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Comparative Analysis of the Rhizosphere and Endophytic Microbiomes across Apple Rootstock Genotypes in Replant Orchard Soils

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ABSTRACT

Apple replant disease (ARD), caused by a complex of soilborne pathogens, negatively impacts tree health and productivity in new orchard plantings at sites previously planted to apple. Use of new disease-tolerant apple rootstock genotypes may diminish growth-limiting effects of ARD; however, the influence of rootstock genotype on modulating the rhizosphere and endophytic microbiome to enable ARD tolerance is not fully understood. Composition of the rhizosphere and root endophytic microbiomes was characterized across a diversity of apple rootstock genotypes. A series of tolerant (G.210, G.41, G.890, and G.935) and susceptible (M.26 and M.9) rootstock cultivars were consecutively planted into orchard replant soil containing a known pathogen complex. Amplicon sequencing was used to determine simultaneously the presence of a broad taxonomic range of organisms and their relative abundance. Microbial communities exhibited significant differences in composition between the rhizosphere and endophytic environments in terms of species

diversity, content, and abundance. Significant differences in composition of the endophytic and rhizosphere microbiomes were observed between rootstock genotypes. Among rootstock endophytic fungal communities, rootstock G.890 samples consistently harbored the highest percentage of arbuscular mycorrhizal fungal species (>5% of total). *Ilyonectria* spp., which may function as a pathogen of apple, were detected at high relative abundance in the endosphere of all genotypes, with the highest relative abundance in M.26. These results provide further insight into rhizosphere and endophytic microbial communities of apple rootstocks, which could be exploited or manipulated to improve tree fruit agricultural management practices with respect to plant nutrition and disease control.

Keywords: amplicon sequencing, apple replant disease, endosphere, microbiome, plant pathology, rhizosphere and phyllosphere, rootstock

The global apple industry has a long history of innovation and development of management practices to increase yield and improve production efficiency. New varieties and rootstocks are developed by breeding programs to meet current market demands and adjust to changing climate and environmental pressures. The process of rapid orchard turnover and replanting is functionally limited by a problem known as apple replant disease

(ARD), symptoms of which include poor establishment of the new planting, reduced growth, and subsequent crop losses (Mazzola and Manici 2012). ARD is a significant barrier to the viability of new plantings, with reductions in productivity and yield resulting in losses ranging from \$70,000 to \$150,000 per acre over the initial 4-year period after orchard establishment (Hewavitharana et al. 2019a). Utilization of innate host resistance or tolerance can provide a cost-effective, durable, and environment-friendly strategy for the control of soilborne plant diseases. Apple rootstocks, though not bred specifically for resistance to pathogens that incite ARD, have been evaluated for tolerance to ARD across diverse geographic regions (Fazio et al. 2004, 2014). However, the combination of microorganisms involved in disease progression and variability in relative pathogen dominance across locations is an impediment in the assessment of rootstock genotype disease susceptibility in a standardized manner.

In perennial cropping systems such as orchard environments, soilborne plant pathogen densities increase over time. Although

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established trees with robust root systems can often withstand the activity of an increasingly elevated pathogen population, young, newly planted trees are more vulnerable to the complex of pathogens that incites ARD (Atucha et al. 2014; Mazzola 1999). ARD is caused by several organisms, including fungi (e.g., *Ilyonectria* and *Rhizoctonia* spp.) (Braun 1995; Mazzola and Manici 2012; Tewoldemedhin et al. 2011); oomycetes (e.g., *Pythium* and *Phytophthora* spp.) (Braun 1995; Mazzola 1998); and a root lesion nematode, *Pratylenchus penetrans* (Mai and Abawi 1981; Mazzola et al. 2009). These causal agents of ARD can be variable in relative abundance or species composition among different sites and regions (Mai and Abawi 1981; Mazzola 1998).

Preplant soil fumigation has commonly been employed as a tool in the management of ARD. However, studies have demonstrated that the benefit of this tactic in terms of pathogen suppression is limited to the initial year of orchard establishment (Mazzola et al. 2015; Wang and Mazzola 2019b). Fumigated soils are not resilient to reinfestation by soilborne pathogens and the practice is generally viewed as a detriment to environmental quality and human health. Similar to fumigation, preplant application of specific mustard seed meal formulations as soil amendments has been shown to effectively reduce ARD incidence and enhance tree growth and yield (Wang and Mazzola 2019b). The capacity of a rootstock to benefit from alternative treatments such as mustard seed meal amendment appears to vary with genotype and is associated with differential composition of the resulting rhizosphere microbiome posttreatment (Wang and Mazzola 2019a,b).

Despite several reports on the relative tolerance of apple rootstocks to ARD (Auvil et al. 2011; Fazio et al. 2012; Isutsa and Merwin 2000), there remains a limited understanding of the dynamics that lead to effective plant defense or susceptibility of rootstocks on known replant sites (Rumberger et al. 2004). Certain rootstocks have been classified as ARD tolerant (Auvil et al. 2011) and functional resistance to specific replant pathogens (e.g., *Pythium ultimum*) has been documented (Zhu et al. 2016); however, these attributes have demonstrated significant site-to-site variability in performance (Auvil et al. 2011; St. Laurent et al. 2010) and have not universally translated to reduced disease incidence under field conditions (Hewavitharana and Mazzola 2020; Wang and Mazzola 2019b). For instance, rootstocks G.41 and G.935, categorized as ARD tolerant, responded to preplant soil fumigation in terms of growth and yield in a manner that was similar to that observed for the susceptible rootstocks M.9 and M.26 (Hewavitharana and Mazzola 2020; Wang and Mazzola 2019b). Studies have shown a genotypic influence on composition of the apple rhizosphere microbial community and linked the genotype-specific nature of the microbiome composition with rootstock tolerance or susceptibility to ARD (St. Laurent et al. 2010). Additionally, differences in microbial endophytic communities in multiple plantings across diverse geographic locations was associated with differences in severity of ARD symptoms (Manici et al. 2013; Turrini et al. 2017).

Soil microorganisms are essential elements of soil health, contributing directly to the productivity of cropping systems through their roles in biochemical cycles and determining nutrient availability (Pimentel et al. 1997). Understanding how plants and microbes form beneficial relationships has a direct impact on crop production and will influence agricultural management strategies (Rey and Schornack 2013). Practices that augment or stimulate mutualistic relationships such as mycorrhizal associations will predictably enhance plant productivity, in this instance by providing improved plant access to phosphorous (Covey 1981). These mycorrhizal associations are achieved through chemical communication between fungi in the rhizosphere and the host plant roots (Leisso et al. 2018; Oldroyd 2013).

Recent insights and discoveries in molecular microbial ecology using metagenomics have improved our understanding of the structure and functional potential of complex microbial communities (Sudarikov et al. 2017). Studies in soil microbial ecology often aim at elucidating the relationship between microbial diversity and function of the community. Improved understanding or insight into this relationship could enhance the capacity to manipulate microbial diversity in a manner resulting in improved plant health and environmental conservation (Mendes et al. 2017). However, a direct relationship between elevated microbial diversity and improved plant health is not a given and, in fact, selective amplification of specific microbial functions rather than enhanced microbial diversity has been demonstrated to be a more meaningful influence on plant disease suppression (Mazzola et al. 2015). Agronomic practices promoting sustainability will benefit from a more comprehensive understanding of the functional ecosystem, and the native microbial communities that inhabit the soil are of paramount importance to that functionality (Wagg et al. 2014). Therefore, understanding the structural composition of microbial communities associated with different plant species and different soil types can provide new strategies to improve plant productivity, while helping to protect the environment through a reduction of mechanical or chemical input (Barret et al. 2014). Advances in high-throughput sequencing technology and methodology for analysis of resulting large datasets have allowed the assessment of highly complex microbial communities in detail. Microbial community sequence data offer great potential to examine linkages or interactions among organisms (Hewavitharana et al. 2019b) as well as those between plants and their associated microbiomes (Myrold and Nannipieri 2014).

The goal of this study was to determine the effect of rootstock genotype on composition of the rhizosphere and root endophytic microbiome in orchard soil systems. This study utilized a diversity of commercially available apple rootstock genotypes reported to be tolerant or susceptible to ARD and were cultivated in two different, previously characterized replant orchard soils, whereas past studies have generally been limited to a few rootstocks in a single soil system. Composition of both the bacterial and fungal communities was assessed to provide a deeper understanding of apple rootstock effects on recruitment and subsequent support of associated microbes.

MATERIALS AND METHODS

Orchard soils. Soils used in this study were obtained from two conventionally managed apple orchards in the Columbia River Valley of Washington State: Columbia View (CV) Research Orchard (Orondo, WA; latitude 47°33'50" N, longitude 120°14'33" W) and the Sunrise (SR) Research Orchard (Rock Island, WA; latitude 47°18'37" N, longitude 120°04'01" W). The soil type at the CV orchard is Burch silt loam possessing a pH of 7.1 and organic matter content of 2.63% (Soil Test Farm Consultants, Moses Lake, WA, U.S.A.). The soil type at the SR orchard is Pogue fine sandy loam possessing a pH of 6.9 and organic matter content of 1.17% (Soil Test Farm Consultants). Both orchard soil systems were confirmed to possess the consortia of soilborne pathogens defined as causal agents of ARD based upon bioassay experiments and previous field trials (Mazzola 1998; Mazzola et al. 2015). The overall nitrogen status of both soils was considered deficient with levels generally ranging from 2.5 to 3.5 ppm (Cascade Analytical, Wenatchee, WA, U.S.A.). Soils were collected from the root zone of established trees at a depth of 10 to 30 cm and 0.5 to 1 m distance from the tree base. Prior to use, soil from each site was mixed thoroughly using a cement mixer (Kobalt; Monarch Industries, Inc., Winnipeg, MB, Canada) to obtain a representative orchard sample.

Greenhouse study design. All apple rootstocks used were obtained in a single shipment from TRECO (Woodburn, OR, U.S.A.). Rootstocks were of uniform size (0.635 cm trunk diameter) and were planted in orchard collected soils in 3-liter pots. Rootstock genotypes included G.41, G.890, G.935, M.9, and M.26 for the CV orchard soil system and the same five genotypes, with the addition of G.210, for the SR orchard soil system. For each trial, five replicates of each genotype were planted and arranged in a completely randomized design on the greenhouse bench. Rootstocks were grown for a period of 12 weeks at 22 to 28°C with supplemental lighting to maintain a 16-h photoperiod and watered three times per week. At completion of the 12-week growing period, the rootstocks were removed while the soil was kept in the pot and utilized for a successive planting of fresh rootstocks of the same genotype. The second planting of rootstocks were grown for a 12-week period under the same conditions. Supplemental nutrients were not employed in the study to avoid the known impact of fertilizer additions on altering orchard soil microbial community composition (Strauss et al. 2014). This design was intended to remove additional variables that might influence the biological system in order to assess the rootstock genotypic effect on the soil and root microbiome. However, in the absence of such fertility additions, somewhat limited root production was attained prior to cessation of tree growth. Thus, the second planting was employed in attempt to capture significant root effects on the soil microbial community.

At completion of the second planting, rhizosphere soils and root samples were collected. Soil adhering firmly to the roots was scraped off and collected in a sealed plastic bag representing the rhizosphere soil sample. The remaining root sample was placed in a separate sealed plastic bag and processed further to assess the root endophytic microbial community (described below). Soil and root samples were stored at -20°C prior to DNA extraction. Growth measurements of rootstocks were made for trunk diameter, root volume, and total rootstock wet weight and dry weight (after a period of drying at 40°C for 2 weeks).

DNA extraction from soil and root samples. DNA was extracted from 0.25 g of rhizosphere soil samples using the DNeasy PowerSoil kit (Qiagen, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions. To obtain a DNA sample representative of the endophytic microbiome, apple rootstock root samples were thoroughly washed with sterile water and the rhizoplane was chemically sterilized with 6% sodium hypochlorite for 2 min (Richter-Heitmann et al. 2016). Treating root samples with NaOCl was determined to be the best method to remove rhizoplane-colonizing cells while retaining the integrity of the root outer cell layer (Richter-Heitmann et al. 2016). After surface sterilization, roots were ground in liquid nitrogen by hand using a sterile mortar and pestle. Ground root tissue was frozen at -80°C until ready for DNA extraction. Total genomic DNA was isolated from 0.5 g of ground root tissue using the DNeasy PowerPlant Pro Kit (Qiagen) with minor modifications, as described below. Before bead beating, the samples were incubated in lysis solution at 60°C for 10 min. Samples were homogenized by a 2-min cycle at 1,800 rpm in a FastPrep instrument (MP Biomedical, Santa Ana, CA, U.S.A.). DNA was purified according to the kit protocol and quantified using a Nanodrop One^C spectrophotometer (Thermo Scientific, Waltham, MA, U.S.A.).

PCR amplification of DNA and Illumina sequencing. The bacterial 16s ribosomal RNA (rRNA) gene amplicons were generated using PCR primers 515F and 806R in the V4 variable region (Caporaso et al. 2011). The fungal internal transcribed spacer (ITS) rRNA gene amplicons were generated using primers ITS1F and ITS4R in the V1 and V4 variable regions (White et al. 1990). PCR

was carried out using HotStarTaq Plus Master Mix Kit (Qiagen) under the following conditions: 94°C for 3 min; followed by 30 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 60 s; after which a final elongation step at 72°C for 5 min was performed. PCR products were visualized on a 2% agarose gel to determine the success of amplification and verification of amplicon size. Multiple samples were pooled together in equal proportion based on molecular weight and DNA concentration, then purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, Beverly, MA, U.S.A.). Pooled and purified PCR product was used to prepare Illumina DNA libraries.

Sequencing was performed at an external facility (Molecular Research, Shallowater, TX, U.S.A.) using the Illumina MiSeq platform following the manufacturer's guidelines. Additionally, a eukaryote blocker was used in the bacterial 16s sequencing reactions. Sequence data were processed using a proprietary analysis pipeline (Molecular Research). In summary, sequences were depleted of barcodes and primers, and short sequences (<150 bp), sequences containing ambiguous base calls, sequences with homopolymer runs exceeding 6 bp, and sequences with an average quality score of <25 were removed. Chimeras were removed using the Uchime algorithm (Edgar et al. 2011). Processed sequences were clustered into different operational taxonomic units (OTUs) at a 97% similarity level. Final OTUs were taxonomically classified using BLASTn against a database derived from RDP II (<http://rdp.cme.msu.edu>) and NCBI (<https://www.ncbi.nlm.nih.gov/>). Data have been deposited at NCBI BioProject (BioProject accession: PRJNA682458).

Data analyses. Rootstock growth measurements were analyzed and visualized using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA, U.S.A.). Sequence data were refined in Microsoft Excel and all eukaryotic and archaeal OTUs were removed from the bacterial 16S rRNA dataset; OTUs matching kingdoms Plantae, Bacteria, and Archaea were removed from fungal ITS datasets. PAST v.4.03 software (Hammer et al. 2001) was used to conduct nonmetric multidimensional scaling (NMDS) of bacterial and fungal species OTU data. Analysis of similarity (ANOSIM) was conducted using the same program to assess relative similarity of bacterial and fungal communities, at the species level, between rootstock genotypes. Explicet software (Robertson et al. 2013) was also used to conduct statistical analysis and visualization of bacterial and fungal species OTU data. The α -diversity indices (Chao1 and Shannon) were calculated in PAST using the minimum library size as the default, with 1,000-bootstrap resampling. The ranks of α -diversity indices were subjected to analysis of variance to assess the differences between genotypes via a permutation of significance. Samples were normalized by taking the relative abundance for each OTU as the proportion of all sequences (tags) in a sample. The Two-Part Test using a nonparametric Wilcoxon rank-based approach was used to identify taxa that were significantly different between rootstock genotypes and between sampling environments (rhizosphere versus endosphere) (Wagner et al. 2011). Comparisons were made using a Benjamini-Hochberg corrected *P* value (Benjamini and Hochberg 2000) threshold of <0.05.

RESULTS

Rootstock growth. In both orchard soil systems, growth of all rootstocks diminished with the successive planting, which likely was exacerbated by a reduction in plant-available nutrients over time. Though measurements for trunk diameter, root volume, and rootstock weight were conducted for each 12-week planting, growth performance of the rootstocks in this system was not the

focus of the study. It must be considered that the rootstocks were not grafted with a scion nor did they receive supplemental nutrients at any time during the course of the study, as noted above, to avoid the known impact of fertilizer additions on altering orchard soil microbial community composition (Strauss et al. 2014).

OTU information and α diversity. In total, 2,538 and 1,662 OTUs, at the species level, were identified in the CV and SR orchard soil systems, respectively (Supplementary Table S1). A large portion of OTUs were identified in both the rhizosphere and endophytic environments within each orchard soil system (Supplementary Table S1); however, there were significant differences between rhizosphere and endophytic communities in terms of relative abundance and species richness, as determined by the Chao1 and Shannon H diversity indices. The endophytic bacterial and fungal communities were significantly less diverse ($P < 0.0001$) in composition relative to the corresponding communities detected in the rhizosphere environment for a given rootstock genotype in both orchard soil systems (Tables 1 and 2).

Microbial community analysis. To gain some perspective on variation of microbial taxa across rootstock genotypes and sample environment, the community of taxa detected in each sample was compared with all others using ANOSIM and visualized by plotting species OTU data in NMDS plots derived by the Dice algorithm. Rhizosphere microbial communities were dissimilar from endophytic microbial communities of the same rootstock genotype in both orchard soil systems ($P_{\text{ANOSIM}} < 0.01$), with an average R_{ANOSIM} value of 0.98, indicating significant differences with

virtually no overlap (Supplementary Tables S2 to S5). The following paragraphs highlight microbial community comparisons of bacterial and fungal species in each orchard soil system.

Bacterial community: CV orchard soil system. The rhizosphere bacterial community of G.890 cultivated in CV orchard soil was significantly different, although with some overlap, in composition from the rhizosphere communities of M.26 and M.9 ($P_{\text{ANOSIM}} < 0.02$, $R_{\text{ANOSIM}} \geq 0.34$) (Fig. 1A). Significant differences in rhizosphere bacterial communities were also observed between M.9 and all other genotypes ($P_{\text{ANOSIM}} < 0.05$) but R_{ANOSIM} values of 0.2 to 0.4 suggest some degree of overlap between the populations (Supplementary Table S2). The endophyte bacterial community of G.41 was significantly different from all other rootstocks ($P_{\text{ANOSIM}} \leq 0.02$, $R_{\text{ANOSIM}} \geq 0.27$) (Fig. 2A), with the endophytic bacterial community exhibiting greatest degree of dissimilarity with that from M.9 ($P_{\text{ANOSIM}} = 0.008$, $R_{\text{ANOSIM}} = 0.76$). Overall, Geneva rootstocks possessed endophytic bacterial communities that were significantly different in structure from the communities detected in Mallring rootstocks ($P_{\text{ANOSIM}} < 0.05$) (Supplementary Table S2; Fig. 2A).

Bacterial community: SR orchard soil system. The rhizosphere bacterial communities of G.210, G.41, and G.890 cultivated in SR orchard soil were significantly different in composition from those of M.26 and M.9 ($P_{\text{ANOSIM}} \leq 0.007$, $R_{\text{ANOSIM}} \geq 0.37$). The G.935 rhizosphere bacterial community did not differ significantly in composition from that of either M.26 or M.9. Within the broad rootstock groups (Geneva and Mallring series), the rhizosphere

TABLE 1
Attributes of bacterial and fungal diversity from the CV soil experiment based upon relative abundance of operational taxonomic units (OTUs)^a

Environment: Genotype	Reads assigned to OTUs	Number of OTUs (97%)	Chao1	Shannon H
Bacteria (16s) data				
Endosphere: G.41	46,595	629 ± 33	793 ± 33	3.56 ± 0.28
Endosphere: G.890	72,491	773 ± 30	955 ± 41	3.77 ± 0.17
Endosphere: G.935	52,347	699 ± 26	860 ± 34	3.60 ± 0.26
Endosphere: M.26	76,416	827 ± 39	966 ± 43	4.51 ± 0.11
Endosphere: M.9	110,166	980 ± 60	1,200 ± 57	4.58 ± 0.28
Rhizosphere: G.41	123,784	1,255 ± 26	1,478 ± 29	4.99 ± 0.02
Rhizosphere: G.890	107,003	1,254 ± 18	1,505 ± 33	4.98 ± 0.02
Rhizosphere: G.935	129,758	1,285 ± 44	1,512 ± 58	4.98 ± 0.02
Rhizosphere: M.26	124,180	1,279 ± 31	1,507 ± 17	5.15 ± 0.03
Rhizosphere: M.9	192,998	1,411 ± 32	1,609 ± 32	5.02 ± 0.02
Fungi (ITS) data ^b				
Endosphere: G.41	16,865	120 ± 11	178 ± 17	2.45 ± 0.19
Endosphere: G.890	30,076	137 ± 12	188 ± 10	2.09 ± 0.16
Endosphere: G.935	15,225	113 ± 10	154 ± 7	2.57 ± 0.12
Endosphere: M.26	48,782	165 ± 12	232 ± 7	2.24 ± 0.16
Endosphere: M.9	32,307	172 ± 26	253 ± 27	2.23 ± 0.10
Rhizosphere: G.41	28,354	303 ± 9	334 ± 10	3.50 ± 0.12
Rhizosphere: G.890	32,895	324 ± 6	350 ± 7	3.59 ± 0.12
Rhizosphere: G.935	25,961	309 ± 7	343 ± 9	3.74 ± 0.15
Rhizosphere: M.26	36,749	354 ± 8	408 ± 7	3.63 ± 0.10
Rhizosphere: M.9	26,518	292 ± 9	333 ± 11	3.10 ± 0.29

^a Data are presented as mean ± standard error of the mean, $n = 5$. OTUs are defined as sequences sharing >97% similarity. The number of reads assigned to OTUs is the mean across the biological samples, with $n = 5$.

^b ITS = internal transcribed spacer.

bacterial community from G.210 differed significantly from G.890, with minimal overlap ($P_{\text{ANOSIM}} = 0.009$, $R_{\text{ANOSIM}} = 0.5$), and M.26 was significantly different from M.9, with a moderate degree of overlap ($P_{\text{ANOSIM}} = 0.016$, $R_{\text{ANOSIM}} = 0.23$) (Supplementary Table S4; Fig. 1C). The endophytic bacterial communities of G.41, G.890, and G.935 were significantly different from M.26 and M.9 but with moderate degrees of overlap ($P_{\text{ANOSIM}} < 0.05$, $R_{\text{ANOSIM}} \geq 0.24$) (Fig. 2C). Additionally, the endophytic bacterial community of G.935 was significantly different from G.41, with some overlap in composition ($P_{\text{ANOSIM}} = 0.008$, $R_{\text{ANOSIM}} = 0.44$). Overall, endophytic bacterial communities from individual Geneva rootstocks differed from the corresponding communities detected in Malling rootstocks, with the exception of G.210 (Supplementary Table S4).

Fungal communities: CV orchard soil system. Sequencing of fungal ITS amplicons for the samples collected from rootstocks cultivated in CV soil was completed in two independent runs. The initial sequencing run included DNA samples from G.890 and M.26, while the second sequencing run contained samples from G.41, G.935, and M.9. There were differences observed that were a factor of the independent sequencing; however, within each sequencing run, significant differences were observed in community

composition among rootstock genotypes. The G.890 and M.26 rhizosphere ($P_{\text{ANOSIM}} = 0.008$, $R_{\text{ANOSIM}} = 0.5$) (Fig. 1B) and endophytic ($P_{\text{ANOSIM}} = 0.045$, $R_{\text{ANOSIM}} = 0.168$) (Fig. 2B) fungal communities differed significantly from each other. The G.41 rhizosphere fungal community differed significantly from the M.9 community ($P_{\text{ANOSIM}} = 0.008$, $R_{\text{ANOSIM}} = 0.5$) (Supplementary Table S3; Fig. 1B). The endophytic fungal community of M.9 was significantly different in composition than that from G.41 and G.935 ($P_{\text{ANOSIM}} < 0.02$, $R_{\text{ANOSIM}} \geq 0.44$).

Fungal communities: SR orchard soil system. Composition of the rhizosphere fungal communities from G.210, G.41, and G.890 rootstocks was significantly different from G.935, M.26, and M.9, with a high degree of separation ($P_{\text{ANOSIM}} < 0.01$, $R_{\text{ANOSIM}} \geq 0.85$) (Fig. 1D). In addition, the G.935 rhizosphere community differed significantly in composition from that of M.9 but not M.26 (Supplementary Table S5; Fig. 1D). The endophytic fungal communities diverged in a similar pattern, in which G.210, G.41, and G.890 were significantly different from G.935, M.26, and M.9, with a high degree of separation ($P_{\text{ANOSIM}} < 0.005$, $R_{\text{ANOSIM}} \geq 0.90$) (Fig. 2D). Additionally, the G.935 endophytic fungal community was significantly different from M.9 ($P_{\text{ANOSIM}} = 0.008$, $R_{\text{ANOSIM}} = 0.56$) but not M.26, and the G.210 fungal endophyte community

TABLE 2
Attributes of bacterial and fungal diversity from the SR soil experiment based upon relative abundance of operational taxonomic units (OTUs)^a

Environment: Genotype	Reads assigned to OTUs	Number of OTUs (97%)	Chao1	Shannon H
Bacteria (16s) data				
Endosphere: G.210	19,515	291 ± 32	443 ± 36	2.08 ± 0.49
Endosphere: G.41	19,548	294 ± 12	446 ± 14	2.30 ± 0.12
Endosphere: G.890	20,473	329 ± 16	474 ± 19	3.01 ± 0.16
Endosphere: G.935	21,499	398 ± 24	533 ± 27	3.57 ± 0.21
Endosphere: M.26	19,357	309 ± 22	457 ± 25	2.74 ± 0.36
Endosphere: M.9	17,069	256 ± 20	375 ± 29	1.98 ± 0.37
Rhizosphere: G.210	25,793	784 ± 13	937 ± 21	5.23 ± 0.04
Rhizosphere: G.41	25,659	807 ± 10	982 ± 4	5.18 ± 0.02
Rhizosphere: G.890	25,980	791 ± 9	951 ± 14	5.11 ± 0.04
Rhizosphere: G.935	25,811	809 ± 7	988 ± 23	5.18 ± 0.02
Rhizosphere: M.26	26,147	782 ± 12	942 ± 22	5.22 ± 0.03
Rhizosphere: M.9	26,342	763 ± 17	930 ± 15	5.18 ± 0.03
Fungi (ITS) data ^b				
Endosphere: G.210	58,852	167 ± 50	258 ± 17	2.32 ± 0.12
Endosphere: G.41	56,716	175 ± 70	257 ± 17	2.50 ± 0.11
Endosphere: G.890	60,795	190 ± 70	265 ± 15	2.73 ± 0.10
Endosphere: G.935	53,930	197 ± 80	251 ± 9	2.72 ± 0.15
Endosphere: M.26	71,231	196 ± 30	273 ± 4	2.07 ± 0.11
Endosphere: M.9	67,800	179 ± 13	243 ± 20	1.65 ± 0.27
Rhizosphere: G.210	56,623	326 ± 13	383 ± 16	3.45 ± 0.13
Rhizosphere: G.41	55,405	331 ± 70	403 ± 19	3.62 ± 0.06
Rhizosphere: G.890	50,055	330 ± 70	427 ± 25	3.36 ± 0.10
Rhizosphere: G.935	63,804	353 ± 90	416 ± 16	3.18 ± 0.23
Rhizosphere: M.26	48,187	316 ± 10	366 ± 11	3.59 ± 0.05
Rhizosphere: M.9	56,665	321 ± 70	369 ± 10	3.17 ± 0.20

^a Data are presented as mean ± standard error of the mean, $n = 5$. OTUs are defined as sequences sharing >97% similarity. The number of reads assigned to OTUs is the mean across the biological samples, with $n = 5$.

^b ITS = internal transcribed spacer.

was significantly different from G.890, with some overlap ($P_{\text{ANOSIM}} = 0.01$, $R_{\text{ANOSIM}} = 0.4$) (Supplementary Table S5; Fig. 2D).

Relative abundance of individual OTUs. The relative abundance of OTUs presented as a percentage of the total OTUs identified for a specific microbial group (bacteria or fungi) was analyzed for each orchard soil system. In the CV orchard soil system, the most abundant bacterial groups detected in the rhizosphere included *Acidobacterium*, *Holophaga*, *Pelobacter*, *Gemmatimonas*, and *Burkholderia* spp., while dominant endophytic bacterial groups included *Arthrobacter*, *Halospirulina*, *Streptomyces*, and *Burkholderia* spp. In the SR orchard soil system, *Acidobacterium*, *Gemmatimonas*, and *Steroidobacter* spp. were dominant in the rhizosphere, while *Halospirulina*, *Streptomyces*,

and *Steroidobacter* spp. were the most abundant rootstock endophytic bacterial entities. Beyond these dominant bacterial taxa, observed at >2% relative abundance, a vast majority (>67%) of bacterial OTUs were observed at significant differences between endophytic and rhizosphere environments (Figs. 3A and 4A), despite being identified in both environments (Figs. 3C and 4C).

An OTU designated as *Chitinophaga* spp. was observed at significantly greater relative abundance in the rhizosphere compared with the endosphere in both CV orchard soil ($P_{\text{adj}} < 0.001$) and SR orchard soil ($P_{\text{adj}} < 0.001$) systems. Within the rhizosphere environment, the *Chitinophaga* spp. OTU was observed at similar relative abundances across all rootstock genotypes. Similarly, a *Burkholderia* spp. OTU also had similar relative abundances across

Fig. 1. Influence of genotype on composition of microbial communities detected in the rhizosphere of apple rootstocks planted in orchard replant soils. Ordination was conducted by nonmetric multidimensional scaling (NMDS) of operational taxonomic units using the Dice similarity index for the Columbia View Research orchard soil system **A**, bacterial and **B**, fungal rhizosphere communities and the Sunrise Research orchard soil system **C**, bacterial and **D**, fungal rhizosphere communities. Symbols signify the following genotypes: filled diamond = G.210, filled square = G.41, filled circle = G.890, filled triangle = G.935, open circle = M.26, and open inverse triangle = M.9. Stress = 0.29, 0.32, 0.25, and 0.16 for plots A, B, C, and D, respectively.

all rootstock genotypes within the rhizosphere environment of both orchard soil systems. In the CV orchard soil system, the OTU designated as a *Frankia* sp. was observed at a significantly greater ($P_{adj} < 0.001$) relative abundance of 0.33% in the endosphere and 0.01% in the rhizosphere. Within the endosphere environment across rootstock genotypes, the *Frankia* sp. OTU was present at 0.20% in M.9 while at $\leq 0.06\%$ in the other rootstocks. The OTU designated as a *Clostridium* sp. was observed at a relative abundance of 0.87% in the endosphere and 0.02% in the rhizosphere of the SR orchard soil system. The genus *Clostridium* has been reported as a widespread plant endophytic bacterium exhibiting nitrogen-fixing capability (Saito and Minamisawa 2006). Within the endosphere environment of the SR system, the relative abundance of the *Clostridium* sp. OTU was higher in the Geneva

rootstocks (1.14%) compared with the Malling rootstocks (0.02%) but the difference was not statistically significant ($P_{adj} = 0.069$).

The fungal community composition differed significantly between the rhizosphere and endophytic habitats (Figs. 3B and 4B), with the rhizosphere community dominated by groups possessing a saprophytic nature while the endophytic community predominantly comprised species having a potentially mutualistic or pathogenic interaction with the host plant. Similar to that observed for the bacterial community, a large proportion (greater than one-third) of fungal OTUs were observed at significant differences between endophytic and rhizosphere environments (Figs. 3D and 4D), despite being identified in both environments. Dominant fungal OTUs in both orchard soil systems included a *Mortierella* sp., *Glomus* sp., *Serendipita vermifera*, *Terfezia* sp., *Nectria* sp., and *Ilyonectria*

Fig. 2. Influence of genotype on composition of microbial communities detected in the endosphere of apple rootstocks planted in orchard replant soils. Ordination was conducted by nonmetric multidimensional scaling (NMDS) of operational taxonomic units using the Dice similarity index for the Columbia View orchard soil system **A**, bacterial and **B**, fungal endosphere communities and the Sunrise orchard soil system **C**, bacterial and **D**, fungal endosphere communities. Symbols signify the following genotypes: filled diamond = G.210, filled square = G.41, filled circle = G.890, filled triangle = G.935, open circle = M.26, and open inverse triangle = M.9. Stress = 0.19, 0.24, 0.2, and 0.22 for plots A, B, C, and D, respectively.

robusta. The OTU designated as *Mortierella* sp. was a significant component of the rhizosphere fungal community in both orchard soil systems, representing 7.82 and 1.11% of the total rhizosphere fungal community in the CV and SR orchard soil systems, respectively. The OTU designated as *Nectria* sp. was abundant in both the rhizosphere and endosphere environments and its relative abundance in the endophytic fungal community from M.26 and M.9 rootstocks cultivated in both CV and SR orchard soil systems was higher (34.07%) than was evident for Geneva rootstocks (8.29%); however, the difference was not statistically significant ($P_{adj} = 0.3$). The relative abundance of the *I. robusta* OTU represented a significantly greater proportion of the endophytic than rhizosphere fungal community in both the CV (5.44 versus 0.63%, $P_{adj} < 0.005$) and SR (3.52 versus 0.40%, $P_{adj} < 0.001$) orchard soil systems. Among rootstocks, M.26 possessed the highest relative abundance of the *I. robusta* OTU detected in endophytic samples in both the CV and SR orchard soil systems.

In the CV orchard soil system, a large portion of members of class Agaricomycetes were represented by the species OTU *Oliveonia pauxilla*, with relative abundances of 1.71 and 4.93% in the

rhizosphere and endophytic environments, respectively. In the SR orchard soil system, a large portion of Agaricomycetes members were represented by the species OTU *Sistotrema sermanderi*, with relative abundances of 8.71 and 0.22% in rhizosphere and endophytic environments, respectively.

Several fungal species known to form mycorrhizal associations with plants were identified and significant differences in relative abundance of these fungal OTUs between the rhizosphere and endophytic environments were observed. These OTUs were designated as *Glomus* sp. ($P_{adj} < 0.01$, CV orchard; $P_{adj} < 0.001$, SR orchard), *Funneliformis mosseae* ($P_{adj} < 0.02$, SR orchard), and *Rhizophagus irregularis* ($P_{adj} < 0.005$, CV orchard; $P_{adj} < 0.05$). Significant differences were observed among rootstocks in the relative contribution of a given taxon to the overall fungal community composition; however, as a group, mycorrhizal fungi represented 7.31 and 14.23% of the total OTUs in the rhizosphere and endosphere environments, respectively, for the CV orchard soil system. In the SR orchard soil system, the same group of mycorrhizal fungi represented 4.62 and 16.92% of total rhizosphere and endophytic fungal community, respectively.

Fig. 3. Differences in the structure and composition of endosphere and rhizosphere bacterial and fungal communities in the Columbia View orchard soil system. **A**, Nonmetric multidimensional scaling (NMDS) of bacterial and **B**, fungal species operational taxonomic units (OTUs) across rootstock genotypes. NMDS ordinations based on Dice similarity index with 95% confidence ellipses. Dots (●) represent endosphere and circles (○) represent rhizosphere. Stress = 0.08 and 0.12 for plots A and B, respectively. **C**, Venn diagram of the bacterial and **D**, fungal OTUs detected in the two environments. Bold numbers represent the number of OTUs detected with the percent relative abundance of total OTUs in italic.

Regarding comparisons between rootstock genotypes within the endophytic environment, taxa belonging to the family Glomeraceae, which included OTUs designated as *Glomus* sp., *F. mosseae*, and *R. irregularis*, had a greater relative abundance in G.890 and M.9 compared with G.41, G.935, and M.26 in the CV orchard soil system. In the SR orchard soil system, G.890 also had the highest relative abundance of Glomeraceae but, among all rootstock genotypes, relative abundance of these fungal taxa was lowest for the M.9 fungal community (Supplementary Fig. S1). Relative abundance of these OTUs exhibited significant variation for a given rootstock genotype between the CV and SR orchard soil systems. In the CV orchard soil system, the relative abundance of the *R. irregularis* OTU in the endophytic fungal community was low in G.890 (0.23%) and highest in M.9 (2.81%) whereas, in the SR orchard soil system, this OTU was equally abundant in both G.890 and M.9 (0.84%). The *F. mosseae* OTU was present at very low relative abundance (<0.02%) for all genotypes in the CV orchard soil system; however, in the SR orchard soil system, the *F. mosseae* OTU had a higher relative abundance in the Geneva rootstocks (average = 2.21%) compared with the Mallings rootstocks

(average = 0.70%), with the highest relative abundance in G.890 (2.88%). Despite not being resolved beyond the genus level, the OTU designated as *Glomus* sp. was observed at 3.92% relative abundance in the G.890 rootstock and <1.5% in the other rootstock genotypes in the CV orchard soil system. In the SR orchard soil system, the relative abundance of the *Glomus* sp. OTU was similar across all rootstocks (0.90 to 2.10%).

Serendipita vermifera and *Terfezia* sp., which have been reported to form mycorrhizal associations in other plant systems (Morte et al. 2000; Ray and Craven 2016; Zitouni-Haouar et al. 2014), displayed similar endophytic patterns in both the CV and SR orchard soil systems. Though not significantly different, the relative abundance of these two fungal OTUs was higher in the endophytic fungal communities from Mallings rootstocks compared with the Geneva rootstocks. In the CV orchard soil system, the average relative abundance of the *S. vermifera* OTU in the endophytic fungal community was 3.44 and 0.72% for the Mallings and Geneva rootstocks, respectively, while the average relative abundance of the *S. vermifera* OTU in the SR orchard soil system was 0.91 and 0.30% for the Mallings and Geneva rootstocks, respectively. Similarly, the

Fig. 4. Differences in the structure and composition of endosphere and rhizosphere bacterial and fungal communities in the Sunrise orchard soil system. **A**, Nonmetric multidimensional scaling (NMDS) of bacterial and **B**, fungal species operational taxonomic units (OTUs) across rootstock genotypes. NMDS ordinations based on Dice similarity index with 95% confidence ellipses. Dots (●) represent endosphere and circles (○) represent rhizosphere. Stress = 0.07 and 0.12 for plots A and B, respectively. **C**, Venn diagram of the bacterial and **D**, fungal OTUs detected in the two environments. Bold numbers represent the number of OTUs detected with the percent relative abundance of total OTUs in italic.

average relative abundance of the *Terfezia* sp. OTU in the CV orchard soil system was 2.42 and 0.63% for the Mallings and Geneva rootstocks, respectively, while the average relative abundance of this OTU in the endophytic fungal community detected in the SR orchard soil system was 2.21 and 0.72% for the Mallings and Geneva rootstocks, respectively.

DISCUSSION

The plant-associated microbiome contributes significantly to the overall health and productivity of cropping systems. Effective use of this biological resource has significant potential but its optimal utilization requires additional knowledge of the factors that influence its composition and activity. Recent studies attributed large differences in composition of the rhizosphere microbiome of apple orchards to the multitude of variables between planting sites such as elevation, soil conditions, management practices, and competitive interactions with the native soil microbial community (Deakin et al. 2019; Turrini et al. 2017). Despite the numerous variables affecting soil and root microbiome composition, rootstock genetics presumably remains constant and, as such, their influence on the microbiome predictable, given a large enough collection of aggregated data. In apple, a comparison across rootstock genotypes demonstrated that host factors will contribute to the ultimate composition of the rhizosphere microbiome (Wang and Mazzola 2019a). The present study sought to examine a more extensive group of rootstock genotypes across multiple replant orchard soils to assess the effect of these genetic factors on composition of the apple rhizosphere and root endophytic microbiomes.

Amplicon sequencing identified bacterial and fungal OTU numbers similar to those of previous microbiome studies in apple (Liu et al. 2018; Tilston et al. 2018). The large percentage of identified OTUs shared across sample environment and apple rootstock genotype may be due to the nuances of microbiome studies such as sample collection, extraction, and sequencing (Figs. 3C and D and 4C and D). However, statistical analyses of rhizosphere and endophytic samples and rootstock genotypes incorporated the relative abundance of identified OTUs across all samples in order to compare microbial community composition. Similar to those observed by Wang and Mazzola (2019a), significant differences in structure and composition of rhizosphere bacterial communities were detected among rootstock genotypes, most notably in comparison of M.9 to the Geneva rootstocks utilized in this study (Supplementary Tables S2 and S4). In both the CV and SR orchard soil systems, rhizosphere bacterial community profiles were highly similar between G.890 and G.935 rootstocks (Supplementary Tables S2 and S4), which could be expected, considering that these two genotypes are both hybrids resulting from a cross between *Malus domestica* ‘Ottawa 3’ and *M. robusta* ‘Robusta 5’ (Fazio et al. 2004). Another consistent observation across both orchard soil systems is the significant difference between rhizosphere bacterial communities of G.890 compared with the Mallings rootstocks (Supplementary Tables S2 and S4; Fig. 1A and C). Differences in relative similarity of the bacterial community among apple rootstocks support a genetic effect in shaping composition of this community, likely through the differential release, both quantitatively and qualitatively, of metabolites by rootstocks as rhizodeposits (Leisso et al. 2017). Similar studies also suggest a plant genetic-based control of the apple root and soil microbiome, and potentially tolerance or susceptibility to ARD via influence on composition of bacterial and fungal communities (Deakin et al. 2019; St. Laurent et al. 2010; Turrini et al. 2017).

Endophytic bacterial communities also differed between rootstocks; again, community profiles primarily diverged between the

Geneva and the Mallings rootstock genotypes. In both orchard soil systems, M.9 endophytic bacterial communities were significantly different from those of G.41, G.890, and G.935, with R_{ANOSIM} values suggesting minimal overlap of composition (Supplementary Tables S2 and S4; Fig. 2A and C). Based upon α -diversity measures, M9 rootstock possessed the least diverse endophytic bacterial and fungal communities when cultivated in the SR orchard soil system, which is consistent with a previous study evaluating endophytic communities of apple rootstocks (Liu et al. 2018). However, differences observed between the CV and SR orchard soil systems suggest that many factors of the native soil environment also appear to play a determining role in endophyte colonization.

The dominant endophytic OTU in the CV orchard soil system, representing *Arthrobacter* spp., was observed at a relative abundance of 6.04%, whereas this OTU was present at 0.30% in the SR orchard soil system. Similarly, the OTU identified as *Burkholderia* spp. was observed as an endophyte at relative abundance of 0.45% in the CV orchard soil system and <0.01% in the SR orchard soil system. Additionally, among rootstocks planted in the CV orchard soil system, the *Burkholderia* spp. OTU was significantly ($P_{adj} = 0.035$) lower in relative abundance (0.10%) in the G.935 endosphere compared with all other rootstocks (0.90%). *Arthrobacter* and *Burkholderia* spp. have been identified in many soils and may play a role in plant growth promotion, nitrogen fixation, siderophore production, and phytopathogen inhibition (Fernández-González et al. 2017; Liu et al. 2018). *Clostridium* sp. is another OTU that had significantly greater relative abundance in the endosphere (0.87%) compared with the rhizosphere (0.02%) in the SR orchard soil system but was observed at <0.01% relative abundance in all samples in the CV orchard soil system. Some species in the genus *Clostridium* are known to be nitrogen-fixing bacteria functioning as endophytes within plants (Saito and Minamisawa 2006). In the CV orchard soil system, the OTU designated *Frankia* sp. was significantly more abundant in the endosphere compared with the rhizosphere and was observed at 0.24% in the M.9 endosphere but $\leq 0.06\%$ in the endosphere of all other rootstocks tested. Although a mutualistic relationship between *Malus* and *Frankia* spp. has not been reported, members of this genus of nitrogen-fixing bacteria can colonize plant roots and initiate the formation of root nodules, resulting in enhanced nitrogen uptake to the plant (Burgess and Peterson 1987). These results indicate both the plant genotype and the soil environment as a factor influencing bacterial diversity and abundance.

Comparisons of the fungal community composition between rootstock genotypes also followed the general trend of separation between the Geneva and Mallings genotypes. Despite the separate sequencing runs of the CV soil system fungal samples, the comparisons between G.890 to M.26 (sequenced together) and G.41 and G.935 to M.9 (sequenced together) follow this trend in both the rhizosphere and endophytic environments (Figs. 1B and 2B). A similar pattern was seen in the SR orchard soil system, with separation between two groups—G.210, G.41, and G.890 compared with G.935, M.26, and M.9—with additional significant differences between G.935 and the Mallings genotypes for both fungal rhizosphere and endophytic communities (Figs. 1D and 2D). The OTU designated *I. robusta* was significantly ($P_{adj} = 0.011$) greater in relative abundance in the Mallings endosphere samples (9.84%) compared with the Geneva endosphere samples (2.51%) in the SR orchard soil environment. In the CV orchard soil environment, G.890 and G.935 had the lowest relative abundances of the *I. robusta* OTU, while M.26 had the highest. Both G.41 and M.9 had similar relative abundances of the *I. robusta* OTU. Studies have identified *I. robusta* as a pathogen involved in the complex causing ARD and found its colonization of apple roots to be negatively

correlated with plant growth (Manici et al. 2013; Mazzola 1998; Tewoldemedhin et al. 2011). Significant reduction in recovery of this fungus from M.26 apple roots in response to effective soil treatments has consistently been observed in orchard replant field trials (Mazzola and Brown 2010; Mazzola and Mullinix 2005). The higher relative abundance of *I. robusta* in the endosphere of M.26 would suggest this genotype to be more susceptible to the pathogenic effects of this fungal endophyte.

Further similarities across both orchard soil systems include the identification of OTUs representative of *Terfezia* sp. and *S. vermifera* as endophytes observed in all rootstocks but generally of greater abundance in the fungal community from Malling rootstocks than Geneva rootstocks. *Terfezia* spp. are common to arid and semiarid environments and are known to form mycorrhizal associations with various plant species (Morte et al. 2000; Zitouni-Haouar et al. 2014). Morte et al. (2000) attributed greater root development and increases in nitrogen, phosphorus, and potassium uptake in *Helianthemum almeriense* to the mycorrhizal association with *Terfezia clavayi*. Overall, the Malling genotypes and G.41 possessed greater relative abundance of the *Terfezia* sp. OTU compared with the other Geneva genotypes. The parentage of G.41 is *M. robusta* 'Robusta 5' and *M. domestica* Borkh. M.27, which may implicate some of the similarities seen between G.41 and the Malling genotypes, as M.9 served as one of the parents for the generation of both M.27 and M.26. The OTU representative of *S. vermifera* was observed as an endophyte in all rootstocks in both orchard soil systems, with the lowest relative abundance in G.210. *S. vermifera* was previously shown to be beneficial in enhancing plant growth and stress tolerance in switchgrass (Ray and Craven 2016). Due to the effectiveness of *S. vermifera* at forming mycorrhizal associations with a diverse range of host plants and subsequently enhancing plant biomass, Weiß et al. (2011) suggested the use of this fungus as a biofertilizer in cropping systems.

Mycorrhizal fungi belonging to the family Glomeraceae are termed arbuscular mycorrhizal fungi (AMF) and have long been known to associate with apple roots (Covey 1981). AMF are commonly found in native microbiomes of cropping systems and are also utilized as soil amendments to promote an increase in plant growth via more efficient uptake of various elements in the soil, especially phosphorus (Covey 1981; Gianinazzi and Vosátka 2004). One study found that plant growth or nutrient uptake of the apple rootstocks MM.111 and MM.106 were enhanced in a similar manner when cultivated in soil that had been augmented by the AMF fungi *F. mosseae* and *R. irregularis* (Berdeni et al. 2018). However, MM.106 and MM.111 are genetically similar, sharing a breeding parent, and the study did not determine AMF species colonization of roots. AMF diversity and community composition in apple are variable across diverse growing regions and altered by management practices (Turrini et al. 2017). Another study aimed at the identification of native microbial communities in apple roots did find differences in relative abundances of species of Glomeromycota between M.M.111 and M.9 (Liu et al. 2018). The AMF taxa identified in this study were observed at significantly different relative abundances in the endosphere environment between rootstock genotypes in both orchard soil systems (Supplementary Fig. S1).

As a family, the Glomeraceae OTUs were observed at greater relative abundances in the G.890 endosphere than the other rootstock genotypes, suggesting an increased ability of G.890 to form associations with these AMF species. In the CV orchard soil system, the *Glomus* sp. OTU was greater in relative abundance in the endosphere of G.890 compared with the other rootstock genotypes. In the SR orchard soil system, two Glomeromycetes OTUs, designated as *Glomus versiforme* and *Paraglomus laccatum*, were also

greater in relative abundance in the endosphere of G.890 compared with the other rootstock genotypes. Turrini et al. (2017) also identified multiple species of AMF in apple roots, with the most abundant genera belonging to *Glomus* and the least abundant belonging to *F. mosseae*, and concluded that the soil environment has a key role in shaping AMF diversity and distribution patterns. The OTUs designated as *R. intraradices* and *R. irregularis*, both belonging to the Glomeraceae family, were present as endophytes at greater relative abundances in the CV orchard soil system compared with the SR orchard soil system. Samples from these two soil systems were not sequenced in the same run but differences within the genotypes were apparent, with a greater relative abundance of both the *R. intraradices* and *R. irregularis* OTUs in the M.9 genotype compared with the other rootstocks in the CV orchard soil system. The *R. irregularis* OTU was also highest in relative abundance in M.9 in the SR orchard soil system. Many studies have also reported on the influence of the host genotype on composition of the associated microbiota. Liu et al. (2018) investigated endophytic communities of two apple rootstocks and concluded a distinct influence of genotype on composition and relative abundance of fungal microbiota. Our findings support both the host genotype and soil environment as influencing fungal community composition in both the rhizosphere and endophytic environments.

Several fungal endophyte species identified here may be of interest based on their known functions in other systems; however, a caveat of relating microbial diversity to function is that identification of a specific microbe in the endosphere cannot be interpreted as the organism directly functioning in a plant-beneficial role (Barret et al. 2014). The results of this study provide foundational information on which to build hypotheses regarding the function and establishment mechanisms of major microbial species specific to commercial and new breeding apple rootstock genotypes under replant orchard conditions. The potential to utilize a genetic-based control of the soil and root microbiome through specific apple rootstock genotypes would be an invaluable part of an integrated approach to mitigate the detrimental effects of ARD.

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