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A Characterization of a Cool-Climate Organic Vineyard's Microbiome

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ABSTRACT

The microbiome, an influential factor affecting plant health and growth, is attracting increasing interest with respect to wine grape production. The purpose of this study was to characterize the microbiome (fungi and bacteria) of the soil, cover crop roots, and grape (*Vitis* spp.) roots across rootstock and depth in a cool-climate, organic vineyard. The cover crop consisted of a fescue (*Festuca* sp.) grass, while grape roots were sampled from New York Muscat, a cool-climate hybrid, across three root types (ungrafted, 3309C and Riparia Gloire) at three root depths (0 to 15, 15 to 30, and 30 to 50 cm). The grape root microbiome was more specialized, with fewer observed amplicon sequence variants for both bacteria (16S) and fungi (internal transcribe spacer) than found in the cover crop and the surrounding soil. Grape roots were dominated by bacterial genera *Pseudomonas*,

Niastella, and *Rhizobium*; most prominent fungal genera were *Plectosphaerella*, *Trichosporon*, and *Ilyonectria*. Although no correlations were found between α -diversity metrics and soil parameters, *Pseudaleuria* relative abundance was correlated with Mn, Fe, and Na levels. Soil depth explained a small portion of bacterial but not fungal variance and taxonomic composition. Rootstock type explained a portion of both bacterial and fungal variance and taxonomic composition, substantiating the role of host plant genetics in the development of the grape root microbiome. This is the first characterization of the grape root microbiome in a cool-climate Canadian vineyard.

Keywords: agriculture, bacteriology, microbiome, mycology, plants

Grapevines (*Vitis* spp.) are readily colonized by both symbiotic bacteria and fungi (Baldan et al. 2014; González and Tello 2011; Linderman and Davis 2001). This microbial activity is fundamental to plant health and development, and to grape production, quality,

and characteristics (Barka et al. 2000; Bokulich et al. 2016; Linderman and Davis 2001; Pinto and Gomes 2016). In particular, the root and soil microbiome affects soil characteristics, nutrient and water uptake, and disease pressure, and may act as a reservoir for microorganisms that go on to colonize the phyllosphere (Zarraonaindia et al. 2015).

Microbes of particular interest include plant-growth-promoting (PGP) bacteria. Promising genera include *Azotobacter*, *Azospirillum*, *Bacillus*, *Pantoea*, *Pseudomonas*, and *Rhizobium* (Ferreira et al. 2019). PGP bacteria may benefit plant growth and health through different modes of action. Biofertilizers can improve the uptake of key nutrients such as nitrogen, sulfur, and phosphorous by transforming the nutrients into forms that are available for uptake by the plant. Biostimulators can enhance plant growth through the synthesis of phytohormones such as auxins and cytokinins. Finally, bio-control microorganisms may protect the plant from pathogens via various mechanisms such as competition or the production of antibiotics or protective enzymes (Ferreira et al. 2019). Similarly, arbuscular mycorrhizae fungi (AMF) are another group of beneficial microbes. These fungi have been shown to improve water and

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nutrient uptake, especially phosphorous, and to improve soil structure. As such, AMF are a common target of viticulture studies that investigate the beneficial relationship between these fungi and vines (Possingham and Obbink 1971; Schreiner and Mihara 2009; Trouvelot et al. 2015; Valentine et al. 2006).

Although the makeup of a plant's microbiome can influence a plant's growth and development, the opposite is also true. The genetics of the plant host, which can influence the nature of the exudates secreted by the root as well as the root structure and morphology, is an influential factor in shaping the host's microbiome (Babalola et al. 2020; Bulgarelli et al. 2013; Pascale et al. 2020). As such, when exploring the microbiome of our cool-climate vineyard, we might expect to find differences between the microbiome in the roots of the cover crop and of that of the grape, or even between grape root types. Additionally, with strong gradients in pH, nutrient levels, and organic matter often existing in the soil column, this also offers an interesting avenue to explore.

Improved protocols and lower sequencing costs have resulted in a moderate though far from excessive number of studies in the literature that already explore the vineyard microbiome, including that of the grape roots (Berlanas et al. 2019; Martínez-Diz et al. 2019; Zarraonaindia et al. 2015). However, more studies are needed as we endeavor to better understand the relationships that exist between grapevines and their microbiome. In much the same way that an ecosystem may vary completely in the particular fauna and flora that make it up but still perform the same key functions such as nutrient cycling and energy flow through the food chain, a plant's microbiome may also be a product of its environment. As such, an examination of the microbiome found in a vineyard located in Nova Scotia would be a unique addition to the studies already found in the literature.

Some of the first vines to be planted in Canada, and among the first in North America, were done so in Nova Scotia. These early vineyards were established by French colonists in the 1600s (Naugler et al. 2004). In the years that followed, grape growing in Nova Scotia was long a sideline devoid of commercial success; however, in recent decades, Nova Scotia's grape industry, buoyed by both a strong buy-local movement and, conflictingly, global warming, has experienced a period of robust expansion and growth, and now contributes over \$218 million annually to Nova Scotia's economy (Rimerman 2015). Nonetheless, with a limited amount of heat (region I according to the heat summation-based Winkler Index), the region remains on the periphery of where wine grapevines are commercially grown. The primary objective of this study was to be the first to characterize the microbiome (bacteria and fungi) in a cool-climate Canadian vineyard, including that of the soil and the roots of both the grapevines and the cover crop. Our secondary objective was to further characterize the grape root microbiome across (i) three different root types (ungrafted, 3309C, and Riparia Gloire) and (ii) at three soil depths (0 to 15 cm, 15 to 30 cm, and 30 to 50 cm).

MATERIALS AND METHODS

Site and plant material. The grapevines were planted at a site located in Avonport, Nova Scotia, Canada, in June 2013 as part of a broader rootstock trial, with the hybrid cultivar New York Muscat grafted to Riparia Gloire and 3309C, as well as grown on their own roots (ungrafted). The 3309C rootstock is the most commonly used in Nova Scotia, as well as the northeastern United States, while Riparia Gloire is either the second or third most common rootstock used. Each scion-rootstock combination was planted in adjacent blocks consisting of two rows each; the rows were approximately 150 m long. Vineyard spacing was 140 cm between vines and 3 m between rows, with four vines per panel (i.e., between posts). The

rows were oriented north-south on a slight, south-facing slope. Weather data from a nearby Environment Canada weather station (Kentville, Nova Scotia), indicate annual averages of 1,158 growing degree days (°C) during the growing season, 158 frost-free days, and 1,208 mm of rain based on the past 10 years (2011 to 2020).

The vineyard was managed under organic or biodynamic principles. The primary sprays used in the vineyard were sulfur and copper based. No herbicides were used; weed control was accomplished via several passes a year with a knife hoe. Rows were hilled (i.e., the graft unions covered with soil) in the fall and dehipped in the spring. For each root type, two panels approximately 100 m apart from each other were randomly selected, one from each row. For each panel, 2 of the 4 vines were randomly selected to be included in this trial, for a total of 12 vines.

Soil analysis. The soil type at this site is a Gleyed Sombric Brunisol (Soil Classification Working Group 1987), locally known as Woodbourne (Holmstrom and Thompson 1989). These soils are moderately well to imperfectly drained, with approximately 50 cm of hard-setting, coarse, loamy-gravelly upper soil material. The lower soil material (>50 cm) is a firm, fine, loamy-gravelly till. Soil samples were taken before bud break in the second week of May 2018; six cores located within 0.5 m of the trunk of each of the 12 vines were taken, in a radial manner, to a depth of 0.5 m using a 1-in. open-faced gouge probe driven with a rubber mallet. Each core was divided into three depths (0 to 15, 15 to 30, and 30 to 50 cm) and used to generate three composite samples for each vine. The samples were mixed thoroughly and sent to the Nova Scotia Department of Agriculture analytical lab (Truro, Nova Scotia) for soil analysis (i.e., macro- and micronutrients, pH, organic matter, and cation exchange capacity).

Terrestrial subsurface microbiome sampling. Tissue sampling occurred at the same time as soil sampling. The soil was carefully removed around the trunk of each vine using a shovel and hand spades. A 1 m-by-1-m square was excavated down to a depth of 50 cm. A representative sample of fine roots for microbiome analysis was obtained from each vine from each of the three soil depths used for the soil analysis described above (i.e., 0 to 15, 15 to 30, and 30 to 50 cm) for a total of 36 samples (12 vines × three depths). Soil and microbiome samples were taken within the same 2-day period. All the root material associated with the vine within the pit was collected and its mass recorded. In addition to this sampling, a bulk soil sample intended for microbiome analysis was also collected at each of the three depths from one representative vine from each of the three root types, for an additional nine samples. Finally, roots were collected from the dominant cover crop species, a perennial fescue grass established at the time of planting. Samples were collected to a depth of 15 cm, which contained the bulk of the cover crop roots, in the aisleway approximately 1.5 m from the nearest vine, adjacent to the six vineyard panels sampled.

Root tissue and bulk soil sample preparation. Larger grape roots (3 to 5 mm in diameter) were cut into 7-cm pieces and placed into 50-ml disposable Falcon tubes with 40 ml of 10% sterile glycerol. The tubes were vortexed vigorously to remove the soil from the roots. The roots were then transferred to a new tube using flamed forceps and washed three times in sterile ultrapure water (Nanopure D11971; Barnstead, Van Nuys, CA, U.S.A.), or until the water cleared. The roots were then further cut into 2.5-mm pieces with a sterile, single-sided razor blade; 250 mg of tissue was placed into 2-ml microcentrifuge tubes with two sterilized 5-mm steel balls and stored at -80°C. Upon removal, the roots were submerged in liquid nitrogen until the boiling stopped. The samples were then macerated (Tissuelyser II; Qiagen, Hilden, Germany) using 2-by-60-s cycles at 30 beats/s. The samples were submerged in liquid nitrogen between cycles. Performing this procedure twice completed sample maceration.

For bulk soil sample preparation, 5 g was passed through a 2-mm sieve and freeze dried for 36 h. A 25-mg sample of the resulting powder was transferred to 2-ml tubes and stored at -80°C prior to DNA extraction.

DNA extraction and sequencing. For root tissue, the DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) using the manufacturer's protocol, with the following modifications. Buffer AP1 with RNase was added to the sample tube and placed on the TissueLyser II for one cycle, as described above. After completion of the cycle, the suspension was incubated at 65°C for 10 min. DNA was eluted from the Mini spin column with a single 100- μl wash of AE buffer.

For bulk soil samples, the DNeasy PowerSoil Kit (catalog number 12888-100; Qiagen, Valencia, CA) was used for the DNA extraction following the manufacturer's protocol, with slight modifications to increase the yield and get high-quality DNA. The buffer from the Power Bead tubes was used for washing the soil slurries into the Power Bead tubes. After adding 60 μl of solution C1 (step 2), the samples were incubated at 65°C for 20 min to inactivate the DNases (Marotz et al. 2017). Samples were then vortexed for 20 min (step 4) and incubated at 4°C (step 7). In step 9 of the protocol, only 600 μl of supernatant was used, with 200 μl of C3 in step 10. All DNA samples were quantified using a Nanodrop 2000C spectrophotometer (Thermo Scientific, Waltham, MA, U.S.A.). Extracted DNA was visualized on a 1% agarose gel (Invitrogen Ultrapure catalog number 16500-500) in $0.5\times$ Tris-borate-EDTA buffer. PCR amplification was carried out with the same primer pairs that were used for subsequent metaamplicon sequencing to ensure that the DNA was of good quality.

A negative control was employed for the DNA-extraction step. PCR was performed on the negative control and samples with the same primers used for sequencing. Because no amplification was observed in the negative control, it was not sent for sequencing.

For sequencing, 20 μl of the extracted DNA sample was sent to the Dalhousie University CGEB-IMR lab (<https://imr.bio>). The V6-V8 region of the 16S ribosomal RNA (rRNA) gene (16S) with bacteria-specific primers B969F (ACGCGHNRAACCTTACC) and BA1406R (ACGGGCRGTGWGTRCAA) and for fungi internal transcribed spacer (ITS) region 2 ribosomal DNA gene (ITS2) with primers ITS86F (GTGAATCATCGAATCTTTGAA) and ITS4 (TCCTCCGCTTATTGATATGC) were used for library preparation and sequencing. The universal ITS2 primers capture a wide range of fungal diversity, including AMF (Berruti et al. 2017; Morgan et al. 2017; Schoch et al. 2012). Samples were multiplexed using a dual-indexing approach and sequenced using an Illumina MiSeq platform with paired-end 300 + 300-bp reads. All PCR procedures and Illumina sequencing details were as described by Comeau et al. (2017). Raw sequencing data are publicly posted on the European Nucleotide Archive (PRJEB45622).

Soil statistical analyses. An analysis of variance (ANOVA) was used to analyze the data in instances where the assumptions of constant variance and a normal distribution of the error term were not violated. In instances where one or both of these assumptions were violated, a transformation was used. These statistical analyses were carried out in R version 3.5.3 (R Core Team 2019).

Bioinformatics statistical analysis. Raw amplicon-sequencing reads were first inspected using FASTQC (v0.11.4; <https://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Primer sequences were then trimmed from the reads using cutadapt v1.11 (Martin 2011). These trimmed reads were then processed with QIIME 2 version 2018.8 (Bolyen et al. 2019) to run several key steps. First, DADA2 v1.6.0 (Callahan et al. 2016) was run to stitch together forward and reverse reads; then, read error models were determined; and final amplicon sequence variants (ASVs) were output using the following options:

p-trunc-len-f 270, p-trunc-len-r 210, and p-max-ee 3. ASVs with a frequency $<0.1\%$ of the total reads for the 16S and ITS datasets were filtered out. This resulted in the exclusion of reads below a threshold of 37 and 7 for the 16S and ITS datasets, respectively. ASVs were aligned with MAFFT v7.310 (Katoh and Standley 2013) to create de novo multiple sequence alignments, which were used to create a tree using FastTree v2.1.10 (Price et al. 2010). Taxonomic classification of the 16S ASVs was performed with a Naïve-Bayes classifier against the SILVA 16S database v132 (Quast et al. 2013) restricted to the V6-V8 region. The ITS ASVs were classified using the same method against the UNITE ITS database v7.2 (Nilsson et al. 2019). All ASVs were classified successfully except for 46 ITS ASVs. Seven ASVs classified as chloroplast and three ASVs classified as mitochondria were removed. These ASVs corresponded to a mean of 0.569% of the relative abundance (RA) per sample (standard deviation = 0.587%). The QIIME 2 command "diversity core-metrics-phylogenetic" was then used to output the default α - and β -diversity metrics with a p-sampling-depth threshold of 4,000.

In addition to running DADA2, deblur was also run to generate ITS-based ASVs (Amir et al. 2017). We did this to investigate how the choice of denoising method impacted the inferred presence of Glomeromycota. To run deblur, forward and reverse reads were paired with VSEARCH and then quality filtered with the quality-filter q-score-joined command (Rognes et al. 2016). These steps were run in QIIME 2 with default options. Deblur was also run in QIIME 2 with default options, with the exceptions of not filtering out ASVs based on depth and a trim length of 263 bp. This trim length represented the bottom 2% of all sequences in the deblur pipeline. We selected this cut-off to be conservative in order to not miss potential Glomeromycota sequences.

All bioinformatic statistical analyses were conducted using QIIME2 and in R (3.6.0; <https://www.R-project.org/>). The CCAs were generated with the vegan R package (version 2.5-4). An Adonis test (permutational multivariate analysis of variance [PERMANOVA]) was used to see to what degree the variance was explained by different factors; Bray-Curtis, unweighted principal coordinate analysis (PCoA), and weighted PCoA methods were used, permutations = 9,999 in the vegan package in R using the formula (distance matrix) approximately rootstock + root depth + vine + rootstock $\times$ root depth. A separate Adonis test (PERMANOVA), based on Bray-Curtis dissimilarity, was used to determine what proportion of the variance in the taxonomic composition was explained by sample type (grape root, cover crop root, and soil). Spearman's correlations used multiple testing corrections within a particular diversity metric. Differential abundance testing of genera was performed using ALDEx2 version 1.24.0 (Fernandes et al. 2013); significant results were based on Benjamini-Hochberg corrected values for the Kruskal-Wallis test.

We have made our key code and datafiles available at this GitHub repository: https://github.com/zoemigicovsky/cool_climate_grape_microbiome.

RESULTS

Microbiome and sample type (cover crop root, grape root, and soil). A breakdown of the resolved taxa, from phylum to species, for the cover crop root, grape root, and soil can be found in Supplementary Files S1, S2, and S3, respectively. Rarefaction curves for both bacteria and fungi for all samples can be found in Supplementary Figure S1. All grape root, cover crop root, and bulk soil samples produced usable 16S sequences. However, for the ITS sequences, although 100% of the cover crop root and bulk soil samples produced usable reads, 41% of the grape-root samples failed to sequence and were excluded from the analyses. For bacteria, the

soil, grape root, and cover crop root were dominated by *Proteobacteria* (30, 68, and 50%, respectively) followed by *Bacteroidetes* (18, 14, and 36%, respectively). *Actinobacteria* and *Firmicutes* were the next most dominant bacteria phyla for the grape and cover-crop roots, while *Acidobacteria* (18%) was next for the soil and was greatly overrepresented compared with the roots. At the species level, *Bacillus simplex* and *Rhizobium leguminosaurus* were overrepresented in the grape roots compared with the soil and the cover crop roots, and *Agrobacterium radiobacter*, *Paenibacillus amylolyticus*, and plant *Pantoea agglomerans* were detected only in grape root but not in soil samples. In terms of fungi, the soil, grape root, and cover crop were all dominated by Ascomycota (59, 80, and 63%, respectively); Basidiomycota was the second-most abundant phylum for the grape and cover-crop roots (18 and 32%, respectively), while Mortierellomycota was the second most common phylum in the soil, followed by Basidiomycota (19 and 17%, respectively). Meaningfully, Glomeromycota figured prominently in the cover crop roots (67% prevalence and 3% RA) and was present in the soil (11% prevalence and RA = 0.02%), but was not detected in the grape roots after filtering out rare ASVs from the DADA2 results (Supplementary Files S1, S2, S3, and S4). Interestingly, if unfiltered DADA2 or deblur were instead used as the denoising algorithm, Glomeromycota was identified as part of the grape-root microbiome, as a minor component. Both unfiltered DADA2 and deblur results indicated Glomeromycota were present in 30% of the grape-root samples, with deblur results indicating a RA of 1.9% (Supplementary File S4).

An Adonis test (PERMANOVA), based on Bray-Curtis dissimilarity, showed significant differences in the taxonomic composition between the root (grape and cover crop) and soil communities for both bacteria ($R^2 = 0.33$, $P < 0.001$) and fungi ($R^2 = 0.40$, $P < 0.001$). Similarly, significant differences in the bacterial ($R^2 = 0.28$, $P < 0.001$) and fungal ($R^2 = 0.29$, $P < 0.001$) communities were also observed between the grape roots and the roots of the fescue cover crop. The α -diversity indices (observed ASVs, evenness, and Shannon index) were all highest in the soil and lowest in the grape roots for the bacteria; the same was true for the fungi, with the exception of evenness, which was lowest in the cover-crop roots (Table 1). The bacterial community showed greater overlap (>40%) in the genera involved between all three sample types (grape root,

soil, and cover crop) compared with the fungal community (<20%) (Fig. 1A and B; Table 1). For both the bacterial and fungal communities, the greatest proportion of specialists was found in the grape soil, while a lower fraction of specialists was observed in the roots of the grapevines and the cover crop (Fig. 1A and B). The top 15 genera across all three sample types can be found in Figure 1C and D. The top 15 classified bacteria and fungi genera found in the grape root systems alone, as well as their known associations as found in the literature, can be found in Tables 2 and 3, respectively.

Grape microbiome, soil parameters, and root phenotypes.

The soil properties associated with each vine consisted of the pH, organic matter (%), and cation exchange capacity (CEC), as well as a range of macronutrients and micronutrients (P_2O_5 , K_2O , Ca, Mg, Na, S, Al, B, Cu, Fe, Mn, and Zn) (Table 4). When a Spearman's correlation was performed between microbiome richness, evenness, and Shannon's phylogenetic diversity and the various soil parameters, no soil parameters were found to be correlated with these α -diversity metrics for either bacteria or fungi. When the same analysis was performed between the soil parameters measured and the top 10 fungi and bacteria features, Benjamini-Hochberg-corrected results suggested that there was little correlation between the two, with one exception: the fungi genus *Pseudaleuria* was found to be positively correlated with Na ($r^2 = 0.54$, $P = 0.02$) and Mn ($r^2 = 0.56$, $P = 0.02$) levels and negatively correlated with Fe levels ($r^2 = 0.50$, $P = 0.03$). A Spearman's correlation showed there was no significant correlation between root phenotype (trunk diameter, secondary root mass, tertiary root mass, and total root mass) and α -diversity metrics (evenness, richness, and Shannon's phylogenetic diversity) for bacteria. However, evenness was found to be positively correlated with total root mass for the fungi ($r^2 = 0.34$, $P = 0.02$).

Grape root microbiome across soil depth (0 to 15, 15 to 30, and 30 to 50 cm). A breakdown of the resolved taxa, from phylum to species for the soil depths of 0 to 15, 15 to 30, and 30 to 50 cm can be found in Supplementary Files S5, S6, and 7, respectively. The results of the soil analyses showed that the different soil depths offered distinct microenvironments (Supplementary Table S1). The soil pH, organic matter, and CEC were all highest in the uppermost soil level. Absolute levels of phosphorous,

TABLE 1 Observed amplicon sequence variants (ASVs), evenness, and Shannon index for both 16S (bacteria) and internal transcribed spacer (ITS) (fungi) across vineyard substrate, root types, and depths ^z						
Niche	Observed ASVs		Evenness		Shannon	
	16S	ITS	16S	ITS	16S	ITS
Sample type						
Soil	847 ± 117 A	109 ± 23 A	0.94 ± 0.01 A	0.71 ± 0.09 A	9.11 ± 0.26 A	4.82 ± 0.58 A
Grape root	436 ± 156 B	37 ± 16 C	0.81 ± 0.07 B	0.62 ± 0.05 AB	7.01 ± 0.94 B	3.21 ± 0.30 B
Cover crop root	690 ± 109 A	57 ± 19 B	0.90 ± 0.02 A	0.58 ± 0.18 B	8.43 ± 0.38 A	3.40 ± 1.30 B
Grape root type						
Ungrafted	366 ± 89 A	31 ± 8.3 A	0.79 ± 0.06 A	0.71 ± 0.03 A	6.68 ± 0.77 A	3.53 ± 0.39 A
3309 C	448 ± 124 A	39 ± 15.7 A	0.80 ± 0.05 A	0.63 ± 0.05 A	7.00 ± 0.71 A	3.23 ± 0.30 A
Riparia Gloire	365 ± 125 A	37 ± 7.9 A	0.79 ± 0.06 A	0.60 ± 0.08 A	6.61 ± 0.85 A	3.12 ± 0.49 A
Grape root depth						
0 to 15 cm	392 ± 148 A	36 ± 9.0 A	0.79 ± 0.05 A	0.65 ± 0.01 A	6.69 ± 0.81 A	3.29 ± 0.25 A
15 to 30 cm	403 ± 101 A	36 ± 8.2 A	0.81 ± 0.05 A	0.59 ± 0.08 A	6.95 ± 0.72 A	3.03 ± 0.59 A
30 to 50 cm	381 ± 111 A	38 ± 15.3 A	0.78 ± 0.07 A	0.64 ± 0.01 A	6.64 ± 0.86 A	3.29 ± 0.40 A

^z Averages, followed by standard deviation, with like letter groupings within a column for a given category are not significantly different according to Tukey's pairwise test ($P < 0.05$).

potassium, calcium, magnesium, boron, copper, iron and zinc, and base saturation K% and Ca% were, likewise, found at their highest levels in the uppermost soil level. The base saturation Na% and Mg% levels were the only two nutrient parameters found to be highest in the lowest soil level (Supplementary Table S1).

Significant overlap in the genera was found in both the bacteria (>65%) and the fungi (>30%) across soil depths (Fig. 2A and B). An Adonis Test (PERMANOVA) showed that root depth did explain a portion of the variance of the taxonomic composition for the bacterial (16S; $r^2 = 0.09$, $P < 0.01$), but not for the fungal (ITS; $r^2 = 0.12$) communities (Table 4). In particular, the *Firmicutes* bacteria was a prominent phylum represented in the shallowest depth (0 to 15 cm), with an RA of 3%, but was less prominent at the deeper soil depths, where the RA was < 1% (Supplementary Files S5, S6, and S7). An ALDEx2 analysis combined with a glm

ANOVA showed that no bacterial or fungal genera had a depth-specific effect. α -Diversity indices from grape roots sampled from the three depths showed no differences (Table 1).

α Diversity from bulk soil samples did not reveal strong trends along the soil column. The exception was fungal evenness: plotting the values suggested that evenness was greater at the deepest soil level sampled (30 to 50 cm) relative to the shallowest (0 to 15 cm) (Supplementary Fig. S2).

Grape root microbiome across root type (ungrafted, 3309C, and Riparia Gloire). A breakdown of the resolved taxa, from phylum to species, for the ungrafted, 3309C, and Riparia Gloire grape root types can be found in Supplementary Files S8, S9, and S10, respectively. There was greater overlap in the genera observed across root type in the bacteria relative to the fungi genera (Fig. 3). With regards to specialization across grape rootstock types, 3309C

Fig. 1. Venn diagrams showing the overlap in **A**, bacterial and **B**, fungal genera found between the grape roots, soil, and cover crop roots and the top 15 most abundant **C**, bacteria and **D**, fungi genera found in the same.

TABLE 2
Top 15 classified bacteria genera detected in grapevine roots using 16S (V6-V8) metaamplicon barcoding^z

Bacteria	Metrics (%)	Taxonomy	Known ecological characteristics	References
1. <i>Pseudomonas</i>	<i>P</i> = 100 <i>RA</i> = 27.9 <i>CV</i> = 68.6	p_Proteobacteria c_Gammaproteobacte o_Pseudomonadales f_Pseudomonadaceae	<ul style="list-style-type: none"> Plant-growth-promoting (PGP) rhizobacteria Siderophores and phytohormones production Nitrogen (N) fixation, phosphate solubilization, biocontrol <i>Pseudomonas syringae</i> plant pathogen 	Ferreira et al. 2019
2. <i>Niastella</i>	<i>P</i> = 100 <i>RA</i> = 3.9 <i>CV</i> = 67	p_Bacteroidetes c_Bacteroidia o_Chitinophaga f_Chitinophagaceae	<ul style="list-style-type: none"> Soil and plant associated 	Kim et al. 2015; Visioli et al. 2018
3. <i>Rhizobium</i> <i>Rhizobium leguminosarum</i> , <i>Agrobacterium radiobacte</i>	<i>P</i> = 100 <i>RA</i> = 3.3 <i>CV</i> = 43	p_Proteobacteria c_Alphaproteobacteria o_Rhizobiales f_Rhizobiaceae	<ul style="list-style-type: none"> PGP rhizobacteria Siderophores and phytohormones production N fixation, phosphate solubilization 	Ferreira et al. 2019; Zarraonaindia et al. 2015
4. <i>Bradyrhizobium</i>	<i>P</i> = 100 <i>RA</i> = 3.1 <i>CV</i> = 55	p_Proteobacteria c_Alphaproteobacteria o_Rhizobiales f_Xanthobacteraceae	<ul style="list-style-type: none"> Grape-root associated PGP rhizobacteria Siderophores and phytohormones production N fixation, phosphate solubilization 	Ferreira et al. 2019; Samad et al. 2017; Zarraonaindia et al. 2015
5. <i>Streptomyces</i>	<i>P</i> = 100 <i>RA</i> = 3.0 <i>CV</i> = 93	p_Actinobacteria c_Actinobacteria o_Streptomycetales f_Streptomycetaceae	<ul style="list-style-type: none"> Grape-root associated Produce siderophores, stimulate fungi growth improving plant phosphorus acquisition Several species are actively being developed as biocontrol agents. 	Franco-Correa et al. 2010; González-García et al. 2019; Zarraonaindia et al. 2015
6. <i>Novosphingobium</i>	<i>P</i> = 100 <i>RA</i> = 2.3 <i>CV</i> = 86	p_Proteobacteria c_Alphaproteobacteria o_Sphingomonadales f_Sphingomonadaceae	<ul style="list-style-type: none"> PGP rhizobacteria Produce siderophores and phytohormones Phosphate solubilization 	Zhang et al. 2016
7. <i>Steroidobacter</i>	<i>P</i> = 100 <i>RA</i> = 2.1 <i>CV</i> = 78	p_Proteobacteria c_Gammaproteobacteria o_Steroidobacterales f_Steroidobacteraceae	<ul style="list-style-type: none"> Brassinosteroids production Role in plant development 	Fahrbach et al. 2008; Zarraonaindia et al. 2015
8. <i>Flavobacterium</i>	<i>P</i> = 94 <i>RA</i> = 2.2 <i>CV</i> = 102	p_Bacteroidetes c_Bacteroidia o_Flavobacteriales f_Flavobacteriaceae	<ul style="list-style-type: none"> Antagonistic to some wine-grape pathogens Enhanced biocontrol activity against Phytophthora ACC deaminase activities 	Kolton et al. 2014
9. <i>Mycobacterium</i>	<i>P</i> = 100 <i>RA</i> = 2.0 <i>CV</i> = 52	p_Actinobacteria c_Actinobacteria o_Corynebacteriales f_Mycobacteriaceae	<ul style="list-style-type: none"> Root-associated 	Bouam et al. 2018
10. <i>Chitinophaga</i>	<i>P</i> = 100 <i>RA</i> = 1.9 <i>CV</i> = 97	p_Bacteroidetes c_Bacteroidia o_Chitinophagales f_Chitinophagaceae	<ul style="list-style-type: none"> Grape-root associated 	Zarraonaindia et al. 2015

(Continued on next page)

^z Genera are ranked according to the product of their prevalence (P) multiplied by their mean relative abundance (RA) in grapevine root samples; the coefficient of variation (CV) is also shown. Species names are listed under genus if classified and found to be present in relatively high abundances.

TABLE 2 (Continued from previous page)

Bacteria	Metrics (%)	Taxonomy	Known ecological characteristics	References
11. <i>Mesorhizobium</i>	$P = 97$ RA = 1.8 CV = 60	p_Proteobacteria c_Alphaproteobacteria o_Rhizobiales f_Rhizobiaceae	<ul style="list-style-type: none"> • PGP rhizobacteria • Siderophores and phytohormones production • N fixation, phosphate solubilization 	Ferreira et al. 2019; Zarraonaindia et al. 2015
12. <i>Arthrobacter</i>	$P = 100$ RA = 1.7 CV = 82	p_Actinobacteria c_Actinobacteria o_Micrococcales f_Micrococcaceae	<ul style="list-style-type: none"> • PGP characteristics • Biostimulation, biofertilization, biocontrol 	Ferreira et al. 2019
13. <i>Bacillus</i> <i>Bacillus simplex</i>	$P = 100$ RA = 1.4 CV = 150	p_Firmicutes c_Bacilli o_Bacillales f_Bacillaceae	<ul style="list-style-type: none"> • PGP rhizobacteria • Produce siderophores and phytohormones • Phosphate solubilization • Ammonia production 	Ferreira et al. 2019
14. <i>Caulobacter</i>	$P = 97$ RA = 1.4 CV = 88	p_Proteobacteria c_Alphaproteobacteria o_Caulobacterales f_Caulobacteraceae	<ul style="list-style-type: none"> • PGP rhizobacteria • Directly or indirectly involved in brassinosteroid metabolism 	Luo et al. 2019
15. <i>Pseudarthrobacter</i>	$P = 97$ RA = 1.2 CV = 118	p_Actinobacteria c_Actinobacteria o_Micrococcales f_Micrococcaceae	<ul style="list-style-type: none"> • PGP rhizobacteria • Produce siderophores and phytohormones • Phosphate solubilization • Ammonia production 	Ferreira et al. 2019

had the most unique genera for bacteria, while *Riparia Gloire* had the most unique fungal genera (Fig. 3A and B). An Adonis Test (PERMANOVA) showed that grape rootstock did explain a portion of the variance of the taxonomic composition for both the bacterial (16S; $r^2 = 0.13$, $P < 0.001$) and fungal (ITS; $r^2 = 0.22$, $P < 0.01$) communities, although the explanatory power of this factor was low (Table 4) and there were no differences in α diversity indices (Table 1). An ALDEx2 analysis combined with a glm ANOVA showed that no fungal genera had a significant root type-specific effect; however, root type was found to have a significant effect for 12 bacterial genera ($P < 0.05$). The relationships between root type and these bacterial genera can be found in Supplementary Figure S3.

The average root mass found in the excavated area for the own-rooted, *Riparia Gloire*, and 3309C vines was 400, 142, and 258 g, respectively. An ANOVA followed by a Tukey multiples means comparison showed that these values were all significantly different from one another. An analysis of soil parameters across the individual vines, as broken down by root type, showed some differences between root type sites; in particular, organic matter (%), CEC, P_2O_5 , K_2O , Na, and Cu levels all showed higher values for the own rooted vines compared with *Riparia Gloire* and 3309C (Supplementary Table S2).

DISCUSSION

Plant microbial specialization. The source material (soil versus root tissue) had the strongest effect on bacterial and fungal microbiome structure and had the most explanatory power among all factors tested. Microbial diversity was greater in soil than in plant roots for both fungi and bacteria (Table 1). This agrees with other agri-plant systems and is explained by a demand for microbial specialization necessary for invasion and survival inside plant tissue (Hacquard et al. 2015; Lundberg et al. 2012; Vandenkoornhuysen et al. 2015; Yurgel et al. 2018). Host plants actively recruit soil PGP microorganisms by secreting root-derived chemoattractants (Babalola et al. 2020; Bulgarelli et al. 2013; Pascale et al. 2020).

Plant species (cover crop versus grape) was the second-strongest factor affecting the structure of the root microbiome. The bacterial grape-root microbiome exhibited lower α diversity than the cover-crop microbiome, which was reflected as a decrease in the microbiome evenness, Shannon index, and observed ASVs, suggesting stronger plant selective pressure by the grape root compared with the cover crop. The effect was not as great in the grape-root fungal microbiome, which was far less rich than the bacteria. However, what was consistent was that the number of observed fungal ASVs in the grape roots was lower than that observed in the cover crop (Table 1). It should be noted that the location of the fescue grass, in the untilled aisleway, and that of the grape, which was in the row, cannot be disentangled from the plant species in this study. To our knowledge, this study is unique in that it compares the microbiome of the grape roots to that of the dominant cover crop, a fescue grass.

Present study's vineyard microbiome as compared with past studies. Because this is the first root microbiome study of a Canadian vineyard, we provide valuable biodiversity data for comparison with other global vineyards, as well as to future studies in our region.

Two previous studies in a Mediterranean climate used universal ITS primers to look at the fungal microbiome in grape (Berlanas et al. 2019; Martínez-Diz et al. 2019). All of the fungal taxa that were identified in this study within grapevine roots were previously identified by different groups (Marzano et al. 2016; Mezzasalma et al. 2018; Miura et al. 2017; Morgan et al. 2017; Portillo et al. 2016; Salvetti et al. 2016; Zarraonaindia et al. 2015).

Ascomycete and Basidiomycete fungi dominated both soil and root fungal assemblages similar to other recent studies (Berlanas et al. 2019; Martínez-Diz et al. 2019; Swift et al. 2021). The second most dominant phylum in our rhizosphere soil, however, was Mortierellomycota, followed by Basidiomycota, which differs from these previous studies. Mortierellomycota has only recently been classified as a unique phylum, which may explain this difference (Tederloo et al. 2018). The grapevine root samples in the present study may be further classified as being dominated by unidentified

TABLE 3
Top 15 classified fungi genera detected in grapevine roots using internal transcribed spacer 2 metaamplicon barcoding^z

Fungi	Metrics (%)	Taxonomy	Known ecological characteristics	References
1. <i>Trichosporon</i>	<i>P</i> = 100 RA = 4.3 CV = 99	p_Basidiomycota c_Tremellomycetes o_Trichosporonales f_Trichosporonaceae	<ul style="list-style-type: none"> Approximately 50 species currently in this genus Most often soil-dwelling May be present on grapevines 	Barata et al. 2012; Comitini and Ciani 2008; Moll et al. 2016; Yurkov 2018
2. <i>Plectosphaerella</i> <i>Plectosphaerella cucumerina</i> (formerly <i>Fusarium tabacinum</i>), <i>Microdochium tabacinum</i> , <i>Monographella cucumerina</i>)	<i>P</i> = 90 RA = 4.6 CV = 107	p_Ascomycota c_Sordariomycetes o_Glomerellales f_Plectosphaerellaceae	<ul style="list-style-type: none"> Soil saprobe, notable plant pathogen (necrotrophic), pathogen of nematodes 	Ramos et al. 2013
3. <i>Ilyonectria</i> <i>Ilyonectria destructans</i> (formerly <i>Cylindrocarpon destructans</i>)	<i>P</i> = 85 RA = 3.0 CV = 141	p_Ascomycota c_Sordariomycetes o_Hypocreales f_Nectriaceae	<ul style="list-style-type: none"> Common soil saprobe Causes root rot, replant and other diseases of cultivated plants including ginseng, pine, and fruit trees Causes black foot disease in grapevine; source of infection may be from nursery or endemic to vineyards; disease ecology is related to soil and climate conditions, and soil microbiome 	Agustí-Brisach et al. 2014; Farh et al. 2018; Halleen et al. 2006; Petit and Gubler 2005; Rego et al. 2000; Traquair and White 1992; Unestam et al. 1989
4. <i>Mycena</i>	<i>P</i> = 40 RA = 3.4 CV = 239	p_Basidiomycota c_Agaricomycetes o_Agaricales f_Tricholomataceae	<ul style="list-style-type: none"> Mainly found in roots of green plants Cycles carbon and nitrogen Some species use formaldehyde as a chemical defense of fruiting bodies 	Grelet et al. 2017; Himstedt et al. 2020; Thoen et al. 2020
5. <i>Gibberella</i> <i>Gibberella tricineta</i> , <i>G. intricans</i>	<i>P</i> = 80 RA = 1.6 CV = 183	p_Ascomycota c_Sordariomycetes o_Hypocreales f_Nectriaceae	<ul style="list-style-type: none"> Associated with Bakanae disease, head blight, foot and crown rot Produce toxic secondary metabolites 	Nirenberg and O'Donnell 1998; Trail and Common 2000
6. <i>Fusarium</i> <i>Fusarium oxysporum</i> , <i>F. tricinctum</i> (formerly <i>G. tricineta</i>), <i>F. solani</i>	<i>P</i> = 95 RA = 1.0 CV = 69	p_Ascomycota c_Sordariomycetes o_Hypocreales f_Nectriaceae	<ul style="list-style-type: none"> Most <i>Fusarium</i> spp. are saprobes; many notable plant pathogens are in this genus. <i>F. tricinctum</i> causes head blight in cereal grains. <i>F. solani</i> causes disease in pea, cucurbits and sweet potato, among other crop plants. 	Bottalico and Perrone 2002; Lombard et al. 2015; Nelson et al. 1994; O'Donnell 2000
7. <i>Cadophora</i>	<i>P</i> = 85 RA = 1.0 CV = 61	p_Ascomycota c_Leotiomycetes o_Helotiales f_Helotiales_fam_Incertae_sedi	<ul style="list-style-type: none"> <i>Cadophora luteo-olivacea</i>, most prevalent species associated with diseased vines in Canada Associated with Petri disease and Esca 	Maldonado-González et al. 2020

(Continued on next page)

^z Genera are ranked according to the product of their prevalence (P) multiplied by their mean relative abundance (RA) in grapevine root samples; the coefficient of variation (CV) is also shown. Species names are listed under genus if classified and found to be present in relatively high abundances.

TABLE 3 (Continued from previous page)

Fungi	Metrics (%)	Taxonomy	Known ecological characteristics	References
8. <i>Tetracladium</i>	$P = 65$ $RA = 1.1$ $CV = 179$	p_Ascomycota c_Leotiomycetes o_Helotiales f_Helotiaceae	<ul style="list-style-type: none"> Plant growth promoter Found in vineyards An endophyte that helps degrade plant debris 	Chen et al. 2020; Liang et al. 2019; Wei et al. 2018
9. <i>Solicoccozyma</i> <i>Solicoccozyma terrea</i> , <i>S. terricola</i> (formerly <i>Cryptococcus terreus</i> , <i>C. terricola</i>)	$P = 75$ $RA = 1.0$ $CV = 114$	p_Basidiomycota c_Tremellomycetes o_Filobasidiales f_Piskurozymaceae	<ul style="list-style-type: none"> Present in soils and leaf litter Isolated from vineyards <i>S. terricola</i> is a psychotolerant yeast 	Bougreau et al. 2019; Stosiek et al. 2019
10. <i>Naganishia</i> <i>Naganishia albida</i> , <i>N. randhawae</i>	$P = 55$ $RA = 1.1$ $CV = 263$	p_Basidiomycota c_Tremellomycetes o_Filobasidiales f_Biolbasidiaceae	<ul style="list-style-type: none"> A yeast Isolated from withered grape berries Resistant to UV, psychotolerant and halotolerant 	Lorenzini and Zapparoli 2019; Schmidt et al. 2017
11. <i>Pseudaleuria</i>	$P = 30$ $RA = 2.0$ $CV = 292$	p_Ascomycota c_Pezizomycetes o_Pezizales f_Pyronemataceae	<ul style="list-style-type: none"> <i>Pseudaleuria</i> contains only two species: <i>Pseudaleuria fibrillosa</i> and <i>P. quinaultiana</i> Presence of <i>Pseudaleuria</i> at the genus level has been found to correlate with low disease severity in pea roots. Presence at the genus level has been detected at high levels in recent soil microbiome research. 	Gkarmiri et al. 2017; Xu 2011
12. <i>Clonostachys</i> <i>Clonostachys rosea</i>	$P = 85$ $RA = 0.7$ $CV = 96$	p_Ascomycota c_Sordariomycetes o_Hypocreales f_Bionectriaceae	<ul style="list-style-type: none"> Common in soils. Found in plants as endophytes, epiphytes, and saprotrophs. A mycoparasitic fungus, usually a biocontrol for plant pathogens or as a facultative pathogens of nematodes <i>C. rosea</i> proliferates better in disturbed and conventionally cultivated habitats. 	Moloinyane et al. 2020; Torcato et al. 2020
13. <i>Alternaria</i>	$P = 65$ $RA = 0.8$ $CV = 303$	p_Ascomycota c_Dothideomycetes o_Pleosporales f_Pleosporaceae	<ul style="list-style-type: none"> <i>Alternaria</i> is a ubiquitous genus isolated from most environments, most often saprobes. Common plant pathogens in many crop plants <i>Alternaria</i> is associated with grape bunch rot and passito style wines. 	Lorenzini and Zapparoli 2014; Woudenberg et al. 2013
14. <i>Heydenia</i> <i>Heydenia alpina</i>	$P = 45$ $RA = 1.0$ $CV = 295$	p_Ascomycota c_Pezizomycetes o_Pezizales f_Pyronemataceae	<ul style="list-style-type: none"> Plant saprobe Few records; one record of isolation from vineyard soil in the Badacsony wine region of Hungary 	Geröcs et al. 2016; Leuchtmann and Cléménçon 2012
15. <i>Filobasidium</i> <i>Filobasidium globisporum</i> , <i>F. magnum</i>	$P = 45$ $RA = 0.9$ $CV = 200$	p_Basidiomycota c_Tremellomycetes o_Filobasidiales f_Filobasidiaceae	<ul style="list-style-type: none"> Yeasts commonly epiphytic populations from grapevines (leaf, sap, berry/ fruit samples rather than soil) 	Bougreau et al. 2019

fungal genera within the family Nectriaceae. However, based on fungi commonly isolated in soil and plant root microbiomes, likely genera include *Bionectria*, *Calonectria*, *Cylindrocarpon/Neonectria*, *Fusarium/Gibberella*, and *Ilyonectria* (Bonito et al. 2014, 2016; Chapelle et al. 2016; Toju et al. 2018). Other Nectriaceae genera of

interest to viticulture are *Campylocarpon*, *Cylindrocladiella*, *Dactylonectria*, and *Thelonectria*; along with members of *Ilyonectria* and *Cylindrocarpon/Neonectria*, species of these genera cause black foot rot in grapevine (Agustí-Brisach and Armengol 2013; Gramaje et al. 2018; Úrbez-Torres et al. 2014). Grapevine root samples also

contained consistent abundances of two Nectriaceae genera that were definitively resolved: *Fusarium* and *Ilyonectria*. Other fungi present across grapevine root samples in relatively high abundances include *Mycena*, *Pseudaleuria*, *Plectosphaerella*, and *Trichosporon* spp. and unclassified members of Pleosporales. The cover-crop roots consist of the largest proportion of “other” fungal genera (genera that do not rank within the 15 most abundant among all samples). Genera of notable abundance in the cover crop root samples were *Gibberella*, *Mycena*, and unclassified members of Pleosporales. When comparing the most abundant fungi we documented in grape roots with taxa documented in other vineyard studies, three fungi stand out. *Clonostachys* sp. was the 12th most abundant fungus observed from grape roots during our study. In other recent studies, the abundance of *Clonostachys rosea* has determined the dissimilarity of fungal assemblages between the rootstock genotypes and between rhizosphere and bulk soil types (Berlanas et al. 2019; Martínez-Diz et al. 2019). *Ilyonectria* sp. was the third most abundant species from grape roots in our study, and determined the dissimilarity of fungal assemblages between rhizosphere and bulk soil types previously (Martínez-Diz et al. 2019). *Filobasidium* sp., which was reported during our study as 15th in abundance from grape roots, has been previously reported as a dominant species of vineyard rhizosphere, bulk soils, and grape roots, and may be responsible for the dissimilarity between the fungal assemblages of these substrata (Martínez-Diz et al. 2019).

Interestingly, different bacteria were recruited by the roots of Merlot grapevines growing in vineyards located in Long Island (Suffolk County, NY, U.S.A.) (Zarraonaindia et al. 2015). This region would be described as region III, according to the Winkler

index; for comparison, the vineyard used in the present study would be considered a much cooler region I. *Xanthomonadales*, *Rhizobiales*, *Saprospirales*, *Cytophagales* and *Actinomycetales* were the most abundant bacterial families enriched in Merlot root microbiomes, while our study identified *Pseudomonadales*, *Steroidobacterales*, *Rhizobiales*, and *Micrococcales* as the most abundant bacterial families enriched in the root microbiome. These differences in root-enriched taxa could be explained by the variation in the rootstock genotypes, though such drastic differences were not apparent in the present study, or by differences in vineyard management or climate.

The PGP rhizobacteria genus *Pseudomonas* was the dominant bacterial genus in the grape-root microbiome. *Pseudomonas* spp. can produce siderophores and phytohormones and are known for their nitrogen-fixation, ammonia-production, phosphate-solubilization, and biocontrol capabilities (Ferreira et al. 2019; Padda et al. 2019; Puri et al. 2020). However, not all species are beneficial; *Pseudomonas syringae* is a known plant pathogen that has also been linked with lowering the freezing tolerance of plant tissues through the generation of ice-nucleation activation proteins (Maki et al. 1974; Morris et al. 2008). The presence of *P. syringae* could have implications in Nova Scotia, where freezing temperatures typically penetrate deep into the soil for several months out of the year. Several *Rhizobiales* and *Actinobacteria* taxa, and *Caulobacter* and *Pantoea* genera with broad spectra of PGP characteristics, including biostimulation, biofertilization, and biocontrol (Ferreira et al. 2019), were enriched in grape roots compared with soil. Overrepresented in root tissue, *Streptomyces* spp. have capabilities to produce siderophores and stimulate fungi growth, improving plant phosphorus acquisition (Franco-Correa

TABLE 4 Adonis test (PERMANOVA) results to determine what proportion of the variance in the taxonomic composition of the grape roots is explained by root depth, rootstock, and the rootstock–root depth interaction ^z						
Grouping	16S (<i>R</i> ²)			Internal transcribed spacer (<i>R</i> ²)		
	Bray-Curtis	Unweighted	Weighted	Bray-Curtis	Unweighted	Weighted
Rootstock	0.13***	0.10***	0.11*	0.22**	0.12*	0.16
Root depth	0.09**	0.08**	0.06	0.12	0.14**	0.11
Vine	0.33***	0.29*	0.31	0.28	0.38***	0.35
Rootstock–root depth	0.10	0.10	0.14	0.12	0.13	0.12

^z Asterisks *, **, and *** indicate *P* < 0.05, 0.01, and 0.001, respectively.

Fig. 2. Venn diagrams showing the overlap in A, bacterial and B, fungal genera across soil depths.

et al. 2010). Similar to a previous report (Zarraonaindia et al. 2015), brassinosteroid-producing *Steroidobacter* spp., which have a potentially important role in plant development (Tang et al. 2016), and endophytic families *Chitinophagaceae* and *Sphingomonadaceae* (Armanhi et al. 2018), were found in higher abundance in grape roots than in soil. Several bacterial species with PGP and biocontrol characteristics were highly enriched in the grape-root microbiome. The RA of *B. simplex*, represented by 1.4% of all 16S rRNA reads in the grape-root microbiome, was 143-fold greater in grape roots compared with soils. In addition to secreting polyamines, producing siderophores, and synthesizing antimicrobial peptides and antibiotics (Maymon et al. 2015), *B. simplex* strains exhibit strong biocontrol activities against *Fusarium* infection (Khan et al. 2018). *Paenibacillus amylolyticus*, also overrepresented in grape roots compared with soils, can have activity against both Gram-positive and Gram-negative pathogens (Anandan and Vittal 2019) and grape-root-specific *Pantoea agglomerans* is a potent biocontrol agent with diverse PGP characteristics (Dutkiewicz et al. 2016). Endophyte colonization can improve plant nutrient uptake and increase plant growth and development (Ferreira et al. 2019). As a result, it is feasible that a long history of grape breeding targeting specific fruit characteristics could potentially promote the ability of the host plant to select for PGP endophytes that may help fulfill these requirements.

AMF are a minor constituent. Notably, AMF were not among the most abundant fungal genera found within any of the sample types (Fig. 1D). However, all three sample types (soil, grape roots, and cover crop roots) showed members of the phylum Glomeromycota in a subset of these samples, at least when an unfiltered denoising algorithm was used (Supplementary File S4). Interestingly, using the deblur denoising algorithm yielded higher RAs compared with unfiltered DADA2 results for all sample types. It was unexpected that AMF were not found to be a major constituent of the grape roots. Grapevines are often highly mycorrhizal and known to depend on AMF for improved water-use efficiency and soil-nutrient uptake (Possingham and Obbink 1971; Schreiner and Mihara 2009; Trouvelot et al. 2015; Valentine et al. 2006). Use of fungal ITS primers rather than AMF-specific primers may have reduced AMF identification power, although Morgan et al. (2017) suggest that the ITS region is the best marker in grapevine microbiome analyses and should sufficiently capture AMF diversity. Other research to date has searched for AMF in grapevine roots using primers optimized for Glomeromycota, and reports higher abundances of AMF (Bouffaud et al. 2016; Holland et al. 2014; Schreiner and Mihara 2009). However, other recent studies have shown that

Glomeromycota may make up only a small percentage of the total fungal species present in grape roots (Berlanas et al. 2019; Swift et al. 2021). It may be that, for the vineyard sampled in this study, high phosphorous levels (Supplementary Table S1) and the ample amount of precipitation provided by the Maritime climate are explanatory factors for the low AMF levels found in this study.

Microbiome throughout the soil column and in relation to soil parameters. Previous studies have shown that microbial composition and diversity of the soil significantly changes with soil depth (Niu et al. 2021; Upton et al. 2020). This study showed that the gradients in the microbial composition within the vertical soil column may also be a factor in the roots, especially for bacteria, albeit with a low degree of explanatory power (Table 4). Alternatively, the subtle influence of the depth from which the root was sampled on the microbiome could also simply be the result of contamination of the root samples from the soil itself.

No individual soil parameters, pH, organic matter, CEC, or a range of macro- and micronutrients were found to be correlated with α -diversity metrics for either bacteria or fungi in this study. However, a lone fungi genus, *Pseudaleuria*, was found to be correlated with a few micronutrients. The reason for this is unknown and is in contrast to the results of Xu et al. (2012), who found no correlation between *Pseudaleuria* and soil pH, P, K, or Mg. Finally, the finding that vine root mass was found to be correlated with fungal evenness may be in keeping with past studies. If root mass may be thought of as a proxy for overall nutrient availability, previous studies found the latter influenced the overall fungal community (Lauber et al. 2008; Santos-González et al. 2011; Sun et al. 2011).

Microbiome across grape rootstock. For the grape root microbiome, rootstock was also a significant factor, albeit with low explanatory power, affecting both bacterial and fungal community structure (Table 4). It should be noted that this study did not endeavor to determine whether the act of grafting itself may be an influential factor because the root types in this study did not include self-grafted vines. The findings of this study are in agreement with past studies (Berlanas et al. 2019; D'Amico et al. 2018; St. Laurent et al. 2010; Marasco et al. 2018; Martínez-Diz et al. 2019; Swift et al. 2021; Vink et al. 2021; Warschefsky et al. 2016). A number of potential PGP and biocontrol bacteria exhibited rootstock-specific distributions in the grape-root microbiome, including *Actinobacteria* taxa. Endophyte colonization can affect the resistance of the plant to pathogen attack, and the ability of the plant to recruit endophytes with biocontrol properties might contribute to rootstock disease resistance. For the fungal assemblage in

Fig. 3. Venn diagrams showing the overlap in **A**, bacterial and **B**, fungal genera between ungrafted, 3309C, and Riparia Gloire grape root types.

particular, Berlanas et al. (2019), who found a strong association between the fungi present and rootstock genotype ($r^2 = 0.86$, $P < 0.001$), showed that this association was greater in older, more-established vines than in younger ones. The young age of the vines in the current study (approximately 5 years) could explain the low degree of explanatory power the rootstock had in its effect on the microbiome. Finally, it should be noted that the differences in bulk root mass as well as soil parameters (Supplementary Table S2) between grape root types may be a factor in the differences found in the microbiome. Although the own-rooted vines had the largest root mass, soil testing showed that these vines also had the highest organic matter and CEC levels, as well as the highest levels of key macronutrients such as P and K as well. However, it is also possible that the microbiome associated with a more extensive root system, and that of a more vegetative plant with higher levels of nutrient cycling, as in the case of the own-rooted vines, may improve the nutrient status of the soil.

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