members of this clade are generally difficult to isolate due to their slow growth rate (Jung et al. 2017). *P.* sp "ohioensis" is not a formally recognized taxa, however it has been reported as species "*P. quercina*-like" by Balci et al. (2007), as contributing to decline in white oak (Balci et al. 2007). *P. versiformis* was first recovered from declining *Corymbia* (Myrtaceae) in western Australia; while it may infect seedlings of this host it generally does not cause mortality (Paap et al. 2017). All members of this clade should be considered pathogenic to Fagaceae; however, we observed no overt disease in association with the detection of the *P. quercina*-OTU.

Balci, Y., Balci, S., Eggers, J., MacDonald, W. L., Juzwik, J., Long, R. P., and Gottschalk, K. W. 2007. *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. Plant Dis. 91:705-710

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.

Jung, T., Cooke, D.E.L., Blaschke, H., Duncan, J.M., and Oßwald, W. 1999. *Phytophthora quercina* sp. nov., causing root rot of European oaks. Mycological Research 103(7):785-798.

Paap, T., Croeser, L., White, D., Aghighi, S., Parber, P., St.J.Hardy, G.E., Burgess, T.I. 2017. *Phytophthora versiformis* sp. nov., a new species from Australia related to *P. quercina*. Australasian J. Plant Pathology DOI 10.1007/s13313-017-0499-7

Jung, T., Jung, M.H., Cacciola, S.O., Cech, T., Bakonyi, J., Seress, D., Mosca, S., Schena, L., Seddaiu, S., Pane, A., di San Lio, G.M., Maia, C., Cravador, A., Franceschini, A., Scanu, B. 2017. Multiple new cryptic pathogenic *Phytophthora* species fromk Fagaceae forests in Austria, Italy, and Portugal. IMA Fungus 8(2):219-244.

Appendix B: Site Statistics and Observations

Following is a summary of the *Phytophthora* detections and our observations at each site, organized by preserve and location. Each location contains any of the following types of sites: reveg site (revegetation project where nursery grown plants were outplanted; prescript RV), planned site (area where a revegetation project is planned but has not been planted; prescript PLND), and/or a disturbed site (area where disturbance being remedied is still present but no revegetation projects are planned; prescript DIST). Each of these classes also has a neighboring control site (an area with relatively minimal disturbance; prescript CON). In some cases, multiple revegetation projects are associated with a single control site.

Each location chart contains which *Phytophthora* species were isolate via baiting or direct leaf plating, as well as the OTU detections which occurred at that site. For OTUs, we listed only those for which within-sample relative abundance is greater than 0.01% (i.e. the OTU was present in at least one sample comprising at least 0.01% of the total number of reads). OTU detections are color-coded by maximum within-sample relative abundance. When applicable, low-level detections (e.g. within-sample relative abundance <0.095%) are discussed in detail but a more broad discussion as to the interpretation of these detections can be found in Appendix E: Interpretation of Illumina Data.

Species and OTU descriptions are kept brief; for more details on the host range and potential impacts of any particular *Phytophthora*, please see Appendix A: *Phytophthora* species recovered and a brief description of diseases they cause. Pictures are provided to illustrate our observations of cases where disease (or lack thereof) may be associated with *Phytophthora*. The pictures are meant to be representative, and do not necessarily indicate the overall plant health status at these sites.

Appendix B. Table. 1. Baiting and Illumina results summary for all sites.

Preserve	Project	Site ID	Baiting summary				Illumina summary			
			No. samples baited	No. of samples in which *Phytophthora* was recovered	No. *Phytophthora* species recovered	Cumulative score[a]	No. samples sequenced	No. samples in which *Phytophthora* was detected[b]	No. *Phytophthora* OTUs detected[b]	Cumulative score[a]
Bear Creek Redwoods	Alma College	CON_BCR_A003	6	2	2	6	6	2	1	2
		PLND_BCR_A003	6	0	0	0	6	3	2	4
	Bear Creek X-mas Tree Farm	CON_BCR_A001	6	0	0	0	5	4	2	5
		PLND_BCR_A001	6	0	0	0	2	0	0	0
	Webb Creek Bridge	CON_BCR_A004	6	1	1	2	5	4	3	9
		RV_BCR_A004	6	1	1	3	0	na	na	na
El Corte De Madera	chinquapin	DIST_CHIN1	3	0	0	0	1	1	1	1
		DIST_CHIN2	3	0	0	0	1	1	1	1
	El Corte de Madera Bridge	CON_CM_C003	6	2	1	4	6	2	2	2
		RV_CM_C003	6	0	0	0	5	1	1	1
	El Corte de Madera Parking Lot	CON_CM_A003	6	0	0	0	6	3	1	1
		RV_CM_A003	6	0	0	0	6	2	3	6
	King Mt. Manzanita	CON_CM_D001	6	0	0	0	6	4	2	5
		PLND_CM_D001	6	0	0	0	6	4	5	11
La Honda	La Honda	CON_LH_F001	6	3	2	2	4	3	4	12
		RV_LH_F001	6	4	4	10	6	5	6	10
Monte Bello	Bridge	CON_MB_A001Bridge	6	0	0	0	6	4	6	13
		PLND_MB_A001Bridge	6	1	1	4	6	4	5	8
	Grassland	CON_MB_A001Grass	12	0	0	0	12	9	9	18
		PLND_MB_A001Grass	6	1	1	4	6	5	5	14

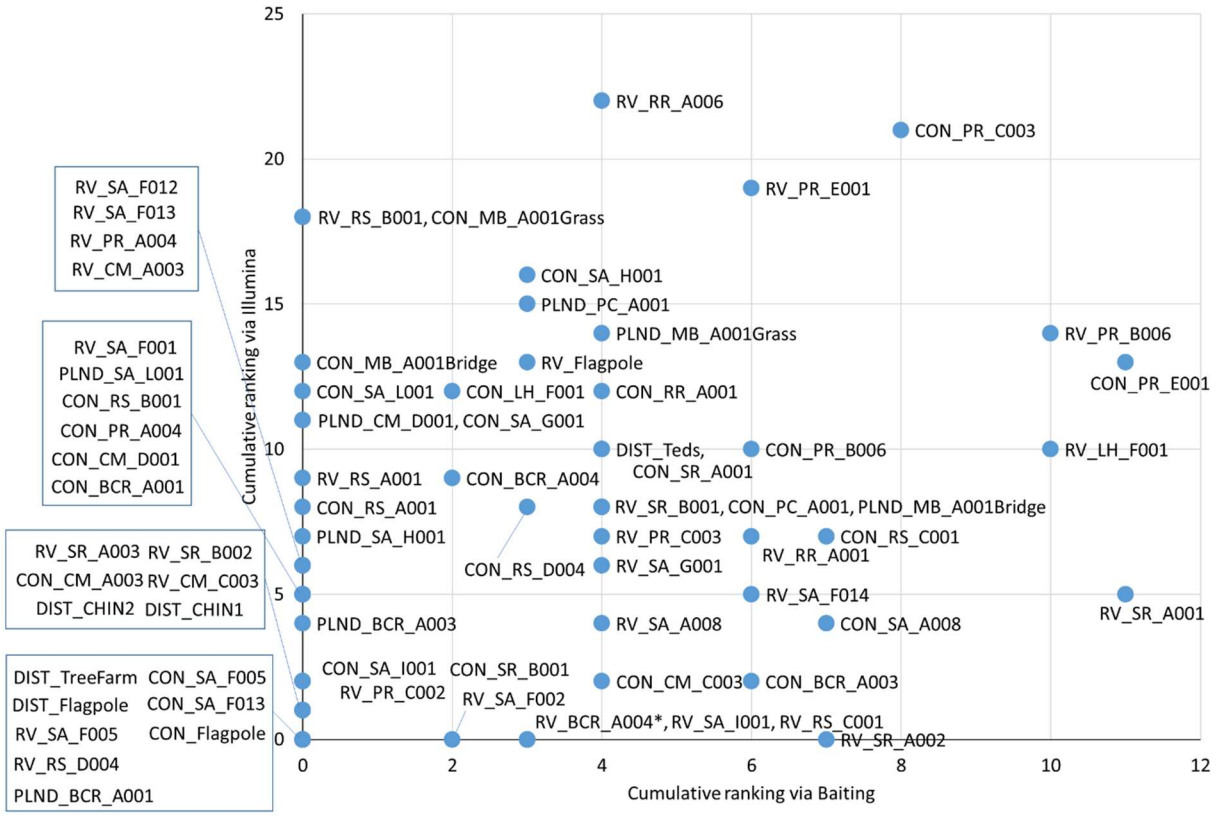
Preserve	Project	Site ID	Baiting summary				Illumina summary			
			No. samples baited	No. of samples in which *Phytophthora* was recovered	No. *Phytophthora* species recovered	Cumulative score[a]	No. samples sequenced	No. samples in which *Phytophthora* was detected[b]	No. *Phytophthora* OTUs detected[b]	Cumulative score[a]
Pulgas Ridge	Blue Oak	CON_PR_E001	17	4	4	11	15	14	5	13
		RV_PR_E001	14	2	2	6	12	12	7	19
	Pulgas forest	CON_PR_B006	12	4	2	6	11	7	5	10
		RV_PR_B006	12	4	3	10	10	6	7	14
	Pulgas Summit A-series	CON_PR_A004	6	0	0	0	4	2	2	5
		RV_PR_A004	6	0	0	0	6	2	2	6
	Pulgas Summit C-series	CON_PR_C003	12	3	2	8	12	10	9	21
		RV_PR_C002	12	0	0	0	4	1	1	2
		RV_PR_C003	12	1	1	4	7	3	3	7
Purisima Redwoods	Purisima Redwoods	CON_PC_A001	6	1	1	4	6	2	2	8
		PLND_PC_A001	12	1	1	3	12	7	6	15
Rancho San Antonio	Annex Garden	CON_RS_B001	6	0	0	0	6	3	2	5
		RV_RS_B001	5	0	0	0	2	2	9	18
	Equestrian Lot	CON_RS_C001	6	2	2	7	1	1	2	7
		RV_RS_C001	6	2	1	3	1	0	0	0
	Rhus Ridge	CON_RS_A001	6	0	0	0	6	3	4	8
		RV_RS_A001	6	0	0	0	6	4	4	9
	Field Office	CON_RS_D004	6	1	1	3	5	3	3	8
		RV_RS_D004	6	0	0	0	4	0	0	0

Preserve	Project	Site ID	Baiting summary				Illumina summary			
			No. samples baited	No. of samples in which *Phytophthora* was recovered	No. *Phytophthora* species recovered	Cumulative score[a]	No. samples sequenced	No. samples in which *Phytophthora* was detected[b]	No. *Phytophthora* OTUs detected[b]	Cumulative score[a]
Sierra Azul	Hendrys	CON_SA_L001	7	0	0	0	7	4	6	12
		PLND_SA_L001	7	0	0	0	7	2	2	5
	Bald Mountain	CON_SA_A008	6	2	2	7	6	2	2	4
		RV_SA_A008	6	1	1	4	6	1	2	4
	Flagpole	CON_Flagpole	12	0	0	0	6	0	0	0
		DIST_Flagpole	12	0	0	0	6	0	0	0
		RV_Flagpole	12	1	1	3	11	5	6	13
		RV_SA_F014	6	3	2	6	6	1	2	5
	Hoita Rd	CON_SA_H001	6	1	0	3	6	5	6	16
		PLND_SA_H001	6	0	0	0	6	3	2	7
	Mt Um Summitt	CON_SA_F013	6	0	0	0	6	0	0	0
		RV_SA_F013	6	0	0	0	6	2	2	6
		CON_SA_I001	12	0	0	0	6	1	2	2
		RV_SA_I001	12	1	1	3	7	0	0	0
		RV_SA_F001	6	0	0	0	6	2	2	5
		RV_SA_F002	6	1	1	2	5	0	0	0
	weather shelter & stairway	DIST_Teds	10	1	1	4	6	3	5	10
		CON_SA_F005	6	0	0	0	5	0	0	0
		RV_SA_F005	6	0	0	0	6	0	0	0
		RV_SA_F012	6	0	0	0	5	2	2	6
	woods trail	CON_SA_G001	6	0	0	0	6	4	5	11
		RV_SA_G001	6	3	1	4	6	2	2	6

Preserve	Project	Site ID	Baiting summary				Illumina summary			
			No. samples baited	No. of samples in which *Phytophthora* was recovered	No. *Phytophthora* species recovered	Cumulative score[a]	No. samples sequenced	No. samples in which *Phytophthora* was detected[b]	No. *Phytophthora* OTUs detected[b]	Cumulative score[a]
Russian Ridge	Mindego Gateway	CON_RR_A001	12	1	1	4	8	6	5	12
		RV_RR_A001	12	5	2	6	8	3	4	7
		RV_RR_A006	12	2	1	4	7	1	7	22
Skyline Ridge	Big Dipper	CON_SR_B001	6	0	0	0	3	1	1	2
		RV_SR_B001	6	1	1	4	4	2	5	8
		RV_SR_B002	6	0	0	0	3	1	1	1
	Skyline	CON_SR_A001	12	1	1	4	11	7	5	10
		DIST_TreeFarm	6	0	0	0	6	0	0	0
		RV_SR_A001	12	3	3	11	9	4	2	5
		RV_SR_A002	12	6	2	7	10	0	0	0
		RV_SR_A003	12	0	0	0	6	1	1	1

[a] Species and OTUs were ranked from 1-4 based on their likely impact on MROSD preserves, representing low to very-high risk. The cumulative score is the sum of the rankings for all species (baiting) or OTUs (Illumina) detected at the site.

[b] To be considered detected an OTU must have comprised a minimum of 0.095% of the number of reads with the sample.



Appendix. B. Fig. 1. Comparison between the cumulative infestation scores as measured by baiting and Illumina for all sites. Those sites in the upper-right quadrant (high scores for both Illumina and baiting) are considered the highest infestation risk due to the large number of high-risk *Phytophthora* species both baited and sequenced.

Given the capacity for *Phytophthora* dispersal and how environmental/host characteristics favoring *Phytophthora* establishment are shared between adjacent sites, overall infestation risk for management purposes should be assessed on a location-basis as presented in Figure 16.

* we were unable to obtain DNA of sufficient quality for sequencing on the Illumina platform for all samples from this site.

Bear Creek Redwoods

Alma College (BCR-A003)

Species isolated: *P. ramorum*, *P.* sp. 'cadmea'

Site ID	CON_BCR_A003	PLND_BCR_A003	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**	*ram, cad*		
No. samples sequenced	6	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. uliginosa-cluster	3.36%	0	3.36%
P. syringae	0.01%	0.71%	0.71%
P. psychrophila	0	0.46%	0.46%
P. cactorum-cluster	0	0.07%	0.07%
P. virginiana	0.06%	0	0.06%
P. citricola-complex	0	0.04%	0.04%
P. nemorosa-cluster	0.03%	0	0.03%
P. hibernalis	0	0.02%	0.02%
P. cryptogea-complex	0.02%	0	0.02%
P. sp. unknown	0.02%	0.01%	0.02%

In addition to native *Q. agrifolia*, we sampled ornamental *Buxus* and *Rosa* in the planned planting areas. The control areas was forested and along the Alma Trail, where we sampled *Rosa californica*, *Q. agrifolia*, and *Rubus ursinus*.

The recovery of *P. ramorum* is consistent with the prevalence of this species in the area. Apart from this, no evidence of *Phytophthora* disease was apparent at either site, however the large number of ornamental plantings at Alma College indicate a high likelihood they have been introduced in the past. We detected strong indications of the presence of *P. syringae*, with other nursery-associated spp. (*P. cactorum*-cluster, *P. citricola*-complex), at the planting site. Detections of the *P. uliginosa*-cluster (of which *P.* sp. 'cadmea' is a member) and the *P. psychrophila* cluster pose no immediate concern.

Bear Creek Redwoods

Christmas Tree Farm (BCR-A001)

Species isolated: *P. ramorum* (foliage only)

Site ID	CON_BCR_A001	PLND_BCR_A001	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**	*(ram)*		
No. samples sequenced	5	2	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	6.36%	0	6.36%
P. cactorum-cluster	0.83%	0	0.83%

For the planned area, we sampled plants naturally regenerated within the Christmas Tree Farm. No *Phytophthora* spp. were isolated or detected via Illumina, however the DNA extract for 4 of the 6 samples taken from this site were not of sufficient quality for submission to Illumina.

Evidence of *P. ramorum* was widespread throughout the forests adjacent to the Christmas Tree Farm planting site. In addition to recovering this species from California bay, we detected a strong signature of the presence of the *P. cactorum*-cluster and the *P. psychrophila*.

We observed branch dieback and flagging on a large number of the madrone, tanoak, and coast live oaks sampled in both areas. Many of the symptoms may be attributed to *P. ramorum*, however soilborne *Phytophthora* (such as *P. cactorum* or *P. pseudotsugae*, both part of the *P. cactorum*-cluster) cannot be eliminated. It is unlikely *P. psychrophila* is causing symptoms to this degree.

Branch dieback on *Arbutus menziesii* (and adjacent dead tanoak). Control point CON_BCR_A001-E2; sampled Dec. 14, 2018

Bear Creek Redwoods

Webb Creek Bridge (BCR-A004)

Species isolated: *P. ramorum* (foliage only), *P. pseudotsugae*, *P. crassamura*

Site ID	CON_BCR_A004	RV_BCR_A004	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*pse, (ram)*	*cra*	
No. samples sequenced	5	0	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	24.54%		24.54%
P. citricola-complex	0.11%		0.11%
P. nemorosa-cluster	0.10%		0.10%
P. cryptogea-complex	0.03%		0.03%
P. sp. unknown	0.03%		0.03%

P. pseudotsugae was recovered at the control area upstream of the Webb Creek Bridge; this species was also detected via Illumina (as a member of the *P. cactorum*-cluster). This species is thought to only infect Douglas-fir, however this host was not present in the area. Recent detections of this species by us and Bourret (2018) indicate other genera may be hosts, though the origin of *P. pseudotsugae* and the impact this species may have on mature plants or future regeneration is unknown. Dieback was only observed on tanoak, consistent with the presence of *P. ramorum* in the area.

Unfortunately, none of the 6 samples collected from RV_BCR_A004 contained DNA of sufficient quality to submit for sequencing with Illumina MiSeq. We were, however, able to recover *P. crassamura* from soil collected from nursery-grown *Oxalis oregano*. This species is sufficiently common in restoration nurseries within the region that a nursery-source should be evaluated, especially since a number of 2018 plantings contained this species.

Bourret, T.B. 2018. Efforts to detect exotic *Phytophthora* species reveal unexpected diversity. PhD. Dissertation, U.C. Davis.

El Corte de Madera Creek

King Mt. Manzanita (CM-D001)

Species isolated: none

Site ID	CON_CM_D001	PLND_CM_D001	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**			
No. samples sequenced	6	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	2.27%	0.29%	2.27%
P. nemorosa-cluster	0	1.64%	1.64%
P. citricola-complex	0.44%	0	0.44%
P. quercina-cluster	0	0.28%	0.28%
P. syringae	0	0.21%	0.21%
P. cactorum-cluster	0	0.15%	0.15%

Both the *P. psychrophila* and *P. nemorosa*-cluster OTUs were detected at high frequency from the King Mt. Manzanita area; both are suspected to be native to Western U.S. and are not associated with substantial disease. Similarly, the *P. quercina*-cluster was detected in multiple sites at high frequency with no apparent disease present and no immediate cause of concern is indicated.

The *P. citricola*-complex and *P. cactorum*-cluster contain known pathogenic species of concern at the site. *P. syringae* may also be pathogenic and of concern, although none of these species were isolated. *Arctostaphylos* and *Arbutus* in the control area, immediately uphill of the proposed planting site, had branch dieback which may be attributable to infection by these species. Given the strong signatures of pathogenic soilborne *Phytophthora* and the presence of symptoms in adjacent vegetation, we recommend against planting vulnerable plant populations in this area.

El Corte de Madera Creek

Parking lot (CM-A003)

Species isolated: none

Site ID	CON_CM_A003	RV_CM_A003	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**			
No. samples sequenced	6	5	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	0	3.84%	3.84%
P. psychrophila	2.18%	0.99%	2.18%
P. nemorosa-cluster	0.06%	0.61%	0.61%
P. sp. unknown	0	0.07%	0.07%
P. hedraiandra	0	0.02%	0.02%

No species of concern were detected within the control area taken along the Sierra Moreno trail. Both the *P. psychrophila* and *P. nemorosa*-cluster OTUs were detected at high frequency from El Corte de Madera Creek; both are suspected to be native to Western U.S. and are not associated with substantial disease.

Among plantings at the revegetation area in the parking lot, the *P. cactorum*-cluster (which may be *P. cactorum* or *P. pseudotsugae*) was detected at a within-sample relative abundance high enough to indicate at least one of these pathogens is established in this area. However, neither *P. cactorum* nor *P. pseudotsugae* were recovered via baiting. The two plants with signatures of this OTU, both coast redwoods, were apparently healthy. *P. pseudotsugae* was recovered from one control site dominated by *Sequoia sempervirens* (BCR-A004) but only in 2018; it is possible this species is present but was not detectable via baiting with the plants were sampled in 2017.

Planting of *Sequoia sempervirens*, which had a strong signature of the *P. cactorum*-cluster but no apparent symptoms of disease. RV_CM_A003-B3; sampled Dec. 23, 2017.

El Corte de Madera Creek

Bridge planting (CM-C003)

Species isolated: *P. ramorum*

Site ID	CON_CM_C003	RV_CM_C003	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*ram*		
No. samples sequenced	6	5	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	0.89%	0.10%	0.89%
P. nemorosa-cluster	0.14%	0.03%	0.14%
P. cactorum-cluster	0	0.05%	0.05%

The presence of *P. psychrophila* and *P. nemorosa*-cluster OTUs should not under most circumstances threaten native plant health at the El Corte de Madera Creek Bridge planting. *P. ramorum* was present at the site, as suspected by the high amount of tanoak mortality observed upstream of the sampled areas. The within-sample relative abundance of the *P. cactorum*-OTU is low relative to that recorded in other samples, notably the detection upstream at CM_A003. Lacking a culture-detection, we cannot discern if this detection indicates an active pathogen is present.

Aside from mortality attributable to *P. ramorum,* which was abundant within the preserve, we found little evidence of *Phytophthora* contamination in this site.

El Corte de Madera Creek

Chinquapin

Species isolated: none

Site ID	DIST_CHIN1	DIST_CHIN2	
Site Status	disturbed	disturbed	
***Phytophthora* sp. isolated**			
No. samples sequenced	1	1	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	0.24%	0.14%	0.24%

These two sites were sampled in 2018 upon recommendation and concern by MROSD staff, who had noticed decline in chinquapin in the area. Two areas were readily identifiable in a brief survey as having dieback symptoms characteristic of *Phytophthora* infection (designated as Chin1 and Chin2). Minor decline of *Arbutus* and *Arctostaphylos* was also noted. Soil baiting and sequencing yielded no cultures and only detections of *P. psychrophila,* which we do not suspect as being a causal agent of the symptoms.

The most likely culprit (if a *Phytophthora*) would be *P. cambivora*, a known pathogen of chinquapin in the western U.S. (Saavedra et al. 2007). *P. cambivora* was commonly isolated in 2017, however this species was not isolated in a single sample in 2018 even from samples in which the pathogen was recovered in year one. This species was similarly overrepresented in the Illumina data from 2017, with few detections in 2018. The pathogen may have been un-detectable when we sampled the site in 2018. Due to time restraints no attempt was made to isolate *P. cambivora* from the roots, and no signs of infection by other agents were observed. Further sampling is necessary to determine if *P. cambivora* is present at the site.

Chinquapin is also a host to *P. ramorum*, which was abundant on tanoak within the area; this pathogen:host combination is reported but uninvestigated.

Saavedra A, Hansen EM, Goheen DJ. *Phytophthora cambivora* in Oregon and its pathogenicity to *Chrysolepis chrysophylla*. Forest Pathology. 2007. 37:409 - 419.

Crown thinning of golden chinquapin, *Chrysolepis chrysophylla*, along the Fir Trail at El Corte de Madera Creek. This symptom is consistent with root disease caused by *Phytophthora.*

La Honda Creek

La Honda Creek (LH-F001)

Species isolated: *P.* taxon *oaksoil*, *P. lacustris*, *P. nemorosa*, *P.* aff. *ilicis* (foliage only), *P. ramorum* (foliage only), *P. syringae*, *P. pseudosyringae*

Site ID	CON_LH_F001	RV_LH_F001	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*oak, lac, (nem), (ili)*	*ram, syr, psy, lac*	
No. samples sequenced	4	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	1.55%	0.04%	1.55%
P. riparia-cluster	0	1.55%	1.55%
P. bilorbang-cluster	1.19%	0.55%	1.19%
P. nemorosa-cluster	0.02%	0.72%	0.72%
P. sp. unknown	0	0.44%	0.44%
P. megasperma-cluster	0.14%	0.37%	0.37%
P. cryptogea-complex	0.15%	0	0.15%
P. syringae	0	0.15%	0.15%
P. siskiyouensis	0.01%	0.09%	0.09%
P. chlamydospora	0	0.05%	0.05%
P. psychrophila	0.04%	0	0.04%
P. ramorum	0	0.04%	0.04%

For the revegetation site we sampled identifiable outplanted plants from the LH_F001 revegetation project and stream-side red alder. Lack of access made an upstream control sampling impossible, hence for the control area we went downstream of planted areas. Both sites had an abundance of clade 6 species (detected via baiting and/or Illumina: *P.* taxon *oaksoil*, *P. lacustris, P. riparia*-cluster, *P. megasperma*-cluster and *P. chlamydospora*) which is consistent with the stream-specialization observed in this clade. With the exception of *P. megasperma*-cluster (indicating the presence of *P. megasperma*, *P. gonapodyides* and /or *P. crassamura*), these species are not thought to significantly threaten riparian forests. All three species in the *megasperma*-cluster have been recovered by us during the course of this study. *P. gonapodyides* was recovered only from stream baits, never soil, but as a common stream-recovered species, it may be the contributing member of this clade in this circumstance.

We additionally detected a large number of clade 3 species from the site including *P. nemorosa, P.* aff. *ilicis*, and *P. pseudosyringae* (via culture) and the *P. nemorosa*-cluster and *P. psychrophila* OTUs (via Illumina). These, too, are not suspected as causing substantial disease in this area.

While the majority of detections may only be weakly pathogenic, their presence indicates a highly conducive environment for the establishment of *Phytophthora*. We cannot determine if pathogenic species indicated by the presence of the *P. cactorum*-cluster, *P. cryptogea*-complex,

and *P. siskiyouensis*-OTUs are present infecting vegetation at the site, or if detections indicate infection upstream. *P. syringae* and *P. pseudosyringae* were baited from a dead outplanting of *Rubus parviflorus*. But again, we cannot determine if these originated from the outplanting up from an upstream source.

Alders in the sample areas appeared, for the most part, healthy. Decay and cankers were found on two mature stems, however a causal agent was not investigated and only *P.* taxon *oaksoil* was isolated from the associated samples. This species is associated with decline of streamside *Rubus anglocandicans* in Australia (Aghighi et al. 2012) and is a commonly recovered from stream water in the U.S., however it is not associated with alder disease (Sims et al. 2015). Rather, Sims et al. (2015) found *P. siskiyouensis*, *P. chlamydospora* and *P. gonapodyides* (but particularly *P. siskiyouensis*), all of which are indicated in Illumina, to be better associated with alder disease in her Oregon surveys.

Aghighi, S., St. J. Hardy, G.E., Scott, J.K., and Burgess, T.I. 2012. *Phytophthora bilorbang* sp. nov., a new species associated with the decline of *Rubus anglocandicans* (European blackberry) in Western Australia. Eur. J. Plant Pathol. 133:841-855.

Sims, L.L., Sutton, W., Reeser, P., and Hansen, E.M. 2015. The *Phytophthora* species assemblage and diversity in riparian alder ecosystems of western Oregon, USA. Mycologia 107(5):889-902.

Stem decay of white alder *Alnus rhombifolia*, causal agent unknown. RV_LH_F001-D1; sampled Dec 14, 2018.

Monte Bello

Future grassland planting (MB-A001Grass)

Species isolated: *P. cambivora*

Site ID	CON_MB_A001Grass	PLND_MB_A001Grass	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**		*cam*	
No. samples sequenced	12	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	38.94%	0.96%	38.94%
P. quercina-cluster	20.67%	0	20.67%
P. cactorum-cluster	0	6.91%	6.91%
P. citricola-complex	0.64%	0.23%	0.64%
P. cambivora-complex	0	0.63%	0.63%
P. virginiana	0.34%	0	0.34%
P. irrigata	0.22%	0	0.22%
P. sp. unknown	0.20%	0.07%	0.20%
P. ramorum	0.19%	0	0.19%
P. lateralis	0.16%	0	0.16%
P. formosa	0	0.16%	0.16%
P. citrophthora-cluster	0.15%	0	0.15%
P. chlamydospora	0.05%	0	0.05%

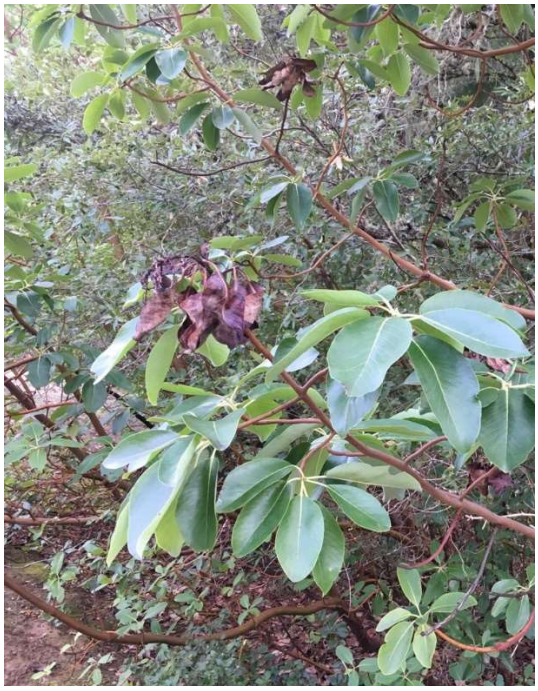
Both sampling sites were composed of woodland forest adjacent to a grassland where a proposed planting is to be located. The planned site was downhill of the proposed area with closer proximity to the Stevens Creek Nature Trail; the control site was uphill from both the site and the trail.

Prior sampling by Phytosphere recovered *P. cambivora* from the preserve in June 2015, September 2016, and June 2017 by baiting the rhizosphere of declining toyon and valley oak. We were able to repeat this detection via baiting and Illumina. There was some evidence of disease activity of madrone, though branch symptoms may be more typical of an aerial pathogen.

There was a high diversity of *Phytophthora* OTUs, indicative of a history of introduction at this location. The *P. psychrophila* and *P. quercina*-cluster OTUs were highly prevalent in our study and are not associated with any disease. They should be considered of low-concern. Other OTUs, however, may be more damaging. The *P. cactorum*-cluster was detected at a high enough abundance to be considered present at the site. This OTU is representative of either *P. cactorum* or *P. pseudotsugae*: *P. cactorum*, though not baited by us or Phytosphere, was recovered in neighboring Skyline Ridge and thus may be present at this site; however, the presence of Douglas-fir at the location makes the presence of *P. pseudotsugae* equally as likely. The *citricola*-complex may be attributable to a number of pathogenic species (*P. pini*, *P. plurivora*, *P. multivora*).

This site had a large number of OTU detections for which a cultured species was never acquired: *P. virginiana* and *P. irrigata* (clade 9), *P. formosa* (clade 7), *P. lateralis* (clade 8), and the *P. citrophthora*-cluster (clade 2). Members of the *P. citrophthora*-cluster have been found infecting nursery *Arbutus* (*P. citrophthora*) and contributing to failed restoration outplantings (*P. occultans*). Clade 7 species include many aggressive plant pathogens, though many are cryptic and cause little apparent disease. *P. formosa* is not reported in North America; likely this OTU represents an undescribed clade 7 species of unknown risk. Lastly, the *P. lateralis*-OTU may be a remnant introduction, as no known hosts were present at the site.

Dead madrone in the understory, associated with sampled plant RV_MB_A001Grass-C5; sampled Dec 23, 2017.



Branch dieback. RV_MB_A001Grass-A5, sampled Dec 23, 2017.

Monte Bello

Future bridge planting (MB-A001Bridge)

Species isolated: *P. ramorum*

Site ID	CON_MB_A001Bridge	PLND_MB_A001Bridge	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**		*ram*	
No. samples sequenced	5	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. quercina-cluster	1.81%	0	1.81%
P. nemorosa-cluster	0.24%	0.95%	0.95%
P. psychrophila	0.43%	0.42%	0.43%
P. chlamydospora	0	0.36%	0.36%
P. sp. unknown	0	0.35%	0.35%
P. cactorum-cluster	0.20%	0	0.20%
P. lateralis	0.17%	0	0.17%
P. ramorum	0	0.15%	0.15%
P. syringae	0.11%	0	0.11%
P. uliginosa-cluster	0	0.04%	0.04%
P. hydropathica	0	0.02%	0.02%

For this site we sampled native vegetation at the bridge site (planned area) and upstream of the site near the junction of the current and old Stevens Creek Nature trail (control area). The high abundance of *P. quercina*-cluster and clade 3 OTUs is typical of the vegetation type at these locations and are not of immediate concern.

Understory tanoak symptoms are consistent with infection by *P. ramorum*. We also observed dieback of madrone consistent with that reported in neighboring MB_A001Grassland. These sites contain detections shared with MB_A001Grassland, notably *P. lateralis* and the *P. cactorum*-cluster. *P. syringae* was also detected at relatively high abundance from the control site, consistent with a large diversity of *Phytophthora* present within Monte Bello. This species has an unknown role in *Phytophthora* decline, although it is relatively common component of the rhizosphere in our and prior surveys.

Understory tanoak symptoms consistent with infection by *P. ramorum*. CON_MB_A001Bridge-E4; sampled Dec 23, 2017.

Dieback of madrone. CON_MB_A001Bridge-D5; sampled Dec 23, 2017.

Dieback of madrone in foreground, with tanoak mortality associated with *P. ramorum* in the background. RV_MB_A001Bridge-A1; sampled Dec 23, 2017.

Pulgas Ridge

Blue Oak Parking Lot (PR-E001)

Species isolated: *P. cambivora*, *P. cinnamomi*, *P.* sp. 'cadmea', *P. cryptogea*-complex, *P. multivora*, *P. pseudosyringae*, *P. ramorum* (foliage only)

Site ID	CON_PR_E001	RV_PR_E001	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*cad, cam, cin, cry*	*mul, psy, (ram)*	
No. samples sequenced	15	12	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. quercina-cluster	5.20%	0.03%	5.20%
P. psychrophila	2.82%	5.09%	5.09%
P. cactorum-cluster	2.74%	3.45%	3.45%
P. citricola-complex	0.05%	3.06%	3.06%
P. cryptogea-complex	2.50%	0.61%	2.50%
P. uliginosa-cluster	1.60%	0	1.60%
P. syringae	0	0.34%	0.34%
P. nemorosa-cluster	0	0.17%	0.17%
P. citrophthora-cluster	0.07%	0.16%	0.16%
P. sp. unknown	0.04%	0.08%	0.08%
P. lateralis	0.05%	0	0.05%
P. irrigata	0.05%	0	0.05%
P. chlamydospora	0.02%	0.03%	0.03%
P. cambivora-complex	0.02%	0	0.02%

Evidence of *Phytophthora* decline was, by far, most apparent in the planted areas adjacent to the parking lot and Blue Oak trailhead. However, a large number of *Phytophthora* spp. were detected in the control area located uphill of the revegetation site. We detected multiple species which may be causing decline in these plants, and the site should be considered highly contaminated. The high-within sample relative abundance of many OTUs indicates biological activity of more *Phytophthora* spp. than detected by baiting.

Both the reveg and control site were sampled both years, with noticable decline in health status for some plants. *Heteromeles* was the only species present and sampled in both locations. Plants in the control area were classified as healthy in both 2017 and 2018, whereas toyons (and madrone) declined substantially in the reveg areas. *Mimulus*, sampled extensively in the control area, has a high diversity of associated species, but did not die between years and showed no specific symptoms other than those which may be attributable to drought stress.

P. multivora and *P. pseudosyringae* were baitied from soil taken from either declining plants. Theses plants also had strong signatures of the *P. cactorum*-complex, *P. citricola*-complex, *P. cryptogea*-complex, and *P. syringae* OTUs (to list only the pathogenic OTUs of

concern). It is possible species such as *P. cambivora* or *P. cinnamomi* may also be contributing to these declines despite not being recovered directly from dead or dying plants.

There was a high number of samples in which species of less concern were detected inclding: *P.* sp. 'cadmea' via baiting, and the *P. psychrophila*, *P. uliginosa*-cluster, *P. quercina*-cluster, and the *P. nemorosa*-cluster via Illumina. The detection of these species, while not themselves alarming, indicate a highly conducive environment for *Phytophthora* at this location. Extreme care should be taken when moving between preserves from and to this area.

Healthy Mimulus seedling from which the *P. cryptogea*-complex was baited. This species also had a strong signature of the *P. cryptogea*-complex OTU. CON_PR_E001-D1; sampled Dec 19, 2017 (pictured). This plant could not be located the second year.

Mature Mimulus presumed to be healthy. No *Phytophthora* sp. were baited and no pathogenic OTUs were detected from soil taken at the base of this plant; the plant appeared to be of consistent health between years. CON_PR_E001-D2; sampled Dec 19, 2017 (pictured) and Dec 11, 2018.

Toyon with classic *Phytophthora* dieback. Note live branches. RV_PR_E001-B1; sampled Dec 19, 2017.

Same toyon in year 2. RV_PR_E001-B1; sampled Dec 11, 2018.

Healthy toyon within control areas. CON_PR_E001-E2; sampled Dec 19, 2017 (pictured) and Dec 11, 2018.

Madrone with classic *Phytophthora* dieback. RV_PR_E001-C1; sampled Dec 19, 2017 (pictured) and Dec 11, 2018.

Pulgas Ridge

Forest site (PR-B006)

Species isolated: *P. cambivora*, *P.* sp. 'cadmea', *P. cinnamomi*, *P. cactorum*, *P. boehmeriae*

Site ID	CON_PR_B006	RV_PR_B006	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*cam, cad*	*cin, cac, boe*	
No. samples sequenced	11	10	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	1.57%	1.00%	1.57%
P. uliginosa-cluster	1.08%	0.14%	1.08%
P. cactorum-cluster	0.71%	0.30%	0.71%
P. irrigata	0	0.50%	0.50%
P. quercina-cluster	0.48%	0	0.48%
P. sp. unknown	0.07%	0.29%	0.29%
P. cryptogea-complex	0	0.12%	0.12%
P. formosa	0.11%	0	0.11%
P. chlamydospora	0	0.10%	0.10%
P. boehmeriae	0	0.02%	0.02%

At the forest site of Pulgas Ridge along the Hassler Loop Trail, we sampled one reveg and one control area. The reveg area had been planted although it was not clear if the sampled plants, which were selected for showing decline symptoms, themselves were outplantings. The reveg area was a neighboring parcel with greater tree coverage for which no obvious outplanting had taken place.

Numerous plant showed dieback in the reveg area, especially *Arctostaphylos* and *Mimulus.* A number of pathogenic species were detected which may be contributing to these symptoms, including *P. cambivora*, *P. cinnamomi*, and *P. cactorum* (all verified via baiting). Closely related to *P. cinnamomi* is *P. formosa*, which was detected via Illumina. *P. formosa* is not reported in North America; likely this OTU represents an undescribed clade 7 species of unknown risk. We also detected and recovered *P. boehmeriae*, which is the first record of this species occurring in North America. This particular species was recovered from a Mimulus with dieback; given the prevalence of other aggressive species in the area, however, we cannot discern if the symptoms are attributable to *P. boehmeriae* or the other species present.

As with the Blue Oak parking lot (PR_E001), some plants in this site declined significantly between year 1 and year 2. This site should be considered highly contaminated.

Arctostaphylos with minor dieback. CON_PR_B006-E2; pictured Dec. 19, 2017.

The same individual 1 year later. CON_PR_B006-E2; pictured Dec. 11, 2018.

Affected Mimulus from which *P. boehmeriae* was recovered. RV_PR_B006-A1; sampled Dec 19, 2017 (pictured) and Dec. 11, 2018.

Healthy toyon from which *P.* sp. 'cadmea' was recovered. CON_PR_B006-F1; sampled Dec 19, 2017 and Dec. 11, 2018 (pictured).

Pulgas Ridge

Summit C-Series (PR-C002 & PR-C003)

Species isolated: *P. cambivora*, *P. cryptogea*-complex

Site ID	CON_PR_C003	RV_PR_C002	RV_PR_C003	
Site Status	unplanted	revegetation site	revegetation site	
***Phytophthora* sp. isolated**	*cam, cry*		*cam*	
No. samples sequenced	11	1	5	
OTU	**Maximum within-sample relative abundance**			**Max detection by area**
P. cactorum-cluster	6.58%	0	8.43%	8.43%
P. psychrophila	6.75%	0	0	6.75%
P. cryptogea-complex	2.99%	0	0	2.99%
P. cinnamomi	1.02%	0	0	1.02%
P. quercina-cluster	0.94%	0	0	0.94%
P. chlamydospora	0	0	0.25%	0.25%
P. nemorosa-cluster	0.21%	0	0	0.21%
P. lateralis	0	0.21%	0	0.21%
P. fallax	0	0	0.16%	0.16%
P. citricola-complex	0.16%	0	0	0.16%
P. sp. unknown	0.10%	0	0	0.10%
P. megasperma-cluster	0.03%	0	0	0.03%
P. cambivora-complex	0.03%	0	0.03%	0.03%
P. syringae	0.02%	0	0	0.02%

Two reveg sites were sampled at the summit of Pulgas Ridge: PR_C002 (first planted 2012) and PR_C003 (first plated 2009). Outplanted plants were identifiable at PR_C002, where they were caged, but not PR_C003. As with other sites in Pulgas Ridge, dieback symptoms were apparent on *Arctostaphylos*. We detected strong signatures of *P. cactorum*-complex in the control and PR_C003, from which we were also able to isolate *P. cambivora*. We also isolated *P. cryptogea*-complex and found a strong signature of *P. cinnamomi* in the control area.

Relative to most of Pulgas Ridge, the PR_C002 planting site was free of *Phytophthora*. While we were only able to sequence one sample (in which we only detected *P. lateralis*, primarily known to infect Cupressaceae), all six plants at this location were baited and were negative both years.

Arctostaphylos with dieback. *P. cambivora* was recovered from this sample. RV_PR_C003-C2; pictured Dec. 19, 2017.

Frangula with minor dieback, though no *Phytophthora* spp. were isolated or detected. RV_PR_C002-A1; pictured Dec. 19, 2017.

Mimulus with dieback. *P. cryptogea* was recovered from this sample in year 1, and a strong signature of the *P. cactorum*-cluster OTU was detected both years (>6%). CON_PR_C003-E1; pictured Dec. 19, 2017.

Mimulus with a strong signature of the *P. cactorum*-cluster OTU (>8%). This plant was classified as healthy in year 1. RV_PR_C003-B1; pictured Dec. 19, 2017.

Pulgas Ridge

Summit (PR-A004)

Species isolated: none

Site ID	CON_PR_A004	RV_PR_A004	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**			
No. samples sequenced	4	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	10.45%	0	10.45%
P. cactorum-cluster	0.49%	1.09%	1.09%
P. uliginosa-cluster	0	0.10%	0.10%
P. hydropathica	0	0.06%	0.06%
P. fallax	0	0.02%	0.02%

PR_A004 (planted in 2005) and the corresponding control area were sampled only in year 2. All plants sampled in both areas had evidence of dieback, though no *Phytophthora* were isolated. Of species of concern, we recorded only strong signatures of the *P. cactorum* complex (all in association with *Mimulus*).

Both *P. hydropathica* and *P. fallax* are Clade 9 species, of which we did not recover any isolates. The former causes crown dieback in Eucalyptus; the later causes damping-off and root rot especially in the Ericaceae. Both are consistent with the history and current appearance of the site.

Arctostaphylos with dieback.
RV_PR_A004-B3; Dec. 11, 2018.

Purisima Creek Redwoods

Purisima Creek (PC-A001)

Species isolated: *P. multivora*, *P. syringae*

Site ID	CON_PC_A001	PLND_PC_A001	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**	*mul*	*syr*	
No. samples sequenced	6	12	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	0.83%	7.83%	7.83%
P. syringae	0	0.42%	0.42%
P. nemorosa-cluster	0	0.39%	0.39%
P. tentaculata	0.03%	0.35%	0.35%
P. citricola-complex	0.16%	0.01%	0.16%
P. sp. unknown	0	0.14%	0.14%
P. siskiyouensis	0.03%	0.11%	0.11%
P. lateralis	0	0.07%	0.07%
P. psychrophila	0	0.03%	0.03%
P. chlamydospora	0.02%	0	0.02%
P. cryptogea-complex	0.01%	0.01%	0.01%

We sampled a proposed project area along the Purisima Creek Trail and Purisima Creek itself. The control area was along Whittemore Gulch Trail. In both areas we found strong signatures of the *P. cactorum*-cluster, although this species was not isolated. The control area contained *P. multivora*, recovered from Mimulus uphill of the Whittemore Gulch Trail, with no apparent symptoms being caused. *P. syringae* was recovered from streamside *Alnus rubra*, again with no apparent symptoms being present. As with the riparian zone sampled at La Honda (LH_F001) we found signatures of *P. siskiyouensis*, which is associated with alder disease although the alders at this site appeared healthy.

Both control and planned areas contained signatures of *P. tentaculata*, which was only detected here and at the Big Dipper Ranch of Skyline Ridge. To confirm this detection, we returned and sampled the planned area in year 2. While we were unable to bait this species, we did repeat the detection of the *P. tentaculata*-OTU, albeit at a lower within-sample relative abundance in year 2.

Rancho San Antonio

Annex Garden (RS-B001)

Species isolated: none

Site ID	CON_RS_B001	RV_RS_B001	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**			
No. samples sequenced	6	2	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. chlamydospora	0.09%	5.87%	5.87%
P. citricola-complex	0	5.02%	5.02%
P. sp. unknown	0.13%	4.29%	4.29%
P. hydropathica	0.09%	3.54%	3.54%
P. cactorum-cluster	1.84%	0	1.84%
P. psychrophila	0.07%	1.57%	1.57%
P. nemorosa-cluster	0	1.34%	1.34%
P. syringae	0	1.31%	1.31%
P. cinnamomi	0	1.04%	1.04%
P. lateralis	0.01%	0.58%	0.58%
P. megasperma-cluster	0.03%	0	0.03%
P. primulae	0.03%	0	0.03%

While we did not isolate any *Phytophthora* spp., the large number of OTU detections is consistent with the yard-like landscaping of nursery-grown plants around the Annex Garden. We cannot determine if the detections are attributable to the RS_B001 planting itself, or are remnants of what was present at the Annex garden prior to the outplanting. A large number of plants at the garden appeared near dead, although it did not appear *Phytophthora* was necessarily indicated.

The control area was located along the Wildcat Canyon and High Meadow trail. Again, we failed to isolate any *Phytophthora* spp. although the *P. cactorum*-cluster was detected at relatively high frequency.

Rancho San Antonio

RSA Field Office (RS-D001)

Species isolated: *P. syringae*

Site ID	CON_RS_D001	RV_RS_D001	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*syr*		
No. samples sequenced	5	4	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. psychrophila	1.38%	0	1.38%
P. cactorum-cluster	0.41%	0	0.41%
P. syringae	0.37%	0.03%	0.37%
P. brassicae	0	0.04%	0
P. citrophthora-cluster	0	0.03%	0
P. citricola-complex	0.02%	0	0.02%

All plants at the RSA Field Office planting appeared healthy; no *Phytophthora* spp. were isolated and we recorded only minor DNA signatures of pathogenic species.

The control area was taken along the Coyote Trail, from which we recorded strong signatures of *P. psychrophila*, *P. cactorum*-cluster, and *P. syringae* OTUs. *P. syringae* was also recovered from a healthy *Mimulus*. No disease was recorded at the site.

Mimulus from which *P. syringae* was recovered via baiting and detected via Illumina. CON_RS_D001-F2; Dec. 16, 2018.

Rancho San Antonio

Rhus Ridge (RS-A001)

Species isolated: none

Site ID	CON_RS_A001	RV_RS_A001	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**			
No. samples sequenced	4	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	1.66%	9.26%	9.26%
P. syringae	0	0.52%	0.52%
P. nemorosa-cluster	0.22%	0.27%	0.27%
P. psychrophila	0.18%	0	0.18%
P. uliginosa-cluster	0.14%	0	0.14%
P. sp. unknown	0	0.14%	0.14%
P. cryptogea-complex	0	0.08%	0.08%
P. lateralis	0	0.07%	0.07%
P. cambivora-complex	0	0.05%	0.05%

At this location we sampled in the planting immediately uphill of the parking lot (reveg site RS_A001) and uphill of the Rhus Ridge Trail (control site). The reveg site was also immediately downhill of a recent housing development. Consistent with other sites within Rancho San Antonio, we found a high frequency of the *P. cactorum*-cluster and *P. syringae* OTUs, however no disease was noted and no *Phytophthora* spp. were isolated.

The *P. nemorosa*-cluster, *P. psychrophila*, and *P. uliginosa*-cluster were widespread OTUs detected throughout our surveys and should not be of immediate concern. Low-level detections of the *P. cryptogea*-complex, *P. lateralis*, and *P. cambivora*-complex were detected. These OTUs were generally abundant at lower frequency throughout MROSD preserves.

Rancho San Antonio

Equestrian Lot (RS-C001)

Species isolated: *P. cactorum*, *P. crassamura*, *P. syringae*

Site ID	CON_RS_C001	RV_RS_C001	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*cac, syr*	*cra*	
No. samples sequenced	1	1	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	2.71%	0	2.71%
P. syringae	0.72%	0	0.72%
P. cinnamomi	0.05%	0	0.05%

We were unable to adequately sequence samples associated with the Equestrian parking lot project (RS_C001) and adjacent control area uphill from the PG&E Trail, with only 1 sample for each area containing DNA of sufficient quality for sequencing on the Illumina platform. However, we baited *P. crassamura* from two samples at the outplanting (from *Quercus lobata* and *Frangula californica*), and *P. cactorum* and *P. syringae* from the control area (from *Heteromeles arbutifolia* and *Mimulus aurantiacus*, respectively). At the time of the sampling, all plants appeared healthy at both sites.

RS_C001 was planted in the months prior to surveys. Likely *P. crassamura* was brought in on the plants, as this species is recently emerging as common within the restoration nurseries in the area. The recovery and detection of *P. cactorum* and *P. syringae* are consistent other detections of these species within the Rancho San Antonio area.

Plants from which *P. crassamura* was recovered at the Rancho San Antonio Equestrian Lot: RV_RS_C001-A2 and RV_RS_C001-B1 . Sampled Dec. 16, 2018.

Russian Ridge

Mindego Gateway

Species isolated: *P. cambivora*, *P. ramorum* (foliage only), *P. cactorum*, *P. nicotianae*,

Site ID	CON_RR_A001	RV_RR_A001	RV_RR_A006	
Site Status	unplanted	revegetation site	revegetation site	
***Phytophthora* sp. isolated**	*cam, (ram)*	*cac, nic*	*cac*	
No. samples sequenced	8	7	5	
OTU	**Maximum within-sample relative abundance**			**Max detection by area**
P. quercina-cluster	7.84%	0	0	7.84%
P. syringae	0	0	5.60%	5.60%
P. cryptogea-complex	0.21%	0	4.31%	4.31%
P. cinnamomi	0.01%	0	3.72%	3.72%
P. cactorum-cluster	0	3.59%	0.33%	3.59%
P. nicotianae	0	3.51%	0	3.51%
P. psychrophila	3.37%	3.30%	0	3.37%
P. lateralis	0	0	2.29%	2.29%
P. nemorosa-cluster	0	0	0.62%	0.62%
P. cambivora-complex	0.59%	0.01%	0.49%	0.59%
P. irrigata	0.40%	0	0	0.40%
P. citrophthora-cluster	0.07%	0	0	0.07%
P. chlamydospora	0	0.04%	0	0.04%
P. clandestine	0	0.04%	0	0.04%
P. cambivora-complex / *formosa*	0.02%	0	0.01%	0.02%
P. drechsleri	0	0.02%	0	0.02%
P. citricola-complex	0.01%	0.02%	0	0.02%
P. sp. unknown	0	0.02%	0	0.02%

The Mindego Gateway plantings had a large assortment of *Phytophthora* spp. detected. Some individuals in the reveg sites had been replanted following the death of the original planting; heat, wind, and cold were attributed to the planting failures, however we detected *Phytophthora* in some of the replanted basins (e.g. RV_RR_A001-B2).

P. cactorum was baited from all site classes, though disease was not apparent on all plants. *P. nicotianae* was also recovered from a single plant both years in RV_RR_A001, again, without causing notable crown symptoms. RV_RR_A006 had an especially large number of pathogenic and non-native OTUs not detected by baiting: *P. syringae*, *P. cryptogea*-complex, *P. cinnamomi*, and *P. lateralis*. While not baited, these detections were of sufficient quantity that

exposure has very likely occurred, we presume at the nursery. Additional baiting should be performed to determine if viable pathogens are present.

Strong detections of the *P. quercina*-cluster and *P. psychrophila* OTUs are consistent with the vegetation type within the control area and are not of immediate concern. *P. cambivora* was isolated and detected in the along the Ancient Oaks Trail. While disease was not apparent, this species could affect woody plants in the area.

P. nicotianae-positive *Arbutus.* RV_RR_A001-B1. Pictured Dec. 16, 2018. *P. nicotianae* was baited both years, however the plant had prolific growth between years.

P. cactorum-positive *Frangula.* RV_RR_A006-A3. Pictured Dec. 24, 2017. This plant had good growth by 2018; defoliated branches are attributable to deer browse. *P. cactorum* was only isolated in year 1.

P. cactorum-positive *Frangula.* RV_RR_A001-A1. Pictured Dec. 24, 2017. The plant was similarly stunted but not dead in year 2. *P. cactorum* was isolated both years.

Skyline Ridge

Skyline Ridge (SR-A001, SR-A002, SR-A003)

Species isolated: *P. cambivora*, *P. ramorum* (foliage only), *P. megasperma*, *P. cactorum*

Site ID	CON_SR_A001	DIST_TreeFarm	RV_SR_A001	RV_SR_A002	RV_SR_A003	
Site Status	unplanted	disturbed	revegetation site	revegetation site	revegetation site	
***Phytophthora* sp. isolated**	*cam, (ram)*		*cam, meg, cac*	*cac, meg*		
No. samples sequenced	11	6	9	8	2	
OTU	**Maximum within-sample relative abundance**					**Max detection by area**
P. cactorum-cluster	0	0.09%	10.62%	0	0	10.62%
P. psychrophila	3.14%	0	0.04%	0	0	3.14%
P. quercina-cluster	2.50%	0.08%	0	0	0	2.50%
P. uliginosa-cluster	1.38%	0	0	0	0	1.38%
P. cambivora-complex	0.55%	0	0.08%	0	0	0.55%
P. formosa	0.27%	0	0.01%	0	0	0.27%
P. clandestine	0	0	0	0	0.26%	0.26%
P. sp. unknown	0	0.03%	0.17%	0	0	0.17%
P. megasperma-cluster	0	0.01%	0.04%	0	0	0.04%
P. lateralis	0	0	0	0	0.04%	0.04%
P. cambivora-complex / *formosa*	0.03%	0	0	0	0	0.03%
P. citricola-complex	0	0.03%	0.01%	0	0	0.03%
P. nemorosa-cluster	0	0	0.02%	0	0	0.02%
P. cryptogea-complex	0	0	0.02%	0	0	0.02%
P. macilentosa	0.02%	0	0	0	0	0.02%

Skyline Ridge cont.

We sampled three planting areas at Skyline Ridge: SR_A001 (planted 2008), SR_A002 (planted 2009) and SR_A003 (planted 2010), a control area along Sunny Jim Trail, and (as the disturbed bur unremediated site) the Christmas Tree Farm still in operation immediately uphill of SR_A003. All but the Christmas Tree site were repeated in year 2.

Prior surveys by Phytosphere in 2016 and 2017 reported *P. cactorum* from all three planting areas, but none from the Christmas Tree Farm. We were able to repeat these detections in RV_SR_A001 and RV_SR_A002, where we also isolated *P. cambivora* and *P. megasperma*. Dieback was apparent at the site, often in association with *Phytophthora* detections. Symptoms include dieback, stunting and death, which we noted in all three planting areas.

In addition to culture-positive detections, we detected a strong signature of *P. cactorum*-OTU in association with a healthy-appearing toyon. *P. clandestine* was also detected via Illumina from the planting area; this species is not known to be present in North America and the detection may be a closely related taxa of unknown risk. Despite two species being isolated from RV_SR_A002, no *Phytophthora* OTUs were detected above 0.01% within-sample relative abundance in that site. Similar to Phytopshere, we found little evidence the Christmas Tree farm is contaminated with *Phytophthora.*

The strongest OTU signatures in the control area are consistent with the habitat type and hosts present, and are of minimal concern. We both detected and baited *P. cambivora*, which is widely distributed on MROSD lands and may cause decline in woody plants. Lastly, we found relatively strong signature of the *P. formosa*-OTU. This species is also not reported in North America and is only weakly pathogenic on the few species it has been tested on; its risk to California natives is unknown.

P. cambivora-positive *Arbutus.* RV_SR_A001-A2. Pictured Dec. 21, 2017.

Frangula, RV_SR_A001-C2. Pictured Dec. 21, 2017. We baited *P. cactorum*, which was detected along with *P. megasperma* via Illumina.

Heteromeles with >10% within-sample relative abundance for the *P. cactorum* OTU, but not crown symptoms. RV_SR_A001-B2. Pictured Dec. 15, 2018.

Skyline Ridge

Big Dipper (SR-B001, SR-B002)

Species isolated: *P. ramorum*

Site ID	CON_SR_B001	RV_SR_B001	RV_SR_B002	
Site Status	unplanted	revegetation site	revegetation site	
***Phytophthora* sp. isolated**	*(ram)*	*ram*		
No. samples sequenced	2	4	2	
OTU	**Maximum within-sample relative abundance**			**Max detection by area**
P. sp. unknown	0	3.25%	15.43%	15.43%
P. chlamydospora	0	2.09%	0	2.09%
P. uliginosa-cluster	1.13%	0	0.02%	1.13%
P. hydropathica	0	1.06%	0	1.06%
P. tentaculata	0	0.16%	0	0.16%
P. hedraiandra	0	0.13%	0	0.13%
P. riparia-cluster	0	0	0.08%	0.08%
P. lateralis	0	0.06%	0	0.06%
P. nemorosa-cluster	0	0.02%	0	0.02%
P. syringae	0	0.02%	0	0.02%

We sampled two planting areas (RV_SR_B001 and RV_SR_B002, both planted in 2011) and a single control area (CON_SR_B001) at the Big Dipper Ranch. The site has very limited public access and minor apparent disturbance aside from grazing and road remediation. Sites were also downhill of a single residence some distance from the sampling locations. As expected, we found very few *Phytophthora* within control areas. We found the *P. uliginosa*-cluster at a large number of control sites in association with *Quercus* and *Notholithocarpus*, where it apparently is not associated with acute disease. Being aerially dispersed and regionally abundant, *P. ramorum* was similarly not a surprising detection given the abundance of California bay laurel and tanoak in the area.

Greater abundance of *Phytophthora* OTUs were detected from the reveg sites, especially RV_SR_B001, which were populated with taxa more strongly associated with the nursery. The most striking of these was the detection of *P. tentaculata*. This was detected from a nursery-grown *Alnus rhombifolia* at SR_B001. We were unable to return to the site in year 2 to validate this finding. Other outplanted alders had canker and poor growth; this may be attributed to the detection of *P. chlamydospora* although this pathogen was not isolated in this location.

Sierra Azul (Mt. Umunhum)

Woods Trial (SA-G001)

Species isolated: *P. cambivora*

Site ID	CON_SA_G001	RV_SA_G001	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**		*cam*	
No. samples sequenced	5	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	1.51%	0.02%	1.51%
P. nemorosa-cluster	1.25%	0	1.25%
P. primulae	0.70%	0.05%	0.70%
P. cryptogea-complex	0	0.48%	0.48%
P. uliginosa-cluster	0.23%	0.01%	0.23%
P. syringae	0.14%	0.02%	0.14%
P. fallax	0	0.12%	0.12%
P. psychrophila	0	0.04%	0.04%
P. porri	0.02%	0	0.02%
P. cinnamomi	0	0.01%	0.01%

The Woods Trail reveg project was planted just prior to our survey in year 1. Consistent with current protocols, the site was seeded when possible and plants were small to reduce residency time in the nursery where *Phytophthora* contamination may occur. Unfortunately, we isolated *P. cambivora* from one outplanted *Frangula* and two outplanted *Heteromeles*. This species was not recovered from the control area, and was not detected via Illumina at either the reveg or control areas. *P. cambivora* was relatively widespread in our surveys despite only being baited in year 1. In many cases it was present without causing overt disease however this species is aggressive on some hosts. Follow-up is warranted to assess the reveg success of these plants, and caution should be exercised should they need to be replaced.

We found strong signatures of the *P. cactorum*-cluster in the control area, as well as other nursery-associated OTUs. *P. fallax* is a Clade 9 species for which we did not recover any isolates, which causes crown dieback in Eucalyptus. The presence of this OTU is consistent with the presence of Eucalyptus at this site.

Sierra Azul (Mt. Umunhum)

Hoita Rd. (SA-H001)

Species isolated: *P.* taxon *asparagi*

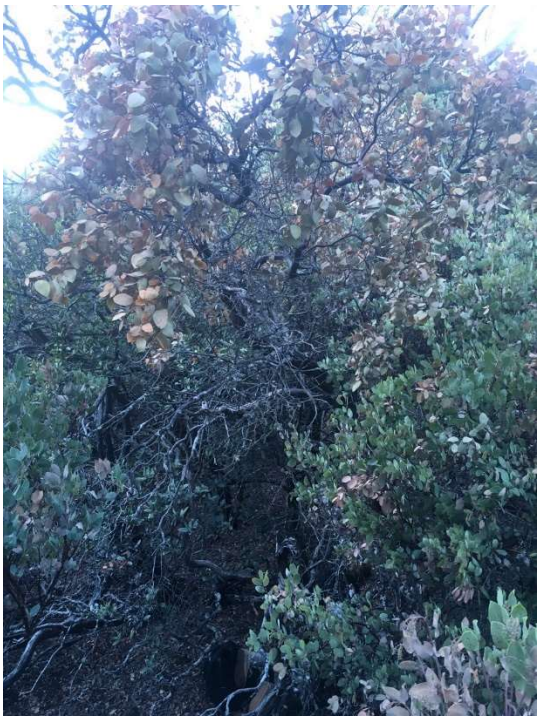
Site ID	CON_SA_H001	PLND_SA_H001	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**	*asp*		
No. samples sequenced	6	6	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. cactorum-cluster	0	35.01%	35.01%
P. asparagi	0.92%	2.32%	2.32%
P. citricola-complex	0.55%	0	0.55%
P. irrigata	0.42%	0	0.42%
P. syringae	0.33%	0	0.33%
P. rosacearum	0.24%	0.04%	0.24%
P. lateralis	0.22%	0	0.22%

Significant dieback was observed in *Arctostaphylos* at the planting site. While there are no reports of *P.* taxon *asparagi* causing infection of members of the Ericaceae (or Rhamnaceae, the isolate coming from soil collected at the base of healthy *Frangula*), the relatively new recognition of this species causing disease in similar habitats and its recovery at the site are cause for concern. Likely the full host-range of this species is not yet described and infection of both *Frangula* and *Arctostaphylos* should be investigated.

A second species concern is the *P. cactorum*-OTU which occurred at a maximum of 35% within-sample relative abundance from around declining plants. As a cluster, we cannot discern if the species present is *P. cactorum*, *P. idaei*, or *P. pseudotsugae*. However, given the abundance and known host range of *P. cactorum* we strongly suspect this species may be contributing to disease at this site. This species attacks a wide range of woody plants, including *Arctostaphylos*, predominantly causing a root or crown rot. Further evaluation of the site and repeat sampling should be undertaken before performing restoration activities.

Many other *Phytophthora* OTUs were detected in high abundance, indicating a history of prior disturbance. Despite the *Phytophthora* spp. abundance at the site, *Heteromeles* and *Frangula* in the area appeared healthy.

Healthy *Frangula* from which *P.* taxon *asparagi* was isolated. CON_SA_H001-E1; Dec. 13, 2018.



Dead and declining *Arctostaphylos* were common, especially downhill of the road. PLND_SA_H001-B2; Dec. 13, 2018.

Flagging of *Arctostaphylos* apparent from Mt. Umunhum Rd. (PLND_SA_H001), as well as unaffected toyon in the foreground; Dec. 13, 2018.

Sierra Azul (Mt. Umunhum)

Bald Mountain (SA-A008)

Species isolated: *P. ramorum*, *P. syringae*, *P. cinnamomi*

Site ID	CON_SA_A008	RV_SA_A008	
Site Status	unplanted	revegetation site	
***Phytophthora* sp. isolated**	*ram, syr*	*cin*	
No. samples sequenced	5	5	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. syringae	2.41%	0	2.41%
P. clandestine	0	0.62%	0.62%
P. sp. unknown	0.21%	0.04%	0.21%
P. megasperma-cluster	0	0.11%	0.11%
P. chlamydospora	0	0.08%	0.08%
P. cryptogea-complex	0	0.06%	0.06%

The planting site was tested by Phytosphere in July 2016, whereby toyon was positive for *P. cactorum*. Infested plants were cut at ground level in 2017 prior to our sampling in year 1, and the basins were not sampled. Despite the recovery of *P. cinnamomi* from outplanted *Q. lobata*, plants within the reveg project appeared healthy. The *P. megasperma*-cluster OTU was widespread on Mt. Umunhum, although no member from the cluster was baited. *P. clandestine* was also detected via Illumina from the planting area; this species is not known to be present in North America and the detection may be a closely related taxa of unknown risk.

We did recover both *P. syringae* and *P. ramorum* from the control area upslope from the Mt. Umunhum Trail. The madrones in the area appeared affected by some agent, showing crown dieback and in some cases basal sprouting.

Arbutus with dieback. CON_SA_B006-E2; sampled Dec. 24, 2017.

Sierra Azul (Mt. Umunhum)

Flagpole (undesignated in 2017; SA-F014 in 2018)

Species isolated: *P. crassamura*, *P. rosacearum*

Site ID	CON_Flagpole	DIST_Flagpole	RV_Flagpole	RV_SA_F014	
Site Status	unplanted	disturbed	Reveg. site	Reveg site	
***Phytophthora* sp. isolated**			*cra*	*cra, ros*	
No. samples sequenced	5	6	11	6	
OTU	**Maximum within-sample relative abundance**				**Max detection by area**
P. syringae	0	0	1.50%	0	1.50%
P. cryptogea-complex	0	0	1.13%	0	1.13%
P. megasperma-cluster	0	0	0.94%	0.72%	0.94%
P. primulae	0	0	0.30%	0	0.30%
P. psychrophila	0	0	0.15%	0	0.15%
P. porri	0	0	0.11%	0	0.11%
P. citrophthora-cluster	0	0	0	0.10%	0.10%
P. lateralis	0.08%	0	0	0	0.08%
P. rosacearum	0	0	0.01%	0.06%	0.06%
P. irrigata	0	0.05%	0	0	0.05%
P. quercina-cluster	0	0	0.05%	0	0.05%
P. chlamydospora	0	0.05%	0	0	0.05%
P. sp. unknown	0	0.04%	0	0	0.04%
P. nemorosa-cluster	0	0	0	0.03%	0.03%
P. citricola-complex	0	0	0	0.03%	0.03%

The flagpole area was sampled both years, in 2017 after the introduction of the first set of plants (RV_Flagpole) and 2018 after the second outplanting (RV_SA_F014). We additionally sampled lupins displaying dieback symptoms in the immediate area (DIST_Flagpole), and control plants uphill of a graded road above the lupins (CON_Flagpole). A significant amount of soil was deposited in the area, and non-native horticultural species were evident indicating prior introduction of nursery plants.

No *Phytophthora* spp. were isolated from soils collected in the control or adjacent disturbed areas or the planted area in 2017. In 2018, we again failed to recover *Phytophthora* from the control and disturbed areas; we did however isolate two species, *P. crassamura* and *P. rosacearum*, from four plants around the flagpole (three of which were added to the plot in 2018), leading to a concern that these two species were introduced in the SA_F014 planting.

The origin on either species is questionable. Neither *P. crassamura* nor *P. rosacearum* were baited in 2017 from any location, indicating these species may not have been biologically active the first year (presuming they were present). Illumina can inform if either OTU was present at the site in 2017, although this too may be influenced by the biological activity of either

species. Both the *P. megasperma*-cluster (of which *P. crassamura* is a member) and the *P. rosacearum* OTUs were detected at the site from a single Flagpole sample at only 0.01% within-sample relative abundance in 2017. At such a low relative abundance we cannot discern if these detections are false positives or are representative of pathogen dormancy.

Of the two, it is more likely *P. crassamura* was present prior to the SA_F014 planting. The two OTUs have different distributions across the Mt. Umunhum summit. Except for one 2017-Flagpole sample (0.01%) and one 2018-SA_F014 sample (0.06%), the *P. rosacearum*-OTU was absent from all other samples taken from the Mt. Umunhum summit (n=87). In contrast, the *P. megasperma*-cluster was more prevalent: in addition to the single 0.01% detection at the flagpole (mentioned above), in 2017 there were also two 0.01% detections at the cube (RV_SA_I001) and one 0.13% detection at Teds. We baited *P. crassamura* from one of these samples at RV_SA_I001 when the baiting was repeated in 2018.

It is also entirely possible *P. crassamura* was already present but was also re-introduced to the flagpole in SA_F014. Notably, *P. crassamura* was also baited from other 2018 projects: Webb Creek Bridge (RV_BCR_A004) and the Rancho San Antonio Equestrian Lot (RV_RS_C001). To investigate this likelihood, we processed soil from the base of nursery plants grown in the same greenhouse as the RV_SA_F014 plants. Plants were designated for two future revegetation projects: SCNT (consisting of 5 *Frangula*, 3 *Arbutus*, and 3 *Ribes* plants) and KMM (consisting of 3 Kings Mountain Manzanita plants). We did not bait any *Phytophthora* spp. from these plants. We also did not detect the *rosacearum*-OTU, however potting mix taken from the SCNT plants contained DNA matching the *megasperma*-cluster (1.24% within-sample relative abundance).

Given the abundance of *P. crassamura*-detections and three positive isolations (two at the flagpole and one at the summit), this species is present at the Mt. Umunhum summit and may common within planted areas.

In regards to the two remaining OTUs detected at the site in high abundance, *P. syringae* and *P. cryptogea*-complex, *P. syringae* was detected both years from the Flagpole location and should be considered at risk of being present. As *P. cryptogea* and *P.* sp. *kelmania* (both being indicated as part of the *P. cryptogea*-complex) are also reported in the Mt. Umunhum summit area, caution should be exercised should these species also be present at the flagpole location.

Lastly, despite concerning symptoms on the lupins, both the control and disturbed areas had very little *Phytophthora* presence as detected by Illumina and by baiting.

Lupinus albifrons with symptoms characteristic of *Phytophthora*, though we found no evidence of contamination. DIST_Flagpole-D5; pictured Dec. 24, 2017.

Sierra Azul (Mt. Umunhum)

Summit (SA-F005, SA-F012, Teds)

Species isolated: *P. cryptogea*-complex, *P. ramorum* (foliage only)

Site ID	DIST_Teds	CON_SA_F005	RV_SA_F005	RV_SA_F012	
Site Status	disturbed	unplanted	revegetation site	revegetation site	
***Phytophthora* sp. isolated**	*cry, (ram)*				
No. samples sequenced	5	5	6	5	
OTU	**Maximum within-sample relative abundance**				**Max detection by area**
P. hydropathica	0.50%	0	0	0	0.50%
P. citrophthora-cluster	0	0.03%	0	0.25%	0.25%
P. brassicae	0.24%	0	0	0	0.24%
P. cryptogea-complex	0.16%	0.06%	0	0.17%	0.17%
P. megasperma-cluster	0.13%	0	0	0	0.13%
P. chlamydospora	0.10%	0	0	0	0.10%
P. sp. unknown	0.08%	0	0	0	0.08%
P. psychrophila	0.02%	0	0	0	0.02%

These reveg sites are below the immediate summit of Mt. Umunhum including the weather shelter (SA_F005) and stairwell plantings (SA_F012), both completed just prior to the 2018 sampling. We also sampled the hillside adjacent to the landing zone and main parking lot (DIST_Teds), which was first assessed by Phytosphere in 2016, recovering *P. cryptogea* and *P.* sp. *kelmania.* In 2017 we were only able to obtained an Illumina detection of these species (both part of the *P. cryptogea*-complex OTU), although we did bait a member of the *P. cryptogea*-complex in 2018. We also isolated *P. ramorum* from lesioned bay laurel located below the parking lot.

Illumina and recovery data indicate the area DIST_Teds is highly contaminated relative to other sites at the Mt. Umunhum summit. It is unclear were the source of the contamination may have come from. *P. crassamura* (a member of the *megasperma*-cluster) is present from other Mt. Umunhum samples and is of management concern. *P. hydropathica*, *P. brassicae*, and *P. chlamydospora* may be legacy detections from the Air Force Station as they do not occur in other plants originating from the nurseries used by MROSD at the

Madrone from which we recovered *P. cryptogea*. The OTU was detected in 2017 with subsequent isolation of the pathogen in 2018. DIST_Teds-H1; pictured Dec. 24, 2017.

time of remediation the and/or are inconsistent with environment and known ecology of the pathogen.

The control area associated with these plantings is relatively free of *Phytophthora* contaminants, with only minor detections of the *P. cryptogea*-complex. The planted areas have similarly low incidence of detections, with only signatures of *P. cryptogea*-complex and the *P. citrophthora*-cluster. We observed no symptoms of *Phytophthora* infestations in the planted or control areas.

DIST_Teds, located below the landing zone and main parking lot at Mt. Umunhum. Note the deposition of soil, indicating high levels of disturbance. *P. cryptogea* was isolated from this area; the bay laurel from which we recovered *P. ramorum* is pictured in the upper right corner.

Sierra Azul (Mt. Umunhum)

Summit (SA-F013, SA-I001, SA-F001, SA-F002)

Species isolated: *P. crassamura*, *P. pseudotsugae*

Site ID	CON_SA_F013	RV_SA_F013	CON_SA_I001	RV_SA_I001	RV_SA_F001	RV_SA_F002	
Site Status	unplanted	revegetation site	unplanted	revegetation site	revegetation site	revegetation site	
***Phytophthora* sp. isolated**				*cra*		*pse*	
No. samples sequenced	6	6	3	5	4	5	
OTU	**Maximum within-sample relative abundance**						**Max detection by area**
P. cambivora-complex / *formosa*	0	0.87%	0	0	0	0	0.87%
P. sp. unknown	0	0	0.19%	0	0.22%	0	0.22%
P. lateralis	0	0.21%	0	0	0	0	0.21%
P. cactorum-cluster	0	0	0	0	0.11%	0	0.11%
P. chlamydospora	0	0	0.10%	0	0.04%	0	0.10%
P. cambivora-complex	0	0.07%	0	0	0	0	0.07%
P. hydropathica	0	0	0.06%	0	0	0	0.06%
P. syringae	0	0	0	0.01%	0.05%	0	0.05%
P. citrophthora-cluster	0.04%	0	0.01%	0	0	0	0.04%
P. cinnamomi	0	0	0.02%	0	0	0	0.02%
P. megasperma-cluster	0	0	0.01%	0.01%	0	0	0.01%
P. uliginosa-cluster	0.01%	0	0	0	0	0	0.01%

Sierra Azul (Mt. Umunhum) Summit cont.

The Mt. Umunhum summit plantings discussed here include the experimental planting (RV_SA_F001, planted 2016), adjacent to the information shelter and immediate summit area (RV_SA_I001, planted 2017), east of the Cube (RV_SA_F002, planted 2018), and a new planting around the ceremonial circle (RV_SA_F013, planted 2018). Control areas include one site adjacent to the experimental planting (CON_SA_F013) and one east of the Cube (CON_SA_I001). CON_SA_F013 and RV_SA_I001 were sampled both years; the rest were either sampled in 2017 (RV_SA_F001) or 2018 (RV_SA_F013 and RV_SA_F002).

With the exception of a strong detection of an unknown *Phytophthora* sp. in CON_SA_I001, the control areas were relatively free of *Phytophthora.* Two *Phytophthora* sp. were recovered from outplanted areas, and two areas RV_SA_F013 and RV_SA_F001, had strong signatures of pathogenic species *P. cactorum* and *P. cambivora / P. formosa* (either may be indicated, however *P. cambivora* is the more likely OTU). The *P. lateralis* OTU was detected in a number of sites, however the identity of these species and risk to native vegetation in the area remains unknown. As a pathogen of Cupressaceae, this pathogen has been detected in nurseries, but no known infections by *P. lateralis* in vegetation outside of ornamental plantings have been reported in this region of California.

The detection of *P. crassamura* in RV_SA_I001 in 2018 was preceded by the detection of this taxa via Illumina in 2017 (as noted in the discussion of this *Phytophthora* under the Flagpole sites). While not detected in other sites at the Mt. Umunhum summit, the *P. megasperma*-cluster OTU was abundant in other nearby locations and should be a high risk for establishment. We also baited *P. pseudotsugae* from a *Penstemon* outplanted at the East Summit planting (RV_SA_F002). This species was thought to only infect Douglas-fir, however we and Bourret (2018) recovered this species in numerous locations in the absence of this host. Its threat to other species is as of yet undetermined.

Monardella RV_SA_I001-B2 from the rock garden at the summit of Mt. Umunhum. The *P. megasperma*-cluster OTU was detected in year 1 via Illumina (0.01% within-sample relative abundance); *P. crassamura*, a member of this cluster, was subsequently isolated in year 2. Pictured Dec. 24, 2017.

Sierra Azul (Hendrys)

Hendrys (SA-L001)

Species isolated: *P. ramorum* (foliage only)

Site ID	CON_SA_L001	PLND_SA_L001	
Site Status	unplanted	planned	
***Phytophthora* sp. isolated**	*(ram)*	*(ram)*	
No. samples sequenced	7	7	
OTU	**Maximum within-sample relative abundance**		**Max detection by area**
P. uliginosa-cluster	13.64%	0.98%	13.64%
P. ramorum	1.01%	0	1.01%
P. syringae	0.92%	0.32%	0.92%
P. chlamydospora	0.55%	0	0.55%
P. sp. unknown	0.40%	0	0.40%
P. nemorosa-cluster	0.19%	0.03%	0.19%
P. lateralis	0.05%	0	0.05%
P. cambivora-complex	0.03%	0	0.03%
P. megasperma-cluster	0	0.02%	0.02%
P. quercina-cluster	0	0.01%	0.01%

The site along Hendrys Creek contained ample evidence of prior disturbance: old retaining walls, foot bridges, and ornamental plantings were present in the area. We sampled natural regeneration within the proposed reveg areas; control samples were taken upstream of these or an adjacent upslope hills. Consistent with the disturbance, numerous *Phytophthora* taxa were detected at low levels via Illumina. We were only able to isolate *P. ramorum*, which is abundant regionally. The other OTU detected at significant abundance was the *P. uliginosa*-cluster which we do not currently suspect as threatening forest health. Streamside sampling of *Alnus rhombifolia* accounts for the detection of *P. chlamydospora*, as consistent with samples taken from alder in other sites. Aside from the abundance of *P. ramorum*, the site appears relatively healthy.

Appendix C: Analysis of factors contributing to site infestation

Introduction

While *Phytophthora* spp. were widespread as detected via baiting and Illumina, substantial variation exists between the overall infestation levels of individual areas (Fig. 15, Fig. 16). Many factors can influence the introduction and establishment of a given *Phytophthora* species, as well as their total diversity. Most notably among these are the plant community and disturbance history: many plant taxa (and their associated communities) have historically been host to *Phytophthora* infestations, and some disturbances, especially the movement of nursery plants and soil, are more strongly associated with the introduction of new species.

Phytophthora diversity was greatest on plant genera identified in prior studies (Fig. 10). We also detected a number of nursery-associated species when we sampled nursery-grown plants within reveg areas (Table 5, Fig. 8). However, these factors alone cannot explain all the variation in infestation between areas. Management of *Phytophthora* is aided by *a priori* information regarding a site's infestation status. To this end, we performed an exploratory regression analysis to assess if multiple disturbance, habitat class, and topographical variables can predict if future sites should be classified as highly contaminated, or relatively free of *Phytophthora*.

Methods

Two analyses were performed: first we determined if disturbance, habitat class, and topographical variables influence our ability to successfully bait a *Phytophthora* from a given sample; a second analysis determined what factors contribute to the overall infestation score of a given site or area. All analyses were performed in R (version 3.5.0).

To calculate the odds of successfully baiting any *Phytophthora* spp., we built a logistic regression model utilizing the two variables we considered most likely to influence baiting success: the site type (control, disturbed/planned, or reveg) and the general habitat class (riparian, upland-evergreen, woodland, chaparral/shrub, or rocky-forb). It was necessary to reduce more detailed habitat descriptions as specified by MROSD to the five broader classes for analysis (Appendix C Table 1). When more than one descriptor could be applied to a given area, we selected the habitat type best matching the genera we sampled. Woodland areas were differentiated from chaparral/shrub habitat by the presence of overstory hardwood, despite often times sharing the same species composition in the understory. Riparian areas were exclusively dominated by an evergreen overstory; upland-evergreen vegetation was distinguished from this by the presence of moving water and in most cases alder. Lastly, the rocky-forb class applies exclusively to the summit plantings of Sierra Azul.

After the importance of site type and habitat class were determined, we added the following variables to see if their inclusion increased our ability to predict baiting success:

1. preserve (which impacts many factors, including habitat type, usage frequency, and disturbance history)
2. gravimetric water content (GCW) of the sample
3. distance to risk factors associated with the spread of *Phytophthora* (trails, streams, and gates)
4. plant health (healthy or unhealthy), and

5. topography (elevation, slope, aspect)

A similar approach was performed to model total infestation score by site and by area (combined reveg, disturbed, or planned site(s) and the corresponding control site). To quantify the disturbance level of a given area, we collated information about each reveg project and preserve (Appendix C. Table 2). Major disturbance classes included the presence or absence of the following: ornamental plantings, restoration plantings, agricultural use (plants), agricultural use (grazing or ranching), activities associated with the movement of soil (grading, roads, excavation, dumping), building construction, and logging. As with *Phytophthora* species, these were ranked by their likelihood for introducing *Phytophthora*, with nursery plantings being considered highest risk and ranching and logging being scored the lowest risk.

Linear regression was used to model total infestation score. Because no combination of variables significantly predicted infestation score of a given area, we focused on modeling infestation score by site. After fitting a model with variables site type (control, disturbed, or reveg) and the general habitat class (riparian, upland-evergreen, woodland, chaparral/shrub, or rocky-forb) we tested these factors:

1. preserve
2. public access (open or closed) and usage frequency (low, moderate, high) of the location
3. distance to risk factors associated with the spread of *Phytophthora* (trails, streams, and gates, averaged across all samples collected in the site)
4. topography (elevation, slope, aspect, each averaged across all samples collected in the area)
5. disturbance measures (total number of disturbances, disturbance ranking, presence/absence of a given disturbance)

Once a final set of explanatory variables were settled upon, we additionally modelled different infestation measures: the number of *Phytophthora* species recovered and number of species / sample as detected via baiting; the number of OTUs detected, the number of OTU detections, and the number of OTUs / sample as detected via Illumina; and, finally, the baiting infestation score and the Illumina infestation score separately.

Results

The odds of baiting *Phytophthora* varied significantly by site type (control, disturbed, or reveg) and the dominant habitat class (riparian, upland-evergreen, woodland, chaparral/shrub, or rocky-forb). The effect was greatest by habitat class, and the effect of site type was statistically consistent across all habitats. Greatest baiting success was observed in the riparian and woodland, chaparral/shrub areas, particularly if the sample was taken from an outplanted nursery plant (Appendix C. Fig. 1).

After the inclusion of these variables, other factors which may influence the establishment of *Phytophthora* were relatively less important or had a negligible effect on our ability to predict the odds of baiting *Phytophthora*: GCW, elevation, slope, aspect, distance to features associated with the spread of *Phytophthora*. The odds of recovery did vary significantly by plant health, however plants were more likely to be positive if they were classified as healthy.

As with the odds of baiting *Phytophthora*, total infestation score of a given site was strongly determined by the dominant habitat class (Appendix C. Fig. 2). Site type was not a significant factor, consistent with reveg/disturbed areas not having consistently greater amounts of infestation than their corresponding control areas. While no topography measures significantly influenced infestation score, distance to the nearest stream or gate did have an impact whereby sites which were further away from these features had overall lower infestation scores.

Total infestation score was the best-predicted response variable, however the model poorly predicted infestation outcomes (multiple $R^2 = 0.3356$). Under no response did any of the three disturbance measures (total number of disturbances, disturbance ranking, presence/absence of a given disturbance) have a significant impact upon model performance.

Preserve was a significant factor in both analyses, indicating that the risk of *Phytophthora* establishment does vary by some condition experienced over a broader geographic range. This was most noticeable for the relatively low baiting success and infestation scores experienced at El Corte de Madera and Bear Creek Redwoods, which had vegetation classes associated with higher infestation scores (Appendix C Table 1).

Conclusion

We emphasize the exploratory nature of this analysis, particularly in light of the generalizations required to summarize vegetation class, disturbance history and other explanatory and response measures. We also emphasize that while variation in infestation and baiting success does vary across the different sites, no areas were free of high-risk or very high-risk species. While some sites may be more receptive to *Phytophthora* or have had infestations for longer periods, all sites meet the minimum requirements for *Phytophthora* establishment.

In general, areas at highest risk of being or becoming contaminated were those in the riparian, woodland, and chaparral/shrub vegetation classes, especially if the site had been revegetated with nursery plants. These three habitat classes were also associated with significantly larger infestation scores. After the inclusion of these variables, other factors which may influence the establishment of *Phytophthora* were relatively less important or had a negligible effect on our ability to predict site risk: plant health, elevation, slope, aspect, usage and distance to features associated with the spread of *Phytophthora*, and prior disturbance history. These factors are still important to consider when assessing the risk an area may be or may become infested by *Phytophthora*; that they are less predictive is largely because of the complex history of the area and the broad distribution of *Phytophthora*, particularly the aggressive species.

Tables & Figures

Appendix C. Table 1. Final habitat classifications from MROSD (FINALTYPE) as extracted from the GIS veg type layers, and final general habitat type used for analysis (habitat-general), for each of the project areas we assessed for *Phytophthora* infestation. The reveg and site IDs associated with each area can be found in Table 1.

Preserve	Area name	FINALTYPE	habitat-general
Bear Creek Redwoods	Bear Crk Xmas Tree	Douglas-fir - Coast Redwood Association	upland-evergreen
	Alma College	Redwood Forest	upland-evergreen
	Webb Crk Bridge	Douglas-fir - Coast Redwood Association	riparian
El Corte de Madera Creek	chinquapin1 / 2	Douglas-fir - / Mixed Hardwoods Mapping Unit	woodland
	ECdM Lot	Redwood / Tanoak Association	upland-evergreen
	ECdM Bridge	Redwood / Tanoak Association	riparian
	King Mt. Manzanita	Douglas-fir - / Mixed Hardwoods Mapping Unit	woodland
La Honda Creek	La Honda Crk	Red Alder Series (mixed willow)	riparian
Monte Bello	Monte Bello Grass	(Mh-L) - Lower Elevation Mixed Broadleaf Hardwoods (California Bay - T	woodland
	Monte Bello Bridge	Douglas-fir - / Mixed Hardwoods Mapping Unit	riparian
Pulgas Ridge	Pulgas Forest	Coast Live Oak Series	woodland
	Pulgas A-series	Coast Live Oak Series; coyote brush - sticky monkeyflower series	chaparral/shrub
	Pulgas C-series	Coyote Brush - California Sagebrush Series; Coast Live Oak series	chaparral/shrub
	Pulgas Blue Oak	Coast Live Oak Series	woodland
Purisima Creek	Purisima Redwoods	Douglas-fir - Coast Redwood Association, red alder series	riparian
Rancho San Antonio	Equestrian Lot	Coast Live Oak Forest / Woodland	woodland
	Rhus Ridge	(Mh-L) - Lower Elevation Mixed Broadleaf Hardwoods (California Bay - T	upland-evergreen
	RSA Field Office	(Mh-L) - Lower Elevation Mixed Broadleaf Hardwoods (California Bay - T	woodland
	Annex Garden	Coast Live Oak Forest / Woodland	woodland
Russian Ridge	Mindego Gateway	grassland, mixed broadleaf	chaparral/shrub

Appendix C. Table 1 cont.

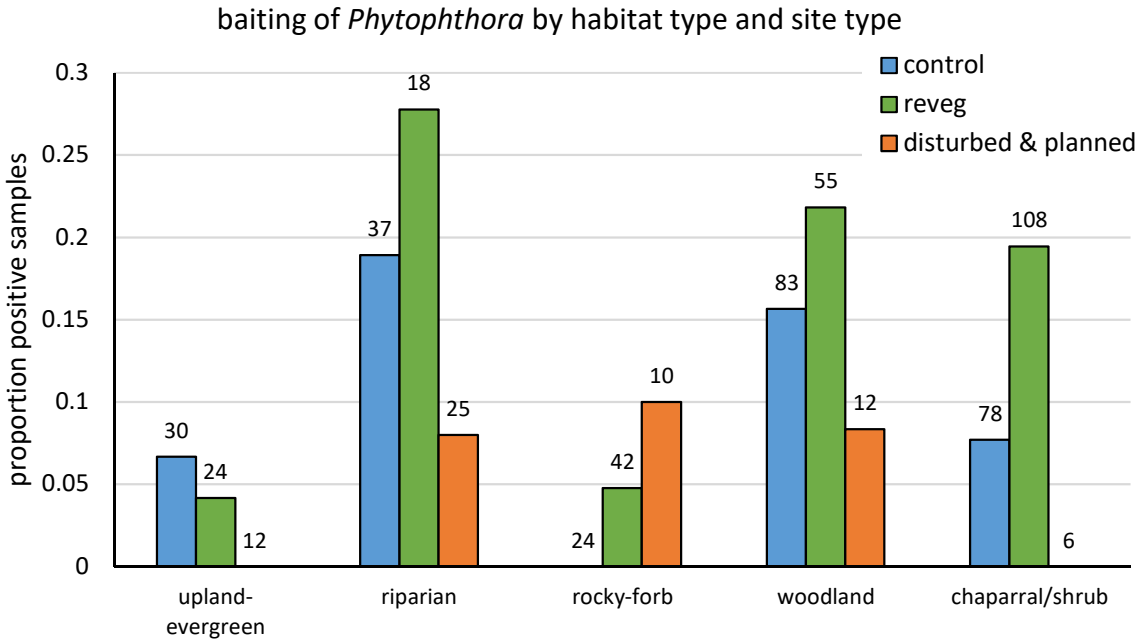
Preserve	Area name	FINALTYPE	habitat-general
Sierra Azul	Hendrys	California Bay Forest	riparian
	Mt.Um. Woods Trail	California Bay - Canyon Live Oak Multiple Series Mapping Unit.	woodland
	Mt.Um. Bald Mt.	California Bay - Canyon Live Oak Multiple Series Mapping Unit.	woodland
	Mt.Um. Hoita Rd	Foothill Pine / Big Berry Manzanita Association	chaparral/shrub
	Flagpole	Moderate Grasslands, urban	chaparral/shrub
	Mt.Um. Shelter & Stairs	California Bay - Canyon Live Oak Multiple Series Mapping Unit.; landslide, cliff, rock outcopes	rocky-forb
	Mt.Um. Summit	California Bay - Canyon Live Oak Multiple Series Mapping Unit.	rocky-forb
Skyline Ridge	Big Dipper	Douglas-fir - / Mixed Hardwoods Mapping Unit	upland-evergreen
	Skyline Ridge	Coyote Brush Open Stands (Coyote Brush / California Annual Grasslands); Higher Elevation Mixed Broadleaf Hardwoods (California Bay -	chaparral/shrub

Appendix C. Table 2. Public access, usage frequency, and disturbances for each of the project areas we assessed for *Phytophthora* infestation. The reveg and site IDs associated with each area can be found in Table 1.

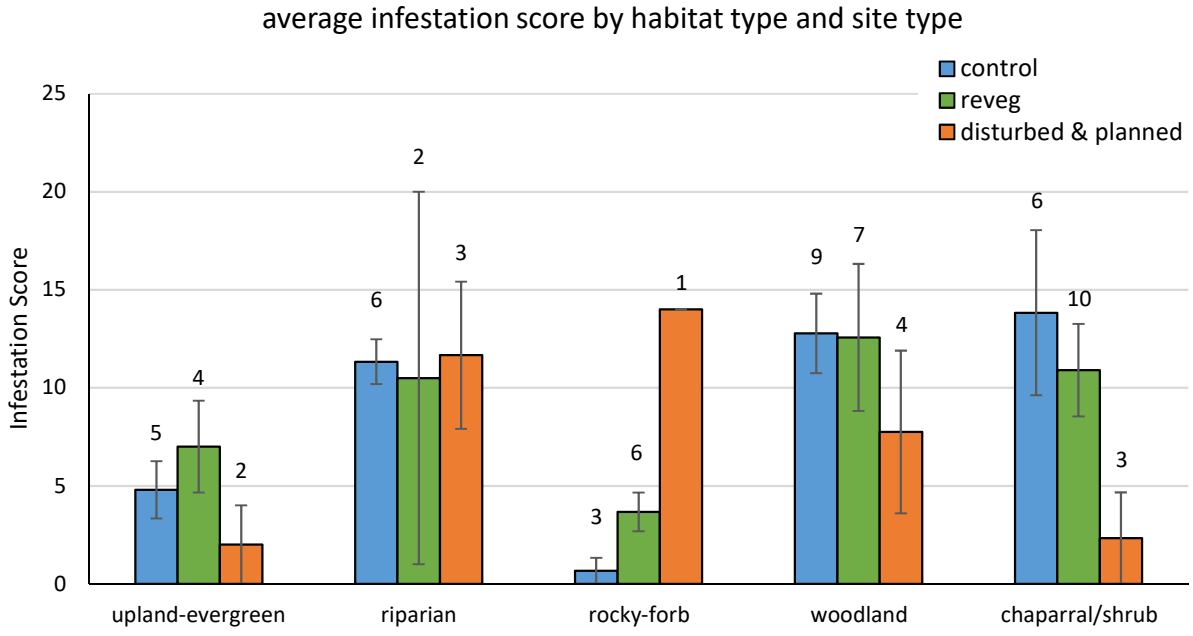
Preserve	Area name	public access	usage frequency	Disturbances (presence/absence)						
				ornamental landscaping	restoration outplanting	agriculture: plants	agriculture: grazing / ranch	grading / roads / excavation / dumping	building	logging
Bear Creek Redwoods	Bear Crk Xmas Tree	closed	light	0	0	1	0	1	0	1
	Alma College	closed	light	1	0	1	1	1	1	1
	Webb Crk Bridge	open	light	0	1	0	0	1	0	1
El Corte de Madera Creek	chinquapin1	open	heavy	0	0	0	0	1	0	0
	chinquapin2	open	heavy	0	0	0	0	1	0	0
	ECdM Lot	open	moderate	0	1	0	0	1	0	1
	ECdM Bridge	open	moderate	0	1	0	0	1	0	1
	King Mt. Manzanita	open	moderate	0	0	0	0	1	0	1
La Honda	La Honda Crk	closed	light	0	1	0	1	1	0	1
Monte Bello	Monte Bello Grass	open	heavy	0	0	0	1	0	0	0
	Monte Bello Bridge	open	heavy	0	0	0	1	0	0	0
Pulgas Ridge	Pulgas Forest	open	moderate	1	1	0	0	1	1	0
	Pulgas A-series	open	moderate	1	1	0	0	1	1	0
	Pulgas C-series	open	heavy	1	1	0	0	1	1	0
	Pulgas Blue Oak	open	heavy	1	1	0	0	1	1	0
Purisima Creek	Purisima Redwoods	open	heavy	0	0	0	0	1	1	1
Rancho San Antonio	Equestrian Lot	open	heavy	0	1	0	1	1	0	0
	Rhus Ridge	open	moderate	1	1	0	0	1	0	0
	RSA Field Office	closed	moderate	1	1	0	1	1	1	0
	Annex Garden	open	heavy	1	1	0	1	1	1	0

Appendix C. Table 2 cont.

Preserve	Area name	public access	usage frequency	Disturbances (presence/absence)						
				ornamental landscaping	restoration outplanting	agriculture: plants	agriculture: grazing / ranch	grading / roads / excavation / dumping	building	logging
Sierra Azul	Hendrys	closed	light	1	0	0	0	1	1	1
	Mt.Um. Woods Trail	open	moderate	0	1	0	0	1	0	0
	Mt.Um. Bald Mt.	open	light	0	1	0	0	1	1	0
	Mt.Um. Hoita Rd	closed	light	0	0	0	0	1	0	0
	Flagpole	open	light	1	1	0	0	1	1	0
	Mt.Um. Shelter & Stairs	open	moderate	1	1	0	0	1	1	0
	Mt.Um. Summit	open	moderate	1	1	0	0	1	1	0
Skyline Ridge	Big Dipper	closed	light	0	1	0	1	1	0	0
	Skyline Ridge	open	heavy	0	1	1	1	0	0	0



Appendix C Fig. 1. Baiting success (proportion of positive samples) of all samples separated by site type and habitat type. Numbers indicate the number of samples baited in each site:habitat combination.



Appendix C Fig. 2. Average total infestation score (+/- s.e.) for all sites within each of the five dominant habitat classes. Numbers indicate the number of sites sampled with each site:habitat combination.

Appendix D: Management of *Phytophthora* within MROSD wildlands

The broad distribution and diversity of *Phytophthora* on MROSD lands reflects the suitability of these habitats for *Phytophthora* establishment and a complex disturbance history contributing to their introduction. High-risk species were found in both outplanted and surrounding areas. Given the high rate of false negatives experienced in baiting, a single positive detection should indicate the possibility the species is present within the general area, especially in light of the wider distribution indicated by Illumina MiSeq sequencing. That being said, most *Phytophthora* spp. are not ubiquitous or uniformly distributed. The methods below are an important means to prevent their further spread and reduce their impacts in wildlands.

It is impractical, if not nearly impossible, to eradicate non-native *Phytophthora* once they are introduced. Therefore, we emphasize the need to manage *Phytophthora* to prevent the continued introduction of new species and to minimize the movement of *Phytophthora* away from heavily infested areas. Disease control principles fall into four categories: **exclusion & prevention** emphasize the need to reduce *Phytophthora* diversity and spread; **resistance** and **eradication** are applicable in limited situations, however the principles of these two methods can inform active management; lastly, **protection & suppression** include methods to manage *Phytophthora* to reduce its impacts on plant health.

Principles of disease control

Exclusion & Prevention

Exclusion is the first defense against *Phytophthora*. The principles of exclusion include any measure aimed at preventing the introduction of a *Phytophthora* species and reducing spread after their establishment. Many *Phytophthora* spp., particularly those which only infect roots and lower stems, require the physical transport of infested soil or plants to colonize new areas. While spread within a site occurs naturally via root to root contact or in overland flow, epidemic expansion of soilborne diseases on the landscape is driven by the movement of these pathogens in infested materials. For this reason, nursery plants, being the source of many new *Phytophthora* spp., are targeted as the single most important way to manage *Phytophthora*; management also focuses on minimizing the movement of infested soil, particularly during wet times of the year when pathogens are more likely to be sporulating.

Exclusion is particularly important for those areas with lower diversity, but prevention protocols are equally as important in heavily infested areas. *Phytophthora* is a diverse group of pathogens, each species having its own host range, temperature tolerances, and other adaptations. Substantial variation in aggressiveness exists even among isolates/strains within a species, and the interaction between species (or lineages or genotypes within a species) may cause more disease than either agent alone. Exclusion prevents their introduction, slows co-mingling of pathogens within an area, and establishes behaviors that minimize the movement of propagules between sites.

Importantly, exclusion principles only delay the introduction of new species, not prevent them completely. During this time management should focus on improving site conditions to suppress *Phytophthora* impact (discussed below under "suppression").

Exclusion principles applicable to MROSD:

- ✓ Direct-seed planting
  - Plants, potting media, and soil are the single most important means by which *Phytophthora* are introduced. Whenever possible, direct-seed in the field.
  - A 10% bleach soak prior to planting is recommended if seed sources are from areas contaminated with aerial *Phytophthora* species of concern (e.g. *P. ramorum*) or if seeds are collected from the soil and not plant canopies. Seeds should be bleached for a minimum of 1 minute, then rinsed with tap water and dried. Note: not all seeds will tolerate the amount of bleaching; shorter periods are less effective but should still be helpful if seeds are collected from high-risk areas.
- ✓ Pathogen-free planting stock
  - Utilize plants from nurseries implementing BMPs for managing *Phytophthora* as outlined by the California Native Plant Society and *Phytophthora* in Native Habitats Working Group (references below).
  - When possible, use smaller pot sizes to reduce nursery residency time.
  - Use a single nursery source and maintain records to track plant movement. Use nursery stock from a single block for individual projects whenever possible.
  - Employ quarantine and pre-planting testing before transport to the site. An excellent demonstration on how to do this is published by Phytosphere Research (reference below).
  - When holding and moving material, follow practices to avoid contact with potentially-infested surfaces, especially soil.
  - Dispose of left-over planting stock, rather than moving plants between locations.
- ✓ Sanitation
  - Keep vehicles on paved surfaces as much as possible, do not bring more vehicles into the planting area than necessary.
  - Remove soil and plant debris from shoes & equipment, ideally as close to the source of the debris as possible.
  - Disinfect shoes, tools, equipment, and vehicles before traveling between areas regardless of infestation status. Disinfectants (10% bleach, 70-90% isopropanol, or quarternary ammonium) must be applied to clean surfaces to be effective. Use recommended exposure times (for labelled products); if not labelled a minimum exposure time of 1 minute is recommended.
  - Use clean water sources for irrigation and cleaning, not untreated surface water.
  - Minimize work during higher-risk wet seasons when pathogens are more biologically active and cleaning is more difficult.
  - Maintain separate sets of tools for different regions when possible.
  - When applicable, bag and dispose of infected plant material from the area, rather than leaving it on site.

- ✓ Manage trails to prevent secondary spread of heavily infested soils
    - Add mulches and gravel to soil surface. Select sources that minimize the risk of contamination or are treated to remove contaminants. For example, as *Phytophthora* spp. are generally more common in moister areas, select upland, non-riparian sources for gravel. Some mulches, for e.g. Alaska yellow cedar, have compounds which may inhibit *Phytophthora* growth. Additional considerations are outlined by Phytosphere (reference below).
    - Divert or seasonally close trails leading through high-risk areas that are heavily infested and persistently saturated.
    - Establish new trails away from less-infested areas or populations of vulnerable species to prevent new introductions.
- ✓ Control drainage and surface water
    - Redirect surface water flows to avoid crossing footpaths, and direct them away from vulnerable plant populations.
- ✓ Planning and implementation considerations
    - Make information regarding infestations readily available to personnel moving between sites, or when working within a heavily infested site.
    - Manage workflow to reduce transport between sites, and move from less infested to more infested areas.

Eradication

Eradication is the complete removal of pathogen after its introduction. It involves the treatment of infested waters, soils, or plants in a manner that either physically removes the pathogen or renders it non-viable. While commonly associated with fungicide or heat applications, eradication may also be obtained by removing the pathogen's host(s).

Eradication is costly to implement and requires continuous and extensive monitoring efforts. There are few successful examples. The biggest impediment to eradication of plant pathogens in a wildlands setting is attributable to the delay between when a pathogen is introduced and when it is detected, during which time it may have spread substantially. *Phytophthora* is inconsistently baited and symptoms may develop slowly or be subtle and confused with water stress. The extent of spread is difficult to impossible to delineate, and under-treatment has resulted in failed eradications even when a pathogen is detected early in an epidemic.

There are, however, circumstances in which eradication may be beneficial or applicable. These are largely restricted to situations where the pathogen can be readily identified, eradication protocols can be easily applied, and there is a large economic burden associated with the development of disease to justify the cost of treatment. The best examples of eradication of *Phytophthora* are the treatment of infested nursery sites when the invasive and quarantined pathogen *P. ramorum* is detected.

Eradication principles applicable to MROSD:

- ✓ Fungicide treatments are an option of last resort.
    - The majority of fungicides available to treat *Phytophthora* post-infection do not kill the pathogen; rather, they just delay development and suppress symptoms for a few weeks after application.
    - Some more recently approved fungicides (e.g. Segovis) are actually curative and are labelled for application to ornamentals in California. Their utility in protecting populations of rare plants areas could be considered.
- ✓ Heat treatments may be applied under some circumstances.
    - The application of heat via steam or solarization is an effective means to kill *Phytophthora* propagules in the soil. *Phytophthora* is generally restricted to the top 20-30 cm of the soil profile and is relatively heat-sensitive. These methods have proved useful in disinfesting nursery soils.
    - The application of steaming and solarizing is often impractical in the field. Prior attempts to solarize infested planting basins at restoration sites have failed due to the difficulty in obtaining conditions required for sufficient and prolonged heating: tight seals at plastic edge, weed management, soil moisture control, and full sun. If these conditions can be met, solarization may be useful to kill *Phytophthora* in a limited area or as pre-planting treatment.
    - Relative to prevention, both steam and solarization treatments may be expensive to operate and implement. They should not be relied upon as a means for long-term management.
- ✓ Pre-emptive or post-invasion host removal within wildlands has benefits
    - Post-infection removal of tanoak has been implemented for the control of *P. ramorum* in Oregon forests. While full eradication was not obtained, this method is thought to have slowed epidemic development and may have had a net economic benefit due to the avoidance of economic burdens which accompany quarantine regulations.
    - Disease control can also be obtained by removing the host or hosts most responsible for sporulation and spread, such as the removal of bay laurel trees around heritage oaks to protect them from *P. ramorum*.
    - Even with the removal of hosts, most *Phytophthora* can persist as dormant spores in the soil for multiple years. The establishment of non-host communities, particularly grasslands, may be beneficial but cannot be relied upon to eliminate the presence of the infective propagules.

Resistance

Resistance breeding is one method being utilized for a limited number of native tree species being impacted by invasive forest pathogens. One of the most successful of these would be the selection of Port-Orford cedar for resistance to the invasive *P. lateralis*. As part of the ongoing efforts to mitigate the effects of SOD on tanoak and coast live oak, studies are being implemented to screen plants for resistance to *P. ramorum*. Efforts to collect and outplant the acorns of surviving trees are also being pursued as one means to aid the recovery of these species.

Natural variation in susceptibility and tolerance to disease does exist for many hosts, even when challenged by non-native pathogens. Resistance is more readily apparent in native populations being impacted by a particularly aggressive pathogen (such as *P. ramorum*), and the buildup of resistance is best accelerated by an active breeding program. However, the principles of resistance also apply to natural regeneration of native communities in areas were the impacts of *Phytophthora* are not so acute. Theoretically, using healthy plants from heavily infested areas as seed sources and encouraging natural establishment could increase the tolerance of those natives to *Phytophthora* infestation.

The buildup of resistance or tolerance in a natural population, however, is a very slow process during which time numerous ecological changes may occur which may be detrimental to a community already impacted by *Phytophthora* infestation. As such, more active management techniques are warranted in most circumstances.

Resistance principles applicable to MROSD:

- ✓ Prioritize the preservation of vulnerable plant populations located in *Phytophthora*-free areas
    - The selection for resistance relies upon the preservation of whatever genetic diversity is naturally inherent in native plant populations.
- ✓ Favor natural resistance in heavily infested areas
    - Monitor species of concern in heavily infested areas, tag and preserve those showing limited disease.

Protection & Suppression

Protection and suppression methods envelop a range of strategies designed to establish a barrier between a pathogen and host or reduce a pathogen's impact once it has been introduced. These methods classically include the use of fungicides, but also includes cultural practices that modify the environment in such a way that either the environment or the host are less conducive for disease. Because soilborne *Phytophthora* are so heavily reliant upon water to complete their life cycle, these practices often include the management of surface waters and wet soils.

Protection & Suppression principles applicable to MROSD:

- ✓ Assess and manage the risk of the site around your restoration objectives.
    - Multiple features of the site (for example, community composition, soil type, the history of the location) and the project objectives (for example, the need to move large quantities of soil) influence the risk of *Phytophthora* establishment, and are known *a priori* to planting. Identify which factors are present, and plan accordingly to minimize whatever risks can be managed. A discussion of risk factors is below.
    - Assess for *Phytophthora* infestation in new planting sites. This can be accomplished with baiting, but you must also include samples from known heavily infested area(s) to gauge your ability to detect *Phytophthora*.
    - Avoid planting vulnerable and valuable species in areas with a high diversity of *Phytophthora* species.
    - Within infested areas, aim to reduce risk factors. For example, plant uphill of foot traffic and in areas with increased drainage, work from upslope areas (which may have lower diversity) towards downslope areas, and only work during dry periods.
- ✓ Manage soil moisture
    - Increase drainage to avoid prolonged saturation that favors soilborne pathogen reproduction and infection. Elevating planting basins, for example, may decrease saturation within the root zone and be beneficial to individual plants; avoiding soil compaction and building swales or tile drains may be used to affect drainage and water accumulation over larger areas.
    - Exercise proper irrigation, allowing planting basins to dry between waterings.
    - When possible, also prevent drought stress as this is generally thought to predispose plants to *Phytophthora* infection.
- ✓ Increase spacing between hosts when host-specific *Phytophthora* spp. are indicated
    - For those species thought to infect a limited number of hosts, increasing the spacing between host genera may slow the spread of propagules. This effect would be heightened by interplanting non-host species.
- ✓ Biological controls are not advised
    - Many microbes (e.g. *Trichoderma*) are antagonistic to *Phytophthora* and constitute many products developed to reduce losses attributable to *Phytophthora* disease. However, we cannot predict how these products may affect the natural microfauna within MROSD preserves. As such, their use is not recommended.
- ✓ Pre-emptive treatment with phosphite-based compounds
    - Phosphorous acid materials (Fosphite, Agri-Fos, Alude) suppress *Phytophthora* mainly by stimulating the plant host's defense mechanism; these materials are less toxic than other fungicides and could be considered in certain wildland applications. Sprays or injections may be utilized, however applications are costly and must be applied at least yearly for full efficacy.

- ✓ Long-term considerations
    - As little is known about the long-term effects of *Phytophthora* infestation in native Californian communities, to inform future management decisions consider performing periodic assessments of plant health, using tagged plants in heavily infested and relatively clean areas.

Risk and *Phytophthora* management

When thinking about the risk posed by *Phytophthora*, it is important to consider a model called the "plant disease triangle". This model states that disease is greatest when three conditions are met:

1. The host is susceptible
2. The pathogen is virulent and able to cause disease, and
3. The environment is suitable for sporulation, infection, and disease development.

Moreover, these conditions must occur at the same time. For example, some *Phytophthora* species only infect one or two hosts in the field; should this species encounter other plant genera, disease will not develop even in a suitable environment. Or, *Phytophthora* needs near-saturation conditions to sporulate; should an otherwise suitable host be present but not producing susceptible tissues during this time, infection will not occur.

Each of these three factors will influence the total risk posed by a particular species. Alternatively, we can consider the risk a plant community is "receptive" and may become invaded by *Phytophthora*. On the part of the pathogen, higher risk species are those with a wide host range (able to infect multiple plant genera), with adaptations allowing them to sporulate readily and abundantly and survive less suitable seasons. Plant communities comprised of hosts able to support multiple *Phytophthora* spp., even if some do not cause substantial symptoms, are at a higher risk than a community comprised of plant species thought to be susceptible to a limited number of *Phytophthoras*; also, if hosts are densely planted, spread and disease development occur more rapidly than if they were more widely spaced. Environments or seasons that are chronically wet are much more conducive for sporulation and spread, and are thus higher risk than drier areas or times.

An excellent guide to risk management has been produced by Phytosphere entitled "Best management practices for preventing *Phytophthora* introduction and spread: trail work, construction, soil impact" (link below). Importantly, the document includes checklists which may be used to evaluate the risks present in MROSD restoration sites, as well as a discussion as to which restoration activities increase the risk of *Phytophthora* introduction and how to manage them. Below is information as it pertains to circumstances or areas which we found to be high or lower risk specific to this study.

Features of a high-risk area

Following are the characteristics of areas considered higher-risk for *Phytophthora* contamination and with a high potential impact. In general, the more risk factors present an overall greater risk for that area.

- ✓ Presence of plant taxa and communities strongly associated with *Phytophthora*.
  - In this study, we found high *Phytophthora* species diversity in numerous plant genera and their associated communities. Greatest diversity was detected from *Alnus*, *Arbutus*, *Arctostaphylos*, *Frangula*, *Heteromeles*, *Mimulus*, *Quercus* and *Rubus* (Table 6, Fig. 10).
  - While *Phytophthora* were not baited from *Lupinus* and *Notholithocarpus*, we did find a large number of *Phytophthora* OTUs associated with these genera, and they should also be considered high-risk.
  - *Ceanothus* was not tested in this study, however it has been associated with *Phytophthora* contamination in other restoration sites and should be considered high-risk as well.
- ✓ Prior baiting success of *Phytophthora* spp., particularly of those species ranked as high or very-high risk (covered in Appendix A).
- ✓ Prior disturbance history strongly associated with *Phytophthora* introduction.
  - Outplanted nursery stock, particularly of stock of unknown origin.
  - Greater public access and multi-use areas.
  - Movement of soil from out of area.
- ✓ Periodic flooding and poor drainage.
  - Prolonged time periods in which the soil is above field capacity increases *Phytophthora* sporulation, dispersal, and infection. Overland sources also increase the geographical range in which *Phytophthora* can disperse to new areas.
  - Of the sites we tested, this was particularly apparent in the La Honda Creek and Purisima Redwoods sites.

Features of a lower-risk area

Just as site-level factors may increase the incidence or diversity of *Phytophthora*, there are situations which indicate lower-risk conditions.

- ✓ Presence of plant taxa weakly associated with *Phytophthora*, or are known to host only limited *Phytophthora* diversity
  - As the goal of the study was to assess overall *Phytophthora* diversity, we targeted those plant genera known to harbor *Phytophthora.* Many other genera were included, but since these were so under-sampled, low diversity as indicated in Fig. 10 should not indicate a genus is a poor host to *Phytophthora*.

- We did, however, find less *Phytophthora* diversity in redwood and Douglas-fir dominated stands, particularly when away from streams, and areas with sparse vegetation (such as the Mt. Umunhum summit).
- Grasses, though not well explored for *Phytophthora* diversity, are thought to be poor hosts to *Phytophthora* in general and are not known to support many of the pathogenic species of concern.

✓ Limited species diversity, or recovery of *Phytophthora* only within a discrete planted area (such as a garden planting)

- Low species diversity, especially if other risk factors are present, could indicate low receptivity of an area. This would occur because the plants present are poor hosts and/or the environment is less conducive for establishment and spread. We expect this was most noticeable for the relatively low infestation rankings of the sites on the Mt. Umunhum summit.
- We found no evidence that areas with greater abundance of native *Phytophthora* species (Clade 3) or weak pathogens (many members of Clade 6) had fewer aggressive species. Rather, the presence of less pathogenic species in Clade 3 or 6 should indicate suitability to *Phytophthora* establishment.

✓ Areas with edaphic factors which suppress *Phytophthora* establishment

- Many chemical and physical properties of a soil may limit the disease attributable to soilborne *Phytophthora*. Soils that readily drain and don't hold saturation over long periods of time, owing to factors such as clay content, bulk density and slope, are less conducive to soilborne species. Increased organic matter may also be suppressive, though multiple mechanisms may be implicated.
- The impact of many factors (for example, soil pH and salinity) depends somewhat on other abiotic conditions. These factors may be inhibitory to some *Phytophthora* spp. but conducive for others, or must be implemented at such an extreme as would affect the physiology of some plant species. As such, a general recommendation cannot be formed when concerning the management of *Phytophthora* in natural ecosystems.

Resources on managing *Phytophthora* in native wildlands and restoration sites

***Phytophthoras* in Native Habitats Work Group guidelines for restoration and fieldwork**
https://www.suddenoakdeath.org/welcome-to-calphytos-org-phytophthoras-in-native-habitats/resources/

- ✓ Guidelines to minimize *Phytophthora* contamination in restoration projects
  https://www.suddenoakdeath.org/wp-content/uploads/2016/04/Restoration_guidance_FINAL-111716.pdf
- ✓ Guidance for plant pathogen prevention when working at contaminated restoration sites or sites with rare plants and sensitive habitat
  https://www.suddenoakdeath.org/wp-content/uploads/2016/04/Sensitive-contam-site-bmp-FINAL-111716.pdf
- ✓ Buying healthy plants: What to look for at a nursery
  http://www.suddenoakdeath.org/wp-content/uploads/2018/01/Buy-in-Guide-December-2017.pdf
- ✓ Guidelines to Minimize *Phytophthora* Pathogens in Restoration Nurseries
  https://www.suddenoakdeath.org/wp-content/uploads/2020/08/Restoration.Nsy_.Guidelines.final_.092216_rv_8.20.20.pdf
- ✓ Best management practices for preventing *Phytophthora* introduction and spread: trail work, construction, soil impact (prepared by Phytosphere)
  https://www.suddenoakdeath.org/wp-content/uploads/2018/06/Phytosphere.GGNPC_.BMPS_.Trails.Construction.Soil_.Import.31Jan2018.pdf

Phytosphere
http://phytosphere.com/

- ✓ Testing procedures for BMPs for producing clean nursery stock
  http://phytosphere.com/BMPsnursery/testingshell.htm
  https://www.youtube.com/watch?v=1OZGxRwuSxc&feature=youtu.be

Appendix E: Interpretation of Illumina Data

Illumina MiSeq sequencing is a powerful and sensitive tool that may detect even rare *Phytophthora* spp. within the rhizosphere. It becomes problematic, however, when the detections occur at a very low frequency and have not be verified in culture. Below is a discussion on the utility of the Illumina dataset, written with the goal of illustrating how to interpret thresholds and low-abundance detections, and help one understand some of the limitations and strengths of this method.

1) On the issues of thresholds, why two levels (0.01 to <0.095% and ≥0.095%) are reported, and the risk of false-positives. Case examples with *P. tropicalis*, *P. tentaculata*, and *P. boehmeriae.*

Illumina does not sequence each sample evenly, resulting in a different number of sequences per sample (range: 1,962 to 537,726 reads). Hence, we cannot compare the absolute number of reads of a particular OTU between one sample and another without converting each OTU to its respective relative abundance within a sample. This alternative, within-sample relative abundance, has drawbacks, namely the relative abundance of one OTU depends upon the abundance of other OTUs in the sample. *Phytophthora*, being such a small component of the soil community, will often comprise only a small fraction of the numdber of reads, especially if other genera (notably *Pythium* and other non-Oomycete sequences amplified by the ITS6 and ITS7 primers) are abundant.

Illumina detections are particularly vexing when no isolations are made to verify the biological activity of the associated species. In general, a higher within-sample relative abundance is likely associated with viability and reproduction of a particular taxa. For that reason, a lower threshold of 0.01% within-sample relative abundance indicates the DNA of a particular taxa might be present, but for analyses and more actionable interpretations higher thresholds are warranted. To be "detected" for analyses, we required a minimum within-sample relative abundance of 0.095%; there were no less than 18 different OTUs with >1% within-sample relative abundance. However, as indicated below, even detections at 0.01% within-sample relative abundance may be meaningful.

There is no consensus on a threshold required for a particular OTU to be truly "detected" and "present" as opposed to it being a false-positive. False-positives occur a number of ways: in addition to cross-contamination between soils during collection or processing, DNA from species being worked on in the laboratory during the extraction process can become incorporated into the DNA extracts used for sequencing. Sequence reads can also "jump" between samples during sequencing on the Illumina MiSeq platform. For the most part these errant reads constitute a very small proportion of each sample's read depth and are eliminated through quality control checks and minimum threshold requirements.

The most-likely instance in which a contaminant was present at above a more conservative threshold of 0.095% within-sample relative abundance occurred with the *P. tropicalis*-OTU. Contaminants of this species may have been introduced two different ways: we were performing assays with this species on a neighboring lab bench while we were extracting the samples for the last of our three Illumina runs; we also used this species as an internal control for the PMA analysis which was sequenced in this last run.

Of the 494 Illumina samples (excluding internal controls) sequenced as part of this project over three separate Illumina runs, *P. tropicalis* was recorded from 64 samples. Of these, 63 samples were part of the last Illumina run. The *P. tropicalis* OTU comprised 52.75% of the total number of reads in the one errant sample which is clearly the result of contamination; this OTU comprised between 0.01 and 3.3% of the total number of reads within the remaining 62 samples. Of the samples sequenced in the first and second Illumina runs, the *P. tropicalis*-OTU was detected in only 1 sample, at a within-sample relative abundance of only 0.01%. For these reasons, we consider the *P. tropicalis*-OTU to be an aberrant OTU and it has been removed from our analysis.

Other instances in which a particular OTU is detected at low frequency are not so easily to discern as being truly present or as a false-positive. A particular OTU may be present only at a low frequency for a number of reasons. The procedures used in this analysis amplifies and sequences all DNA in the soil for which there are primer sites; this includes remnant or "free" DNA from cells which are no longer viable. DNA is incredibly stable in soils even without an intact cell; while detection of these DNA fragments no longer indicates the presence of a live phytopathogen, it does indicate a history of this taxa's introduction. Thusly, a low-frequency species may either be a relatively new introduction to the site, in which case the *Phytophthora* hasn't had time to build up its population relative to the amount of DNA already present there, or it may be the result of a failed, but remnant, introduction. Biases in the Illumina platform, as documented in sequencing of mock-communities, are also prevalent whereby some species are underrepresented in some samples depending upon the mixture of other species used. (Correspondingly, some species are overrepresented.)

There are two notable instances in which OTUs were indicated at below the 0.095% within-sample relative abundance detection threshold, but are very likely present at the site.

The first of these occurs in the case of *Phytophthora boehmeriae*. *P. boehmeriae* has never been detected in the United States, yet in 2017 the *P. boehmeriae*-OTU comprised 0.01% of the DNA reads in a single sample taken from Pulgas Ridge Open Space (sample RV_PR_B006-C1). In 2018 we again only recorded this OTU from a single sample, located in the same area as 2017 albeit from a different plant (sample RV_PR_B006-A1). Again, at only 0.02% the within-sample relative abundance was low and below conservative detection thresholds. We did, however, recover an isolate from this sample with an ITS sequence matching *P. boehmeriae*. We are currently investigating the morphological characteristics of this isolate and sequencing the COX region to verify this particular isolate is *P. boehmeriae* and not a novel, closely related species.

The second case occurs with the potential detection of *P. tentaculata*. This particular OTU is of concern given its recent introduction to wildlands via the outplanting of nursery stock during restoration, and its apparent virulence on a number of native plant genera. The *P. tentaculata*-OTU was present in 2017 in two samples at Purisima Creek Redwoods (PLND_PC_A001-A1, 0.35%; CON_PC_A001-D2, 0.03%) and in one sample at the Big Dipper Ranch in Skyline Ridge (RV_SR_B001-B2, 0.16%). We were unable to return to Big Dipper in 2018, however we did return to PLND_PC_A001 where *P. tentaculata* was detected from 3 samples (including PLND_PC_A001-A1) with within-sample relative abundances ranging between 0.03-0.05%. The *P. tentaculata*-OTU was not recorded in any other samples above 0.01% within-sample abundance. The only other instance in which there was any record of this OTU being present occurred in a single sample from CON_SA_H001, where by it constituted

only 2, or 0.0007%, of the 287,358 total reads in this sample (and was subsequently eliminated). We were unable to isolate *P. tentaculata* in either year, however the repeatability and limited distribution of these detections leads us to conclude that *P. tentaculata* was introduced at PLND_PC_A001 and likely RV_SR_B001. We cannot however, determine at this time if *P. tentaculata* is actively propagating at the site or if these detections are the result of a failed introduction.

2) Internal Controls

To aid the interpretation of the Illumina dataset, we added internal positive and negative controls to each Illumina run: mock-communities in which PCR products of known species were combined in known proportions, and a water-blank sample in which no *Phytophthora* DNA should be present. We performed three distinct Illumina runs as part of the analysis: one in year 1 (run 1, including all samples collected in Dec. 2017), and two in year 2 (run 2, including all samples collected only in Dec. 2018, plus some samples repeated from year 1; and run 3, including some repeat soil samples and extracts from the PMA-nonPMA analysis). Each run has its own set of controls, which are analyzed separately.

The final output of these controls may differ from the expected results for numerous reasons: laboratory contaminants, particularly of species being grown in the vicinity, are notoriously difficult to eliminate completely and may be introduced over numerous stages during the process; PCR may not amplify all member species with equal efficiency; lastly, incorrect assignment of sequences to samples during the final sequencing stage (also known as “index-hopping”, a phenomenon in Illumina sequencing technology).

Mock-community (positive control)

The addition of a mock-community, as a positive control, fulfills two functions: it tests our ability to accurately determine the relative abundance of those taxa within the sample, and it assesses what threshold should be used to screen out unexpected, contaminant sequences. Contaminant Illumina sequences are usually rare and comprise the lowest relative abundances within each sample. The relative abundance threshold is set, then, to a point that eliminates all such unexpected OTUs while keeping those which were added to the sample.

Our mock community was comprised of ITS6 and ITS7 amplified PCR products from 7 *Phytophthora* and 3 *Pythium* species. All PCR products were normalized to 5 ng/ul concentration, and combined in various concentrations. Three different compositions of mock communities were created: OMC0 in which PCR products were added in equal proportions, and two different cocktails, OMC1 and OMC2, in which these PCR products were added in different proportions (Appendix E. Fig. 1).

No OTU was present in greater than the original proportion. Some taxa, notably the *Pythium mamillatum*-complex, were consistently under-represented (Appendix E. Fig. 1). Of the *Phytophthora* OTUs added, the *P. cambivora*-complex was the least

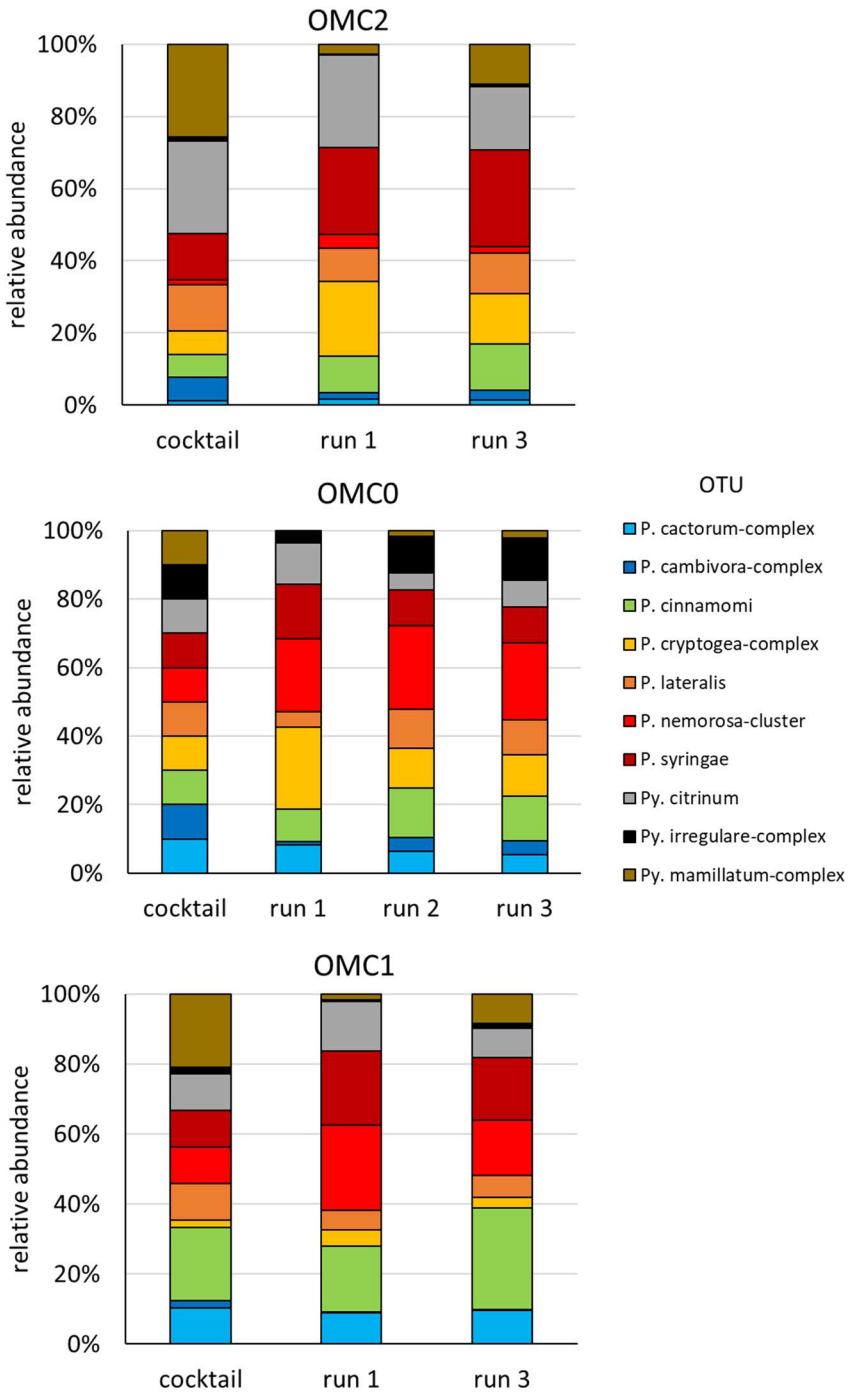
represented in the final output (Appendix E. Fig. 1). OTU signatures of taxa which were not added to the cocktail mix were present in the Illumina output, however the majority of these were eliminated when a 0.01% within-sample relative abundance threshold was applied; all were eliminated under a 0.095% within-sample relative abundance threshold. Nearly all *Phytophthora* OTUs were retained as "detected" when we applied the more conservative threshold of 0.095%. The single exception to this occurred with the *P. cambivora*-complex OTU, which comprised 0.014% of the total number of reads within 1 sample, community-OMC1 in run 1 (of which it originally comprised 2.08% of the total PCR product in the sample).

Water-blanks (negative control)

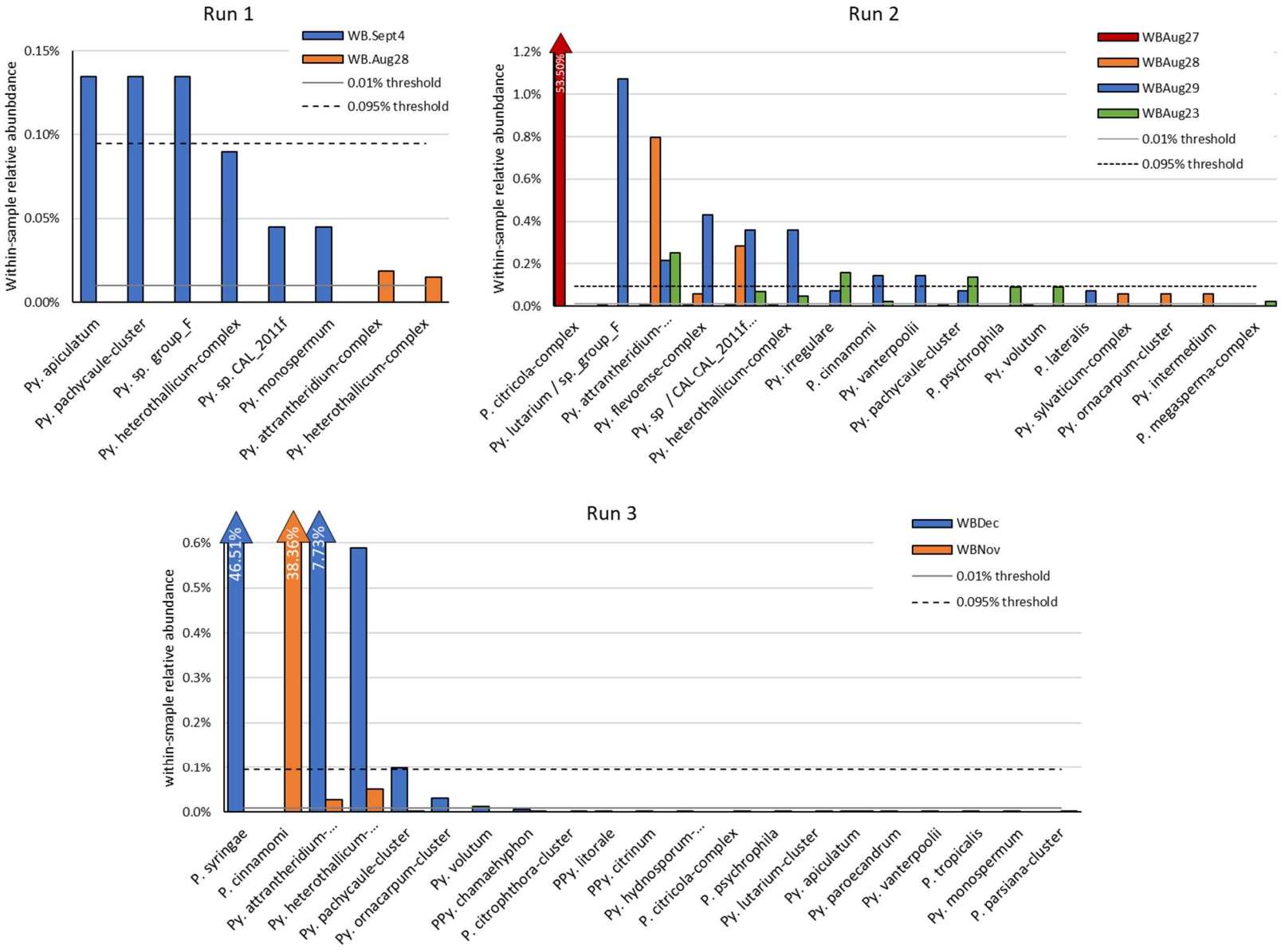
We added one water-blank to each PCR performed during the extraction process, each comprised of DNA-free water. Any sequences if present in these samples due to index hoping, should fall below the threshold relative abundance as determined in the mock-community. Because the samples are devoid of DNA, any contamination occurring during the extraction process will comprise the vast majority of sequences recorded for that sample.

Most of the reads within the water blanks are attributable to noise resulting from the Illumina process, being largely composed of OTU reads which were eliminated when a 0.095% within-sample relative abundance threshold was applied (Appendix E. Fig. 2). We observed notable contaminates in Run 2 and Run 3. In Run 2, 53.50% of the reads within water-blank sample from the PCR performed on August 27 (WBAug27) were attributable to the *P. citricola*-complex OTU; Run 3 (WBDec and WBNov) had large proportions of *P. syringae*, *P. cinnamomi*, *P. attrantheridium*-complex, and *P. heterothallicum*-complex OTUs (Appendix D. Fig. 2).

These species could have been introduced from the lab bench or via cross contamination during extraction and PCR, much as the *P. tropicalis*-OTU was detected in soil samples during Run 3. *P. multivora* (one member species of the *P. citricola*-complex), *P. syringae*, *P. cinnamomi*, *Py. attrantheridium*, and *Py. heterothallicum* were all recovered via baiting during year 2 and were thus being actively cultured and extracted during the soil extractions and PCR. The presence of contaminants should be taken into consideration when interpreting the Illumina dataset, however these OTUs would not comprise as large a proportion of the DNA within the actual samples.



Appendix E. Fig. 1. Compositions of the mock communities used as positive controls in the Illumina runs, as well as the subsequent relative abundance of these OTUs as measured in each of the 3 Illumina runs. Cocktails (OMC2, OMC0, and OMC1) were comprised of ITS6 and ITS7 amplified PCR products from 7 *Phytophthora* and 3 *Pythium* species, combined in different quantities to form a single "community". For clarity the relative abundance in each of the runs is standardized as a percentage of the total numbers of reads of only those OTUs (not total number of reads within each sample).



Appendix E. Fig. 2. Recorded OTU signatures in the water blank controls for each of the 3 Illumina runs. The majority of detections fell below a 0.095% within-sample relative abundance threshold (dashed line), however some OTUs comprised a substantial number of reads within their sample, particularly in Run 3. When noted, the WSRA exceeded the displayed scale for clarity.

3) Limitations of databases. Case examples with the *P. quercina*-cluster and the *P. uliginosa*-cluster.

Even for OTUs within high within-sample relative abundances, the limitations of the read lengths (100 to 250 bp) and deficiencies in *Phytophthora* databases can make it difficult to discern which species may be present. Some species cannot be differentiated by sequencing the ITS region, resulting is species complexes or clusters in which case multiple species may be indicated. Even then, ITS matches are only made for known species with entries in existing databases.

An excellent examples of this includes the *P. quercina*-clsuter. *P. quercina*-cluster includes only *P. quercina*, *P. versiformis*, and/or *P.* sp. "ohioensis". Of these, only *P. quercina* is thought to be present within the region, being first detected in the United States in 2016 from an outplanted *Quercus lobata* seedling in neighboring Santa Clara County. As a potential cause of oak decline in central Europe, this species is of concern to oak restoration in the United States. This *P. quercina*-cluster was abundant (>5% within-sample relative abundance in some samples) and widespread (27 samples across 7 preserves), though we obtained no isolate matching this taxon. We also failed to amplify longer reads of *P. quercina* via qPCR using *P. quercina*-specific primers from samples containing strong *P. quercina*-cluster detections. Longer sequence reads (1,034 bp in length) provided by the MinION sequencer indicates the OTU matching the *P. quercina*-cluster is neither *P. quercina, P.* sp. "ohioensis", or *P. versiformis*; rather, the OTU is likely an unidentified clade 12 species.

Similarly abundant and widespread was the *P. uliginosa*-cluster. The known members of this OTU (*P. uliginosa* and *P. europaea*) were not recovered via baiting, however we did recover the provisionally described *P.* sp. 'cadmea'. This species was first recovered and described by Tyler Bourett in surveys in Santa Clara County. Likely, the *P. uliginosa*-OTU is attributable to the presence of this species, which was not present in our database, and not *P. uliginosa* and *P. europaea*.

4) Conclusion

Despite some of the drawbacks of Illumina, the sensitivity of this method provides a glimpse into *Phytophthora* diversity and legacy that is near impossible to determine with culture-based methods alone.

In many cases both methods are in agreement, such as occurred for both baiting and Illumina detections of rare *P. nicotianae*, *P. boehmeriae*, and *P.* taxon *asparagi*; both methods were also in agreement in regards to the abundant and widely distributed *P. cactorum*.

More often than not though, a species was recovered when the associated OTU was not detected at any level, or only comprised 0.01 to <0.095% of the total number of reads within a sample. This speaks to the some of the strengths of baiting. Being rare, capturing a *Phytophthora* is often easier when using a larger volume of soil (1.5 L in comparison to the 10 g used by Illumina). A bait also functions as a biological filter to eliminate competing taxa from detection, notably the non-Oomycete taxa that dominated the sequence reads.

Similarly, there were numerous taxa which were better detected by Illumina being abundant in our sequence data but completely absent from cultures (e.g. *P. quercina*), isolated in

2018 only after 2017 detections (e.g. *P. boehmeriae*, *P. cryptogea* on Mount Umunhum, *P. crassamura*), or were isolated infrequently relative to their abundance (Clade 3 species). As a method, baiting requires the pathogen be biologically active and capable of infecting the bait. The species recovered are also biased towards those that grow well in culture. These limitations result in a large number of false-negatives and an under-representation of *Phytophthora* diversity.

We propose Illumina and baiting are best used in tandem with an understanding of both their limitations. Baiting *Phytophthora* validates the establishment of some species and is a clear indication of the potential for *Phytophthora* spp. to survive on-site. Illumina is useful in identifying areas where further baiting efforts are needed to verify the presence of biologically-active pathogens of concern; it can better identify those areas with greater diversity as an indication *Phytophthora* has historically being introduced; lastly, Illumina may provide the resolution needed to perform landscape-level analyses on metrics conducive to *Phytophthora* establishment.

Appendix F: PMA analysis

Introduction

Illumina MiSeq sequencing of amplified DNA (amplicon sequencing) extracted directly from soil has proved a useful tool in describing the diversity of *Phytophthora* on MROSD preserves. This approach detects the many species we were not able to bait. It may also be used to gauge the overall infestation level of a site to more certainty than baiting alone. As performed, however, we cannot distinguish between 'relic DNA' – DNA that exists outside of intact cells or cell-free DNA – from DNA extracted from viable hyphae or spores. Relic DNA may be remnants of nursery-infestations that failed to establish in the field; those detections that occurred at a very high frequency (for e.g. the presumed native *P. nemorosa*-cluster) may be attributable to long residency times. Relic DNA may persist in soils for years, if not decades. Because of this, actual levels of those *Phytophthora* which can cause disease are likely overestimated.

The addition of a propidium monoazide (PMA) to samples during DNA extraction is one means to determine if DNA detected in Illumina MiSeq is present in intact cells or as relic DNA. PMA binds to DNA located outside of cells when activated with light, preventing its amplification and detection in Illumina sequencing. As PMA cannot cross cell membranes, any DNA retained within cells is unaffected. Paired PMA and nonPMA samples can then be compared to determine to what extent reads are relic detections.

PMA analyses have never been performed for oomycetes in native soil. In this analysis, we added an additional PMA treatment to a select number of samples collected in 2018. Determining the proportion of oomycete DNA which is relic may give us a sense of the activity of *Phytophthora* in heavily infested vs. lightly infested areas. It may aid the interpretation of the Illumina MiSeq dataset by determining which OTUs are unviable and therefore of less actionable concern.

Methods

Samples for PMA analysis

During our sample period of 2018, we returned to sites which had a high and low frequency of OTUs detected in 2017. Additional sites were resampled because of detections of concern (e.g. the detection of *P. tentaculata* at Purisima Creek Redwoods). These samples were processed as with the other 2018 samples, via baiting and extracting for Illumina MiSeq sequencing. An additional 10g of soil was allocated for PMA analysis. In total, 105 soil samples were collected and processed with PMA.

PMA application and processing

To obtain a soil DNA in solution for PMA activation, we created a soil suspension containing 10g of soil, 25 mL of 10x PBS buffer, and 225 mL of DI water. This was shaken and allowed to settle as we pulled off 20 mL for the PMA sample and 20 mL for the nonPMA sample, which were placed in separate 50 mL Falcon tubes. These were done in 5 mL increments, alternating between the two tubes. In a dark room we added 40 µl PMA to each of the PMA tubes, and 40 µl DNA-free water to each of the nonPMA tubes. These were then

vortexed for 2 minutes, then arranged in clear box for light activation. The box was secured to an orbital shaker set at 50 rpm set below a light source (250-watt halogen lamp placed 20 cm away from the sample). The light was turned on and off in 30 second intervals for a period of 20 minutes in total. Light-dark intervals were required to avoid overheating the samples.

The solutions were then filtered through 5 µm cellulose nitrate filters (Sartorius Stedium Biotech, Germany), using disposable pipettes such that the solutions did not touch the sides of the filtering apparatus. In many cases, multiple filters were required to filter the entire solution. Each filter(s) was placed in 2.0 mL screw top tubes containing 50 µm garnet shards and one 6 mm Zirconium bead tube (Benchmark Scientific, New Jersey). To this we added 1 mL CTAB with PVP and placed in -20°C before extraction of DNA retained on the filter.

As a control to test the efficacy of the PMA, we spiked samples with two species not detected in the first year and not suspected to be present on MROSD preserves: *P. tropicalis* (as encysted zoospores) and *P. kernoviae* (as free DNA extract). Instead of 10 g of soil, we added 20 g of clean sand to 25 mL of 10x PBS buffer and 225 mL of DIwater. Additional sand was required to mimic the opacity of the soil samples. Three sand slurries were created, representing three replicate controls. For each we pulled off 19.11 ml for the PMA sample and 19.11 ml for the nonPMA sample, which were placed in separate 50 ml Falcon tubes. Each tube additional received 884 µl of zoospore suspension (*P. tropicalis*, 2.78 x 10^5 zoospores/ml) and 1.65 µl DNA extract (*P. kernoviae*, 14.2 ng/µl). The PMA and nonPMA pairs were processed as described above. As *P. tropicalis* was added as intact cells, we expected to see the *P. tropicalis*-OTU in both the PMA and nonPMA samples; the *kernoviae*-OTU should only be present in the nonPMA sample.

DNA extraction and sequencing data analysis

DNA was extracted from filters using standard chloroform-phenol extraction method. This method involves disintegration and homogenization of filters submerged in 1 mL CTAB with PVP followed by separation of protein and lipids from DNA content using a phenol:chloroform solution. Proteins and lipids usually denature in phenol and remains at the bottom half of the tube with phenol, while DNA dissolves in chloroform (aqueous phase) and remains in the top half of the tube. The top aqueous phase containing DNA was then transferred into a new tube and chilled isopropyl alcohol was added to initiate DNA precipitation. After centrifugation, precipitated DNA formed a pellet at bottom of the tube, which was further cleaned with 80% alcohol. Alcohol-free DNA pellet was then dissolved in TE buffer. We measured DNA concentration with Nanodrop spectrophotometer. DNA concentration was then normalized to 25 ng/ul for all samples. DNA was amplified with ITS6 and ITS7 primers that are known to amplify ITS1 region from mostly oomycete DNA targets. The amplified DNA was then sequenced using Illumina MiSeq sequencer. This step generated over 30 million sequences (or reads) in total. Every sequence was checked against a database containing ITS1 sequences from known oomycete species. This included species of *Phytophthora, Pythium, Phytopythium, Saprolegnia*, *Achlya, Aphanomyces*, etc. These results of this analysis are reported in terms of operational taxonomic unit (or OTU), a group of similar sequences that matched a given taxon within our database.

Analysis for comparison between PMA and nonPMA pairs

Because the number of reads within a sample is not consistent, we first calculated the within-sample relative abundance (WSRA) of each OTU, which standardizes the number of reads of an OTU as a proportion of the total number of reads within the sample. To be included in the analysis we removed any detections comprising less than 0.095% of the number of reads within that sample (i.e. a minimum threshold of 0.095% WSRA).

To compare paired PMA and nonPMA samples, we took the WSRA of any given OTU in the nonPMA-sample and subtracted the WSRA in the corresponding PMA sample. In this calculation, positive values indicate the WSRA is greater in the nonPMA sample, as consistent with the removal of relic DNA by PMA; negative values indicate DNA (relic and intact) was more abundant in the PMA sample. For further clarification, see "Conceptualization of potential outcomes resulting from PMA-nonPMA comparisons" at the end of this section.

To investigate the proportion of relic DNA present in our samples by genus, we additionally pooled the number of reads placed in the three most commonly detected genera: *Phytophthora*, *Pythium*, or *Phytopythium*. Reads were only included if they comprised a minimum of 0.095% WSRA. We then calculated the WSRA of each genus and performed the analysis as done for individual *Phytophthora* OTUs.

Results

Controls

The controls had 29 OTU signatures recorded, however the vast majority of these OTUs were eliminated when a 0.095% within-sample relative abundance (WSRA) threshold was applied. The kernoviae-OTU and tropicalis-OTU comprised between 66-70% of the total number of reads within their samples (Appendix F Fig. 1). PMA was effective at reducing *P. kernoviae* DNA from the PMA sample, however signatures of the OTU were present in both PMA and nonPMA samples (Appendix F Fig. 1). No non-*Phytophthora* DNA was detected in the control samples above a 0.095% WSRA.

Soil Samples

Of the 105 soil samples processed, only 24 had DNA of sufficient quantity and quality for sequencing in both the PMA and nonPMA sample. Similar numbers of *Phytophthora* OTUs were detected in the nonPMA, PMA, and corresponding soil samples (Appendix F Fig. 2). More OTUs were detected in the nonPMA sample for 6 nonPMA-PMA pairs, however more OTUs were detected in the PMA sample for 4 pairs (Appendix F Fig. 2).

In comparing individual *Phytophthora*-OTUs in PMA-nonPMA pairs, some OTUs were, on average, more abundant in the nonPMA sample (positive values, blue) while others were more abundant in the PMA sample (negative values, orange) (Appendix F Fig. 3). However, for most OTUs the average was not significantly different than 0 indicting the within-sample relative abundance values were equally positive and negative (i.e. one pairing might have greater WSRA in the PMA sample, but it was balanced by another pairing with a greater WSRA in the nonPMA sample).

When present, the vast majority of OTUs were detected only one of the samples pairs (i.e. only in the PMA sample or only in the nonPMA sample; Appendix F Fig. 4, Appendix F Fig. 5). This was observed regardless of which threshold was applied (Appendix F Fig. 5). A *Phytophthora* OTU was detected in both PMA and nonPMA samples in only four instances: three *citricola*-complex detections and one *parsiana*-cluster detection (Appendix F Fig. 4). In all four, the OTUs comprised less than 0.4% of the total number of reads within their respective samples.

The within-sample relative abundance of three most common genera – *Phytophthora, Pythium*, and *Phytopythium* – were consistent with prior Illumina results: *Pythiums* were by far the most commonly detected genus in all sample types, followed by *Phytophthora* then *Phytopythium* (Appendix F Fig. 6). There was no difference in the average WSRA between PMA and nonPMA samples for any genus.

Similar to *Phytophthora*, in comparing *Pythium*-OTUs in PMA-nonPMA pairs some OTUs were more abundant in the nonPMA sample (positive values, blue) while others were more abundant in the PMA sample (negative values, orange) (Appendix F Fig.7). Instances in which a genus was only detected in the PMA sample were still relatively common for *Phytopythium* and *Phytophthora* when data are combined as a genus (Fig. 8, yellow); *Pythium* was more commonly detected in both PMA and nonPMA samples, however the WSRA was larger in the PMA sample for a large number of pairs (Fig. 8, Fig. 9). When *Pythium* were detected only in one of the sample pairs, *Pythium* comprised a small proportion of the total number of reads in the samples (WSRA < 4%), however many samples contained *Pythium* at high levels only in the PMA sample (Appendix F Fig. 9). All genera were, on average, more abundant in the PMA samples, though the average difference between the PMA and nonPMA samples was not significantly different than 0 for any genera (Appendix F Fig. 10).

Discussion

Propidium monoazide is one promising treatment to determine what proportion of relic DNA is contributing to the detections of OTUs from environmental samples. Unfortunately, we found inconsistencies in our results which make direct PMA-nonPMA comparisons difficult to interpret. Notably, the high number of instances in which OTUs were detected only in the PMA sample indicate each nonPMA-PMA pair did not contain an identical (or even near-identical) representation of which OTUs were present in the soil.

Numerous observations conformed with expected results: we expected to see a greater abundance of a given OTU or taxa in the nonPMA samples relative to that in the PMA sample (as indicated in blue for Appendix F Fig.s 3-5 and Appendix F Fig.s 7-10). Instances in which an OTU or taxa is recorded only in the nonPMA sample (light blue) can occur, indicating all DNA for that taxa was present as relic DNA. We hypothesized most taxa would be present in both PMA and nonPMA samples (dark blue) either because there would be a mix of relic and intact DNA, or because PMA would likely not eliminate 100% of the relic DNA from the sample, as observed in the controls.

It is also expected, to some degree, that some samples would have a slightly greater abundance of a given OTU in the PMA sample relative to the nonPMA sample. This would occur for a combination of reasons: the PMA sample may have more DNA initially because of

random sampling error, or the within-sample relative abundance may be suppressed in the nonPMA sample because of an overabundance of other genera (correspondingly, an OTU may be overrepresented in the PMA sample because of the lack of other taxa). We still expected, under these scenarios, that the difference between the nonPMA sample and the PMA sample, while negative, would be near 0. Under no circumstance did we expect the number of cases in which a taxon was detected only in the PMA sample (indicated in orange for Appendix F Fig.s 3-5 and Appendix F Fig.s 7-10). Nor did we expect the degree to which taxa would be so over-represented in the PMA sample.

As a result, very few OTUs were detected predominantly in nonPMA samples, and the difference between the two rarely deviated from 0. This was observed when comparing individual *Phytophthora* OTUs (Appendix F Fig. 3), individual *Pythium* OTUs (Appendix F Fig. 7), or when combining all reads into a genus-level analysis (Appendix F Fig. 10). We also attempted analyses on data screened with a lower threshold (0.01% within-sample relative abundance) or no threshold. The results (e.g. Appendix F Fig. 5) where similarly inconclusive whereby there was a large number of detections occurring only the PMA sample. A minimum 0.095% within-sample relative abundance was required to eliminate erroneous reads in the control samples and lowering the threshold did not improve the sensitivity of the PMA analysis. Therefore, to minimize erroneous detections, we limited the analysis and discussion to those detections occurring at greater than 0.095% WSRA.

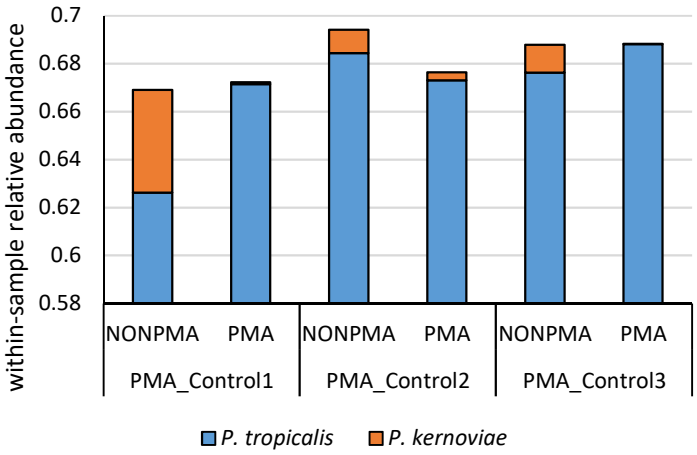
Such results strongly suggest that the PMA-nonPMA pairs did not contain a similar DNA profile after being separated into different tubes. We attribute this to the incredibly small proportion of DNA which may be attributed to oomycetes (let alone just *Phytophthora*) in the soil relative to other genera. When DNA extracts were taken from 10g of soil we found oomycetes comprised roughly a quarter of the total DNA reads amplified by the ITS6 and ITS7 primers, of which *Phytophthora* comprised only 1.3% of the total number reads and *Phytopythium* only 0.04%. Because ITS6 and ITS7 are specific to only a subset of organisms present in the soil (notably they do not amplify bacteria), the oomycetes comprise an even smaller proportion of DNA in each sample. As PMA requires light for activation, we had to rely on an extremely dilute subsample of each 10g of soil. As such, it is highly likely this subsample would miss rare OTUs. Unfortunately, this was observed even for the most common oomycete genera, *Pythium.*

With the evidence OTUs were present only in the PMA sample, we similarly must presume some pairs contained OTUs only within the nonPMA sample. As such, pairs with detections predominantly in the nonPMA sample (light and dark blue) cannot be interpreted as having worked as intended. But while we cannot say what proportion of DNA of a given OTU or genus was present as relic vs. intact DNA, we do have evidence of *Phytophthora* and *Pythium* are present in the samples in intact cells. Numerous *Phytophthora* OTUs were detected in PMA samples at a high within-sample relative abundance, including many taxa we were unable to bait (indicated in orange, yellow and dark blue in Appendix F Fig. 4). These include members of the *P. parsiana*-cluster, *P. citrophthora*-cluster, and *P. psychrophila* OTUs.

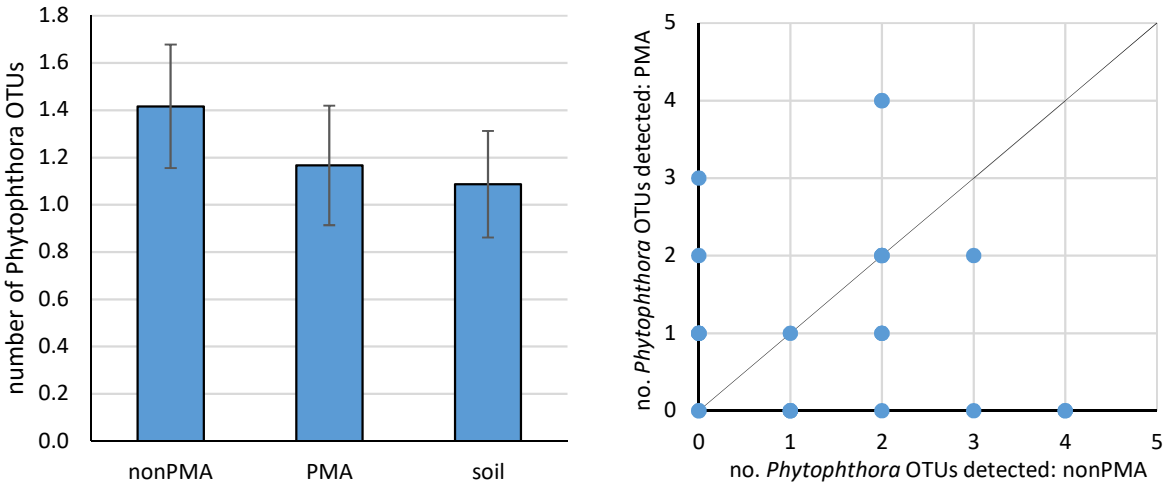
Conclusion

PMA was an effective treatment to differentiate relic, cell-free DNA from intact cellular DNA, as shown in controls. Relic DNA was contributing to the OTU signals in soil samples, however, quantifying relic DNA proportions in the environmental samples is tricky due to following reasons: (a) getting soil samples with an identical oomycete composition for nonPMA-PMA paired comparisons is not feasible; (b) no two taxa have identical relic:intact DNA proportions in a given sample that might interfere with relic DNA reduction with PMA treatment; (c) incredibly small proportion of oomycete exists in the soil relative to other microbes, which could lead to excess PMA consumption to reduce relic DNA of non-oomycete microbes from soil sample.

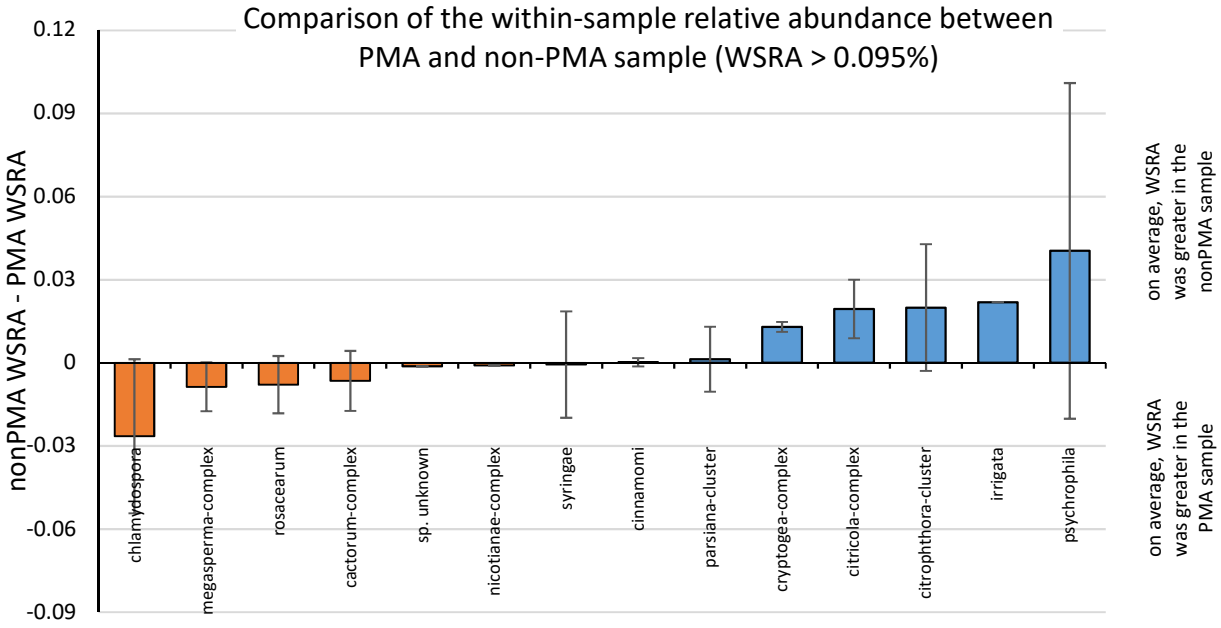
Appendix F Figures



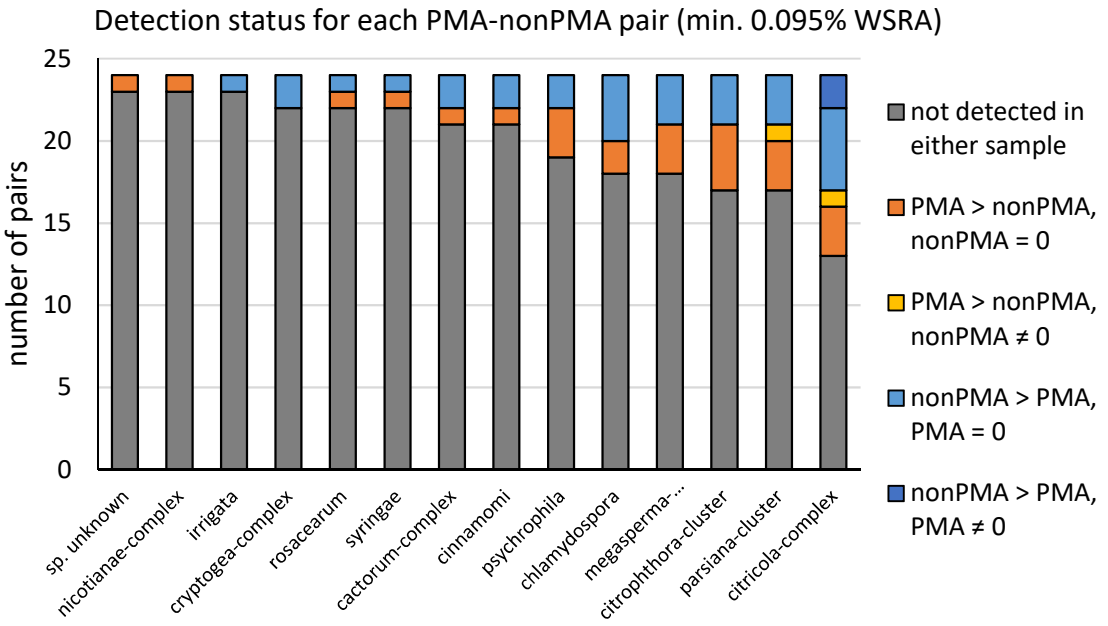
Appendix F. Fig. 1. Comparison between the within-sample relative abundance of the *tropicalis*-OTU and the *kernoviae*-OTUs in the PMA and nonPMA control samples. *P. tropicalis* was added as zoospores and thus should not be affected by the addition of PMA; *P. kernoviae* was added as DNA extract and should have been reduced or eliminated with the addition of PMA.



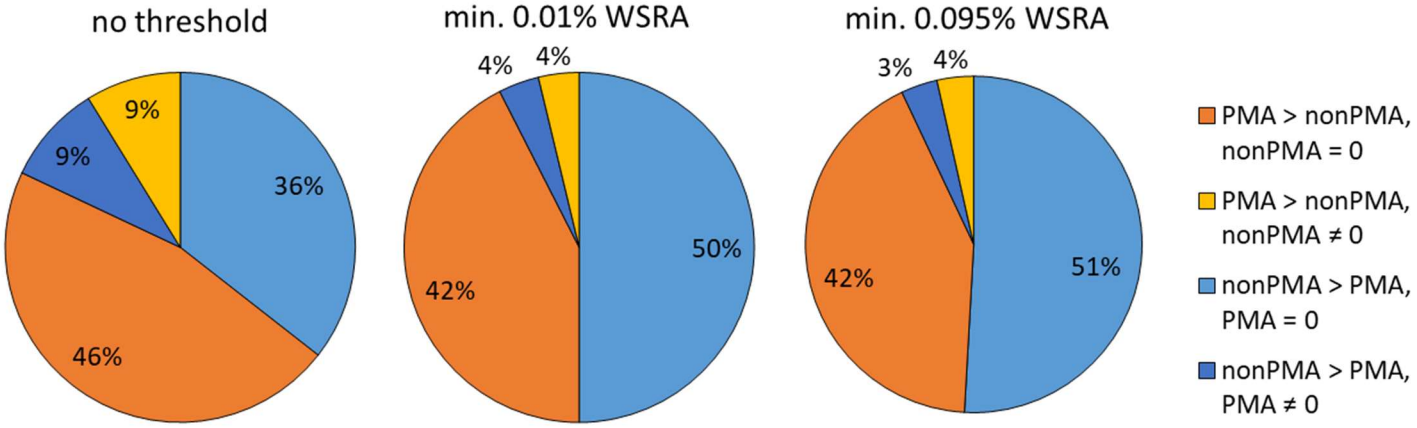
Appendix F. Fig. 2. Average (± s.e.) number of *Phytophthora* OTUs detected in the nonPMA, PMA, and corresponding soil samples (left), and comparison between the number of *Phytophthora* OTUs detected in paired nonPMA and PMA samples (right). To be considered detected, an OTU must have comprised a minimum 0.095% within-sample relative abundance.



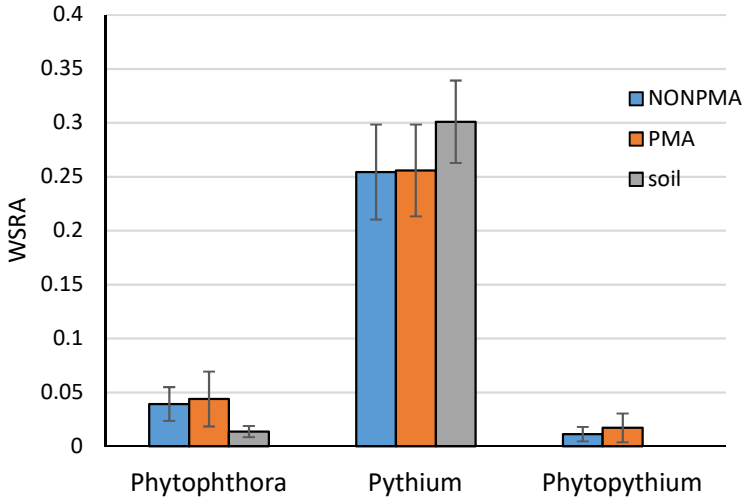
Appendix F. Fig. 3. Average (± s.e.) difference between the WSRA of each *Phytophthora* OTU in paired nonPMA and PMA samples. Included are all reads for which the within-sample relative abundance was greater than or equal to 0.095%; average was calculated only for samples in which there was a detection in at least one of the nonPMA-PMA pair.



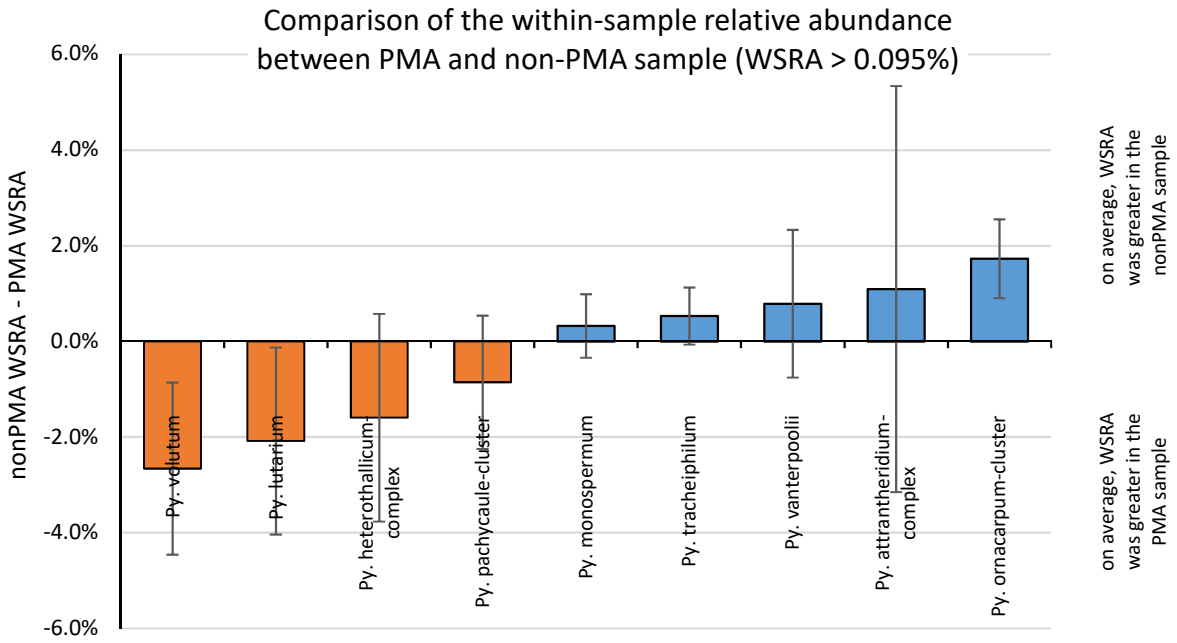
Appendix F. Fig. 4. Number of PMA-nonPMA pairs in which an OTU was not detected (gray), detected but WSRA was greater in the PMA sample (orange / yellow), or detected but WSRA was greater in the nonPMA sample (light blue / dark blue).



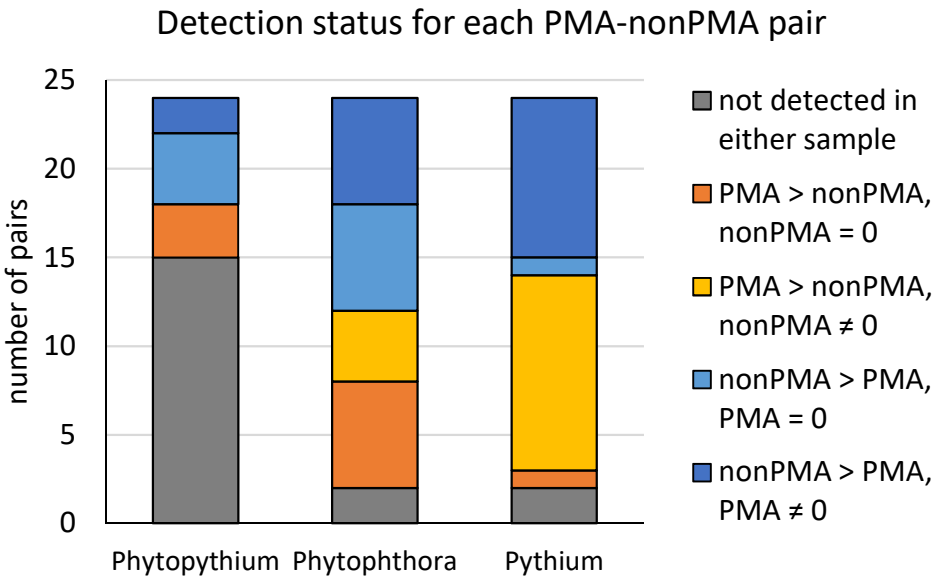
Appendix F. Fig. 5. Proportion of *Phytophthora*-OTU detections within each of the four different detection classes, as calculated under three different detection thresholds: no threshold, a minimum of 0.01% within-sample relative abundance, and a minimum 0.095% within-sample relative abundance.



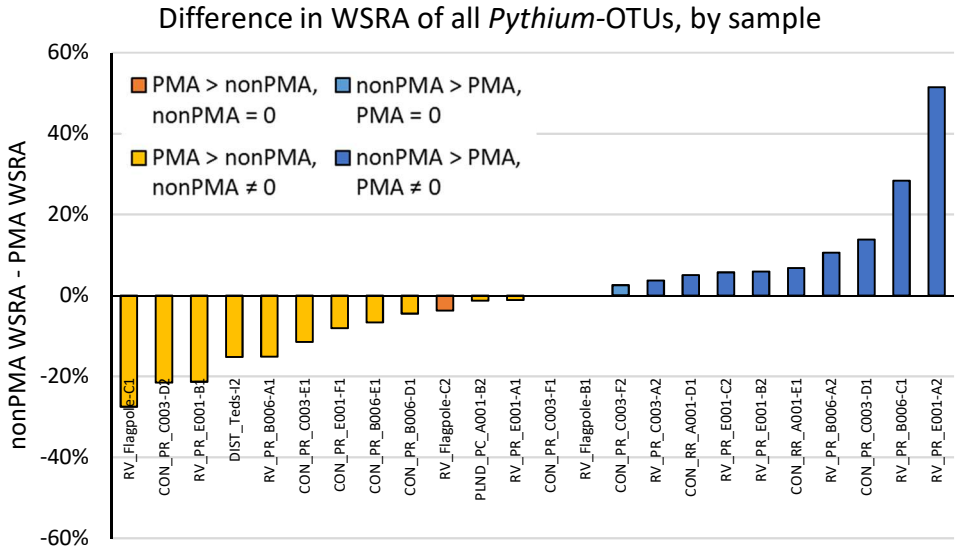
Appendix F. Fig. 6. Average (± s.e.) WSRA (for all WSRA ≥ 0.095%) for the three most commonly detected genera – *Phytophthora, Pythium*, and *Phytopythium* – in the in the nonPMA, PMA, and corresponding soil samples.



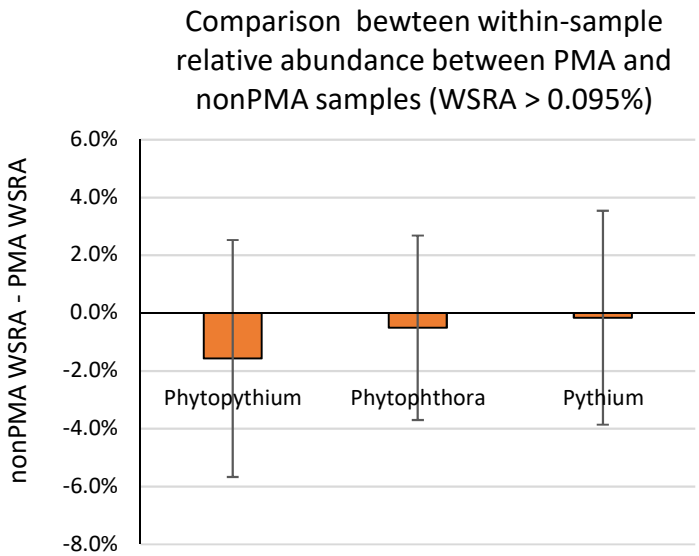
Appendix F Fig. 7. Average (± s.e.) difference between the WSRA of *Pythium* OTUs in paired nonPMA and PMA samples. Included are all reads for which the within-sample relative abundance was greater than or equal to 0.095%; average was calculated only for samples in which there was a detection in at least one of the nonPMA-PMA pair. Data are presented only for *Pythium* OTUs detected in a minimum of 12 samples.



Appendix F Fig. 8. Number of PMA-nonPMA pairs in which each genus was not detected (gray), detected but WSRA was greater in the PMA sample (orange / yellow), or detected but WSRA was greater in the nonPMA sample (light blue / dark blue).



Appendix F Fig. 9. Difference between the *Pythium*-WSRA in PMA and non-PMA pairs, for each sample sequenced in the PMA analysis. Negative values indicate *Pythium* DNA was more abundant in the PMA sample; positive values indicate *Pythium* was more abundant in the nonPMA sample. *Pythium* was not detected in either the PMA or nonPMA samples for CON_PR_C003-F1 and RV_Flagpole-B1.



Appendix F. Fig. 10. Average (± s.e.) difference between the WSRA of paired nonPMA and PMA samples, when reads are pooled by genus. Included are all reads for which the within-sample relative abundance was greater than or equal to 0.095%; average was calculated only for samples in which there was a detection in at least one of the nonPMA-PMA pair.

Conceptualization of potential outcomes resulting from PMA-nonPMA comparisons.

Mock data, containing the number of reads of a hypothetical *Phytophthora* OTU in a paired PMA and nonPMA sample, under 4 different scenarios. To calculate the within-sample relative abundance, each hypothetical sample contains a total of 35 reads.

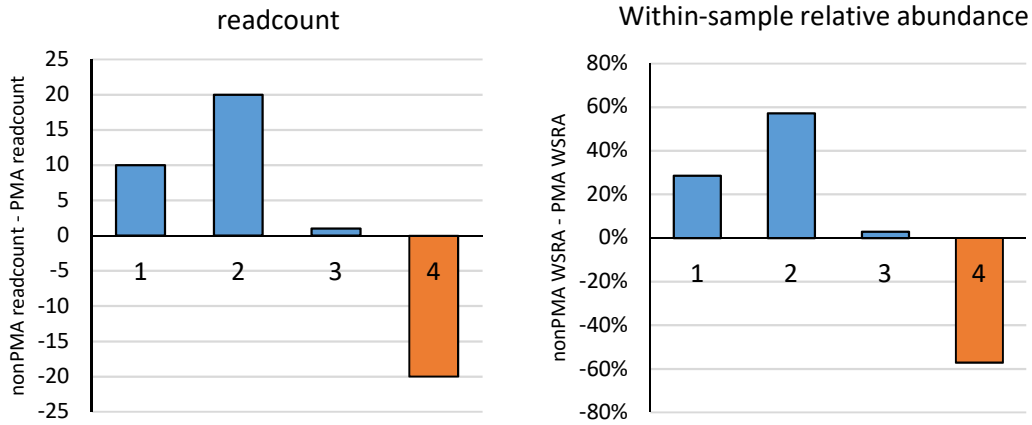
	scenario			
sample	1	2	3	4
nonPMA	20	20	11	0
PMA	10	0	10	20
nonPMA-PMA	10	20	1	-20

Scenario 1. Expected result: mix of relic-DNA and intact-DNA. Likely, there would be more relic-DNA than intact DNA due to buildup of DNA over time, as indicated by the greater number of reads in the nonPMA sample.

Scenario 2. Expected result: much greater proportion of relic-DNA due to a failed introduction. No viable pathogen present.

Scenario 3. Expected result: large proportion of intact-DNA is present relative to the amount of non-viable DNA. This scenario shouldn't be common as it indicates a recent active infection which hasn't had time to populate the soil with relic DNA. Note: the DNA from these intact cells should be detected in both the nonPMA and PMA samples.

Scenario 4. Unexpected result: much greater proportion of intact-DNA, or where DNA is only detected in PMA sample. Intact-DNA should be detected in both the nonPMA and PMA samples. We would observe this result only if the PMA sample contained an OTU which the nonPMA sample lacked (i.e. the pairs weren't identical at the onset of the processing with PMA).



Expected appearance of analysis displaying the difference between the nonPMA and PMA samples under four different scenarios. Scenarios 1-3 are expected, but no. 4 is an unexpected result. Presented is the difference between the absolute read counts (left) or the within-sample relative abundance (right).

Appendix G: Stream Surveys

Introduction & Methods:

Waterways provide a landscape-level picture of *Phytophthora* abundance as inoculum is washed into streams and may be recovered a great distance from the inoculum source. Representing a wider geographical area, monitoring of streams may detect species of interest and direct future sampling needs.

To assess the utility of baiting as a means to survey MROSD lands, we baited 14 stream locations along 8 distinct waterways associated with soil sampling locations (Appendix G Fig. 1). Some (Guadalupe Creek, Stevens Creek and Peters Creek) were baited at multiple locations. Criteria for stream selection included access, proximity to outplanting sites, and geographic representation targeting MRSOD preserves of interest. Baiting was completed only during the first sampling year, December 2017.

At each location we baited waters using two methods: *in-situ* baiting and the bottle o' bait (BOB) baiting. For *in-situ* baiting, 5 leaves of both rhododendron and coast live oak were enclosed in mesh bags constructed of fiberglass window screen material. These were floated in waters between five and nine days (Appendix G Fig. 2). When we collected the *in-situ* baits, we performed the BOB baiting whereby we collected 2 1-L water samples in plastic Nalgene containers. Leaves (2 each of rhododendron and tanoak) were added to each bottle which were allowed to sit for two to four days at room temperature. Rhododendron leaves were obtained from *Phytophthora*-free plants maintained in OSU greenhouses, which were stored in moist paper towels in a cooler or fridge prior to their deployment; both the coast live oak and tanoak leaves were taken from residential areas in Santa Clara or San Mateo County, respectively, with no evidence of prior phytopathogen infection. For all leaves, we re-trimmed the petioles prior to their deployment to create fresh wounds amiable to the colonization by *Phytophthora*. We additionally gently bent the rhododendron leaves in half perpendicular to the midrib to create a new wound.

After recovery, all leaves (n = 258) were wrapped in moist paper towels and transported back to OSU where they were monitored for symptom development at 20°C (Appendix G Fig. 3). We plated two lesions per leaf onto PARPH selective media to isolate *Phytophthora* and other closely related genera. If there were no lesions, we plated two disks: one disk from where the petiole meets the leaf blade (all species) and one disk from the midrib 1 cm from the leaf tip (coast live oak and tanoak) or the break point where the leaf was bent (rhododendron). During this time we placed non-deployed rhododendron, coast live oak, and tanoak leaves in five containers of DI-water (two leaves per species per container). These we incubated at 20°C for 4 days before being removed and monitored for symptoms.

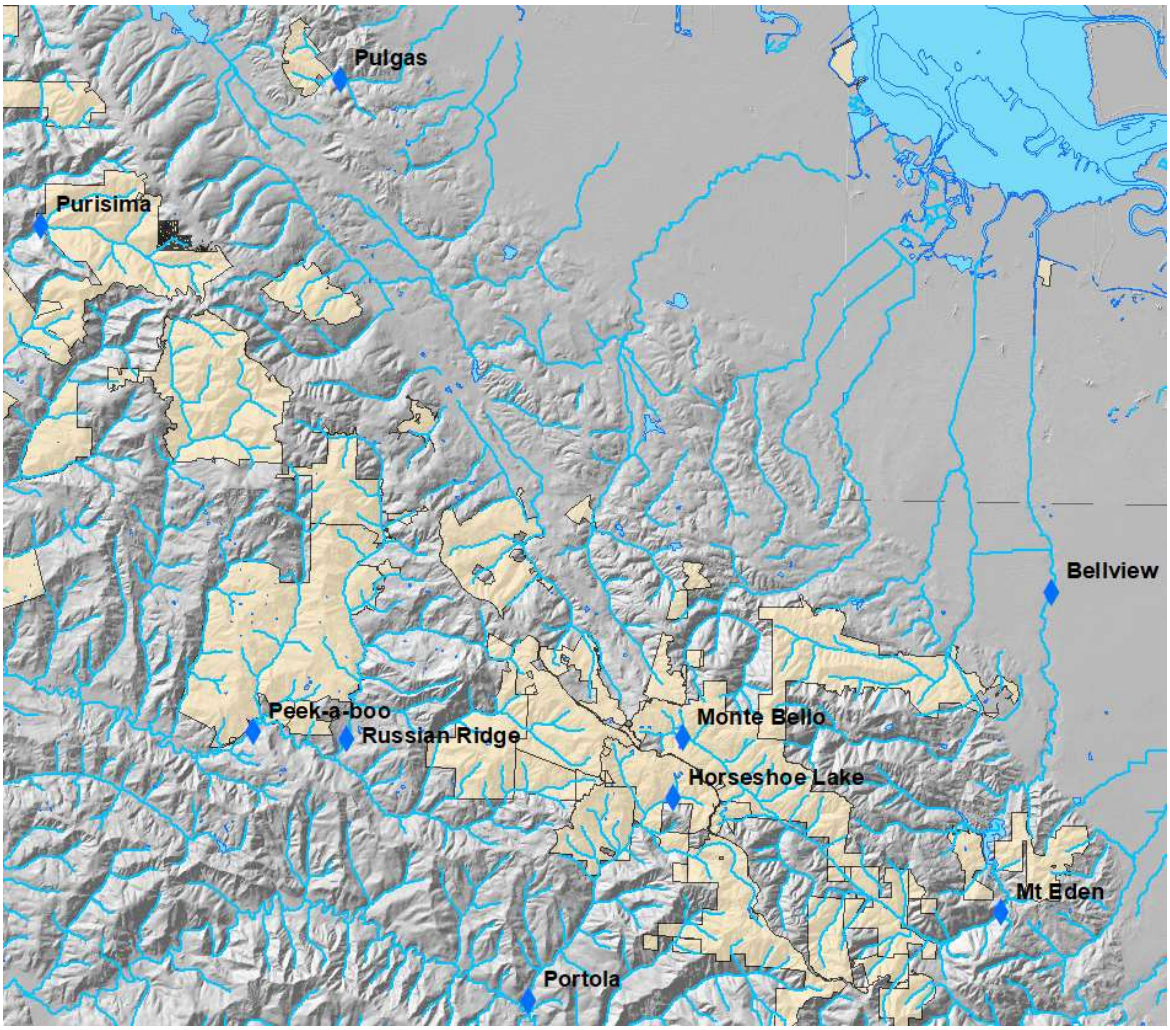
For each bait, we transferred at least three isolates onto new selective media for further isolation and identification, preferentially selecting those most characteristic of *Phytophthora*. These were organized into morphotype based off hyphal and spore (if present) morphology. To identify the isolates, we extracted DNA from all morphotypes from a single bait location and method, which was sequenced via sanger sequencing.

Results

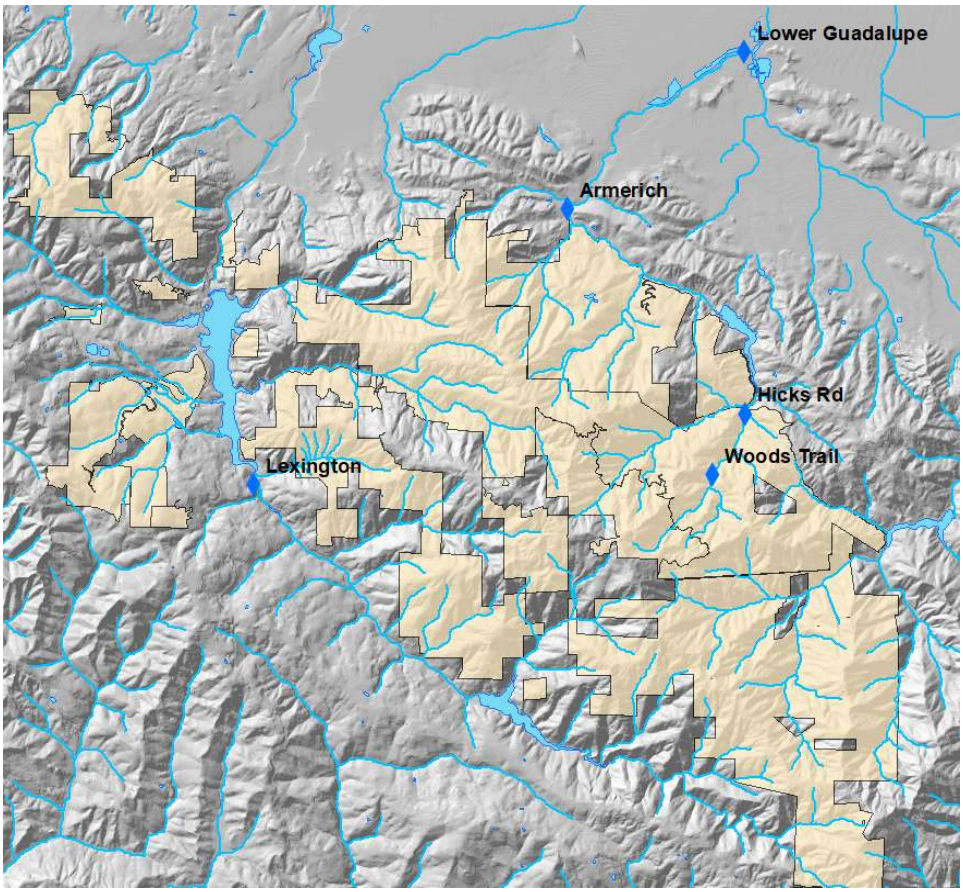
Phytophthora was recovered from all streams (Appendix G Table 1). We successfully sequenced 105 pure-isolate cultures. Of these, nearly all are Clade 6 species commonly found in streams and bodies of water. These include: *P. amnicola*, *P. chlamydospora*, *P. gonapodyides*, *P. lacustris*, *P.* taxon *oaksoil*, *P. riparia*, and *P. thermophila*. These are considered weakly pathogenic relative to other species, although under certain circumstances some can cause disease.

The only pathogenic species of concern were *P. ramorum*, which is known to be widely distributed throughout the area, and *P. syringae*, which is a widely dispersed horticultural pathogen also recovered by baiting directly from soil.

No cultures were obtained from leaves baiting DI-water.



Appendix G. Fig. 1a. Location of stream baits (Northern streams).



Appendix G. Fig. 1b. Location of stream baits (Southern streams).

Appendix F. Fig. 2. *In-situ* bait.

Appendix F. Fig. 3. Symptoms on rhododendron (left 2 leaves) and tanoak (right 2 leaves).

Appendix G. Table 1. All *Phythophthora* species recovered from either *in-situ* baits and BOB baits in the 14 stream locations. See Appendix G. Fig. 1 for locations of each stream site.

Site	**Target Reserve**	***Phytophthora* species recovered**[4]
Lexington	Sierra Azul	*P.* taxon *oaksoil*, *P. chlamydospora*, *P. ramorum*
Woods Trail[1]	Sierra Azul	*P.* taxon *oaksoil*, *P. chlamydospora*, *P. gonapodyides*, *P. ramorum*
Hicks Rd[1]	Sierra Azul	*P. chlamydospora*, *P. gonapodyides*, *P. ramorum*
Armerich[1]	Sierra Azul	*P. chlamydospora*, *P. gonapodyides*, *P. ramorum*, *P. riparia*, *P. thermophila*
Lower Guadalupe[1]	Sierra Azul	*P. lacustris*, *P. riparia*
Monte Bello[2]	Monte Bello	*P. gonapodyides*, *P. ramorum*
Mt Eden[2]	Monte Bello	*P. lacustris*
Bellview[2]	Monte Bello	*P. lacustris*, *P. riparia*, *P. thermophila,*
Portola[3]	Skyline Rdige	*P. chlamydospora*, *P. gonapodyides*
Horseshoe Lake[3]	Skyline Rdige	*P. amnicola*, *P. chlamydospora*, *P. gonapodyides*, *P. ramorum*
Pulgas	Pulgas Ridge	*P. lacustris*, *P. riparia*
Purisima	Purisima Redwoods	*P. chlamydospora*, *P. gonapodyides*, *P. syringae*
Peek-a-boo	La Honda & Russian Ridge	*P.* taxon *oaksoil*, *P. gonapodyides*
Russian Ridge	La Honda	*P. chlamydospora*, *P. gonapodyides*, *P. lacustris*

[1] All sites are along the same waterway of Guadalupe Creek
[2] All sites are along the same waterway of Stevens Creek
[3] All sites are along the same waterway of Peters Creek
[4] Note: Prior to formal naming, *P. chlamydospora* was called *Phytopthora* taxon PgChlamydo; there is dispute as to whether *P. bilorbang* is the same as *Phytophthora* taxon *oaksoil*, which has not been formally named.

Guidelines for Minimizing *Phytophthora* Contamination at Midpeninsula Regional Open Space District Preserves

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The goal of these guidelines is to minimize the contamination of Midpeninsula Regional Open Space District (MROSD) preserves with *Phytophthora*, a soil pathogen that kills plants. Once a site is contaminated, this soil pathogen can spread farther into wildland areas and can be difficult to eradicate. Prevention is the lowest cost and easiest method to manage contamination.

The best way to prevent the spread of this disease is to not move soil from one location to another by cleaning tools, equipment, and footwear.

Part of the District's mission is to protect and restore the natural environment. Within the last few years, planted restoration sites have unintentionally exposed preserves to soil pathogens brought in by nursery plants that were later found to be contaminated. Testing of former restoration sites on District preserves is now underway to determine which sites are contaminated and the necessary remedial actions.

Who should use these guidelines?
These guidelines are intended for use by field staff and Natural Resource (NR) staff who pose the highest chance of spreading soil *Phytophthora* via equipment and footwear. Several methods are provided on how and when to decontaminate tools and equipment depending on the site conditions (contaminated versus clean site) and staff activities (planting, other). Guidelines for contractors, consultants, volunteers and preserve visitors are under development. Consult NR staff (Amanda Mills, amills@openspace.org or x558, or Coty Sifuentes-Winter, csifuentes@openspace.org or x560) on which guidelines are best for your project.

When to use these guidelines?
Use these guidelines for any activity that contacts soil, water or plants on a known Phytophthora-contaminated site, on a formerly planted site, on a site with rare plants, or when preparing or planting a new restoration site.

Table of Contents

1. Overview

Remember to **Arrive Clean and Leave Clean**. The best way to prevent the spread of *Phytophthora* is to leave soil at its original location in the field. Equipment and footwear should be clean and sanitized before entering a site, especially for planting events where extra precautionary steps will be taken. Before leaving a site, especially at contaminated sites, it's crucial to clean and sanitize footwear and equipment.

Definitions:

Clean - remove soil and organic debris from tools and footwear

Sanitize - Use disinfecting agent such as alcohol or chlorine bleach.

Phytosanitary- control of plant pests and diseases especially in agricultural crops

1.1 What is Phytophthora?

1.1.1 *Phytophthora* (Fie-tof-thora) is a group of water molds that infect plants. There are many species, mostly notably *P. ramorm* (Sudden Oak Death), *P. infestans* (potato blight/ Irish potato famine) and *P. tentaculata* (nursery root rot).

1.1.2 Symptoms are similar to drought, making diagnosis difficult without testing.

1.1.3 Symptoms include leaf spots, branch die-back, cankers, trunk bleeding and death of whole plant.

1.1.4 Hosts include many native and nursery plants including oaks, bay laurel, madrones, sticky monkeyflower.

1.1.5 Brought to California through imported camellia and rhododendron nursery plants.

1.1.6 Mainly spreads from contaminated nursery stock, pots and soil. Can spread by foot traffic from contaminated footwear.

1.2 General Steps:

1.2.1 **What -** Items to be cleaned: Anything that comes into contact with soil, water or plants. This includes tools (shovels, hand trowels, hori-horis, rakes, tree cages, plant protection tubes etc.), footwear, equipment, wheeled equipment and vehicles.

1.2.2 **When -** Prior to the project day, field staff will be notified what items need to be cleaned and by which method. In general, tools and equipment should be cleaned at the field office before bringing them to the field site, and soil should be removed from footwear beforehand and more thoroughly cleaned at the entrance to the field site.

1.2.3 **Transportation** - Cleaned equipment should be transported in a truckbed from which all soil has been washed out, or cleaned equipment can be wrapped in a clean tarp before placed in a dirty truck.

1.3 Proper Disinfectants

All recommended disinfectants are considered pesticides. Personal protective equipment required by the State of California for anyone using disinfectants is eye protection with wrap-around and brow protection and 14 mil chemical resistant gloves. You can use smaller mil gloves if handling chemicals for 15 minutes or less.

1.3.1 The disinfectants listed in Table 1 are recommended by standard phytosanitary guidelines.
1.3.2 Other disinfecting agents or methods, such as Lysol or heat treatments, must be reviewed and approved by NR staff before use.
1.3.3 Disinfectants are most effective when surfaces are clean of soil and user follows label instructions.

Disinfecting Agent	**Active ingredient**	**Contact time**	**Product shelf life**	**Proper Disposal**	**Health Risk**	**Personal Protective Equipment**
Granular Chlorine Bleach (Leslies Chlor Brite, EZ Chlor)	Sodium dichloroisocyanurate dihydrate	2 min	Long if undiluted	Neutralizer (Vita-D-Chlor)	High	Eyewear, gloves; do not inhale
Liquid Bleach (Clorox)*	Sodium chloride	2 min	3-5 months	TBD	High	Eyewear, gloves; do not inhale
Rubbing Alcohol	Ethanol or Isopropyl Alcohol	1 min	Long	TBD	Med	Eyewear, gloves; flammable
Quaternary ammonium compounds (Quat 128 or Physan 20)	Dodecyl dimethyl ammonium chloride	10 min	Long if undiluted	TBD	Med	Eyewear, gloves; toxic to fish

Table 1: List of approved disinfecting agents. Always follow chemical label instructions.
*Liquid bleaches are generally not recommended as a disinfectant because they lose potency in storage.

2. Cleaning at the Field Office

Clean equipment, tools and footwear at the field office **before** arriving to the project site. This is the easiest way to prevent soil contamination. For those occasions where equipment and footwear must be cleaned at a field site, see Cleaning at Field Site (page 7).

2.1 Remove Soil from Equipment and Footwear

2.1.1 At the field office, scrape, brush, and wash off any soil or organic material. Take care to remove soil trapped in treads or cracks.

2.1.2 Pathogens can survive inside soil clods even after soaking because disinfectants may not completely penetrate large or clayey masses. Therefore, it is important to remove large clods of soil before soaking or otherwise treating with disinfectants.

2.2 Disinfect Tools With Bleach

Several disinfecting agents are available for treating Phytophthoras (Table 1). When many tools need treatment, use granular chlorine bleach at the field office. Spraying with rubbing alcohol is more appropriate for spot treatment at remote field locations.

NEVER MIX DIFFERENT DISINFECTING AGENTS.

ALWAYS FOLLOW LABEL DIRECTIONS.

FOLLOW REQUIREMENTS FOR PERSONAL PROTECTIVE EQUIPMENT WHEN USING DISINFECTING AGENTS.

List of Equipment for Disinfecting Tools:

- **Disinfectant** – most frequently, we expect to be using granular chlorine bleach such as EZ Chlor or Leslie's Chlor Brite when cleaning multiple tools at the field office. Carefully follow the directions below when using any [?] of the bleach disinfectants.
- **Vita-D-Chlor (chlorine neutralizer) -** This neutralizing product is only required if you used chlorine bleach as a disinfectant.
- **Waterproof container -** A large [minimum size?] plastic trashcan or waterproof pop-up garden trimming container in which to mix the water-based disinfectant and soak the tools.
- **Hard bristled scrub brushes and paint scrapers** - Grill brushes with scrapper attachment are handy tools to loosen soil from both flat surfaces and narrow cracks.
- **Personal Protective Equipment** Close-toed shoes, apron or coveralls, protective eyewear, 14 mil chemical resistant gloves (not leather or cloth).
- **Clean water source** - should not be cloudy or with a lot of organic material in it**.** Pressure washers or nozzles are helpful to remove soil quickly and get into small cracks.

2.2.1 Before using the disinfectant, remove soil as described in above section.

2.2.2 Fill waterproof container with 10 gallons of water. Use label instructions to add the right amount of disinfecting agent. For granular bleach, use one teaspoon in 10 gallons to get the desired 0.525% dilution.

2.2.3 Dunk tools in solution for required soaking time (see Table 1). For granular bleach, this is 2-minutes. Just getting tools wet does not mean they will be disinfected. Think of it as chemical cooking.

2.2.4 If you used chlorine bleach as a disinfectant, it needs to be neutralized after soaking. This 'rinse cycle" will deactivate the bleach so it does not corrode metal and so that it is safer to dispose of the soak water. Equipment sprayed with alcohol does not require this neutralization step.

2.2.5 In addition to tools, remember to disinfect the sanitation kit, gloves, tarps, or other miscellaneous items that have come into contact with soil.

2.2.6 Let tools dry. The hose lay is great for drying tarps.

2.3 Disinfect Wheeled Equipment/ Vehicles

Anything with wheels, including wheel barrels, ATV's, motorized carts that will be used at the field site needs to be cleaned and this is best done at the field office before the project. Vehicles that stay at the staging area do not have to be cleaned and sanitized. However, it is good phytosanitary practice to remove soil from wheels every time you leave a site.

2.3.1 Scrub down tires either by hand scrubbing or using a pressure spray wash.

2.3.2 Sanitize using disinfecting spray such as bleach (must be made weekly) or rubbing alcohol.

3. Cleaning at Field Site

Remember to **Arrive Clean and Leave Clean**. If equipment was cleaned and treated with a disinfectant at the field office and delivered in a clean truck, then on-site cleaning of equipment will only be required when leaving at the end of a work day. We recommend that everyone be encouraged to thoroughly clean their footwear of soil before arrival at the site, and then footwear be treated with alcohol upon arrival. Volunteers may not always be aware of this recommendation and may arrive with boots that need to be cleaned of foreign soil at the field site. Scraping all soil off equipment and footwear is required before leaving site, and sanitation of all footwear is usually recommended when leaving a site, especially for known contaminated sites. Rubbing alcohol is usually the preferred disinfectant in the field. Bleach products can be used in the field, but it is harder to mix and dispose of them properly in the field. See details below.

3.1 Cleaning at Start of Field Day

Tools:

Portable sanitation kits include the following items in a bin: 2 tarps, boot brush with scraper, 2 spray bottles of 70% isopropyl alcohol, 2 long-handled brushes, 2 paint scrapers, and instructions. On muddy days, also bring a basin and 2 jugs of water.

Alcohol 70% Ethyl alcohol (Ethanol) or 90% Isopropyl alcohol is fine. Called rubbing alcohol at drug stores.

Spray bottle - we take the nozzles from chemical resistant spray bottles and screw them directly into the rubbing alcohol bottle. Sometimes the stem needs to be trimmed. This allows you to have a spray bottle that is properly labeled with rubbing alcohol information and precautions.

3.1.1 Any equipment or footwear not cleaned and sanitized at the field office must be cleaned and sanitized before entering the site. Off-site soil should be considered contaminated.

3.1.2 Using the items in the portable sanitation kit, set up a staging area where equipment and footwear will be cleaned and sanitized. A paved parking lot or surface near the entrance to the work site is preferred.

3.1.3 Lay out 2 tarps, one labeled 'dirty' and one labeled 'clean'.
Remove any off-site soil from footwear and equipment onto the 'dirty' tarp. Try not to use water. If water is used, DO NOT dump potentially contaminated water onto on-site soil. Water can be dumped onto non-permeable pavement such as a road or parking lot in a low traffic area. This will UV-sterilize the dirty water (24 hr daylight cycle) as long as no clumps exist. Potentially contaminated soil in the 'dirty' tarp should be bagged in a trash bag and thrown away. DO NOT dispose of off-site soil at the new site.

3.1.4 Use the 'clean' tarp to sanitize soil-free footwear and equipment. Standing on the tarp, spray cleaned footwear and tools with 70% isopropyl alcohol, thoroughly wetting the surface. If the surface of your footwear or tools is already wet, spray extra alcohol to displace the water and allow the alcohol to soak the surface. Spray the footwear from the top down to avoid contamination.

3.1.5 Allow alcohol to evaporate (approx. 1 min) before starting work. You can stand on the tarp until your shoes are dry.

3.1.6 Footbath Alternative - we are investigating sanitizing mats where sanitizing only requires stepping on the mat. Gemplers.com, sanistride.com, and nelsonjameson.com sell both sponge mats and footbath mats for disinfecting shoes. Either chlorine bleach or non-evaporating disinfectants are used in these footbaths and the solution is changed weekly or as needed. Chemical strips are available to test if disinfectants are still effective. Caution should be taken if footbaths and solutions are transported to avoid spills.

3.1.7 Bleach alternative in the field. We are currently recommending that the bleach alternative be used at the field office and alcohol be used in the field. Bleach may be a better alternative in the field under some circumstances (large amounts of tools that must be disinfected in field), but will require special processes for safety and to properly dispose of the chlorine treatment water. Consult with the NR Department to determine best methods under these conditions.

3.2 Cleaning at End of Field Day

Tools:

Portable sanitation kits include the following items in a bin: 2 tarps, boot brush withscraper, 2 spray bottles of 70% isopropyl alcohol, 2 long-handled brushes, 2 paint scrapers, and instructions. On muddy days, also bring a basin & 2 jugs of water.

3.2.1 Sanitation of equipment and shoes is important for known or suspected contaminated sites. More leniency can be given for 'clean' sites.

3.2.2 Remove all soil and organic material from footwear and equipment. Leave soil onsite. Use the boot scraper, paint scraper and a stiff brush to remove any soil and plant material on both the top and bottom of footwear and from tools including the digging ends and handles. Make sure to clean out crevices. On muddy days, fill the basin with water to assist in rinsing off excess soil once the majority of debris has been removed.

3.2.3 Water helps in removing dried clods of soil. This water can be dumped on-site only if the soil originates from on-site.

3.2.4 Standing on the 'clean' tarp, spray cleaned footwear and tools with 70% isopropyl alcohol, thoroughly wetting the surface and allowing it to dry (approx. 1 min). If the surface of your footwear or tools is already wet, spray extra alcohol to displace the water and allow the alcohol to soak the surface.

3.2.5 Before leaving the site, shake soil off the scrapers, brushes and tarp.

3.2.6 At the field office, thoroughly clean the portable sanitation kit by washing out, spraying with alcohol and drying the container and all contents before storage. The portable sanitation kit must be clean before moving to a new site.

4. FAQ

Q. What do we do with left over soil?
A. Depends on the soil. Soil from off-site should be disposed of in a trash bag and thrown away--there's no knowing if off-site soil is contaminated or not. On site soil can be disposed of on-site back where it came from.

Q. What do we do with dirty water?
A. Pouring on pavement or another non-porous surface should disperse the contaminated soil enough to UV (sun) sterilize the water. If using bleach, use neutralizer and the water can be considered clean and safe enough to pour out anywhere. Don't pollute! Other disinfectants need proper disposal that isn't safe for dumping on the ground. Contact Natural Resources Department (Amanda Mills/Coty Sifuentes-Winter) or EH&S for safe disposal procedures.

Q. How do we use the tarps?
A. Two tarps, two purposes. Dirty tarp: use as a containment area to clean off soil clogs, especially offsite soil, for later disposal. Clean tarp: provides users a clean surface to sterilize (with alcohol or other sanitation liquid) shoes and equipment not cleaned at the Field offices.

Q. When will we need to sanitize or use the kits?

A. 1. Contaminated sites (list TBD) 2. Planting events-NR staff lead 3. When NR Staff recommend sanitation. Most of these will be NR staff lead, otherwise a leading crew member will advise on Phytosanitary BMP.

Q. Can we use hot water to sterilize?
A. Hot water can be used only if equipment bathes in 120-125° water for 30 minutes in order to be effective at killing both surface contaminants and internal infections.

Q. What about large equipment and Ranger lead projects?
A. TBD. Field staff will be trained on phytosanitary measures. For field crew lead projects, a crew member should be in charge of facilitating phytosanitary compliance.

Q. Why does this take so much time?
A. It's best to prevent rather than respond to contamination by *Phytophthora*. Once a natural area has been exposed to this soil disease, it can slowly spread and kill other plants. It is very difficult and expensive to kill all the pathogens in the soil of a natural area.

5. Sources

CalPhytos.org. "Guidelines to minimize *Phytophthora* Pathogens in Restoration Nurseries". Suddenoakdeath.org. http://www.suddenoakdeath.org/wp-content/uploads/2016/04/Restoration.Nsy_.Guidelines.final_.092216.pdf

Kurowki, Chet. "Control Pathogen Spread through use of Disinfectants". Calseed.org. http://www.calseed.org/documents/Disinfectants%2004-22-14a.pdf

Cornell University Institutional Animal Care and Use Committee "Cleaning and sanitizing equipment used in the transport of animals." https://ras.research.cornell.edu/care/documents/ACUPs/ACUP532.pdf

http://agriculture.mo.gov/animals/pdf/animalag_guide4.pdf

6. Future Methods

Let us know how these guidelines worked for your project! We may adjust guidelines based on feedback.