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The Composition of the Fungal and Oomycete Microbiome of *Rhododendron* Roots Under Varying Growth Conditions, Nurseries, and Cultivars

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ABSTRACT

The microbiome of agricultural crops influences processes such as nutrient absorption, drought stress, and susceptibility to pathogens. Interactions between a plant's genotype and its environment influence the composition of the microbiome, but these interactions are not well understood. We compared how the fungal and oomycete microbiomes of rhododendrons from Oregon nurseries differed among cultivars, growth conditions, and nurseries. Roots were sampled from randomly selected container and field-grown plants of three cultivars of rhododendron at four nurseries. The internal transcribed spacer 1 (ITS1) barcode was sequenced with the Illumina MiSeq using two sets of primers specific to fungi and oomycetes, respectively. Sequences were used to infer community composition using VSEARCH and a custom reference database combining curated fungal and

oomycete sequences. Comparisons of diversity and community composition were conducted in R using the vegan and metacoder packages. Organism lifestyle was inferred using the FUNGuild database. Few oomycetes were found and fungal communities were dominated by saprobes and mutualists. Nurseries that grew plants in containers and in-field had a significantly higher diversity of fungi than those that only grew plants in containers. Microbiome composition differed significantly among growth conditions and nurseries, but not among cultivars. This suggests that, among these cultivars of rhododendron, environment is important in structuring the root microbiome, but cultivar is not.

Keywords: microbiome, mycology, plant pathology, rhizosphere and phyllosphere, soil ecology

Given major advances in microbiome research, it might soon become possible to engineer the microbiome of plants to improve nutrient absorption and health. Plants influence the composition and function of their microbiome by selectively recruiting a subset of the microbes from the surrounding environment that tend to provide benefits to the host (Berendsen et al. 2012). Plant genotype can affect this process, influencing the composition of the microbiome (Bálint et al. 2013; Panke-Buisse et al. 2015; Wagner et al. 2016).

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Different cultivars of maize are known to respond differently to inoculation with the nitrogen-fixing bacteria *Azospirillum*; in some cultivars, addition of *Azospirillum* is equivalent to 100 kg ha⁻¹ of nitrogen, whereas other cultivars are unaffected (De Salamone et al. 1996). Different cultivars of wheat and grape have been observed to harbor distinct microbiomes (Bokulich et al. 2014; Sapkota et al. 2015). For fermentation substrates like grapes, the microbiome has additional relevance due to its effects on the sensory qualities of the finished product (Swiegers et al. 2005). Common garden experiments of wild plants also suggest that the fungal microbiome differs among genotypes of the same species (Bálint et al. 2013; Wagner et al. 2016). There is evidence that microbiome differences in natural stands of European beech correlate more with genotypic differences than with geographical distance (Cordier et al. 2012). Agricultural management practices have a major influence on the plant environment, which determines which microbes can be recruited and which pathogens will be present. For example, how a nursery recycles irrigation water could influence the community of oomycete pathogens (Redekar et al. 2019). Given a mechanistic understanding of the factors required to assemble a microbiome and achieve certain functions, one can envision a future where direct management of the plant microbiome can improve crop production.

However, before a microbiome can be managed it has to be well characterized including how different hosts, cultivars, and production conditions can affect the microbiome.

High-throughput sequencing allows for rapid, affordable, and comprehensive characterization of the diversity found in microbial communities compared with sequencing by cloning or culturing (Ji et al. 2013). For example, obligate symbionts and pathogens that have important implications for agriculture are difficult to culture and are therefore less likely to be detected by traditional culture-based microbial surveys (Yarza et al. 2014). New techniques, such as metabarcoding (i.e., amplicon metagenomics) and shotgun metagenomics, rely only on sequencing mixtures of PCR products or raw genomic DNA derived from environmental samples of complex communities, such as soil, and are therefore less biased by organismal lifestyle. Metabarcoding is a particularly powerful technique that involves extracting DNA from complex samples, amplifying a common barcode gene with PCR, and sequencing amplicons using high-throughput sequencing (Taberlet et al. 2012). Sequences can then be used to estimate community diversity and compared with reference databases to estimate community composition (Cole et al. 2008). However, metabarcoding also has its own set of biases, such as differential PCR efficiency and limited taxonomic resolution (Nichols et al. 2018). Recently, there have been attempts to use metabarcoding to characterize the communities and the distribution of pathogenic organisms, such as *Phytophthora* spp. in agricultural settings (Prigigallo et al. 2016; Riddell et al. 2019).

Rhododendron is a major ornamental crop in the Pacific Northwest and is known to host both mycorrhizal symbionts and plant pathogens (Farr et al. 1996; Knaus et al. 2015; Parke and Grünwald 2012; Parke et al. 2014). The nursery and greenhouse industry is a leading agricultural sector in the Pacific Northwest, with gross sales in Oregon of \$948 million in 2017 and rhododendrons are one of the leading ornamental plants sold (Losh 2017). Plants in the family Ericaceae, including *Rhododendron*, are known to form a distinct type of mutualism with fungi known as ericoid mycorrhizae. Ericoid mycorrhizae are known to aid their host in nutrient absorption and survival in poor and polluted soils (Cairney and Mehrag 2003). Rhododendrons in nurseries are also known to be vectors of the sudden death pathogen *Phytophthora ramorum* (Grünwald et al. 2008; Werres et al. 2001) and other oomycetes such as *Phytophthora plurivora* (Weiland et al. 2018). The presence of well-known mutualists and multiple pathogens combined with its economic importance make *Rhododendron* a good system to study the effects of management and cultivar on phytobiome composition (Jones and Benson 2001).

Root pathogens and symbionts described on rhododendron include both fungi and oomycetes. Oomycete pathogens generally cause root rot or damping off. Commonly found oomycetes on *Rhododendron* include *Phytophthora plurivora*, *Phytophthora cinnamomi*, and *Pythium cryptoirregulare* (Weiland et al. 2018). Fungal pathogens include *Cylindrocladium scoparium*, *Cylindrocladium theae*, *Rhizoctonia solani*, *Armillaria mellea*, and *Thielaviopsis basicola* (Dreistadt 2001; Jones and Benson 2001). Common ericoid mycorrhizal fungi isolated from ericaceous plants include ascomycetes such as *Rhizoscyphus ericae*, *Oidiodendron maius*, and dark septate endophytes, such as *Phialocephala fortinii*. Some basidiomycetes also occur, including *Clavaria* and members of the order Sebaciales (Dighton and Coleman 1992; Vohník and Albrechtová 2011; Vohník et al. 2005).

The goal of this study is to characterize the fungal and oomycete root microbiome of *Rhododendron* in Oregon nurseries and determine what factors might influence its composition. Therefore, we evaluated the relative importance of host genotype, environment,

and management practices on structuring *Rhododendron* microbiomes in the rhizosphere using high-throughput sequencing. Specifically, we used metabarcoding to survey fungal and oomycete rhizosphere communities from three cultivars in four nurseries. We tested the hypothesis that microbiomes would differ among cultivars, nurseries, and production systems (container versus field-grown). We also expected to detect well-known *Rhododendron* pathogens, such as *Phytophthora cinnamomi* or *Phytophthora plurivora*, and well-known *Rhododendron* symbionts, such as *Rhizoscyphus ericae* or *Phialocephala fortinii* (Jones and Benson 2001; Knaus et al. 2015; Parke et al. 2014; Weiland et al. 2018). Our work provides novel insights into the makeup of oomycete and fungal communities in *Rhododendron* roots and is one of the few studies to apply Illumina sequencing to oomycete metabarcoding.

MATERIALS AND METHODS

Sample collection. The rhizosphere of *Rhododendron* was sampled to determine the effect of nursery, production system (container versus field-grown plants), and cultivar. Plants that appeared healthy were sampled in four nurseries from three cultivars in the Willamette Valley, Oregon, United States during May 2014. The nurseries varied in size and management practices. Nurseries A and B grew plants in both field and container systems while nurseries C and D grew plants only in containers. Both field-grown and container-grown plants were sampled. *Rhododendron* cultivars Nova Zembla (RHS 58), Roseum Elegans (RHS 58), and PJM (ARS874) were sampled in all nurseries and in both growing conditions. Five plants were sampled at random from each combination of nursery, cultivar, and production system (i.e., field-grown versus container-grown). Two nurseries only grew the selected cultivars in containers, so field-grown plants were only sampled from the other two nurseries. Each sample consisted of four equally-sized subsamples, each roughly 50 cm³, taken from opposite sides of the root ball. Root balls were sampled by hand using sterile gloves and transported to the lab on ice. In the case of field-grown plants, a hand trowel was used to expose the root ball on four sides and this trowel was sterilized between plants by rinsing in distilled water and 10% bleach.

Sample processing. Root ball samples were broken apart by hand using sterile gloves and excess dirt or potting media was shaken off to obtain rhizosphere samples. Gloves were changed between samples to avoid cross-contamination. Each frozen rhizosphere sample was ground by hand using a clean mortar and pestle with liquid nitrogen. Mortars and pestles were autoclaved for 1 h and soaked in 10% bleach for at least 4 h between uses (Prince and Andrus 1992). Ground rhizosphere samples were stored at -80°C in prechilled Falcon tubes (Corning, MA) until DNA extraction of approximately 150 mg of the sample using the Fast DNA Spin Kit (MP Biomedicals, Santa Ana, CA). The samples were not allowed to thaw at any time during this process.

PCR. We characterized both fungal and oomycete diversity using two internal transcribed spacer 1 (ITS1) primer pairs specific to each group. The fungal PCR used primers ITS1F (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNCAAACTTGTCATTTAGAGGAAGTAA 3') and ITS2 (5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCGTTCTTCATCGATGC 3') (White et al. 1990). The underlined sequences are the Illumina adapters and the italic sequence is a spacer added to increase the annealing temperature to what is recommended in the Illumina 16S sample preparation guide (Illumina Inc., San Diego, CA). The spacer was designed to be complementary to fungal DNA based on alignments of the primer to a random selection of sequences downloaded from GenBank. Each reaction consisted of

1× PCR buffer, 0.2 mM dNTP mixture, 1 µM of each primer, 0.15 µl of GeneScript Taq polymerase (GenScript, Piscataway, NJ), and 2 µl of template DNA extract in a total volume of 15 µl. The thermocycler profile was 3 min at 94°C, followed by 30 cycles of 30 s at 94°C, 45 s at 60°C, and 1 min at 72°C with a final elongation for 7 min at 72°C. The oomycete PCR was seminested and used ITS6 (GAAGGTGAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) without the MiSeq adapters followed by ITS6 (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGKGAAGGTGAAGTCGTAACAAGG) and ITS7 (GTCCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCGTTC TTCATCGATGTGC) with the MiSeq adapters (Sapkota and Nicolaisen 2015). The reactions for the first PCR consisted of 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTP mixture, 0.2 µM of each primer, 0.04 units Platinum Taq polymerase (ThermoFisher Scientific, Waltham, MA), and 7.5 µl of template DNA extract in a total volume of 15 µl. The thermocycler profile was 2 min at 94°C, followed by 25 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C with a final elongation for 2 min at 72°C. The second PCR had the same reaction composition and thermocycler profile, except that 1.5 µl of a 1:10 dilution of the first PCR was used as the template.

Sequencing. The pooled oomycete and fungal libraries were sequenced on the Illumina MiSeq (Illumina) at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University. Fungal and oomycete PCR products were mixed in equal volumes and used by the CGRB Core Lab to create sequencing libraries. In brief, the mixture of PCR products was cleaned with AMPure XP beads (Beckman Coulter, Brea, CA). Sample indexes were added using the Illumina Nextera XT kit and cleaned again with AMPure XP beads. The samples were then quantified using an Agilent Bioanalyzer (Agilent, Palo Alto, CA), diluted to the same concentration, and pooled. A total of 25% PhiX was added to the pooled samples to correct for low diversity sequence bias. This library was then run on the CGRB's Illumina MiSeq using 250 bp paired-end sequencing.

Data analysis. An abundance matrix of operational taxonomic units (OTUs) versus samples was generated in order to compare the diversity and composition of the different cultivars, nurseries, and management practices. Primers and low-quality sequences were removed with *cutadapt* (Martin 2011). Any sequences with greater than 10% mismatch to the primers or more than two “N” ambiguity codes were filtered out. All sequences from the ends of each read that had a phred score of less than 20 were removed. VSEARCH (Rognes et al. 2016) was then used to merge forward and reverse reads. Pairs with less than 15 bp overlap or more than three mismatches were filtered out. Unique reads were found using the *derep_fulllength* command and singletons were filtered out. Predicted chimeric sequences were filtered out using the *uchime_de-novo* command. The remaining sequences were then clustered into OTUs using the *cluster_size* command at 99 and 97% to approximate species-scale differences for oomycetes and fungi respectively. Finally, the *usearch_global* command was used to assign taxonomic classifications to OTUs by comparing them to a custom reference database composed of the UNITE database (Koljalg et al. 2005) for fungi, and *Phytophthora*-ID (Grünwald et al. 2011), *Phytophthora*-DB (Park et al. 2008), and the sequences from Robideau et al. (2011) for oomycetes. OTUs with less than 10 reads were filtered out. The taxonomy assigned to OTUs was used to infer organism lifestyle using FUNGuild (Nguyen et al. 2016). The FUNGuild results presented are those for OTUs that had at least a 97% identity with the reference sequence supplying the taxonomy and that were considered “Probable” or “Highly probably” by FUNGuild.

The diversity of communities was compared with alpha (diversity within a sample) and beta (the compositional dissimilarity between two samples) diversity statistics and ordination techniques using the R packages *vegan* (Dixon 2003), *taxa* (Foster et al. 2018), and *metacoder* (Foster et al. 2017). The inverse Simpson index was calculated for each sample as a measure of alpha diversity and the Bray-Curtis index was calculated for each pair of samples as a measure of beta diversity. Differences in alpha diversity among factors were determined using a Tukey's honest significant difference (HSD) test following analysis of variance (ANOVA). Differences in beta diversity were visualized using nonmetric multidimensional scaling (NMDS) using the *metaMDS* function from *vegan* (Kruskal 1964). Permutational multivariate analysis of variance (PERMANOVA), as implemented by the *adonis* function in the *vegan* package, was used to test which factors (cultivars, nurseries, and management practices) might be important for explaining differences in beta diversity (Anderson 2001). PERMANOVA is a nonparametric approach allowing partitioning of variance among factors, analogous to a factorial ANOVA. All factors and interactions were included in the model.

To test for taxa with differential abundance between factors, nonparametric Wilcoxon rank-sum tests (Mann and Whitney 1947) with a false discovery rate (FDR) correction for multiple tests were performed on the median read proportions for each taxon, at all taxonomic ranks. The results were visualized with differential heat trees using *metacoder*. For experimental factors that had more than two types (i.e., cultivar and nursery), differential abundance tests were conducted for each pairwise combination of types.

Data availability. All supplementary materials including R scripts to reproduce the analysis and figures, FASTQ files, and the OTU abundance matrix were deposited at Open Science Framework (Foster and Grünwald 2019). The raw MiSeq sequences are also deposited in NCBI's Sequence Read Archive (BioProject PRJNA561631).

RESULTS

Sequencing. Raw reads were filtered and grouped into OTUs to simulate species-level differences. After processing the raw sequences with VSEARCH, a total of 3,120,565 reads were assigned to OTUs. At the primer removal step, approximately 6.3% of the reads were derived from the oomycete primers and 93.7% of the reads were derived from the fungal primers. After quality filtering, 3.6% of the reads were assigned to oomycetes and 96.4% of the reads were assigned to fungi. The number of raw reads per sample ranged from 8,869 to 82,015. There were 1,915 OTUs in the raw data and 731 remaining after removing OTUs with fewer than 10 reads. The number of OTUs per sample ranged from 68 to 259 for raw counts and from 29 to 98 for filtered counts.

Alpha diversity. Differences in alpha diversity (i.e., the diversity within a sample) were determined using ANOVA followed by a Turkey's HSD test. Overall, differences in alpha diversity among factors were small or not significant (Fig. 1). There were minor, but significant, differences in diversity among the three cultivars. Nursery B had significantly higher alpha diversity than the others. Although there was no difference in alpha diversity between container-grown and field-grown plants, the species diversity of container-grown plants in nurseries that also grew plants in-field was greater than from container-grown plants in nurseries that only grew rhododendrons in containers ($P < 0.05$). The same analyses were done at the OTU and genus levels with similar results (Supplementary Figs. S1 to S3).

Beta diversity. Differences in rhizosphere community composition correlated with differences in nursery and production system,

but not with differences in cultivar. NMDS revealed distinct communities associated with each combination of production system and nursery when using two (Fig. 2) or three dimensions (Supplementary Fig. S4). Production systems (e.g., container versus field-grown) separated communities based on axis NMDS1, whereas axis NMDS2 separated nurseries (Fig. 2). There was little,

if any, clustering associated with cultivar. PERMANOVA supported the NMDS results, showing highly significant associations between community similarity and production system ($R^2 = 0.149$; $P < 0.001$) or nursery ($R^2 = 0.234$; $P < 0.001$) (Table 1). Cultivar was also significantly correlated, but the effect size was small ($R^2 = 0.025$; $P = 0.001$). The same analysis on other levels of the

Fig. 1. Alpha diversity of the combined fungal and oomycete species in the *Rhododendron* rhizosphere in different cultivars (Nova Zembla, PJM, and Roseum Elegans), nurseries (A, B, C, and D), nursery types (plant grown in containers and field soil versus nurseries growing only in container soil), and production systems (container versus field-grown). Letters represent significantly different distributions as determined by analysis of variance followed by a Tukey's honest significant difference test.

Fig. 2. Two-dimensional nonmetric multidimensional scaling of Bray-Curtis distances between samples based on operational taxonomic unit relative abundance for both oomycetes and fungi. Ellipses represent 95% confidence intervals, assuming a multivariate t-distribution. Samples colored by **A**, container versus field-grown *Rhododendron*, **B**, the four nurseries sampled, and **C**, the three *Rhododendron* cultivars sampled.

taxonomic hierarchy (e.g., OTU, species, genus, and family) showed similar results (Supplementary Fig. S5).

Only the comparison between container and field-grown samples indicated significant differences in median read abundance for numerous taxa, as determined by Wilcoxon rank sum tests followed by a false discovery rate correction (Fig. 3 and Supplementary Fig. S6). Taxa with a greater proportion of reads assigned to them in field-grown samples than container-grown samples include: *Phialocephala fortinii*, *Cladophialophora chaetospora*, *Galerina atkinsoniana*, *Solicoccozyma terrea*, and *Trichoderma crassum*. Taxa with a higher read abundance in container-grown samples than in field-grown samples include: *Coniochaeta lignicola*, *Lecythophora fasciculata*, *Pleurostoma richardsiae*, *Sporothrix lignivora*, and *Exophiala heteromorpha*. No significant differences between experimental factors were observed for any oomycete taxa, probably due to the infrequency of detecting individual oomycete taxa relative to the number of samples for each factor.

Organismal diversity. Saprobies and beneficial fungi dominated the fungal and oomycete communities in rhododendron roots, according to FUNGuild results. Most sequence reads were assigned to saprobies and beneficial organisms (Supplementary Fig. S7). Pathogens were much less common than either saprobic or beneficial fungi. The three most common putative mutualists were OTUs matching reference sequences ($\geq 99.5\%$ identity) for *Lecythophora fasciculata*, *Trichoderma pubescens*, and *Phialocephala fortinii*, appearing in 74, 68, and 53% of samples, respectively. The 10 most common saprobies occurred individually in 38 to 18% of samples and included OTUs that matched reference sequences ($\geq 99.5\%$ identity) of *Xenopolyscytalum pinea*, *Humicola grisea*, *Cladophialophora chaetospora*, *Scytalidium lignicola*, and *Trichocladium opacum*. We also found taxa typically associated with ericaceous plants to be common, including taxa in the genera *Rhizoscyphus*, *Meliniomyces*, *Oidiodendron*, *Pezoloma*, *Hymenoscyphus*, *Phialocephala*, and the order Sebaciniales (Fig. 4 and Supplementary Fig. S7). *Trichoderma* (some with purported biocontrol properties) and the saprophytic genera *Galerina* and *Mortierella* were also common and diverse.

Putative plant pathogens were generally much less common than saprobies or mutualists. Some potential fungal pathogens found include *Pestalotiopsis unicolor*, *Pestalotiopsis rhododendri*, and *Ophiostoma bragantinum*, which were found in 23, 10, and 8% of samples, respectively. The genus *Microbotryum* was also found. There were much fewer OTUs assigned to oomycetes than fungi

and these OTUs occurred in much fewer samples than the common fungal OTUs. Due to the nature of oomycete ITS1 sequences, OTUs that differ by more than 1% in sequence identity from their closest reference sequences are very likely different species than the one assigned and even exact matches do not always resolve individual species (Redekar et al. 2019). The reference sequences that were matched well ($>99\%$ identity) by the most common oomycete OTUs include *Pythium irregulare*, *Pythium sylvaticum*, *Phytophthora cactorum*, *Phytophthora citricola*, and *Phytophthora infestans*. These putative pathogens appeared in 11 to 2% of samples. Less frequently, sequences matching *Pythium macrosporum* and *Pythium dissotocum* were found. Other OTUs found had no close match to a reference sequence or occurred in only one sample. These likely represent either technical error or species not in the reference database.

DISCUSSION

The environment of plants has a strong influence on the composition, and therefore function, of the microbiome (Bonito et al. 2014; Wagner et al. 2016). The plants sampled in this study differed by nursery and production system (container versus field-grown), both of which constitute different environments that are expected to change the composition of associated microbiomes. Of all the factors tested, production system and nursery were the two best predictors of differences in the root microbiome according to NMDS (Fig. 2) and PERMANOVA (Table 1). The effect of production system is likely due in part to the difference in growing media: container-grown plants were grown in nearly 100% Douglas fir bark chips whereas field-grown plants were grown in soil. Surprisingly, there was no significant difference in alpha diversity between the two types of production systems (Fig. 1). We expected that field soil would support a more diverse community of fungi than a potting mix largely composed of bark and that would be reflected in the diversity of the rhizosphere community. However, it could be that rhododendrons select a subset of the bulk soil community, as happens in other plants (Uroz et al. 2010), and that both environments have more diversity than is selected, even if the potting media is less diverse. Since we did not analyze bulk soil samples, we cannot confirm this. It could also be that the soil in the fields had relatively little organic matter and thus supported less saprotrophic diversity than the potting mix that was almost entirely organic matter. The composition of rhizosphere communities also varied among nurseries (Fig. 2), even though production system was the dominant effect. For example, the container-grown plants from nurseries A and B clustered together in NMDS ordination, as to a lesser degree did the field-grown plants. This suggests that there could be microbiomes characteristic of container-grown and field-grown plants regardless of where they are grown, even though the location also has an effect. If this is true, it would mean that cultivation practices can have predictable effects on the microbiome of *Rhododendron* and that there is potential for optimizing these practices to maximize the benefits received from the microbiome. However, a study of a much greater number of nurseries, representative of a larger geographical area and greater diversity of cultivation practices, would be needed to verify that this result applies to nursery-grown *Rhododendron* in general.

In many agricultural plant species, slight genotypic differences influencing interactions with the plant's microbiome can have a large impact on plant productivity (De Salamone et al. 1996). Surprisingly, we observed little, if any, differences between the microbiome compositions of the three cultivars of *Rhododendron* sampled (Fig. 2). This suggests that either the cultivars we selected

TABLE 1 Results of permutational multivariate analysis of variance (PERMANOVA) of Bray-Curtis distances between samples ^a		
Factor	<i>R</i> ²	<i>P</i>
Cultivar	0.025	0.0012
Production system	0.149	0.0001
Nursery	0.234	0.0001
Cultivar × production system	0.025	0.0007
Cultivar × nursery	0.076	0.0001
Production system × nursery	0.045	0.0001
Cultivar × production system × nursery	0.028	0.0003
Residuals	0.418	–
^a PERMANOVA was conducted to test for correlations between community similarity and combinations of cultivar, production system (field versus container), and nursery factors. A total of 9,999 permutations were performed.		

are not very distinct genotypically or that their differences are not relevant for structuring the microbiome under the conditions sampled. If the microbiomes of other cultivars of *Rhododendron* are similarly indistinct, attempts at breeding for beneficial microbial communities will likely be ineffective. A study of the perennial herb *Boechera stricta* revealed an association between genotype and leaf microbiome but no association between genotype and root microbiome, although a genotype by environment interaction was significant (Wagner et al. 2016). In our results, interactions between environmental factors (production system and nursery) and cultivar were also more significant than cultivar alone according to PERMANOVA, but the effect size was small relative to the environmental factors alone (Table 1). In addition, the relative effects of environment and genotype can be different for bacteria and fungi (Bonito et al. 2014), so it is possible that bacterial communities, which we did not characterize, have a stronger association with genotype.

Saprobies and symbionts are the most ubiquitous organisms observed in the *Rhododendron* rhizosphere. Some of the most commonly observed organisms, *Humicola grisea* and *Cladophialophora chaetospora*, have been isolated from *Rhododendron* in other studies and *Trichoderma opacum* has been isolated from other ericaceous plants (Bruzzone et al. 2017; Kowalik et al. 2015; Vanó et al. 2011). *Cladophialophora chaetospora* has been observed to form intracellular structures in root cells of *Rhododendron*, similar to ericoid mycorrhizae (Vanó et al. 2011). *Scytalidium lignicola* is an anamorphic ascomycete generally found in wood or compost and is related to pathogens of *Citrus* and *Manihot* (Büttner et al. 2018). The common ericoid mycorrhizal fungus *Pezoloma ericae* (aka *Hymenoscyphus ericae*) was found often (Vrålstad et al. 2002). Ericoid mycorrhizal fungi, such as *Pezoloma ericae*, have been previously reported on *Rhododendron* and as root endosymbionts shown to aid in the breakdown of organic debris, releasing mineral

Fig. 3. Differential heat tree (i.e., taxonomic tree) showing significant differences in median read proportion between fungal operational taxonomic units found in the rhizosphere from container-grown (green) versus field-grown (brown) rhododendrons. Significance was determined using Wilcox rank-sum tests with a false discovery rate correction for multiple comparisons. Only taxa that appear in at least five samples are shown.

forms of nutrients that are available to the plant host (Smith and Read 2010). The dark septate endophyte *Phialocephala fortinii* was also common and is known to aid in the breakdown of organic compounds, potentially leading to increased growth (Surono and Narisawa 2017). The commonly found genus *Trichoderma* is known to contain many plant rhizosphere symbionts with bio-control applications (Harman 2006). The frequent occurrence of individual beneficial symbionts and saprobes combined with the negative results of differential taxon abundance analysis among nurseries and cultivar suggest that there might be a subset of the fungal community that is typical of rhododendron.

Plant pathogens were much less common than saprobes and mutualists. The most common pathogen found was *Pestalotiopsis unicolor* (99.6% identity), which appeared in 23% of samples. The genus *Pestalotiopsis* contains a diverse group of anamorphic plant pathogens that causes a variety of diseases such as leaf spots, blights, and cankers in many agricultural hosts, including blueberry (Maharachchikumbura et al. 2014). An OTU similar to *Pestalotiopsis rhododendri* (99.1% identity) was also observed in 9% of

samples. *Pestalotiopsis rhododendri* was originally described on *Rhododendron sinogrande* in 2013 in China (Zhang et al. 2013). The only oomycete pathogen that was commonly predicted and had a high sequence similarity to its reference was an OTU matching *Pythium irregulare*, which occurred in 11% of samples. The next most common *Pythium* observed was *Pythium sylvaticum*, occurring in 3% of samples. Oddly, an OTU matching *Phytophthora infestans* was also found in 3% of samples. However, it should be noted that we only sequenced ITS1 and even with the entire ITS sequence, it is not always possible to differentiate oomycetes species, so even a 100% sequence match to a reference sequence does not necessarily imply that an OTU is from that species. In particular, *Pythium irregulare* has the same sequence as *Pythium cryptoirregulare* and *Phytophthora infestans* has the same sequence as *Phytophthora andina*, *Phytophthora mirabilis*, and *Phytophthora ipomoeae* in this region of the ITS (Redekar et al. 2019). The OTU matching *Phytophthora infestans* could also be from an undescribed species, considering that the known species sharing a sequence with *Phytophthora infestans* are not known to occur on

Fig. 4. A heat tree (i.e., taxonomic tree) of fungal taxa with unambiguous classifications found in at least five samples. Edge width is proportional to the number of samples a given taxon was found in. The color and size of nodes is the relative standard deviation of operational taxonomic unit read proportions and the mean read proportion, respectively. Cooler colors indicate less variation between samples and hotter colors indicate more.

Rhododendron. The rare OTU classified as *Phytophthora citricola* could also be *Phytophthora plurivora*, which was found to be common in diseased rhododendrons in Oregon (Carleson et al. 2018). Notably, we did not detect some pathogens commonly found when sampling symptomatic tissue, such as *Phytophthora syringae* and *Phytophthora citrophthora* (Parke et al. 2014). We found a rare OTU in two samples that most closely matched *Phytophthora cinnamomi* with 98.9% sequence identity, but for the previously mentioned reasons, this level of similarity does not indicate that *Phytophthora cinnamomi* was actually present. We also found fewer *Phytophthora* species compared with some other oomycete metabarcoding studies, which find around 20 species total, albeit in diseased plants for a greater diversity of sites (Prigigallo et al. 2016; Riddell et al. 2019). The relative infrequency of pathogens and the different pathogens found compared with some previous research is probably due to sampling apparently healthy plants, whereas many studies target symptomatic plants (Weiland et al. 2018).

This study used high-throughput amplicon sequencing to characterize how the oomycete and fungal rhizobiome of rhododendrons in Oregon nurseries is structured by plant cultivar and environmental factors. The overall diversity of samples varied little between production system, nursery, and cultivar, although plants in nurseries that had both container and in-field production systems were significantly more diverse than those in nurseries that only grew plants in containers. The differences between the compositions of microbiome communities were correlated with production system and nursery, but not cultivar. Communities were dominated by saprobes and symbionts. This study provides novel insights into potential factors influencing the rhizosphere microbiome of rhododendrons in nurseries, and more generally, how plant genotype and environment impact the makeup of the rhizobiome of woody plants.

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