

# Soilborne *Phytophthora* Species at Restoration Sites in the Midpeninsula Regional Open Space District<sup>1</sup>

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## Abstract

The inadvertent spread of *Phytophthora* species from nurseries into native ecosystems has increased interest in assessing *Phytophthora* diversity in native plant communities. Of major concern is the inadvertent movement of *Phytophthora* spp. from native plant nurseries into vulnerable habitats during restoration outplantings. Root-infecting *Phytophthora* spp. are abundant within plant nurseries; their introduction can result in failed plantings and further spread of *Phytophthora* into surrounding habitat. To assess *Phytophthora* diversity, we surveyed restoration sites within the Midpeninsula Regional Open Space District (MROSD) to determine the presence and distribution of *Phytophthora* pathogens.

In December 2017 and 2018, we collected and baited a total of 579 soil samples from the base of native shrubs and seedlings at 30 planted restoration sites, 12 planned restoration sites, and 29 non-planted areas adjacent to restoration projects. We also extracted DNA from each sample and submitted ITS1 amplicons (250 base pairs in length) for sequencing on the Illumina MiSeq platform. Distinct sequences, or operational taxonomic units (OTUs), were assigned to a species when the amplicon was a  $\geq 99\%$  match to known sequences. 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. To reduce the inclusion of false-positives we required an individual OTU comprise a minimum of 0.05% of the within-sample relative abundance.

*Phytophthora* was equally prevalent in planted and non-planted areas, however many species were only found in areas in which nursery plants were introduced. Soil baits yielded 18 *Phytophthora* species (73 samples, comprising 13% of the 562 samples baited). Common species of concern include *P. ramorum*, *P. cinnamomi*, *P. cambivora*, and *P. cactorum*, all of which are associated with plant decline in native plant communities. Metabarcoding revealed a high

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diversity of *Phytophthora* OTUs; 57% of the 220 samples sequenced contained at least one *Phytophthora* OTU detection above minimum thresholds. This method also detected ITS1 sequences matching the *P. quercina*-cluster (which may be *P. quercina* and/or *P. versiformis* or closely related taxon not in our database) and *P. tentaculata*, species of concern not detected by baiting. While the short read lengths and deficiencies in existing databases make positive identification of OTUs difficult, Illumina MiSeq sequencing is a sensitive tool able to detect prior introductions and describe *Phytophthora* diversity.

*Phytophthora* spp. are widespread within MROSD preserves, although some preserves had noticeably greater species diversity and detection frequency. Importantly, DNA-only detections may be remnants of prior introductions and may not indicate substantial disease at the site. However, the long-term outlook of *Phytophthora* establishment is poorly understood. *Phytophthora* can persist in soils and may cause disease later or on different hosts; disease development may be slow; and disease may only occur at specific stages like regeneration. For these reasons, future management of MROSD preserves and restoration projects should utilize best management practices to limit the spread of *Phytophthora* to surrounding environs.