Soil Microbial Ecology of Oregon White Oak in an Urban Landscape

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In a series of storms during the winter of 1995, several trees were lost from a grove of approximately 100 native Oregon white oaks (*Quercus garryana* Dougl.) on the Linfield College campus in McMinnville, Oregon. Inspection of the fallen trees revealed the absence of major structural roots, suggesting infection by *Inonotus dryadeus*, and evident symptoms of *Armillaria* root rot. Subsequently, horticultural practices within the grove were modified (summer irrigation and fertilization were eliminated and mowing was reduced to once/month) to minimize further damage by pathogenic fungi and to restore the health of the grove. In 1995, we began a study to quantify and describe changes in soil microflora as management practices were changed. Here, we describe baseline measurements of soil bacteria and fungi in the grove and adjacent sites. We describe antagonism of *Streptomyces* spp., which were isolated from the samples, against Armillaria, and mycorrhizal colonization of oak seedlings germinated in soil samples.

Materials and Methods

Collection of samples — Soil collections were made in December 1995 and January 1997 and in the summers of 1996 and 1997. We collected soil and root samples from six oaks located in the interior of the grove and along its perimeter. The specimens included one large savanna oak that serves as the visual symbol of the College (the "old oak"); two sites in a large lawn area

(the "graduation green") lying between the grove and the savanna oak; and two oaks located downslope from the graduation green (Table 1). Except during January 1997, when the Cozine Creek, which runs through the campus, was flooded, we also collected from four oaks growing on banks alongside this creek. Here, the oaks are growing in an unmanaged, relatively undisturbed riparian stand dominated by Oregon ash, *Fraxinus latifolia* Benth.

At each site, except the two in the graduation green where there are no trees, soil samples were taken from the base and six meters to the south of each tree. The two sites in the graduation green were chosen from the center of the lawn area away from any trees. Each sample consisted of five soil cores approximately 5-7 cm deep, which were mixed to give a composite sample of approximately 1,000 g of soil. Subsamples were combined again to form a total of 13 composited samples, which were stored at 5° C until processed (Van Elsas and Smalia 1997, Wollum 1994). Soil pH was determined using a Kelway® Soil pH and Moisture Tester. Soil types were obtained from the Soil Survey of Yamhill Area, Oregon (USDA SCS 1974).

Dilution plating — The soil was thoroughly mixed and sifted through a two mm screen to remove rocks and plant segments. Ten grams of each composite sample were added to 90 ml of sterile water, dispersed by agitation for 20 minutes, and serially diluted in deionized water. Triplicate samples of appropriate dilutions were made using the pour plate method (Wollum 1994, Zuberer 1994) in either tryptic soy agar (TSA) to isolate bacteria or rose bengal agar (RBA) containing chloramphenicol (0.1 mg/ml) to selectively isolate fungi (Atlas 1995). Plates were incubated at 25° C and counted after 48 hours. Counts were corrected for soil moisture content (Zuberer 1994).

Statistical analysis — All statistical analyses were performed using Microsoft Excel 5.0. Means of triplicate plate counts are expressed as colony forming units (CFU) per gram of dry soil. The 95 percent confidence intervals were defined as plus or minus two standard errors of the mean (± 2 SE). Standard regression analysis was used to test for relationships between plate counts and the environmental parameters of soil moisture and pH.

Mycorrhizal colonization bioassay — Attempts to use acorns from oaks collected from the oak grove for our mycorrhizal colonization bioassy failed when a hard winter frost killed every seedling. Subsequently, acorns collected from two sites within the city of McMinnville were obtained from the Oregon State University Extension forester and used to bioassay for mycorrhizal colonization. The acorns were surface-cleaned and sown in Monarch plant bands filled with soil from each site. The seedlings were harvested after seven months and measured for height and weight, number of leaves, number of root branches, and root biomass. Visual evaluation for the presence of ectomycorrhizae was conducted using a dissecting microscope. Root segments were removed from each seedling, thin sectioned, and stained with Trypan blue (Cox and Sanders 1974, Jarstfer and Sylvia 1997) in order to enable direct observation of ectomycorrhizal Hartig nets and mantles and arbuscular-mycorrhizal hyphae (AM) and arbuscules.

We searched for soil propagules (spores) of AM fungi by passing a slurry (100 g soil in 1 l water) through a nested series of sieves (1000 uM to 45?uM). Residues from the 350, 150, and 45 uM sieves were centrifuged in a sucrose gradient and the resulting pellet was searched under a dissecting microscope for the presence of spores in the AM fungal order *Glomales*.

Antibiosis — Actinomycete isolates were used in a cross-inoculation assay method (Hutchins and Rose 1984) to test for antagonism of *Streptomyces* spp. to the root rot fungus, *Armillaria mellea*. In December 1995, we isolated strep-

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tomycetes from the soil subsamples based on gross colonial characteristics (e.g., size of hyphae, size of colony, general colony appearance) and inoculated them onto sodium albuminate agar, a selective medium that allows for the rapid growth of *Streptomyces*. We grew *Armillaria mellea* for 10 days on yeast malt agar (YMA) and placed a subculture of the streptomycetes on the test plates. After incubation for two weeks at 25° C, we counted zones of inhibition between pathogen and antagonist and inspected the pathogen for hyphal abnormalities and altered morphology, which are indicators of antagonism between *Streptomyces* spp. and *Armillaria*.

Results

Soil bacteria and fungi — Total aerobic counts per gram of dry soil were approximately 107 CFU for bacteria and 105 for fungi (Figs. 1 and 2). No significant differences in microbial population counts were evident among sites (ANOVA $F = 1.29$, $p = 0.248$ for bacteria; $F = 1.36$, $p = 0.21$ for fungi) but significant seasonal differences were found for both bacteria and fungi ($F = 22.46$, $p < 0.05$ for bacteria; $F = 6.2$, $p = < 0.05$ for fungi). In both cases, microflora numbers were lower in winter than in summer. Low bacterial populations were also present in June 1997, when soil conditions were drier than in previous years.

The study area encompassed three soil types: Woodburn Silt Loam, Terrace Escarpment, and Wapato Silty Clay Loam. Soil pH averaged across the collection sites was generally lower in winter than in summer, averaging 5.6 in winter and 6.7 in summer ($t= 9.2$, $p<0.01$). Both bacteria and fungal numbers increased with increasing moisture content ($r2 = 0.08$, $p<0.05$ and $r2 = 0.07$, $p<0.05$ respectively). The relationship between pH and microflora populations depended on microfloral type: bacterial counts increased significantly with increasing pH ($r2 = 0.24$, $p<0.05$); fungal counts did not vary with pH ($r2 = 0.003$, $p = 0.67$).

Mycorrhizal colonization — Forty-one percent of the oak seedlings bioassayed supported ecotomycorrhizal fungal structures. None displayed any AM (endomycorrhizal) structures; however, soil sievings from all sites revealed spores from the AM genus *Glomus*.

Antibiosis — Twelve percent of our *Streptomyces* isolates displayed some antagonism toward *Armillaria*. However, we did not find a reduced incidence of antagonism in soils associated with diseased trees nor did we detect any relationship between the presence of antagonistic *Streptomyces* and site or management regime.

Discussion

The goal of our study was to quantify and describe soil microbiota in the rhizosphere of a grove of Oregon white oaks and in adjacent sites under different management regimes. The rhizosphere includes a narrow band (usually 5 mm) of soil surrounding plant roots where soil microorganisms, such as bacteria, fungi, algae and protozoans are found in higher numbers than in non-rhizosphere soil. We hypothesized that the total rhizospheric concentrations of soil bacteria and fungi would differ among sites and seasons, and change with improved horticultural practices in the grove.

The abundance and diversity of soil microorganisms reflects the species composition of above-ground vegetation, and the microorganisms affect growth and development of the

plants. For example, bacteria and fungi play a critical ecological role as decomposers in ecosystems, and approximately 90 percent of all plants assessed thus far support mycorrhizal fungi, indicating the important role they play in plant nutrition (Gray and Williams 1971, Sylvia et al. 1998). On the other hand, some microorganisms, such as *Armillaria*, are pathogenic. Actinomycetes are aerobic soil bacteria that form a mycelium composed of branching filaments, and **Streptomyces**, which produce antibiotics that can inhibit plant pathogens such as *Armillaria,* are often dominant members of the actinomycete population (Hutchins and Rose 1984).

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Quercus garryana *Douglas ex. Hook*

Soil type and other environmental factors may affect the distribution and diversity of microorganisms. Environmental influences on microbial diversity and distribution include season, temperature, pH, and soil depth and moisture (Alexander 1977, Killham 1994). In western Oregon, where winters are very wet and summers are essentially rain free (Franklin and Dyrness 1973), one would expect to find seasonal differences in soil microbiota. Horticultural practices may also affect the distribution and types of soil microorganisms. Native species, such as Oregon white oak, are adapted to summer drought and suffer in environments where summer watering, fertilizing, and mowing are common horticultural practices (Hopkins 1998). Such was the case in the oak grove on the Linfield College campus where the lawn underlying the oaks was irrigated for 14 weeks during the summer (June through September) with one inch of water per week. Subsequently (June 1995), summer watering, fertilizing, and mowing have been halted within the grove, but they continue in the graduation green, which affects both the large savanna oak and the oaks situated downslope from the graduation green.

Soil bacteria and fungi — Colony forming units of 107 for bacteria and 105 for fungi are within the range of values commonly reported for soil bacteria and fungi; bacteria are typically 100 times more numerous than fungi (Alexander 1977, Tate 1995). Although soil microflora varied considerably in space and time, we were unable to detect consistent significant differences in microbial population counts among sites. For example, no differences were detected between the microflora at sites along the periphery of the oak grove (with summer irrigation) and sites in the interior of the grove (without summer irrigation). Several years of sampling may be necessary to discern changes in the soil microflora in those sites where horticultural practices have improved recently. The highest numbers of bacteria were found at those sites where no changes have been made in horticultural practices, such as along the periphery of the grove and beneath the Old Oak.

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Bacterial populations were generally lower in June 1997, when soil conditions were drier than in previous years, suggesting that microflora at our sites may eventually respond to reduced summer irrigation.

Surprisingly, despite the differences in soils in the study area and the fact that both fungal and bacterial counts increased with increasing moisture, we were not able to detect differences in soil microflora associated with the three soil types. Woodburn Silt Loam is the most extensive soil found on Willamette Valley terraces and is moderately well-drained. Terrace Escarpments are found along small streams, such as the Cozine Creek, that have cut deeply into Willamette Valley terraces. Like Woodburn Series soils, Terrace soils are well drained, but tend to be more sloping and to include small seep spots. Both of these soil types typically support Oregon white oak. On the other hand, Wapato Silty Clay Loam consists of poorly drained soils in bottom lands along streams and supports a vegetation consisting primarily of Oregon ash (USDA SCS 1973). Thus, we anticipated that summer bacterial and fungal counts in Wapato soils would differ from those in Woodburn and Terrace soils. Because winter precipitation in Oregon is abundant and all soils are saturated, we did not expect to detect microflora differences in this season.

The dilution plate method detects only those microorganisms capable of multiplying in TSA and RBA culture media, aerobic conditions, and within 48 hours at 25° C (Johnson 1998, Tate 1995). Thus, the cultural conditions under which our study was performed precluded our detecting certain microflora, such as anaerobic bacteria that likely are present in greater numbers in the hydric Wapato soils. These same conditions may contribute to inflated estimates of fungal numbers in winter soils because spores, dormant in anaerobic soils, would germinate under the conditions of this experiment.

Correlations with soil pH — Johnson (1998) compared seasonal differences at our sites and two additional sites for one year (June 1997 and January 1998). She also found that bacteria were more abundant in winter than in summer possibly because they prefer a pH range of 6.5 to 7.5, which is well above the average winter pH of 5.6 that we document at our sites. In contrast to our findings, she did not detect any seasonal differences in numbers of fungi, which she attributes to their ability to remain functional over a wide range in pH and, thus, make up a larger percentage of the microflora community than bacteria in winter (Griffen 1972, Alexander 1977).

Mycorrhizal colonization — More than 95 percent of all plants support mycorrhizae, and roots colonized by mycorrhizal fungi are less susceptible to infection by root pathogens than non-mycorrhizal roots (Marx 1971). Although most of their interactions are with ectomycorrhizae, oaks support AM (endo-) and ectomycorrhizae (Bagyaraj 1991, Johnson 1998). Our finding that 41 percent of the oak seedlings bioassayed supported ecotomycorrhizal fungal structures is within the range of colonization detected in greenhouse experiments in oaks (Riffle and Tinus 1979). Arbuscular mycorrhizae do not grow well in culture (Bowen 1987) even though their spores are often isolated from soil samples (Johnson 1998). Thus, it is not surprising that we were unable to detect AM structures on our oak seedlings even though were able to identify

spores from the AM genus *Glomus* in our soil sievings.

We did not examine mature oaks for mycorrhizal structures, but a single observation of the root system of a fallen oak revealed dichotomously branding roots typical of ectomycorrhizae and rhizomorphs characteristic of *Armillaria*. Thus, both symbionts are most likely present at most, if not all, of our sites.

Antibiosis — Actinomycetes numbers are typically higher in summer when conditions are dry (Sylvia et al. 1998) and the pH is 6 or above. Thus, our winter soil samples, which were collected from wet, acidic soils, probably represent a low estimate of the total numbers present throughout the year. On the other hand, horticultural practices may, in fact, reduce actinomycete abundance in summer. In addition to increasing the presence of pathogens, such as *Armillaria* and to adversely affecting the inoculum potential of mycorrhizal fungi, intensive fertilizer use, which was typical in our study sites, decreases antagonist abundance (Sylvia et al. 1998).

A number of studies have reported actinomycete antagonism to root pathogens found in our region (Hutchins and Rose 1984) and have demonstrated their ability to inhibit the growth of *Armillaria mellea* and *Fomes annosus* under laboratory conditions (Gunderson 1963). Although we found that 12 percent of our *Streptomyces* isolates displayed some antagonism toward *Armillaria mellea* cultures, we do not know if this antagonism occurs at our study sites, nor do we know if the antibiotic substances are stable under field conditions. We are now attempting to isolate water soluble antimicrobial substances from the antagonistic *Streptomyces*, and we are using SEM and biochemical tests to identify the species.

In addition to antagonistic *Streptomyces*, mycorrhizal fungi may also inhibit *Armillaria mellea*. In healthy stands, mycorrhizal fungi compete favorably with root pathogenic fungi for colonization zones on the root surface.

Summary

In this paper, we discuss preliminary results of a long-term study to quantify and describe seasonal and site diifferences in soil microflora in a grove of Oregon white oak and adjacent sites under different management regimes. Although the results of our study have not yet been fully analyzed, we report the following preliminary findings:

-Total aerobic counts per gram of dry soil are within the range typically reported for soil microflora (107 CFU for bacteria and 105 for fungi). We found no statistically significant differences in microflora numbers across sites, but both fungal and bacterial counts are lower in winter than in summer. The highest numbers of bacteria were found at those sites where horticultural practices remain unchanged.

-Soil pH was generally lower in winter than in summer, averaging 5.6 in winter and 6.7 in summer. The relationship between pH and microflora populations depended on microfloral type: bacterial counts increased significantly with increasing pH; fungal counts did not vary with pH. Soil moisture varied seasonally and among sites. Both bacteria and fungal numbers increased with increasing moisture content.

-Forty percent of oak seedlings surveyed supported ectomycorrhizae, but we were unable to detect any AM structures. However, spores of the AM genus *Glomus* were found in all soil samples. Thus, mycorrhizae are present in the study area.

-Twelve percent of the *Streptomyces* isolated from the soil samples displayed some antagonism against *Armillaria*. Although we did not find a correlation of antagonistic *Streptomyces* to diseased trees, we suspect that the change in horticultural practices will benefit the oaks by altering the relationship between the root pathogen *Armillaria*, mycorrhizal fungi, and the *Streptomyces* populations.

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We plan to continue monitoring these sites over the next several years, comparing soil microflora between seasons and among sites where managment conditions differ or change over time.

This paper is based on a poster reporting on preliminary results. A manuscript, which will include an additional year of data and a complete analysis of our results, is in preparation.

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