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Research Article

RESEARCH ARTICLE

Endophytic microbial assemblage in grapevine

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One sentence summary: This study investigates the origin and assembly of the microbial endophytes inhabiting grapevine, and highlights bacteria and fungi that may play a role in the host environmental fitness.

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ABSTRACT

The plant vascular system has remained an underexplored niche despite its potential for hosting beneficial microbes. The aim of this work was to determine the origin of the microbial endophytes inhabiting grapevine. We focused on a single commercial vineyard in California over a two-year period and used an amplicon metagenomics approach to profile the bacterial (16S–V4) and fungal (ITS) communities of the microbiome across a continuum of six grapevine compartments: bulk soil, rhizosphere, root, cordon, cane and sap. Our data supported that roots are a bottleneck to microbial richness and that they are mostly colonized with soilborne microbes, including plant growth-promoting bacteria recruited by the host, but also saprophytic and pathogenic fungal invaders. A core group of taxa was identified throughout the vine; however, there was clear partitioning of the microbiome with niche adaptation of distinct taxonomic groups. Above- and belowground plant tissues displayed distinct microbial fingerprints and were intermixed in a limited capacity mostly by way of the plant sap. We discuss how cultural practices and human contact may shape the endosphere microbiome and identify potential channels for transmission of its residents.

Keywords: grapevine; endosphere; rhizosphere; microbiome; endophyte; *Vitis*

INTRODUCTION

Characterizing healthy microbiomes for crop production systems has become the new frontier in plant science with the hope to identify beneficial microorganisms that could be further marketed into novel agricultural bioproducts. To date, the field of plant microbiome research has focused on the soil/rhizosphere/root interface (Bulgarelli *et al.* 2012; Edwards *et al.* 2015; Fitzpatrick *et al.* 2018a). One major reason is the fundamental role that roots play in nutrition and its analogy to the human gut. Plants must actively and selectively recruit organisms from their surrounding environment through chemical signaling. They provide food substrate in the form of photosynthetically fixed carbon through rhizodeposits in exchange for increased nutrients assimilation and improved tolerance against abiotic and biotic stresses (Compant, Clement and Sessitsch 2010; Bulgarelli *et al.* 2013). Bulgarelli *et al.* (2013) described the root microbiota assembly as a dynamic two-step selection process that involves an initial acquisition of microbes from the soil to the rhizosphere and a sorting step that involves a host-driven mechanism of subsetting specific microbes into the root. As a result, microbial diversity is reduced spatially along the soil–endorhiza continuum and microbial profiles are markedly differentiated between rhizocompartments (Bulgarelli *et al.* 2012; Edwards *et al.* 2015; Fitzpatrick *et al.* 2018a). Endophytic microbes are required to be highly specialized organisms that can migrate to the root zone, attach to the rhizoplane, move past the endodermis and pericycle, and colonize the central cylinder (Compant, Clement and Sessitsch 2010). In addition, they must be fitted to survive under the internal conditions of the plant roots that are vastly different from the external conditions of the rhizosphere. Subsequently, some root endophytic microbes can move systemically and colonize other plant organs, using the vascular system as a freeway (Compant, Clement and Sessitsch 2010; Deyett and Rolshausen 2019).

It has been hypothesized that the plant endosphere is mainly composed of rhizospheric organisms that systemically colonize the host. However, most of the research has focused on

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either the below- or aboveground compartments of the plant, but few studies have adopted a wholistic approach looking at the continuum between the two niches (Cregger *et al.* 2018). The plant vascular system plays a key role of connecting all the plant organs and internally transport water, nutrients and signals both acropetally and basipetally across biocompartments. Characterizing plant endophytic microbes has gained traction because they are more likely to have bioactive functions including plant growth-promoting capabilities and protection against phytopathogens (Lugtenberg, Caradus and Johnson 2016; Santoyo *et al.* 2016; Ek-Ramos *et al.* 2019). This enticing research axis has been actively pursued in plant model systems and annual crops, but it has been limited in perennial cropping systems due in part to the lignified nature of the host's vascular system.

Grapevine (*Vitis*) is an excellent plant model system to study the microbiome of woody perennial crops. It is one of the most cultivated fruit plants in the world. The International Organization of Wine and Vine estimated the wine growing surface area at ∼7.5 million hectares in 2016 (http://www.oiv.int/en/statistiques/). Metagenomics tools have been actively used to map the microbiome of the rhizocompartments, phyllosphere, carposphere and anthosphere of grapevine. Studies have clearly established that microbial communities are an integrated part of the identity of a viticulture region that defines the terroir and showed that it is influenced by several factors, including the host genotype and viticulture practices as well as climatic and edaphic factors (Bokulich *et al.* 2014; Zarraonaindia *et al.* 2015; Marasco *et al.* 2018; Berlanas *et al.* 2019; Coller *et al.* 2019; Gupta *et al.* 2019). The endophytic microbes of the grapevine vascular system have been mostly studied in the context of diseases, including crown gall (Faist *et al.* 2016), Pierce's disease (Deyett *et al.* 2017) and esca (Del Frari *et al.* 2019a). Deyett and Rolshausen (2019) showed that the sap microbiome is a dynamic entity that responds to environmental cues (i.e. host phenology or disease condition). However, questions about the origin of microbial endophytes have remained unanswered.

The goal of this study was to address this gap in the knowledge with regard to the origin of endophytic microbes residing in the plant vascular system. We provide a microbial map of six distinct compartments (bulk soil, rhizosphere, root endosphere, cordon, cane and sap) of vines in a single commercial vineyard over a two-year period to determine the proportion of rhizospheric microbes capable of colonizing the host vasculature. This study enables a better understanding of the assemblage of microbes in the grapevine endosphere and dynamics along the below- and aboveground continuum, and identifies potential channels for transmission of microbes.

MATERIALS AND METHODS

Plant sampling and processing

The experiment was conducted in a cordon-pruned conventional commercial vineyard cv. 'Syrah' on 1103P rootstock in Temecula, California. The vineyard was planted in 2010 and all samples were collected after harvest (Fall) in 2017 and 2018. Cane, cordon, root and rhizosphere samples were collected from 30 grapevines in the vineyard. Plant sap was extracted from a subset of 10 of the 30 vines. Five soil samples were also collected in 2018 from the four corners and the middle of the vineyard. Cordon pieces (10 cm in length) were removed from the vine with two-hand pruners. Root samples were dug out with a shovel at a 10–30 cm soil depth. Four canes (two from each side of the vine's cordon) were mechanically removed from the vine using pruners. Samples were placed in a chilled cooler on ice and transported back to the laboratory.

All samples were processed within 24 h as follows. Sap was extracted from two canes of 10 vines using a Scholander pressure bomb as previously described (Deyett and Rolshausen 2019). For all 30 vines, two canes were selected and petioles and leaves were stripped away. One internode per cane was cut off (two total per vine) and bark peeled off using a sterile scalpel. The internodes were surface sterilized by flaming, cut into smaller pieces (∼5 mm^3) and stored in a 15-mL conical tube at −70°C for further processing. Root and rhizosphere samples were processed as described by Lundberg *et al.* (2012). Briefly, roots were placed in sterile 50-mL conical tube with 25-mL of PBS with 200-μL L^{-1} Silwet® L-77 surfactant. Samples were vortexed for 15 s. Roots were then transferred to a clean 50-mL conical tube with 25 mL of PBS. The first tube was centrifuged at 3200 g for 15 min and the aqueous layer was removed. The pellet was retained as the rhizosphere fraction. The roots continued to be vortexed and were moved to a clean PBS tube until PBS remained clear after vortexing. Roots were then sonicated using a Branson Sonifier 450 at a low frequency for 5 min (five 30 s bursts followed by 30 s breaks). Roots were then stored at −70°C for further processing. Cane, cordon and roots were then lyophilized in the FreeZone 2.5-L benchtop freeze dry system (Labconco, Kansas City, USA) for 48–72 h. Samples were then ground to a powder using the MM300 grinder (Retsch, Haan, Germany) in a 35-mL stainless steel grinding jar with 20-mm stainless steel balls at 25 oscillations per second in 45-s increments until sample was fully pulverized.

Microbiome library preparation

DNA was extracted from all samples using the ZymoBIOMICS DNA miniprep kit per manufacturer's protocol, using 250 μL of sap, 100 mg of dried tissue or 250 mg of wet rhizosphere (Zymo Research, Irvine, USA). DNA was assessed for quality and quantity using the Synergy HTX multi-mode reader (Biotek Instruments, Winooski, USA). Both bacterial 16S–V4 and fungal ITS rRNA regions were amplified from all samples using the Earth Microbiome protocol and primers (http://www.earthmicrobiome.org/). Briefly, primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) were used for bacterial microbiomes and ITS1f (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) for fungal ITS amplification (Caporaso *et al.* 2010). PCR reactions of 25 μL were performed in triplicate and pooled for each sample using 10 μL of Phusion hot start flex 2× master mix, 0.5 μL of each primer (10 μm) and 2 μL of DNA. In bacterial woody tissue, universal pPNA and mPNA clamps were added at a starting concentration of 1.25 μL (25 μm). These clamps were designed to reduce the amplification of host chloroplasts and mitochondria while having no effect on bacterial amplification (Fitzpatrick *et al.* 2018b). Every PCR was also accompanied by a negative control to ensure barcodes and master mix were not contaminated. Successful amplification was verified on a 1% agarose gel and DNA quantification was checked using the Synergy HTX multi-mode reader (Bioteck Instruments). Equal quantities of each sample in a library were combined into an Eppendorf tube and cleaned using the AMPure XP PCR purification system (Beckman Coulter, Brea, USA) per manufacturer's protocol. Final concentration of libraries were determined using both qPCR and bioanalyzer before being sequenced on the MiSeq instrument (Illumina, San Diego, USA) using 600-cycle run (2 × 300 paired end) for fungal reads and 500-cycle run (2 × 250 paired end) for bacterial

microbiome at the UC Riverside Genomics Core facility. Fungal and bacterial sequences were deposited in NCBI under the accession number PRJNA610418.

Computational analysis

Trimmomatic (Bolger, Lohse and Usadel 2014) was used as an initial quality filtering with a sliding window 5:20. Primers and PhiX reads were removed from sequences and demultiplexed using QIIME v 1.9.1 (Caporaso *et al.* 2010). Most processing for the reads were done in DADA2 v 1.14.0 (Callahan *et al.* 2016) including further quality control (no ambiguous base calling, no more than two errors), dereplicating, sample inference using learned error rate algorithm, merging of paired reads, removal of chimeric sequences and construction of sequence tables. Taxonomy identification was assigned using the SILVA SSU r132 reference database for bacterial taxa and Unite database v 10.10.2017 for fungal taxa. With DADA2's learn error rate algorithm, authors claim sequences as ASVs (amplicon sequence variants) and has the capacity to resolve sequences difference to a single nucleotide, allowing for more robust identification and capability of identifying taxa, in some cases to the species level (Callahan *et al.* 2016).

Phyloseq v 1.30.3 (McMurdie and Holmes 2013) was used for much of the graphical and statistical analyses of the data. Unidentified microbes at the kingdom or phylum level, or microbes that occurred less than three times were removed from the full dataset. The bacterial dataset totaled 126 samples (21 cane, 23 cordon, 33 rhizosphere, 31 root, 13 sap and 5 soil samples) and the fungal dataset totaled 119 samples (24 cane, 26 cordon, 27 rhizosphere, 24 root, 13 sap and 5 soil samples) after filtering out poor quality reads, chloroplast (1% of ASVs), mitochondria (13% of ASVs), taxa with unidentified phyla (bacteria and fungi represented 0 and 9% of ASVs, respectively) and samples with fewer than 500 ASVs. After removal of singletons and doubletons (bacteria and fungi represented 25 and 40% of ASVs, respectively), the total ASVs were of 18 305 (soil = 3050; rhizosphere = 12 825; root = 3320; cordon = 2900; cane = 1908; sap = 995) and 3991 (soil = 432; rhizosphere = 2924; root = 830; cordon = 290; cane = 198; sap = 277) for the bacterial and fungal datasets, respectively.

Shannon diversity index was used as a metric of taxa diversity within the communities. Kruskall–Wallis and pairwise Wilcoxon tests were run to verify statistical differences among groups. Phylum pie charts were constructed by aggregating taxa at the Phylum level and samples by tissue compartments and transforming to relative abundance. Phyla occurring at <1% were removed before creating pie charts for clarity purpose. Bray–Curtis dissimilarity was used to calculate the compositional similarities between samples and was visualized with PCoA (Principal Coordinates Analysis) plots using the Vegan package v 2.5-6. To determine statistical significance of beta diversity, Adonis tests were run. Venn diagrams were created using UpSetR v 1.4.0 by transforming to relative abundance and filtering taxa to those that occur greater than 0.1% and are prevalent in at least two samples of that tissue type. For prevalent Venn diagrams, data was aggregated by genus and transformed to relative abundance. Taxa were denoted as prevalent in each biocompartment if they occurred in at least 50% of the samples of that biocompartment. Taxa that occurred <1% in the dataset were removed before visualizing Venn diagrams using UpSetR v 1.4.0. Graphs were generated using VennDiagram v 1.6.20. For concentric pie charts representing core microbiome, data was aggregated to the ASV or genus level and transformed to relative abundance. ASVs/genera were filtered based on core microbiome as previously defined. To find microbes associated with a biocompartment and above- and belowground sections, DeSeq2 v 1.26.0 was utilized and visualized using ComplexHeatmap v 2.2.0. Taxa were filtered by P value and log2 fold change, keeping only taxa with $P < 0.01$ and having a log2 fold change >5 or <−5. Taxa were also filtered based on relative abundance >0.1%. Heat maps represent the relative abundance of the data and the log2 fold change as determined through DeSeq2.

RESULTS

Shannon diversity index indicated that the plant bacteriome was overall more diverse than the plant mycobiome (Fig. 1). The

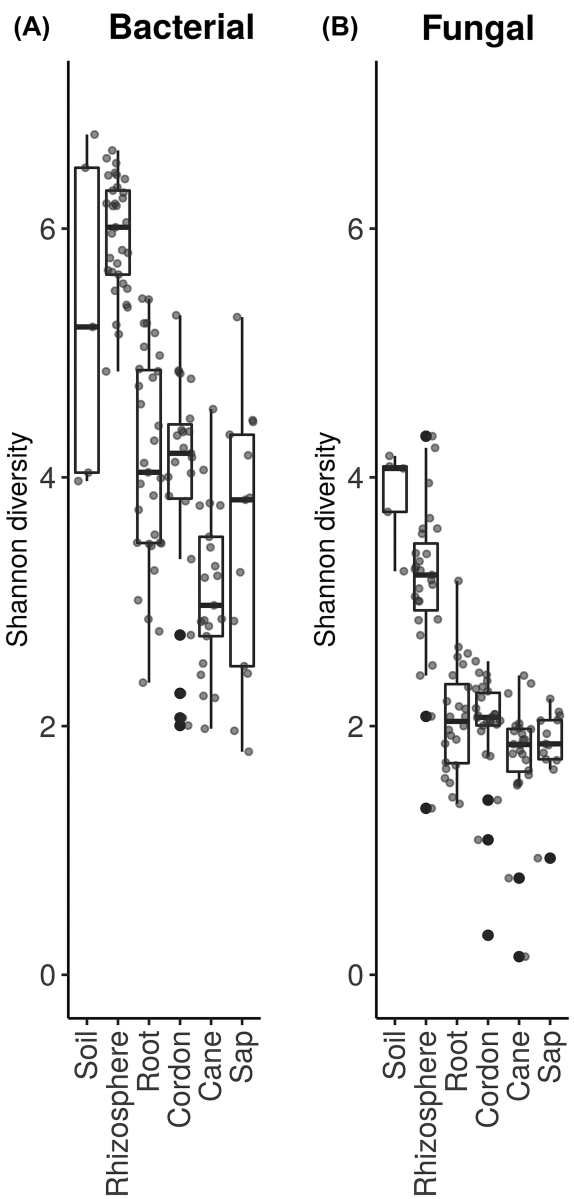

Figure 1. Shannon alpha-diversity plots for **(A)** bacteria and **(B)** fungi within six different grapevine biocompartments (soil, rhizosphere, root, cordon, cane and sap).

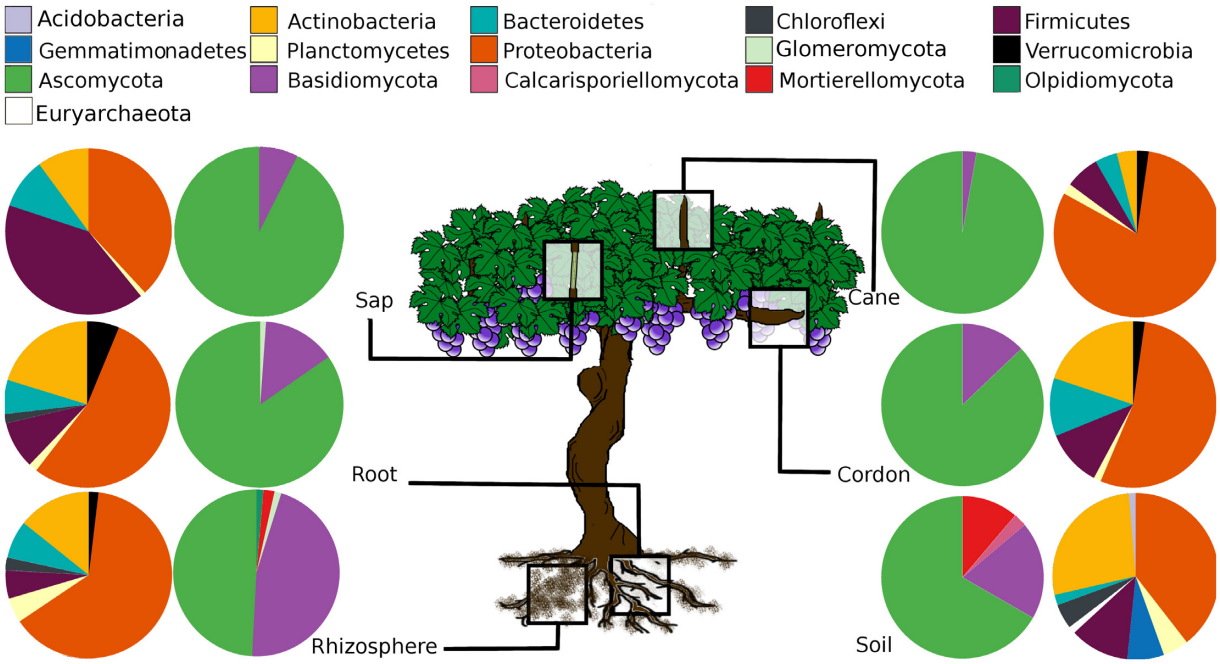

Figure 2. Relative abundance of bacterial and fungal phyla within individual grapevine biocompartment (soil, rhizosphere, root, cordon, cane and sap). Only phyla occurring at ≥1% relative abundance are displayed.

soil mycobiome was significantly richer than all other biocompartments, including rhizosphere ($P < 0.05$ [pairwise Wilcox]), whereas the soil bacteriome was only significantly different from the cane ($P < 0.01$ [pairwise Wilcox]). In contrast, the rhizosphere bacteriome displayed significant higher Shannon diversity index as compared to all endophytic tissues ($P < 0.0001$ [pairwise Wilcox]). Within the plant endosphere, Shannon index was significantly higher in the root vs the cane for the bacterial dataset ($P < 0.001$ [pairwise Wilcox]) and in the cordon vs the cane for both datasets ($P < 0.01$ [pairwise Wilcox]). Of all the biocompartments, the cane microbiome had the lowest average Shannon diversity index although not significantly different from the sap.

Proteobacteria and *Ascomycota* were the most abundant phyla within the entire dataset representing on average 54.2 and 79.3% of all taxa, respectively (Fig. 2). Phyla *Basidiomycota*, *Actinobacteria* and *Firmicutes* were also important phyla as they occurred in greater than 10% on average across the entire datasets. *Proteobacteria* ranged from ~80% in relative abundance of the cane bacteriome to <40% in the sap and soil. It was the most abundant phylum in all tissue types except for the sap (37%), which harbored a higher relative abundance of *Firmicutes* (39.7%). Likewise, *Ascomycota* ranged from over 80% in relative abundance of the endosphere mycobiome to below 50% in the rhizosphere. In contrast, several phyla with abundance above 1% were limited to a few compartments. Hence, *Mortierellomycota*, *Calcarisporiellomycota*, *Olpidiomycota*, *Gemmatimonadetes* and *Acidobacteria* were mainly found in the rhizosphere and soil.

Bray–Curtis beta-diversity metrics with PCoA were used to visualize how biocompartments impacted fungal and bacterial community composition (Fig. 3). Our data showed distinct clustering between above- (cane and cordon) and belowground (root, soil and rhizosphere) microbiomes with a significant segregation of the two habitats, while plant sap microbiome clustered in between the two habitats ($P < 0.01$ [Adonis]). Year also had a significant effect ($P < 0.01$ [Adonis]) in the clustering pattern, most noticeably in the bacterial cane and cordon datasets.

We used Venn diagrams with a filtering of the bacterial and fungal datasets to determine the proportion and name of the most common and abundant ASVs shared amongst compartments (Fig. 4). The filtering consisted of ASVs present in two or more samples and with a relative abundance >0.1% within each biocompartment (not across the entire dataset). The majority of all belowground ASVs (root, rhizosphere, soil) were found in the soil/rhizosphere (342 ASVs = 68%), and 21% (102 ASVs) of the rhizosphere ASVs were able to colonize the root endosphere, whereas 11% (57 ASVs) of the remaining root ASVs were not soilborne (Fig. 4A). When looking at the root microbiome, 64% (102 of 159 ASVs) of the filtered endophytic ASVs were of soil/rhizosphere origin (Fig. 4A). However, when looking at the totality of the plant microbiome, 28% (102 of 364 ASVs) of the filtered endophytic ASVs were of soil/rhizosphere origin (Fig. 4B). In addition, we found that only 4% (14 ASVs) of the filtered endophytic ASVs was shared among all biocompartments belonging to ten genera with two fungal (*Cladosporium* and *Mycosphaerella*) and eight bacterial (*Devosia, Rhizobium, Bacillus, Novosphingobium, Steroidobacter, Bradyrhizobium, Pseudomonas* and *Streptomyces*) taxa (Supporting Information 1 and 2). In contrast, 55% (200 ASVs) of filtered endophytic ASVs were specific to each compartment (root, sap, cordon or cane). A prevalence Venn diagram with a 50% cutoff was also used to identify genera often associated with a specific tissue type as well as genera overlapping between biocompartments (Fig. 5). Eight genera emerged as dominant taxa as they inhabited all the biocompartments of the grapevine endosphere and included one fungal genus (*Cladosporium*) and seven bacterial genera (*Escherichia/Shigella, Novosphingobium, Pseudomonas, Rhizobium, Sphingomonas, Bacillus* and *Steroidobacter*). *Streptomyces* was also abundant taxa commonly found in all the vine lignified tissues (root, cordon and cane), and could also be found frequently in the sap (although

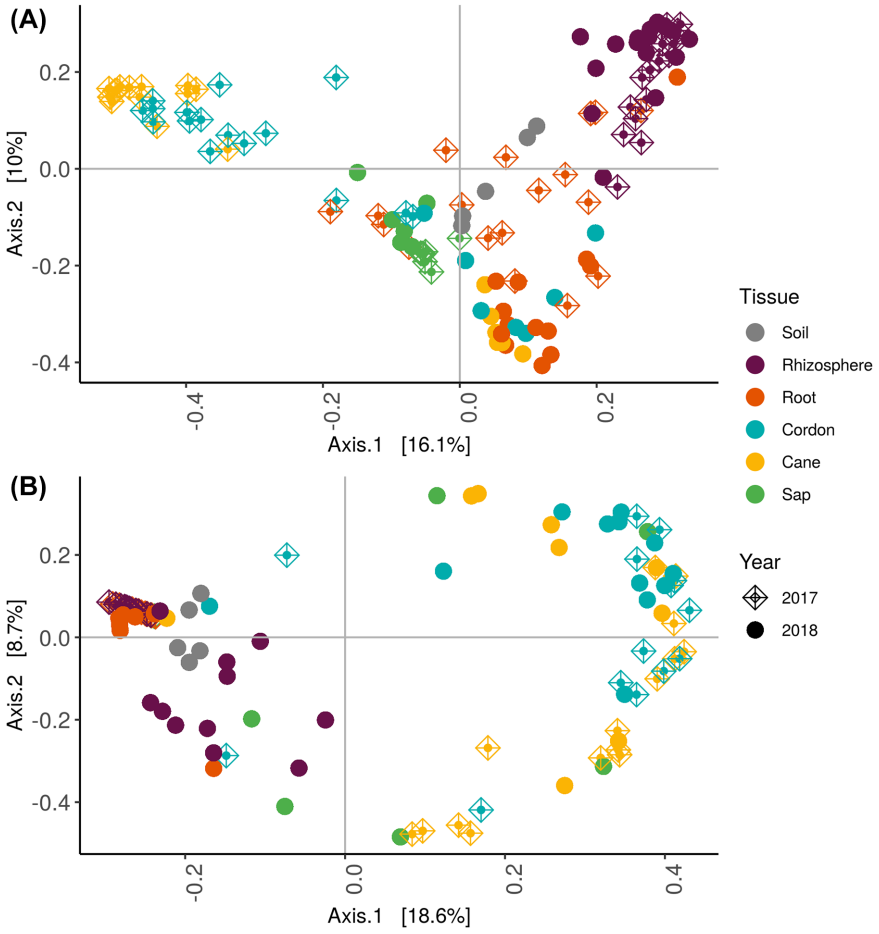

Figure 3. Bray–Curtis beta diversity for **(A)** bacteria and **(B)** fungi. Points represent individual sample communities for one biocompartment from one vine at one year. Points are colored by biocompartment and shaped by year collected.

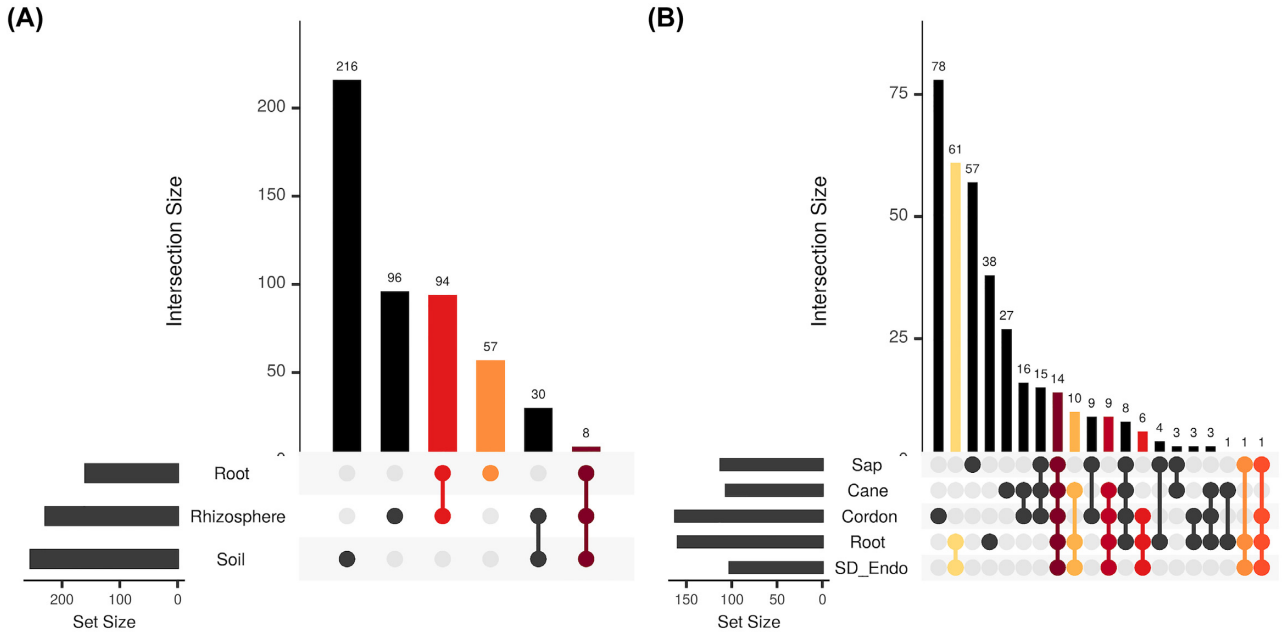

Figure 4. ASV Venn diagrams depicting overlapping taxa. **(A)** Belowground: soil, rhizosphere and root and **(B)** endosphere: cane, cordon, sap, root and soil-derived endosphere (SD_Endo). Taxa were filtered based on a sample prevalence >1 and relative abundance of >0.1% for each biocompartment.

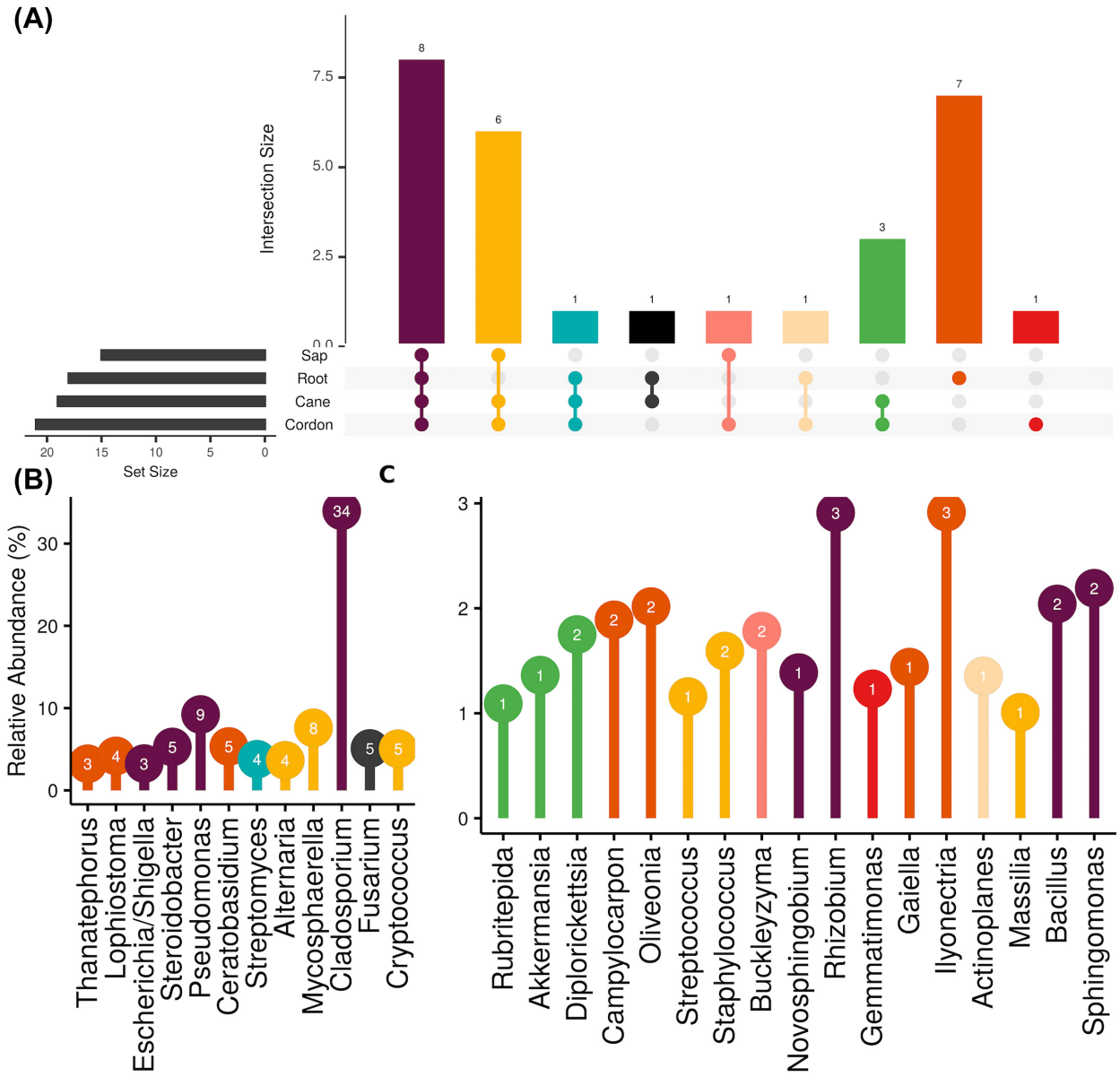

Figure 5. Prevalence Venn diagrams: genera that occur in ≥50% of all samples from each biocompartment. **(A)** Intersections of genera associated with endospheric biocompartment (sap, cordon, cane and root) combination. **(B)** High and **(C)** low relative abundant for the genera colored by intersections in (A).

in <50% of our samples). *Mycosphaerella* was also found in all of the aboveground tissues (sap, cordon, cane) and was also found frequently in the roots (although in <50% of our samples). *Devosia* and *Bradyrhizobium*, the remaining core microbes as defined by ASV Venn Diagrams, did not pass prevalence and abundance filters. Based on the combined results from the Venn diagrams (Figs 4 and 5), we identified two fungal (*Cladosporium* and *Mycosphaerella*) and six bacterial (*Rhizobium, Bacillus, Novosphingobium, Steroidobacter, Pseudomonas* and *Streptomyces*) taxa as members of the grapevine core microbiome. Our results also highlighted the shifts in microbial abundance for core taxa organisms across biocompartments between the two years of sampling (Supporting Information 3), supporting beta diversity metrics data (Fig. 3).

We used DeSeq2 analyses to indicate enrichment/rarefaction patterns of individual taxa across the different biocompartments (Fig. 6). Results clearly indicated above- and belowground microbial signature profiles. We measured an enrichment pattern for bacteria (*Rhizobium, Devosia, Novosphingobium, Steroidobacter* and *Streptomyces*), and fungi (*Campylocarpon, Ilyonectria, Ceratobasidium, Thanatephorus* and *Lophiostoma*) in the root zone. In contrast, core (*Cladosporium, Mycosphaerella* and *Pseudomonas*) and non-core (*Methylobacterium, Alternaria*) microbiome genera were differentially abundant in aboveground tissues, with specific enrichment of bacteria in the sap (*Staphylococcus, Streptococcus* and *Escherichia/Shigella*).

DISCUSSION

This study focused on a single commercial vineyard in California, and we spatially profiled the grapevine microbiome across a continuum of six biocompartments from the host ectospshere to its endosphere. Our data is in line with other grapevine studies that measured greater microbial diversity

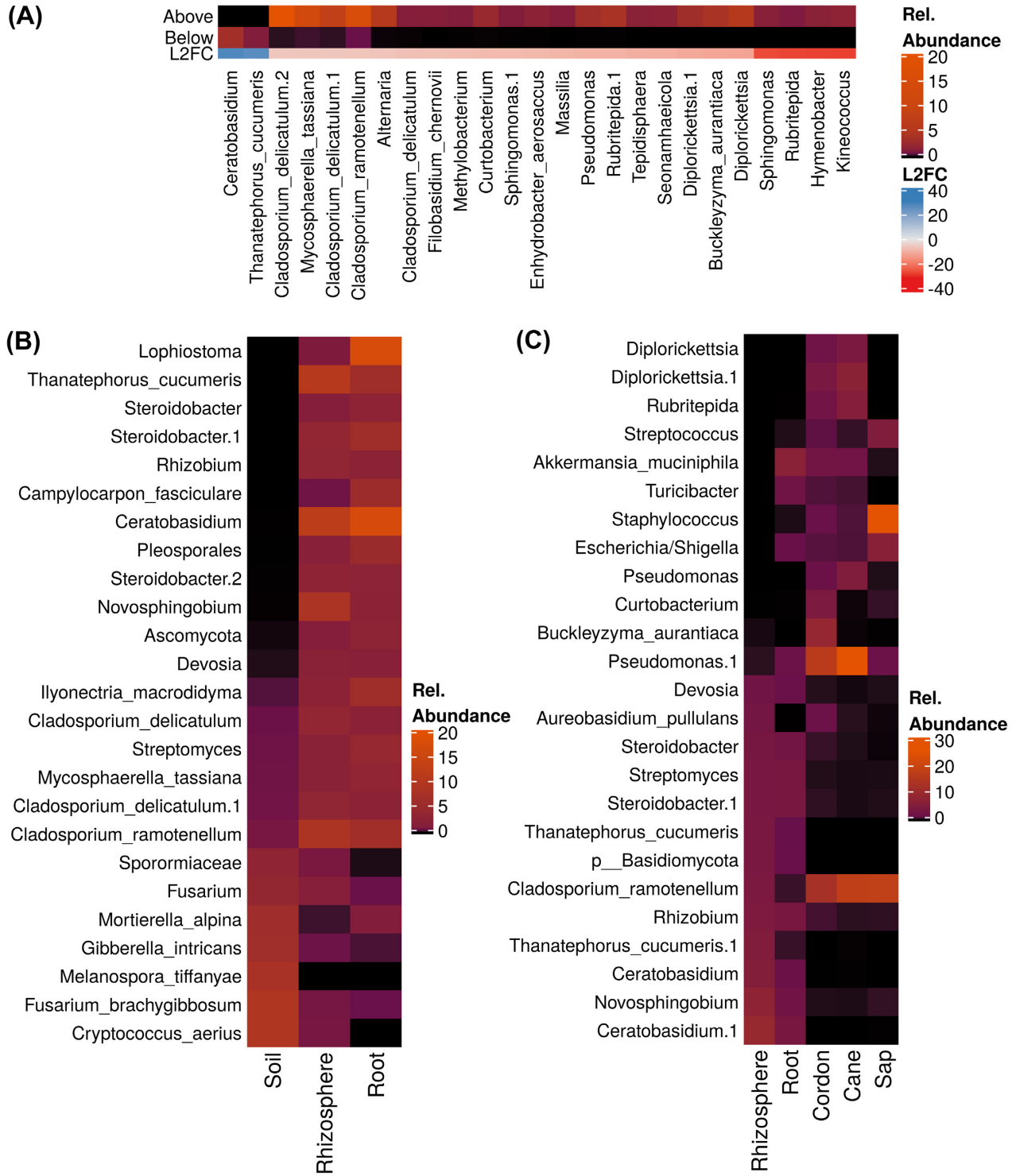

Figure 6. Relative abundance heat maps of significant taxa as determined through DESeq2 analyses. **(A)** Data is grouped by above- (cane, cordon) and belowground (root) habitats, and log2 fold change (L2FC) as determined through DESeq2 is displayed in a colorimetric bar. Tissue-specific DESeq2 results for **(B)** belowground compartments and **(C)** endosphere biocompartments. Data was filtered to a $P < 0.01$ cutoff and log2 fold change of >5 or <−5. The top 25 most abundant taxa are displayed. Black squares represent absence of taxa.

in below- vs aboveground (Zarraonaindia *et al.* 2015; Morrison-Whittle, Lee and Goddard 2017) and that bacterial richness was overall superior to fungal richness (Coller *et al.* 2019; Gupta *et al.* 2019) including inside the host vascular system. Fungal soil communities in vineyards display strong biogeographical patterns driven by dispersal limitations and are dominated by few generalist taxa capable of wind dissemination (Coller *et al.* 2019). It has also been proposed that farming practices, host species and diversity are selection drivers to fungal community composition (Verbruggen *et al.* 2014; Urbanova, Snajdr and

Baldrian 2015). Vineyards are monoculture cropping systems and the lack of host genotypic diversity across viticulture areas may be a bottleneck to microbial diversity. In addition, the cumulative inputs of synthetic and organic fungicides deployed for managing grapevine diseases have likely a negative impact on fungal biodiversity in the soil because of chemical residue runoffs but also inside the grapevine vasculature due to the systemic properties of some of those fungicides (Pancher *et al.* 2012; Morrison-Whittle, Lee and Goddard 2017; Del Frari *et al.* 2019b).

Our data supports Bulgarelli *et al.* (2013) hypothesis of a two-step selection model for root bacteria differentiation. Hence, the rhizosphere bacteriome profile was distinct from the soil profile with evidence of enrichment of specific bacteria (*Rhizobium, Devosia, Streptomyces*, *Pseudomonas*) with some members known for promoting plant growth. Zarraonaindia *et al.* (2015) also measured an enrichment of *Rhizobiales, Proteobacteria* and *Actinobacteria*, while Bona *et al.* (2019) showed that *Streptomyces, Pseudomonas* and *Rhizobia* were the most active bacteria involved in phosphorus and nitrogen metabolisms in the grapevine rhizosphere. In addition, we measured a noticeable enrichment of *Novosphingobium* and *Steroidobacter* in rhizocompartments. Some members in these taxonomic groups may play a role in bioremediation as they are known to metabolize aromatic compounds (Fahrbach *et al.* 2008; Gan *et al.* 2013). *Novosphingobium* was also found across viticulture areas and has been proposed to promote, by way of enhancing the quorum-sensing signal, the spread of pTi plasmid among *Agrobacterium/Allorhizobium* the causal agents of crown gall (Gan *et al.* 2019). We should point out that there was no sign of crown gall in our vineyard, but *Allorhizobium* was found in our dataset. In contrast, we measured a drop in fungal diversity from soil to rhizosphere combined with a colonization of root with some taxa reported to be pathogenic (*Ilyonectria, Campylocarpon* and *Thanatephorus/Rhizoctonia*) and saprophytic (*Ceratobasidium, Mycosphaerella* and *Lophiostoma*) suggesting that the acquisition model proposed by Bulgarelli *et al.* (2013) is not well supported with fungi, at least in a vineyard setting. Saprophytic fungi likely colonize the root zone in an opportunistic manner and decay products of the root exudates, while necrotrophic pathogens could also break-down root cell walls to gain access to the endorhiza. Martinez-Diz *et al.* (2019) made similar observations with both saprophytic and pathotrophic fungi colonizing the rhizocompartments of wine grapes in Spain. Surprisingly, we measured very low abundance of mycorrhizae (*Rhizophagus*, *Glomus*) in our dataset, that could be partly attributed to the ITS markers used for this analysis as the small subunit rRNA region is commonly preferred to characterize arbuscular mycorrhizal fungal communities (Lekberg *et al.* 2018). In agricultural tree and vine cropping systems, mycorrhizae play an essential role in supporting tree health by way of a mutualistic interaction with the host roots (Cheng and Baumgartner 2004; Mercado-Blanco *et al.* 2018). Those interactions are sensitive to soil management practices and the in-between rows mechanical weeding couples with the lack of cover cropping as implemented in our commercial vineyard are perhaps determinants for the low mycorrhizae incidence (Trouvelot *et al.* 2015; Mercado-Blanco *et al.* 2018).

As expected, we measured a significant decrease in both fungal and bacterial richness from the rhizosphere to the endorhiza, and 64% of the root endophytes were derived from the rhizosphere. The conditions encountered in those vastly different habitats select for a narrow group of microbes with plastic traits capable of adapting to environmental changes. It has been reported that culturable bacteria decrease from 10^7–10^9 CFU g^{-1} of soil in the rhizosphere to 10^5–10^7 CFU g^{-1} of fresh weight in the root (Compant, Clement and Sessitsch 2010), which is in line with data from culture-independent studies conducted for several cropping systems (Edwards *et al.* 2015; Cregger *et al.* 2018; Fitzpatrick *et al.* 2018a) including grapevine (Zarraonaindia *et al.* 2015; Marasco *et al.* 2018; Martinez-Diz *et al.* 2019). The vine endosphere collective microbiome was dominated by *Proteobacteria* and *Ascomycota*, but also displayed substantial abundance of *Basidiomycota*, *Actinobacteria*, *Bacteroides*, *Verrucomicrobia* and *Firmicutes* in the root that were in the range of previous reports (Zarraonaindia *et al.* 2015; Marasco *et al.* 2018; Martinez-Diz *et al.* 2019). A subgroup representing ~4% of the filtered ASVs residing in the vine endosphere and originating from the soil/rhizosphere was able to systemically colonize the grapevine vasculature and shape its core microbiome backbone including two fungal (*Cladosporium* and *Mycosphaerella*) and eight bacterial (*Streptomyces*, *Bacillus*, *Devosia*, *Novosphingobium, Pseudomonas, Rhizobium, Bradyrhizobium* and *Steroidobacter*) taxa. Most of these taxa have been identified on or in grapevine organs both above- and belowground, supporting the theory that some rhizospheric microbes may use the host vascular system as a transportation pathway (Compant, Clement and Sessitsch 2010; Compant *et al.* 2011; Zarraonaindia *et al.* 2015). We propose that only *Streptomyces*, *Bacillus*, *Pseudomonas, Novosphingobium* and *Rhizobium* should be viewed as keystone taxa due to their confirmed cosmopolitan distribution across viticulture areas combined with their high incidence in our dataset and across tissue habitats. In grapevine, some members of *Streptomyces*, *Bacillus*, *Pseudomonas* and *Rhizobium* are well described plant growth promoters and have been shown to maintain environmental fitness against biotic and abiotic stressors (Ait Barka *et al.* 2002; Compant *et al.* 2011; Baldan *et al.* 2015; Rolli *et al.* 2015; Andreolli *et al.* 2016; Jiao *et al.* 2016; Zhao *et al.* 2016; Alvarez-Perez *et al.* 2017; Deyett *et al.* 2017; Ma *et al.* 2017; Nigris *et al.* 2018), while some *Novosphingobium* species may also play a role in abiotic stress tolerance through bioremediation of aromatic compounds (Gan *et al.* 2013). The two nitrogen-fixing *Devosia* and *Bradyrhizobium* (Rivas *et al.* 2002; Bona *et al.* 2019), although present throughout the vine endosphere, were niche specific as they displayed higher prevalence in roots, and also were only detected in a small subset of samples and thus were not considered as part of the core microbiome. Moreover, the biological function of the two fungal members, *Cladosporium* and *Mycosphaerella*, is more ambiguous. Both have been found on the surface and inside grapevine (Zhang *et al.* 2017; Dissanayake *et al.* 2018; Singh *et al.* 2018; Deyett and Rolshausen 2019). Species of *Cladosporium* have also been reported as pathogenic of grape berries or act as a biocontrol agent on pruning wound surface against a causal agent of grapevine wood disease (Munkvold and Marois 1993; Briceno and Latorre 2008; Iasur-Kruh *et al.* 2015; Zhang *et al.* 2017), while Li *et al.* (2019) showed that *Cladosporium sphaerospermum* could promote plant growth. Additional research will need to determine if these taxa have a functional role in grapevine.

Our results also supported that within the host and across its different habitats there was clear partitioning of the microbiome with niche adaptation of distinct taxonomic groups (Zarraonaindia *et al.* 2015; Cregger *et al.* 2018). The microbiome of both below- and aboveground habitats intermixed in a limited capacity and mostly by way of plant sap flow. The sap microbial fingerprint was unique because it was colonized with yeasts (*Aureobasidium*, *Sporobolomyces*, *Cryptococcus/Filobasidium*) commonly found on grape berry surface (Barata, Malfeito-Ferreira and Loureiro 2012) and bacteria (*Staphylococcus, Escherichia/Shigella* and *Streptococcus*) commonly associated with human (Zoetendal *et al.* 2012; Lloyd-Price, Abu-Ali and Huttenhower 2016), and plant

(VanderZaag *et al.* 2010; Barata, Malfeito-Ferreira and Loureiro 2012; Yousaf *et al.* 2014; Zarraonaindia *et al.* 2015) systems. Campisano *et al.* (2014) provided evidence of horizontal interkingdom transfer of a human opportunistic pathogen (*Propionibacterium acnes*) to domesticated grapevine. Grapevine constantly interface with humans throughout its lifespan, during the propagation phase in nursery and the production phase within vineyard, which may be the source of cross inhabitation. Deploying metagenomics tools could further unfold the traceability and reveal the degree of host specificity of these bacterial populations. We found that only 28% of the total taxa residing in the host vascular system stem from the rhizosphere. In fact, the majority of the host endosphere microbiome likely originates either from aboveground introduction or from native microbes that were already present in grapevine at the time of planting. The communities residing in the aboveground compartments were predominantly colonized with *Pseudomonas* and *Cladosporium* and were structured around single ASVs capable of colonizing all the biocompartments and with a complex of strains/species adapted to a specific habitat. They both especially thrived in the young cane tissue and were previously detected as early as bloom (Deyett and Rolshausen 2019), suggesting that they may be pioneers of young and developing green organs. In addition to *Pseudomonas*, *Methylobacterium* and *Sphingomonas* were signature taxa of the aboveground vascular system as previously recognized, and some members could play a role in phytohormone production and defense against vascular pathogens occupying the same niche (Zarraonaindia *et al.* 2015; Lai *et al.* 2016; Asaf *et al.* 2017; Deyett *et al.* 2017). Interestingly, we also found that grapevine sap, cordon and cane were colonized by a wide range of bacteria found in grape berry and involved in malolactic fermentation (*Lactobacillus*), wine spoilage (*Gluconobacter*), or of unknown effect to wine making (*Acinetobacter, Enterobacter*), but could shape to some degree the identity of a wine region (Barata, Malfeito-Ferreira and Loureiro 2012; Belda *et al.* 2017).

The origin of microbes residing in the aboveground compartments of grapevine may originate from several horizontal transmission routes including wounds (Munkvold and Marois 1995), natural openings such as stomatas (Compant *et al.* 2011) and vector-assisted transmission (Lopez-Fernandez *et al.* 2017). For example, we found evidence of *Xylella*, the causal agent of Pierce's disease, in several of our samples likely because of introduction events from the glassy-winged sharpshooter, an important vector of the pathogen in southern California (Redak *et al.* 2004). Another alternative route for microbial introduction is prior to vineyard planting, during the propagation phase in nurseries. In perennial cropping systems, plants are not grown from seeds but are propagated from wood cuttings. Plant propagation practices are known to spread many fungal vascular pathogens (Gimenez-Jaime *et al.* 2006) and one can suspect that it also spreads other microbial endophytes (Waite *et al.* 2013). We also anticipate that a fraction of the host microbiome is inherited from the mother vine in nursery and passed on to commercial plants. Further experiments will help determine the fraction of the native communities that persist in the vine endosphere following planting and characterize which microbes are introduced during the propagation phase at the nursery and the production phase in the vineyard.

This study provides insightful information about the origin of the microbes in the vine endosphere and a perspective with regard to potential inheritance from mother vines in nurseries and horizontal acquisition during the propagation and production phases of the host's life. The profiling of microbial communities highlights target organisms with plant growth-promoting capabilities. Whole genome metagenomics will aid in refining the population structure for some of the key organisms identified here and address the missing gaps about their source and habitat range. Metaproteomics and metabolomics will also aid in moving the scientific field from a descriptive phase to assigning functions to members of the microbiota. This fundamental knowledge may fuel the engineering of novel technological bioproducts with commercial applications or help with the implementation of cultural practices that support presence and abundance of key beneficial microbes associated with the host plant.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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