ORIGINAL RESEARCH ARTICLE

The transcriptionally active bacterial communities of grapevine rhizosphere in dependence on rootstock and scion variety

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ABSTRACT

The rhizosphere is where crucial processes for the productivity of viticultural systems occur. The composition of the bacterial communities associated with the rhizosphere of grapevines is known to depend on plant genotype. However, the genotype of grafted grapevines differs between scion and rootstock; the role of each genotype is unclear. To untangle the effect of scion and rootstock, the rRNA (V4–V5 region of 16S rRNA) extracted from the rhizosphere of the grape varieties Riesling and Mueller-Thurgau ungrafted vs grafted on different rootstocks was sequenced. The bioinformatic analysis with tools designed to be robust for compositional data showed that the investigated rootstocks or scions or combinations, respectively, recruited bacterial communities with distinguishable traits. Statistical differences were revealed between ungrafted Riesling vs Mueller-Thurgau, between grafted Riesling vs ungrafted Riesling, and between ungrafted Mueller-Thurgau vs grafted Mueller-Thurgau. Thus, confirming the role of scion and rootstock genotype as a driver of the structure and composition of bacterial communities in the rhizosphere of grapevines.

INTRODUCTION

Plants are colonised below and above ground by a variety of microbes that serve their mutualistic benefits. The rhizosphere microbiome is often described as a positive interaction between plants and microorganisms (Ryan *et al.*, 2009; Taye *et al.*, 2019). In particular, the microbiota associated with the plant rhizosphere is involved in important processes such as growth modulation, defence responses, and nutrient uptake (Berendsen *et al.*, 2012; Durán *et al.*, 2018; Hu *et al.*, 2018; Li *et al.*, 2020; Schlaeppi and Bulgarelli, 2015; Zhang *et al.*, 2019). To date, the majority of rhizobiome studies has focused on model plants such as *Arabidopsis thaliana* (Alegria Terrazas *et al.*, 2016) or annual crop plants such as barley (Bulgarelli *et al.*, 2013), canola, wheat, pea, and lentil (Cordero *et al.*, 2020), oilseed rape (Etesami and Alikhani, 2016) and maize (Peiffer *et al.*, 2013). However, it is also remarkably important to consider the rhizosphere microorganisms of perennial plants, such as grapevines (Marasco *et al.*, 2018), for supporting crop growth, especially under difficult conditions (Timmusk *et al.*, 2014).

Microbial communities associated with grapevines and wine, respectively, have already been extensively studied (Burns *et al.*, 2016; Hendgen *et al.*, 2018; Holland *et al.*, 2014). In vineyard soil, the microbial communities have been described, for example, as a function of spatial distribution or management practices (Bokulich *et al.*, 2014; Hendgen *et al.*, 2018; Holland *et al.*, 2014; Vega-Avila *et al.*, 2015). Most of these studies were based on soil samples taken near the grapevines, not on veritable rhizosphere samples. Nonetheless, the rhizosphere is the location where crucial processes for the productivity of agricultural systems take place, mediated by microorganisms. Therefore, the processes and microorganisms in the rhizosphere need to be considered to study the direct interaction between the grapevine root and the surrounding soil.

The choice of grapevine rootstock variety impacts the microorganisms in the grapevine rhizosphere (Berlanas *et al.*, 2019; Marasco *et al.*, 2018). Since the 19th century, *Vitis vinifera* cultivars have been grown as scions grafted onto *Phylloxera*-tolerant *Vitis* sp. rootstocks. However, understanding the mechanisms underlying grafted vines and the interactions between scion and rootstock is still beginning (as reviewed by Gautier *et al.*, 2019). For future research, the selection of the rootstock is, thus, inevitable, also with regard to its microbial community (Zarraonaindia *et al.*, 2015). Vink *et al.* (2021) investigated differences in the microbial communities in the rhizosphere of an 11-year-old grapevine of four scion cultivars and four rootstock types regarding alpha and beta diversity indices, concluding that bacterial diversity is affected by both scion and rootstock variety. However, this effect depends on the diversity measures and the specific rootstock-scion combinations considered (Vink *et al.*, 2021). Further studies on this specific topic are needed (Berlanas *et al.*, 2019). Therefore, a study was conducted under controlled conditions to reduce the variability resulting from all factors except the genotype of the rootstock and scion with potted grapevines. In three different experimental designs, the rhizobiome of two ungrafted *Vitis vinifera* varieties (Riesling and Mueller-Thurgau) were compared with varieties of Riesling and Mueller-Thurgau grapevines grafted on different rootstocks. A metabarcode analysis was performed on the extracted RNA (Carvalhais *et al.*, 2012; Garoutte *et al.*, 2016; Turner *et al.*, 2013) to get a better insight into the active microbiome of the grapevine rhizosphere. To the best of our knowledge, no compositional data analyses of 16S rRNA data of the grapevine rhizosphere microbiome with different scions and rootstock cultivars have been carried out before. Data derived from high-throughput sequencing of biological samples must be considered as compositions rather than counts, as ratio-based analyses can lead to qualitatively incorrect conclusions (Fernandes *et al.*, 2014; McLaren *et al.*, 2019; Quinn *et al.*, 2019). The effect of the grape variety and rootstock, respectively, on the bacterial communities in the rhizosphere could be investigated due to the controlled experimental conditions.

MATERIALS AND METHODS

2.1 Plant material

The independent experiments were located at Hochschule Geisenheim, University, Germany. The grapevines were planted in pots (15 cm × 15 cm × 18.5 cm) with soil (Einheitserde Typ ED 73, H. Nitsch & Sohn GmbH & Co. KG, Kreuztal, Germany) in 2019 and 2020, respectively (Table 1). The grapevines for time point May 2019 were grown in an open greenhouse under field conditions for three months. The grapevines for time points October 2019 and October 2020 were grown in an open greenhouse under field conditions for six months. They were watered whenever it was required. Additionally, the grapevines were managed according to Good Agricultural Practices. In May 2019, ungrafted Mueller-Thurgau und Riesling grapevines, as well as soil-filled pots without grapevines used as controls, were examined. In the same year, additional Riesling grapevines, grafted on four different rootstocks (*Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. SO4; *Vitis riparia* × *Vitis cinerea* Engelm. cv. Boerner; *Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. 125AA; and *Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. Teleki 8 B) were investigated. In 2020 ungrafted Mueller-Thurgau and Riesling grapevines, soil-filled pots without grapevines as control, Riesling grapevines with four different rootstocks (*Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. SO4; *Vitis riparia* × *Vitis cinerea* Engelm. cv. Boerner; *Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. 125AA; and *Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. Teleki 8 B) and Mueller-Thurgau with three different rootstocks (*Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. SO4; *Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. 125AA; and *Vitis berlandieri* Planch. × *Vitis riparia* Michx. cv. Kober 5 BB) were examined.

TABLE 1. Grape varieties and rootstocks were used for rhizosphere sampling at three different experimental time points. Number of plants sampled (n).

time point	grape variety (n)	rootstock
May 2019	Mueller-Thurgau (4)	ungrafted
	Riesling (4)	ungrafted
	No plant (4)	Control
October 2019	Riesling (5)	SO4
	Riesling (5)	125AA
	Riesling (5)	Boerner
	Riesling (5)	8B
October 2020	Riesling (9)	ungrafted
	Riesling (3)	SO4
	Riesling (3)	125AA
	Riesling (3)	Boerner
	Riesling (3)	8B
	Mueller-Thurgau (9)	ungrafted
	Mueller-Thurgau (3)	SO4
	Mueller-Thurgau (3)	125AA
	Mueller-Thurgau (3)	Kober 5BB
	No plant (9)	Control

2.2 Rhizosphere sampling

Rhizosphere sampling was performed at three different time points for the three experimental setups: May 2019, October 2019, and October 2020. A total of four Riesling, four Mueller-Thurgau, and four controls were sampled in May 2019. Five samples per grapevine rootstock were collected in October 2019. Additionally, nine controls, nine ungrafted Riesling and Mueller-Thurgau, and three samples per grapevine rootstock were collected in October 2020. Only soil attaching to the roots was considered as rhizosphere soil for sampling and microbiome analyses. For controls ("No plant"), bulk soil was sampled in the same horizons of the pot as where the roots of the vines grow in the pots with a plant.

2.3 RNA extraction, Reverse-Transcriptase-PCR, and Ion Torrent Sequencing

RNA extraction from the rhizosphere soil and cDNA amplification were done according to Rosado-Porto *et al.* (2021). First Ion Torrent PCR was performed with a KAPA HiFi Polymerase kit (VWR International GmbH, Darmstadt, Germany), amplifying the partial sequence of the hypervariable regions (V4 and V5) of the 16S rRNA gene with the primer 520F (5'-AYTGGGYDTAAAGNG-3', (Claesson *et al.*, 2009)) and 926R (5'-CCGYCAATTYMTTTRAGTTT-3', (Engelbrektson *et al.*, 2010)). Amplification parameters were 3 min at 95 °C followed by 35 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, and finally, 72 °C for 5 min. Second Ion Torrent PCR with primers, including barcodes and Ion Torrent sequencing adapters, was conducted with the

PCR product from the first Ion Torrent PCR, as suggested by Berry *et al.* (2011). The PCR was performed with the following amplification parameters: 3 min at 95 °C followed by 10 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, and finally, 72 °C for 7 min. The PCR products were applied to a 1 % agarose gel and subsequently purified using NucleoSpin® Gel and PCR Clean-up (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Further, this product was purified with DNA purification beads NucleoMagVR NGS clean-up kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The Ion Torrent Sequencing was done according to Kaplan *et al.* (2019).

2.4 Bioinformatic analysis of the sequencing data

Bioinformatic analysis was performed with QIIME 2 2020.11 (Bolyen *et al.*, 2019). The obtained raw sequences were demultiplexed using cutadapt (Martin, 2011) with no errors allowed in the barcode sequences. Quality control, sequence denoising, clustering to amplicon sequence variants (ASVs), dereplication, and removal of chimaera sequences were conducted with DADA2 (Callahan *et al.*, 2016) (via q2-dada2). Thereby, the first 15 base pairs (bp) were removed, and the sequences were cut to a length of 312 bp (May 2019), 317 bp (October 2019), and 312 bp (October 2020), respectively. Sequences that did not pass the quality control were removed. Therefore, two samples of 125AA (Timepoint October 2019) and one sample of SO4 (Timepoint October 2019) were not considered in the following analysis. Afterwards, the taxonomy was assigned as described previously by Dries *et al.* (2021a), and all ASVs belonging to chloroplasts and mitochondria were removed. All ASVs were aligned with

MAFFT (Katoh & Standley, 2013) (via q2-alignment) and used for constructing a phylogeny with fasttree2 (Price *et al.*, 2010) (via q2-phylogeny). The feature table was filtered for the 20 dominant bacterial families, and taxabarplots were created with GraphPad Prism version 9.3.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Alpha diversity metrics (Shannon, Simpson, and observed features) and significances were calculated with Kruskal-Wallis or PERMANOVA (Anderson, 2017), respectively. Beta diversity was measured using DEICODE (Martino *et al.*, 2019) with a filtered beta diversity ordination file. For this purpose, all feature counts below 10 and all sample counts below 500 were removed. Beta diversity for all time points was visualised within a compositional biplot, displaying the eight most important features. From the ASVs shown as arrows in the DEICODE graphs, a more accurate taxonomic affiliation was done with a pairwise alignment at the online database EzBioCloud (Yoon *et al.*, 2017). The distance matrices were also analysed by the PERMANOVA test, using 999 permutations. The significance of differential abundance was determined using ALDEx2 (Fernandes *et al.*, 2013). Therefore, all feature frequency counts below 10 and all sample counts below 2 were removed.

RESULTS

3.1. Bacterial alpha diversity across the different experimental set-ups

The 20 dominating bacterial families for the three-time points are shown in Figures 1 and 2. Control soil showed clear differences in the 20 dominating bacterial families compared to the ungrafted Mueller-Thurgau and Riesling (Figure 1A). In the rhizosphere of Riesling grapevine grafted onto four different rootstocks (SO4, 125AA, Boerner, 8B), the dominating bacterial families in the rhizosphere showed no clear differences of the 20 dominating families comparing the four rootstocks (Figure 1B). For time point October 2020 (Figure 2), some differences were found between Riesling and Mueller-Thurgau ungrafted compared to the grafted varieties and the control of the 20 dominating bacterial families.

For ungrafted grape varieties Mueller-Thurgau and Riesling, significant effects of the grape variety were found in alpha diversity indices Shannon and observed features (Table 2). No statistical differences were found regarding the alpha diversity Simpson index. However, while the Shannon–Wiener index is strongly influenced by species richness and

TABLE 2. Results of Kruskal-Wallis pairwise tests of alpha diversity indices per ungrafted grape variety or control, respectively, for time point May 2019.

Time point	grape variety	Shannon	Simpson	Observed features
	Mueller-Thurgau vs Riesling	0.03 *	1.00 ns	0.03 *
May 2019	Mueller-Thurgau vs Control	0.25 ns	1.00 ns	0.56 ns
	Riesling vs Control	0.03 *	1.00 ns	0.03 *

Significant differences (corrected p-value < 0.05) are indicated with *, and no differences are indicated with ns = not significant. For time points October 2019 and October 2020, no statistical differences were measured, thus, they are not listed in this table.

TABLE 3. Bacterial beta diversity results of PERMANOVA pairwise tests.

time point	grape variety	PERMANOVA
	Mueller-Thurgau vs Riesling	0.03 *
May 2019	Mueller-Thurgau vs Control	0.03 *
	Riesling vs Control	0.03 *
	Mueller-Thurgau ungrafted vs Mueller-Thurgau grafted	0.001 *
	Mueller-Thurgau ungrafted vs Riesling ungrafted	0.002 *
	Mueller-Thurgau ungrafted vs Riesling grafted	0.001 *
	Mueller-Thurgau ungrafted vs Control	0.001 *
	Mueller-Thurgau grafted vs Riesling ungrafted	0.001 *
October 2020	Mueller-Thurgau grafted vs Riesling grafted	0.21 ns
	Mueller-Thurgau grafted vs Control	0.001 *
	Riesling ungrafted vs Riesling grafted	0.001 *
	Riesling ungrafted vs Control	0.001 *
	Riesling grafted vs Control	0.001 *

Significant differences (corrected p-value < 0.05) are indicated with *; pairs with no statistical differences are not shown in this table. In October 2019, no significant differences were measured.
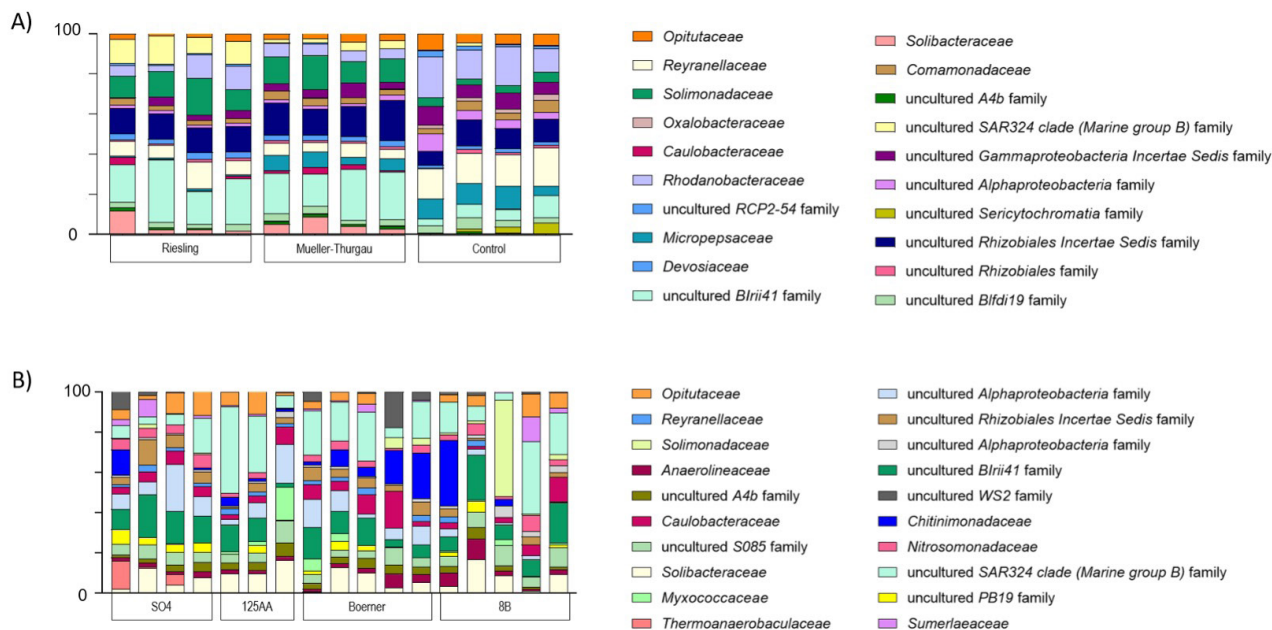
FIGURE 1. Relative abundance of different bacterial families (in %) in the rhizosphere of grapevine (time point May 2019 and October 2019). A) The 20 most dominating bacterial families in the rhizosphere of ungrafted Riesling, ungrafted Mueller-Thurgau, and a control (time point May 2019). B) The 20 most dominating bacterial families in the rhizosphere of the Riesling grapevine grafted onto four different rootstocks (SO4, 125AA, Boerner, 8B, time point October 2019).
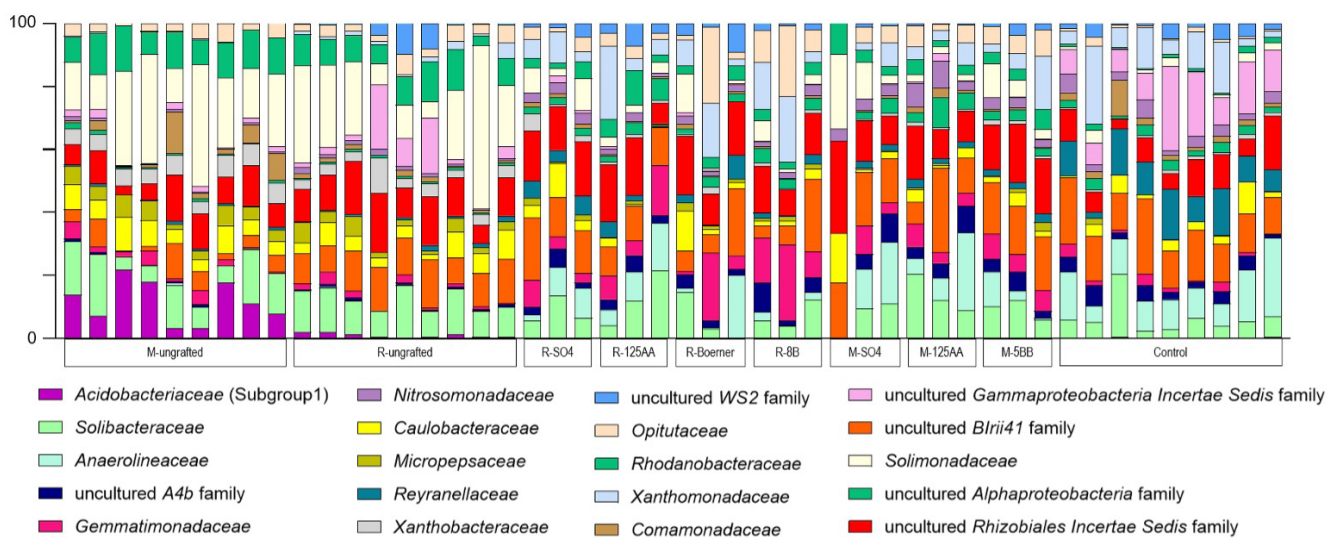

FIGURE 2. Relative abundance of different bacterial families (in %) in the rhizosphere of grapevine (time point October 2020). The 20 most dominating bacterial families in the rhizosphere of ungrafted Mueller-Thurgau, ungrafted Riesling, Riesling grafted onto different rootstocks (SO4, 125AA, Boerner, and 8B), Mueller-Thurgau grafted onto different rootstocks (SO4, 125AA, 5BB), and a control.

by rare species, the Simpson index gives more weight to evenness and common species. For the grape variety Riesling with four different rootstocks, examined in October 2019, and for time point October 2020, no statistical differences were found in the alpha diversity (Shannon, Simpson, and observed features).

3.2. Bacterial beta diversity across the different experimental set-ups

The compositional beta diversity metric was calculated using a robust Aitchison PCA via DEICODE. Aitchison distance is a Euclidean distance between samples after centre log ratio (clr) transformation. For all experimental set-ups and time points,
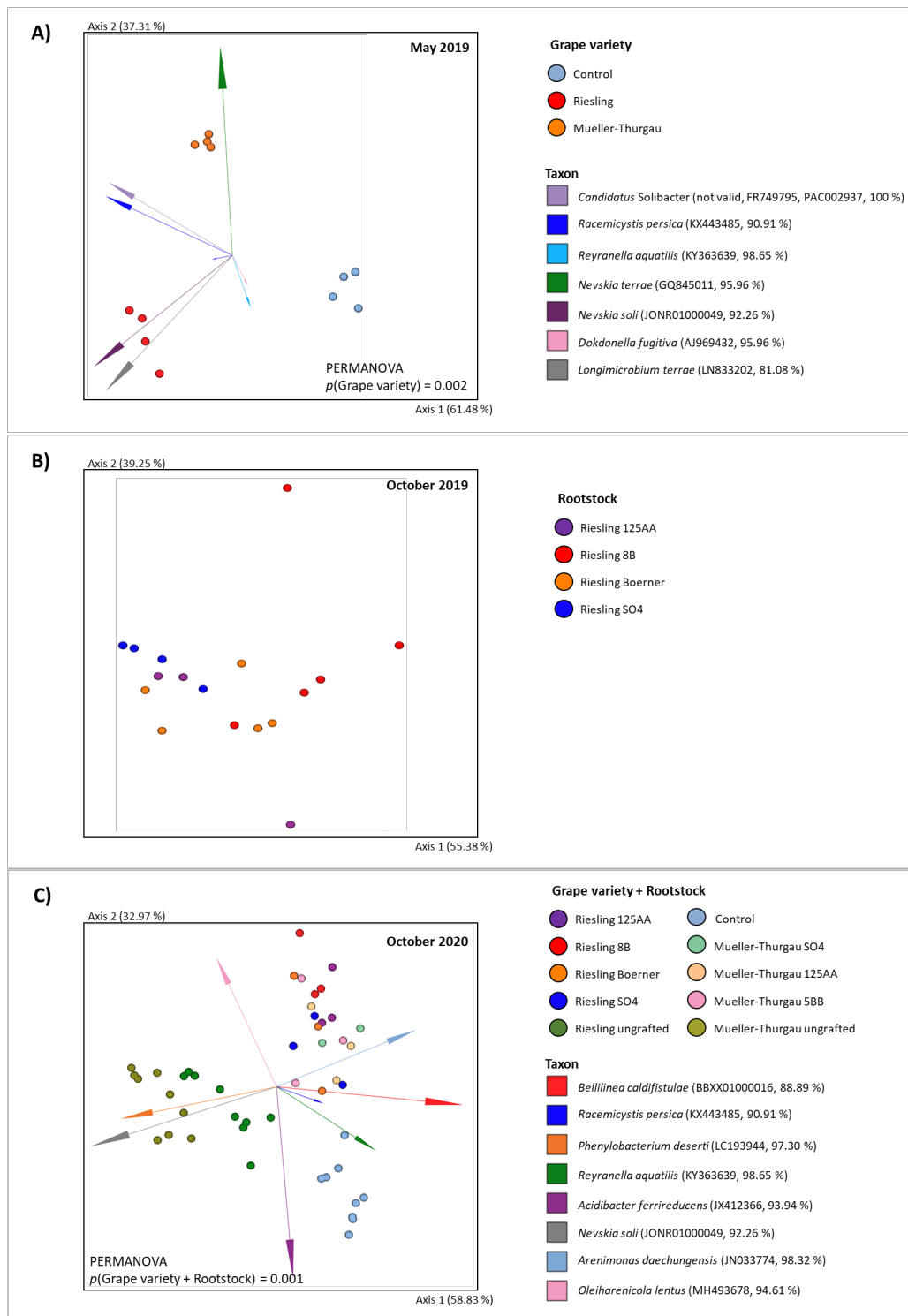
FIGURE 3. PCoA biplots are calculated based on a robust Aitchison community dissimilarity distance matrix with arrows illustrating the ASVs strongly influencing the principal component axis. Taxonomic affiliations of the arrows showed the next related sequences with accession numbers found by pairwise alignment at EzBioCloud Database. Numbers in the brackets show the percentage of identity of the ASV sequence with the next related sequence. A) Beta diversity of bacterial rhizosphere communities for ungrafted Riesling and ungrafted Mueller-Thurgau grape variety and a control (time point May 2019). Statistical differences were detected between all groups (PERMANOVA, $p = 0.002$). B) Beta diversity of bacterial rhizosphere communities for the Riesling grape variety with four different rootstocks (time point October 2019). No statistical differences were detected (PERMANOVA, $p > 0.05$). C) Beta diversity of bacterial rhizosphere communities for the Riesling grape variety ungrafted and with four different rootstocks, the Mueller-Thurgau grape variety ungrafted and with three different rootstocks, and a control (October 2020). Statistical differences were detected (PERMANOVA, $p = 0.001$).

significant differences in the beta diversity of the bacterial communities were detected (Figure 3). The determinant of the bacterial communities in the rhizosphere is the grape variety and rootstock, or grape variety and rootstock combination, respectively. PERMANOVA pairwise results reveal statistical differences (Table 2) for the time point May 2019 between Mueller-Thurgau and Riesling (p = 0.03), Mueller-Thurgau and control (p = 0.03), and Riesling and control (p = 0.03). No statistical significances for time point October 2019 were detected between the rootstocks (p > 0.05), so no arrows illustrating ASVs strongly influencing the principal component axis are visible. Moreover, statistical differences were detected for time point October 2020 between Mueller-Thurgau ungrafted vs Mueller-Thurgau grafted onto different rootstocks, Mueller-Thurgau ungrafted vs Riesling ungrafted, Mueller-Thurgau ungrafted vs Riesling grafted onto different rootstocks, and Mueller-Thurgau ungrafted vs the control (p < 0.05, Table 3). Statistical differences were also detected between Mueller-Thurgau grafted vs Riesling ungrafted, Mueller-Thurgau grafted vs the control, Riesling ungrafted vs Riesling grafted onto different rootstocks, Riesling ungrafted vs the control, and Riesling grafted vs the control (p < 0.5, Table 3). However, no statistical differences in beta diversity were detected between Mueller-Thurgau grafted and Riesling grafted (p = 0.21, Table 3).

DEICODE allows the display of a biplot by showing not only the Aitchison distances but also the taxa (in the form of an arrow) that most strongly influence principal component axes. The eight most important taxa influencing the principal component axes shown in each figure are members of the phyla *Verrucomicrobia*, *Proteobacteria*, *Acidobacteria*, and *Chloroflexi*. According to the taxonomic classification, *Verrucomicrobia*, *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* also form the main composition of the predominant phyla in the rhizosphere of the different rootstock or shoot genotypes or combinations. A bacterium related to *Nevskia terrae* influenced the rhizosphere microbiome of Mueller-Thurgau, whereas *Nevskia soli* and *Longimicrobium terrae* influenced the rhizosphere microbiome of Riesling (Figure 3). A sequence next relative to the bacterium *Racemicystis persica* (KX443485, 91.06 %) belonging to the *Proteobacteria* phylum and *Polyangiaceae* family is one of the eight most important features in two of the three experimental set-ups (Figure 3).

3.3 Changes in the rhizosphere microbial community

Compositional differential abundance analyses indicated that several bacterial genera in the rhizosphere were affected. ALDEx2 demonstrated that for the time point of May 2019, in total, 26 bacterial genera differed according to the grape variety. Bacterial genera with the highest fold changes belonged to *Rhodospirillaceae*, *Opitutaceae*, *Burkholderiaceae*, *Polyangiaceae*, and *"Solibacteraceae"* (Tables 1–3, Supplementary Material). An uncultured bacterium relative to the *Candidatus* Solibacter showed the highest fold changes (fold change 1640.65 Control vs Mueller-Thurgau and fold change 862.6 Control vs Riesling). This bacterial genus is also displayed in Figure 3A) as an

ASV strongly influencing the principal component axis. For time point October 2019, no genera differ according to the different rootstocks and Riesling grape variety. For time point October 2020, ALDEx2 demonstrated 560 bacterial genera in total differing according to the grape variety or rootstock or combinations, respectively. The highest log fold changes showed *Nevskiaceae* (also displayed in Figure 3C), *Acidobacteriaceae*, *"Solibacteraceae"*, *Comamonadaceae*, *Caulobacteraceae* (*Phenylobacterium deserti*, also shown in Figure 3C), *Opitutaceae*, and *Steroidobacteraceae*. Statistical different genera were detected for time point October 2020 between Mueller-Thurgau ungrafted vs Mueller-Thurgau grafted onto different rootstocks, Mueller-Thurgau ungrafted vs Riesling ungrafted, Mueller-Thurgau ungrafted vs Riesling grafted onto different rootstocks, Mueller-Thurgau ungrafted vs the control, Riesling ungrafted vs Riesling grafted onto different rootstocks, and Riesling grafted vs the control (Tables 4–9, Supplementary Material). Between Riesling grafted vs Mueller-Thurgau grafted, no statistical differences regarding the bacterial genera were detected.

DISCUSSION

The experimental design of this study aimed at minimizing the variability coming from all factors except grape variety and rootstock genotype using grapevines in pots under the same environmental conditions. The differences in the bacterial communities between the two grape varieties, Riesling and Mueller-Thurgau, ungrafted and grafted onto different rootstocks, were investigated using compositional data analyses. The observed ASVs revealed that the rootstock and scion rhizosphere, respectively, recruited complex bacterial communities mainly composed of *Proteobacteria*, *Verrucomicrobiota*, *Chloroflexi*, *Myxococcota*, *Acidobacteria*, and *Gemmatimonadota*. A similar study by Dries *et al*. (2021a) with ungrafted grapevine rootstocks also showed the phyla *Proteobacteria*, *Acidobacteria*, and *Gemmatimonadota* as some of the predominant bacterial communities in the rhizosphere. Other studies have come to comparable conclusions, independent of factors such as grape variety and rootstock (Berlanas *et al*., 2019; Coller *et al*., 2019; Marasco *et al*., 2018; Novello *et al*., 2017; Torres *et al*., 2021; Vink *et al*., 2021; Zarraonaindia *et al*., 2015). Gobbi *et al*. (2022) showed in their study that *Proteobacteria* occurred with the highest relative abundances, and *Actinobacteria* and *Acidobacteria* were the second-most or third-most abundant bacterial phylum. In this study, we also detected *Actinobacteria* in the samples (Supplementary Material), but not as one of the most abundant bacterial phyla, which could be due to the commercial soil used for the experiments. The dominating families give a higher resolution on the taxonomic level which is more suitable for comparison than the phyla level. During all experimental time points, *Opitutaceae*, *Reyranellaceae*, *"Solibacteraceae"*, and *Solimonadaceae* were found as dominating families, among others (Figures 1 and 2). This is also consistent with a study conducted by Marasco *et al*. (2018). They revealed *"Solibacteraceae"* as one of the families shaping the

topology of the bacterial network in the grafted root system (Marasco *et al.*, 2018). *Comamonadaceae* (Figures 1A and 2) were also found in a study by D'Amico *et al.* (2018) in the rhizosphere of grapevine rootstocks 5BB and Paulsen 1103. As a wider variety of bacteria is found in soil, it can be assumed that soil serves as a primary reservoir for potential plant-associated bacteria (Zarraonaindia *et al.*, 2015). However, soil, as well as the rhizosphere, can also form a path of infection for soil-borne pathogens (Berlanas *et al.*, 2019). For grapevines, this includes pathogens such as *Fusarium oxysporum* (Freire Cruz and Carvalho Pires, 2014) or *Sorosphaera viticola* (Neuhauser, 2009).

The alpha diversity indices in this study revealed higher observed features for ungrafted Riesling grape varieties in May 2019 (Table 2). This may indicate that ungrafted Riesling grapevines could recruit a higher number of bacteria in their rhizosphere than Mueller-Thurgau grapevines. The underlying reason for this varying colonisation could originate from the root exudates. Indeed, root exudates are strongly influenced by the cultivar, and they are considered among the most important factors in the recruitment of the microbiome (Kusstatscher *et al.*, 2021; Marasco *et al.*, 2018; Wei *et al.*, 2019). As a key role in the rhizosphere ecosystem, it is essential to understand the root exudation patterns to unravel the subsequent effects on the surrounding soil and microbial communities (Yee *et al.*, 2021). Berlanas *et al.* (2019) investigated in their study the grapevine rootstock variety as one of the factors shaping the vineyard microbiome. In a study conducted by Vink *et al.* (2021), the authors observed highly specific cultivar–rootstock interaction effects on the microbiome only occurring for a few specific rootstock–cultivar combinations. In a recent study by Marasco *et al.* (2022), the authors showed that the interaction of rootstock and scion resulted to be more important in shaping the root system microbiome than the rootstock and scion considered separately. They investigated four different rootstocks and three scions in seven combinations (Marasco *et al.*, 2022).

The alpha diversity indices for Riesling and Mueller-Thurgau grafted onto different grapevine rootstocks revealed no statistical differences (Table 2). However, three out of four rootstocks used for this experiment have emerged from the same breeding: *Vitis berlandieri* Planch. × *Vitis riparia* Michx., which may be an explanation of the statistical same bacterial communities in the rhizosphere. The fact that most cultivated grapevines are genetic chimaeras with two different genotypes (Marín *et al.*, 2021) complicates the separation of the genotypes from each other regarding their related microbial communities. Hence, all the studies indicate that certain taxa are always apparently present at all times in the rhizosphere of the grapevine. However, no statistical differences regarding alpha diversity indices were found between ungrafted Mueller-Thurgau and ungrafted Riesling for the time point October 2020, which could be due to differences between May 2019 and October 2020 regarding the time of growth in the greenhouse under field conditions. Since grapevines for May 2019 were grown only

four months instead of six months, alpha diversity indices could have been changed.

The beta diversity revealed statistical differences in the three experimental setups. Pairwise PERMANOVA results showed statistical differences between ungrafted Mueller-Thurgau and ungrafted Riesling grape varieties but no statistical differences between different rootstocks. The results indicate that the grape variety may be a driving factor of the bacterial communities in the rhizosphere, while the combination of grafted vine and rootstock may have a minor effect. This is also in accordance with a former study, showing the ungrafted grapevine rootstocks as a driver of the bacterial communities (Dries *et al.*, 2021a). Thus, it might be concluded that grafting grapevines onto rootstocks implies a change in bacterial communities. Moreover, Vink *et al.* (2021) revealed in their study that the main determinant of the bacterial communities was scion variety for the alpha diversity and a significant interaction between scion and rootstocks regarding the beta diversity. However, the authors did not refer to ungrafted grape varieties. In another study conducted by Berlanas *et al.* (2019), they described the rootstock genotype as the most important factor in shaping the microbiome. Wright *et al.* (2022) revealed that the rootstock was a significant factor driving the root microbiome with the grape variety New York Muscat ungrafted and grafted onto two different rootstocks.

Comparing the ASVs strongly influencing the principal component axis from the PCoA biplot (Figure 3) with the results of the compositional differential abundance analyses (ALDEx2, Tables 1–3, Supplementary Material) for time point May 2019 revealed ASVs next relative to *Nevskia terrae*, uncultured *Candidatus* Solibacter, and uncultured Deltaproteobacteria as those taxa appearing in both analyses. *Nevskia terrae* was already described as a bacterium isolated from soil in Korea (Kim *et al.*, 2011), belonging to the *Xanthomonadaceae*. Some members of this family are already described as plant-growth-promoting (Cutiño-Jiménez *et al.*, 2020). *Candidatus* Solibacter was described previously as a bacteria inhabiting the rhizosphere of walnut trees (Bai *et al.*, 2020) and Deltaproteobacteria in rice (Zhang *et al.*, 2018). For time point October 2020, all eight displayed ASVs strongly influencing the principal component axis from the DEICODE beta diversity also appeared in the compositional abundance analyses (ALDEx2, Tables 4–9, Supplementary Material). The next relative *Phenylobacterium deserti* was first isolated from desert soil (Khan *et al.*, 2017), and other *Phenylobacterium* were also isolated from different soil samples (Khan *et al.*, 2018; Li *et al.*, 2019). The next relative of uncultured "*Acidibacter*" belongs to Gammaproteobacteria; members of this class are known for plant growth-promoting traits, inhabiting the rhizosphere (Madhaiyan *et al.*, 2017), root nodules (Ibáñez *et al.*, 2009), or plant tissue (Madhaiyan *et al.*, 2020).

However, further research on this topic is needed to reveal all effects of rootstock and grapevine scion combinations on the bacterial communities in the rhizosphere. Here, it should be emphasised again that in practice, *Vitis vinifera* cultivars are usually grown as scions grafted onto *Phylloxera*-tolerant

Vitis sp. Darriaut *et al.* (2022) indicate that grapevines can select different but potentially beneficial microorganisms via rootstock and scion genotypes. The effects of rootstock-scion interactions should, therefore, be considered primarily from a practical perspective. Nevertheless, it is remarkable that the bacterial communities change with grafting. Therefore, the next approach should be to find out whether this change brings advantages or even disadvantages. Moreover, it is important to understand the complexity of the grapevine holobiont as a crucial issue for the future of the wine industry (as reviewed by Bettenfeld *et al.*, 2021). In addition, the effects on the growth and health of the vine must be revealed from a practical perspective. A thorough understanding of microorganisms in vineyard soil and the complex relationships between microbial communities, soil properties, and plants are crucial for enhancing plant productivity, grape production, biogeochemical processes, and vineyard management practices (Di Liu *et al.*, 2019; Dries *et al.*, 2021b; Holland *et al.*, 2014; Liang *et al.*, 2019; Yee *et al.*, 2021). Inferring, the colonisation of the microorganisms in the rhizosphere and the root exudation patterns of grapevine must be investigated. Future research in this field is inevitably required to provide a better understanding of the rhizospheric grapevine microbiome in the context of root exudates, grape variety, and rootstock, as well as different environmental conditions.

CONCLUSION

The results from these experimental designs reveal differences in the bacterial communities in the rhizosphere of grafted or ungrafted grapevine varieties, respectively. The bacteria in the rhizosphere of grapevine are affected by both grapevine variety and scion-rootstock combination. While differences were observed between ungrafted vs grafted grape varieties, especially in terms of beta diversity, no differences were observed between the different rootstocks. Thus, the grapevine cultivar appears to have a predominant role compared with the rootstock in shaping the rhizosphere microbiota. Further research is needed to provide a better understanding of the different microorganisms in the grapevine rhizosphere regarding the scion-rootstock combinations. Moreover, the effects on the grapevine growth and health, and also on the wine quality, have to be revealed.

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REFERENCES

Alegria Terrazas, R., Giles, C., Paterson, E., Robertson-Albertyn, S., Cesco, S., Mimmo, T., Pii, Y., & Bulgarelli, D. (2016). Plant-Microbiota Interactions as a Driver of the Mineral Turnover in the Rhizosphere. *Advances in Applied Microbiology*, *95*(95), 1–67. https://doi.org/10.1016/bs.aambs.2016.03.001

Anderson, M. J. (2017). Permutational Multivariate Analysis of Variance (PERMANOVA). *Wiley Statistics Reference Online*, 1–15. https://doi.org/10.1002/9781118445112.stat07841

Bai, Y.-C., Chang, Y.-Y., Hussain, M., Lu, B., Zhang, J.-P., Song, X.-B., Lei, X.-S., & Pei, D. (2020). Soil Chemical and Microbiological Properties Are Changed by Long-Term Chemical Fertilizers That Limit Ecosystem Functioning. *Microorganisms*, *8*(5). https://doi.org/10.3390/microorganisms8050694

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012) The rhizosphere microbiome and plant health. *Trends in plant science*, 17, 478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J. F., Sagües, A., & Gramaje, D. (2019). The Fungal and Bacterial Rhizosphere Microbiome Associated With Grapevine Rootstock Genotypes in Mature and Young Vineyards. *Frontiers in Microbiology*, *10*, 1142. https://doi.org/10.3389/fmicb.2019.01142

Berry, D., Ben Mahfoudh, K., Wagner, M., & Loy, A. (2011). Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Applied and Environmental Microbiology*, *77*(21), 7846–7849. https://doi.org/10.1128/AEM.05220-11

Bettenfeld, P.; Cadena i Canals, J.; Jacquens, L.; Fernandez, O.; Fontaine, F.; van Schaik, E.; Courty, P.-E.; Trouvelot, S. (2021). The Microbiota of the Grapevine Holobiont: A Key Component of Plant Health. *J. Adv. Res*. 40, 1–15. https://doi.org/10.1016/j.jare.2021.12.008

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., & Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(1), E139-48. https://doi.org/10.1073/pnas.1317377110

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J.,... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Loren Themaat, E. ver, & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, *64*, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106

Burns, K. N., Bokulich, N. A., Cantu, D., Greenhut, R. F., Kluepfel, D. A., O'Geen, A. T., Strauss, S. L., & Steenwerth, K. L. (2016). Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation by vineyard management. *Soil Biology and Biochemistry*, *103*, 337–348. https://doi.org/10.1016/j.soilbio.2016.09.007

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). Dada2: High-resolution sample

inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Carvalhais, L. C., Dennis, P. G., Tyson, G. W., & Schenk, P. M. (2012). Application of metatranscriptomics to soil environments. *Journal of Microbiological Methods*, *91*(2), 246–251. https://doi.org/10.1016/j.mimet.2012.08.011

Claesson, M. J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J. R., Smidt, H., Vos, W. M. de, Ross, R. P., & O'Toole, P. W. (2009). Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS ONE*, *4*(8), e6669. https://doi.org/10.1371/journal.pone.0006669

Coller, E., Cestaro, A., Zanzotti, R., Bertoldi, D., Pindo, M., Larger, S., Albanese, D., Mescalchin, E., & Donati, C. (2019). Microbiome of vineyard soils is shaped by geography and management. *Microbiome*, *7*(1), 140. https://doi.org/10.1186/s40168-019-0758-7

Cordero, J., Freitas, J. R. de, & Germida, J. J. (2020). Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. *Canadian Journal of Microbiology*, *66*(1), 71–85. https://doi.org/10.1139/cjm-2019-0330

Cutiño-Jiménez, A. M., Menck, C. F. M., Cambas, Y. T., & Díaz-Pérez, J. C. (2020). Protein signatures to identify the different genera within the Xanthomonadaceae family. *Brazilian Journal of Microbiology: [Publication of the Brazilian Society for Microbiology]*, *51*(4), 1515–1526. https://doi.org/10.1007/s42770-020-00304-2

D'Amico, F., Candela, M., Turroni, S., Biagi, E., Brigidi, P., Bega, A., Vancini, D., & Rampelli, S. (2018). The Rootstock Regulates Microbiome Diversity in Root and Rhizosphere Compartments of Vitis vinifera Cultivar Lambrusco. *Frontiers in Microbiology*, *9*, 2240. https://doi.org/10.3389/fmicb.2018.02240

Darriaut, R., Lailheugue, V., Masneuf-Pomarède, I., Marguerit, E., Martins, G., Compant, S., Ballestra, P., Upton, S., Ollat, N. & Lauvergeat, V. (2022) Grapevine rootstock and soil microbiome interactions: Keys for a resilient viticulture. *Horticulture research*, 9. https://doi.org/10.1093/hr/uhac019

Di Liu, Zhang, P., Chen, D., & Howell, K. (2019). From the Vineyard to the Winery: How Microbial Ecology Drives Regional Distinctiveness of Wine. *Frontiers in Microbiology*, *10*, 2679. https://doi.org/10.3389/fmicb.2019.02679

Dries, L., Bussotti, S., Pozzi, C., Kunz, R., Schnell, S., Löhnertz, O., & Vortkamp, A. (2021a). Rootstocks Shape Their Microbiome-Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks. *Microorganisms*, *9*(4). https://doi.org/10.3390/microorganisms9040822

Dries, L., Hendgen, M., Schnell, S., Löhnertz, O., & Vortkamp, A. (2021b). Rhizosphere engineering: leading towards a sustainable viticulture? *OENO One*, *55*(2), 353–363. https://doi.org/10.20870/oeno-one.2021.55.2.4534

Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P. & Hacquard, S. (2018). Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. *Cell*, 175, 973–983.e14. https://doi.org/10.1016/j.cell.2018.10.020

Engelbrektson, A., Kunin, V., Wrighton, K. C., Zvenigorodsky, N., Chen, F., Ochman, H., & Hugenholtz, P. (2010). Experimental factors affecting PCR-based estimates of microbial species richness and evenness. *The ISME Journal*, *4*(5), 642–647. https://doi.org/10.1038/ismej.2009.153

Etesami, H., & Alikhani, H. A. (2016). Rhizosphere and endorhiza of oilseed rape (*Brassica napus* L.) plant harbor bacteria with multifaceted beneficial effects. *Biological Control*, *94*, 11–24. https://doi.org/10.1016/j.biocontrol.2015.12.003

Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G., & Gloor, G. B. (2013). Anova-like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLoS ONE*, *8*(7), e67019. https://doi.org/10.1371/journal.pone.0067019

Fernandes, A. D., Reid, J. N., Macklaim, J. M., McMurrough, T. A., Edgell, D. R., & Gloor, G. B. (2014). Unifying the analysis of high-throughput sequencing datasets: Characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome*, *2*, 15. https://doi.org/10.1186/2049-2618-2-15

Freire Cruz, A., & Carvalho Pires, M. de (2014). Soil-Borne Plant Pathogens Associated to Decline of Grapevine Grown in Greenhouse. *Journal of Plant Physiology & Pathology*, *02*(01). https://doi.org/10.4172/2329-955X.1000115

Garoutte, A., Cardenas, E., Tiedje, J., & Howe, A. (2016). Methodologies for probing the metatranscriptome of grassland soil. *Journal of Microbiological Methods*, *131*, 122–129. https://doi.org/10.1016/j.mimet.2016.10.018

Gautier, A. T., Chambaud, C., Brocard, L., Ollat, N., Gambetta, G. A., Delrot, S., & Cookson, S. J. (2019). Merging genotypes: Graft union formation and scion-rootstock interactions. *Journal of Experimental Botany*, *70*(3), 747–755. https://doi.org/10.1093/jxb/ery422

Gobbi, A., Acedo, A., Imam, N., Santini, R. G., Ortiz-Álvarez, R., Ellegaard-Jensen, L., Belda, I., Hansen, L. H. (2022). A global microbiome survey of vineyard soils highlights the microbial dimension of viticultural terroirs. *Communications biology, 5, 241.* https://doi.org/10.1038/s42003-022-03202-5

Hendgen, M., Hoppe, B., Döring, J., Friedel, M., Kauer, R., Frisch, M., Dahl, A., & Kellner, H. (2018). Effects of different management regimes on microbial biodiversity in vineyard soils. *Scientific Reports*, *8*(1), 9393. https://doi.org/10.1038/s41598-018-27743-0

Holland, T. C., Bowen, P., Bogdanoff, C., & Hart, M. M. (2014). How distinct are arbuscular mycorrhizal fungal communities associating with grapevines? *Biology and Fertility of Soils*, *50*(4), 667–674. https://doi.org/10.1007/s00374-013-0887-2

Hu, L., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M. G. A., Schlaeppi, K. & Erb, M. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature communications*, 9, 2738. https://doi.org/10.1038/s41467-018-05122-7

Ibáñez, F., Angelini, J., Taurian, T., Tonelli, M. L., & Fabra, A. (2009). Endophytic occupation of peanut root nodules by opportunistic Gammaproteobacteria. *Systematic and Applied Microbiology*, *32*(1), 49–55. https://doi.org/10.1016/j.syapm.2008.10.001

Kaplan, H., Ratering, S., Felix-Henningsen, P., & Schnell, S. (2019). Stability of in situ immobilization of trace metals with different amendments revealed by microbial 13C-labelled wheat root decomposition and efflux-mediated metal resistance of soil bacteria. *The Science of the Total Environment*, *659*, 1082–1089. https://doi.org/10.1016/j.scitotenv.2018.12.441

Katoh, K., & Standley, D. M. (2013). Mafft multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Khan, I. U., Habib, N., Xiao, M., Huang, X., Khan, N. U., Im, W.-T., Ahmed, I., Zhi, X.-Y., & Li, W.-J. (2018). *Phenylobacterium terrae* sp. Nov., isolated from a soil sample of Khyber-Pakhtun-Khwa, Pakistan. *Antonie Van Leeuwenhoek*, *111*(10), 1767–1775. https://doi.org/10.1007/s10482-018-1064-2

Khan, I. U., Hussain, F., Habib, N., Wadaan, M. A. M., Ahmed, I., Im, W.-T., Hozzein, W. N., Zhi, X.-Y., & Li, W.-J. (2017).

Phenylobacterium deserti sp. Nov., isolated from desert soil. *International Journal of Systematic and Evolutionary Microbiology*, *67*(11), 4722–4727. https://doi.org/10.1099/ijsem.0.002366

Kim, S.-J., Weon, H.-Y., Kim, Y.-S., Park, I.-C., Son, J.-A., & Kwon, S.-W. (2011). *Nevskia terrae* sp. Nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, *61*(Pt 5), 1226–1229. https://doi.org/10.1099/ijs.0.021238-0

Kusstatscher, P., Adam, E., Wicaksono, W. A., Bernhart, M., Olimi, E., Müller, H., & Berg, G. (2021). Microbiome-Assisted Breeding to Understand Cultivar-Dependent Assembly in *Cucurbita pepo*. *Frontiers in Plant Science*, *12*, 642027. https://doi.org/10.3389/fpls.2021.642027

Li, J., Mavrodi, O. V., Hou, J., Blackmon, C., Babiker, E. M. & Mavrodi, D. V. (2020) Comparative Analysis of Rhizosphere Microbiomes of Southern Highbush Blueberry (*Vaccinium corymbosum* L.), Darrow's Blueberry (*V. darrowii* Camp), and Rabbiteye Blueberry (*V. virgatum* Aiton). *Frontiers in microbiology*, 11, 370. https://doi.org/10.3389/fmicb.2020.00370

Li, X., Yu, Y., Choi, L., Song, Y., Wu, M., Wang, G., & Li, M. (2019). *Phenylobacterium soli* sp. Nov., isolated from arsenic and cadmium contaminated farmland soil. *International Journal of Systematic and Evolutionary Microbiology*, *69*(5), 1398–1403. https://doi.org/10.1099/ijsem.0.003325

Liang, H., Wang, X., Yan, J., & Luo, L. (2019). Characterizing the Intra-Vineyard Variation of Soil Bacterial and Fungal Communities. *Frontiers in Microbiology*, *10*, 1239. https://doi.org/10.3389/fmicb.2019.01239

Madhaiyan, M., Poonguzhali, S., Saravanan, V. S., Selvapravin, K., Duraipandiyan, V., & Al-Dhabi, N. A. (2017). *Pseudomonas sesami* sp. Nov., a plant growth-promoting Gammaproteobacteria isolated from the rhizosphere of Sesamum indicum L. *Antonie Van Leeuwenhoek*, *110*(7), 843–852. https://doi.org/10.1007/s10482-017-0859-x

Madhaiyan, M., Saravanan, V. S., Blom, J., Smits, T. H. M., Rezzonico, F., Kim, S.-J., Weon, H.-Y., Kwon, S.-W., Whitman, W. B., & Ji, L. (2020). *Phytobacter palmae* sp. Nov., a novel endophytic, N2 fixing, plant growth promoting Gammaproteobacterium isolated from oil palm (*Elaeis guineensis* Jacq.). *International Journal of Systematic and Evolutionary Microbiology*, *70*(2), 841–848. https://doi.org/10.1099/ijsem.0.003834

Marasco, R., Rolli, E., Fusi, M., Michoud, G., & Daffonchio, D. (2018). Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome*, *6*(1), 3. https://doi.org/10.1186/s40168-017-0391-2

Marasco, R., Alturkey, H., Fusi, M., Brandi, M., Ghiglieno, I., Valenti, L., Daffonchio, D. (2022). Rootstock–scion combination contributes to shape diversity and composition of microbial communities associated with grapevine root system. *Environmental Microbiology*, *24*(8), 3791-3808.

Marín, D., Armengol, J., Carbonell-Bejerano, P., Escalona, J. M., Gramaje, D., Hernández-Montes, E., Intrigliolo, D. S., Martínez-Zapater, J. M., Medrano, H., Mirás-Avalos, J. M., Palomares-Rius, J. E., Romero-Azorín, P., Savé, R., Santesteban, L. G., & Herralde, F. de (2021). Challenges of viticulture adaptation to global change: tackling the issue from the roots. *Australian Journal of Grape and Wine Research*, *27*(1), 8–25. https://doi.org/10.1111/ajgw.12463

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. https://doi.org/10.14806/ej.17.1.200

Martino, C., Morton, J. T., Marotz, C. A., Thompson, L. R., Tripathi, A., Knight, R., & Zengler, K. (2019). A Novel Sparse Compositional Technique Reveals Microbial Perturbations. *MSystems*, *4*(1). https://doi.org/10.1128/mSystems.00016-19

McLaren, M. R., Willis, A. D., & Callahan, B. J. (2019). Consistent and correctable bias in metagenomic sequencing experiments. *ELife*. Advance online publication. https://doi.org/10.7554/eLife.46923.001

Neuhauser, S., Huber, L., Kirchmair, M. (2009). *Sorosphaera viticola*, a plasmodiophorid parasite of grapevine. *Phytopathol Mediterr.*(48(1)), 136–139.

Novello, G., Gamalero, E., Bona, E., Boatti, L., Mignone, F., Massa, N., Cesaro, P., Lingua, G., & Berta, G. (2017). The Rhizosphere Bacterial Microbiota of *Vitis vinifera* cv. Pinot Noir in an Integrated Pest Management Vineyard. *Frontiers in Microbiology*, *8*, 1528. https://doi.org/10.3389/fmicb.2017.01528

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., Buckler, E. S., & Ley, R. E. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(16), 6548–6553. https://doi.org/10.1073/pnas.1302837110

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 - Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE*(5(3)), 1–10. https://doi.org/10.1371/journal.pone.0009490.t001

Quinn, T. P., Erb, I., Gloor, G., Notredame, C., Richardson, M. F., & Crowley, T. M. (2019). A field guide for the compositional analysis of any-omics data. *GigaScience*, *8*(9). https://doi.org/10.1093/gigascience/giz107

Rosado-Porto, D., Ratering, S., Cardinale, M., Maisinger, C., Moser, G., Deppe, M., Müller, C., & Schnell, S. (2021). Elevated Atmospheric CO_2 Modifies Mostly the Metabolic Active Rhizosphere Soil Microbiome in the Giessen FACE Experiment. Microbial Ecology. 83(3), 619-634. Advance online publication. https://doi.org/10.1007/s00248-021-01791-y

Ryan, P. R., Dessaux, Y., Thomashow, L. S. & Weller, D. M. (2009). Rhizosphere engineering and management for sustainable agriculture. *Plant and Soil*, 321, 363–383. https://doi.org/10.1007/s11104-009-0001-6

Schlaeppi, K. & Bulgarelli, D. (2015). The plant microbiome at work. *Molecular plant-microbe interactions*: MPMI, 28, 212–217. https://doi.org/10.1094/MPMI-10-14-0334-FI

Taye, Z. M., Helgason, B. L., Bell, J. K., Norris, C. E., Vail, S., Robinson, S. J., Parkin, I. A. P., Arcand, M., Mamet, S., Links, M. G., Dowhy, T., Siciliano, S. & Lamb, E. G. (2019). Core and Differentially Abundant Bacterial Taxa in the Rhizosphere of Field Grown *Brassica napus* Genotypes: Implications for Canola Breeding. *Frontiers in microbiology*, 10, 3007. https://doi.org/10.3389/fmicb.2019.03007

Timmusk, S., Abd El-Daim, I. A., Copolovici, L., Tanilas, T., Kännaste, A., Behers, L., Nevo, E., Seisenbaeva, G., Stenström, E., & Niinemets, Ü. (2014). Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS ONE*, *9*(5), e96086. https://doi.org/10.1371/journal.pone.0096086

Torres, N., Yu, R., & Kurtural, S. K. (2021). Inoculation with Mycorrhizal Fungi and Irrigation Management Shape the Bacterial and Fungal Communities and Networks in Vineyard Soils. *Microorganisms*, *9*(6). https://doi.org/10.3390/microorganisms9061273

Turner, T. R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., Osbourn, A., Grant, A., & Poole, P. S. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *The ISME Journal*, *7*(12), 2248–2258. https://doi.org/10.1038/ismej.2013.119

Vega-Avila, A. D., Gumiere, T., Andrade, P. A. M., Lima-Perim, J. E., Durrer, A., Baigori, M., Vazquez, F., & Andreote, F. D. (2015).

Bacterial communities in the rhizosphere of *Vitis vinifera* L. Cultivated under distinct agricultural practices in Argentina. *Antonie Van Leeuwenhoek*, *107*(2), 575–588. https://doi.org/10.1007/s10482-014-0353-7

Vink, S. N., Dini-Andreote, F., Höfle, R., Kicherer, A., & Salles, J. F. (2021). Interactive Effects of Scion and Rootstock Genotypes on the Root Microbiome of Grapevines (*Vitis spp*. L.). *Applied Sciences*, *11*(4), 1615. https://doi.org/10.3390/app11041615

Wei, F., Zhao, L., Xu, X., Feng, H., Shi, Y., Deakin, G., Feng, Z., & Zhu, H. (2019). Cultivar-Dependent Variation of the Cotton Rhizosphere and Endosphere Microbiome Under Field Conditions. *Frontiers in Plant Science*, *10*, 1659. https://doi.org/10.3389/fpls.2019.01659

Wright, A.H., Ali, S., Migicovsky, Z., Douglas, G.M., Yurgel, S., Bunbury-Blanchette, A., Franklin, J., Adams, S.J., Walker, A.K. (2022). A Characterization of a Cool-Climate Organic Vineyard's Microbiome. *Phytobiomes Journal, 6, 69–82,* https://.doi.org/10.1094/PBIOMES-03-21-0019-R

Yee, M. O., Kim, P., Li, Y., Singh, A. K., Northen, T. R., & Chakraborty, R. (2021). Specialized Plant Growth Chamber Designs to Study Complex Rhizosphere Interactions. *Frontiers in Microbiology*, *12*, 625752. https://doi.org/10.3389/fmicb.2021.625752

Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology*, *67*(5), 1613–1617. https://doi.org/10.1099/ijsem.0.001755

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., van der Lelie, D., & Gilbert, J. A. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio*, *6*(2). https://doi.org/10.1128/mBio.02527-14

Zhang, J., Zhang, N., Liu, Y.-X., Zhang, X., Hu, B., Qin, Y., Xu, H., Wang, H., Guo, X., Qian, J., Wang, W., Zhang, P, Jin, T., Chu, C., & Bai, Y. (2018). Root microbiota shift in rice correlates with resident time in the field and developmental stage. *Science China. Life Sciences*, *61*(6), 613–621. https://doi.org/10.1007/s11427-018-9284-4

Zhang, X., Di Zhang, Sun, W. & Wang, T. (2019). The Adaptive Mechanism of Plants to Iron Deficiency via Iron Uptake, Transport, and Homeostasis. *International journal of molecular sciences*, 20. https://doi.org/10.3390/ijms20102424.