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Prokaryotic diversity of the rhizosphere in Argentine wine-producing regions

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ABSTRACT

Argentina is the seventh-largest wine producer globally, with its vast geographical and climatic diversity contributing to wines that reflect distinctive regional characteristics. Microorganisms associated with vines play a crucial role in shaping wine quality. Recent studies have highlighted significant differences in microbial communities across grape varieties and vineyard locations. This study conducted a comprehensive analysis of the diversity of prokaryotic microorganisms in the rhizosphere of vineyards located in three prominent wine regions of Argentina, using next-generation sequencing methods. The research focused on the two most iconic Argentine cultivars: Malbec and Cabernet-Sauvignon. The physicochemical properties of the vineyard soils were assessed using principal component analysis, revealing distinct differences among the regions. While no significant differences in microbial diversity were found between Malbec and Cabernet-Sauvignon vineyards, notable variations were observed among the vineyard locations. Taxonomic identification showed distinct microbial compositions across regions, with substantial differences in abundance at the family level. Canonical correspondence analysis further demonstrated correlations between soil physicochemical properties and microbial families, underscoring the influence of soil characteristics on microbiota composition. These variations in microbial populations established a site-specific prokaryotic profile that could serve as a unique identifying signature. Understanding these interactions is critical for refining vineyard management practices and shaping the sensory characteristics of wines produced in diverse regions.

KEYWORDS: rhizosphere, prokaryote, grapevine

INTRODUCTION

Internationally ranked as the seventh-largest wine producer in 2022, Argentina has a vast area of grapevine crops (International Organization of Vine and Wine, 2023). The diversity of Argentine terroirs is made up of climatically and topographically distinct regions composed of diverse climates, soils, altitudes and native flora and fauna. All of the latter factors contribute to the expression of the unique characteristics of the wines from each geographical location, including the adaptation of specific grapevine cultivars. Terroir is defined by the OIV as being the collective interaction of identifiable physical and biological factors in a given area that, together with the winemaking practices, give the products originating from that area their distinctive characteristics (Resolution OIV/Viti 333/2010). Argentina's emblematic Malbec (MA) wines and their versatile Cabernet-Sauvignon (CS) varietals are the best example of this concept. Harsh climatic conditions in important national winemaking regions can have an economic impact due to low yield or a low-quality season; this has led the scientific community to carry out research on the subject in order to ultimately help improve the future economic value of the country's wine industry.

Early microbial studies in grapevine research have found that vine-associated microbial communities are shaped by topography, climatic conditions, soil physicochemical characteristics and agricultural techniques. These communities particularly stimulate the plant productivity, as well as host resistance to pests and diseases (Belda *et al.,* 2017; Bokulich *et al.,* 2016; Zarraonaindia *et al.,* 2015). Additionally, microbial interactions result in the release of aromatic precursors or chemical compounds and secondary metabolites that have been produced in these exchanges, impacting the sensorial attributes of wines (Borneman *et al.,* 2013), and further contributing to the distinct regional characteristics of the terroir expressed in the final product (Liu *et al.,* 2019). Along with other biotic and abiotic factors, the biological characteristics of the soil enable complex metabolic interactions with plant hosts, which contribute significantly to the factors that intrinsically coexist and shape the regional attributes of local wines (Belda *et al.,* 2017; Burns *et al.,* 2016).

In Argentina, research on these grapevine microbial communities and their complex dynamics is scarce. A few studies (mentioned hereafter) have covered areas in the main wine producing regions of Mendoza and San Juan, still leaving a lot of scope for investigation in the other important wine-growing regions of the country; for example, Alto Valle del Rio Negro and the Atlantic coast of Patagonia, and the most north-western province of Salta, where grapevines are grown at > 2000 m asl (National Institute of Viticulture, 2023).

Associations of microorganisms with plants is a widespread phenomenon that comprises mutually beneficial, commensalistic or host-pathogen interactions (Kogel *et al.,* 2006; Newton *et al.,* 2010). Rhizosphere and endosphere communities are enriched by a specific subset of organisms from the soil microbiome (Pieterse *et al.,* 2016). In the context of wine production, the most prevalent microbial populations in soil have a major influence on plant health, soil texture, nutrient cycling and biodiversity (Wang *et al.,* 2024), influencing the quality of the fruit in the vineyard and thus of the produced wine (Liu *et al.,* 2019; Rivas *et al.,* 2021). Grape health (Mueller & Sachs, 2015), grapevine genotype, geographical area and climatic factors all contribute to the differences between microbial populations in the grapevine phyllosphere (Singh *et al.,* 2018). Studies on these biological features have contributed to the understanding of biodiversity patterns influencing microbial populations variations according to geographic features like climate, vintage, and topography in association with the plant genotype and soil edaphic factors (Burns *et al.,* 2016; Gobbi *et al.,* 2022; Griggs *et al.,* 2021; Zarraonaindia *et al.,* 2015).

Numerous studies have shown that the structure of microbial communities in grape berry and grape juice samples are similar, indicating that under ideal conditions the communities present in grapes prior to fermentation can remain relatively stable (Belda *et al.,* 2016; Bokulich *et al.,* 2016; Knight *et al.,* 2015; Zarraonaindia *et al.,* 2015). Meanwhile, the microbial communities associated with roots, root zone and bulk soil are often similar, differing from those of aboveground plant organs (Zarraonaindia *et al.,* 2015). Soil health in vineyards is conditioned by both direct and indirect microbial associations, including the mineralisation of soil organic matter, activation of plant defence mechanisms and production of antibiotics against phytopathogens; together, these affect plant productivity and plant health (Bacon & White, 2016; Sanchez *et al.,* 2009).

The rhizosphere is the region of soil around the plant root whose biological and chemical properties is significantly influenced by root activity. This area is a hotspot for complex biological ultrastructures, and microbial activity is responsible for the highly complex ecosystems found here. The combined populations of filamentous fungi, yeasts and a large subset of prokaryotes constitute part of the grapevine (Darriaut *et al.,* 2022; Xie *et al.,* 2024). The microbiome is also known as the microbiota, which contains microorganisms belonging to different kingdoms, such as Prokaryotes (Bacteria, Archaea), Eukaryotes (*e.g.,* Protozoa, Fungi, and Algae), whose "theatre of activity" comprises microbial structures, metabolites, mobile genetic elements (*e.g.*, transposons, phages and viruses) and relic DNA embedded in a given environment (Berg *et al.,* 2020).

Since the development and availability of Next Generation Sequencing technologies (NGS), research involving *V. vinifera* grapevine cultivars has increasingly focused on the study of plant-microbe interactions. This approach aims to understand the implications of the microbiome on crop health and the link between grape quality and geographic location. Such technology, in parallel with classical microbial techniques, can help detect the non-culturable fraction of microbes, especially those in complex microbial ecosystems, allowing researchers to fully assess the complexity of the grapevine microbiome (Burns *et al.,* 2016), and to provide better assessment of the use of biological products in the field.

TABLE 1. Sampling sites of rhizosphere-associated prokaryotes.

Region	Location	Grape variety	Acronym	Soil type	Samples	
					Obtained	Sequenced
San Juan	Ullum, Finca Norte	MA	SJ1MA	Loam	3	3
		CS	SJ1CS	Loam	3	3
	Ullum, Finca Arriba	MA	SJ2MA	Loam	3	3
		CS	SJ2MCS	Loam	3	3
Mendoza	Agrelo, Lujan de Cuyo	MA	MZAMA	Silty Clay	3	3
		CS	MZACS	Silty Clay Loam	3	3
Rio Negro	Mainque, Alto Valle del Rio Negro	MA	RN1MA	Clay Loam	3	3
	Viedma	CS	RN2CS	Sandy Clay Loam	3	3
		MA	RN2MA	Sandy Clay Loam	3	3
Salta	Molinos	MA	SAL1MA	Sandy Loam	3	3
		CS	SAL1CS	Sandy Loam	3	3
	Cafayate	MA	SAL2MA	Sandy Loam	3	3
		CS	SAL2CS	Sandy Loam	3	3
	Cachi	MA	SAL3MA	Loamy sand	3	2

In this context, studies using an NGS approach to characterise regional microbial communities associated with vineyards in Argentina have been conducted over the past decade. Vega-Avila *et al*. (2015) analysed the diversity of vine-associated prokaryotes in vineyards located in San Juan province, where they found that the changes observed in the bacterial communities could not be explained by variations in the physical and chemical properties of the rhizosphere (Vega-Avila *et al.,* 2015). Through the DNA amplification of the bacterial 16S rRNA and fungal ITS1 regions using the NGS approach, our group analysed the soil prokaryotic and eukaryotic composition of MA and CS grapevine cultivars from two different vineyards located in the San Juan Province of Argentina (Oyuela Aguilar *et al.,* 2020). We showed significant differences in microbial community composition between grape varieties and vineyard locations, *Proteobacteria, Firmicutes* and *Bacteroidetes* being the most representative prokaryote groups found. Additionally, Rivas *et al.* (2022) characterised the microbial communities of MA vineyards recently established in a re-emerging wine region of Argentina (the Southwest Buenos Aires province). They found that *Proteobacteria* and *Actinobacteria* were the predominant phyla, in which the order *Rhizobiales* stood out in the soil and rhizosphere samples (Rivas *et al.,* 2022). Previously, they had studied the variations in soil and wine bacterial diversity in three consecutive vintages, and the effect of climatic conditions on this diversity (Rivas *et al.,* 2021). By demonstrating that soil microbiota is widely adapted to harsh conditions and can withstand even prolonged droughts, they found a core of microorganisms in soil and in wines, belonging to different phyla that are dominant and remain throughout different vintages (Rivas *et al.,* 2021). Recently, Paolinelli *et al*. (2023) investigated bacterial and fungal communities in arid vineyard soils from two sites in Mendoza (Paolinelli *et al.,* 2023). They found that certain taxonomic groups are linked to the sampling location, which also influences the metabolic pathways present in these soils.

Although NGS has been frequently used to analyse the microbial diversity associated with wine production, comparative studies on how geography, soil and cultivar influence the grapevine microbiome in Argentine vineyards had yet to be performed. Consequently, the aim of this study was to analyse prokaryotic diversity in vineyards of the main Argentine wine-producing regions by carrying out a comparative study of microbial communities in two grape cultivars across four distinct wine-producing regions. To this end, we sampled soils from MA and CS vineyards in Río Negro, Mendoza, San Juan and Salta to evaluate the effect of Argentine geography on prokaryotic diversity. Using Illumina MiSeq 16S rRNA sequencing, we identified microbiological patterns associated with microbial families, locations and soil physicochemical properties. This study provides insights into the microbiological characteristics of vineyards across Argentina, establishing a foundation for understanding prokaryotic role in the diverse microbial terroirs that contribute to the unique qualities of regional Argentine wines.

MATERIALS AND METHODS

1. Soil sampling

Soil samples were collected from ungrafted grapevines of MA and CS cultivars one week prior to the harvest period in 2016. We sampled fourteen vineyards in four wine-producing regions of Argentina: the southern region (Río

Negro Province, Patagonia), the western central region (San Juan and Mendoza Provinces), and the northwestern region (Salta Province) (Figure 1A). Information regarding the sampled sites is summarised in Table 1.

In the San Juan (SJ) Province (western central wine region), the samples came from two vineyards located in the Ullum Valley, 6 km from each other: Finca Norte (SJ1) and Finca Arriba (SJ2). In the Mendoza (MZA) Province (also western Central wine region), the samples came from vineyards with vines planted based on a vertical shoot positioning system in Agrelo (Luján de Cuyo); all the vineyards were on loamy-clay soils, and the climate of both San Juan and Mendoza is arid.

Samples from the Río Negro (RN) Province (southern wine region) were collected from Mainque (RN1) (upper Valley of Río Negro) and Viedma (RN2) (near the Atlantic Ocean), which both have a semi-arid climate. Annual rainfall in Mainque ranges from 120 to 180 mm, and the temperatures range from 30 to 34 °C in summer and from 10 to 14 °C in winter. The climate in Viedma is influenced by its proximity to the Atlantic Ocean: in the summer, maximum temperatures can reach 30 °C, while in winter they drop to between 2 °C and 12 °C. Samples were taken from two plots on with loamy-sandy soils and located 7 m above sea level (masl).

The Salta province (northwestern wine region) has a warm and dry climate. Samples of MA and CS cultivars were collected from three sites in the Calchaquí Valleys (southwest of the province): Molinos (SAL1), Cafayate (SAL2) and Cachi (SAL3). The elevation of the sites varied between 1100 and 1800 masl, that of Molinos being the lowest and Cachi the highest.

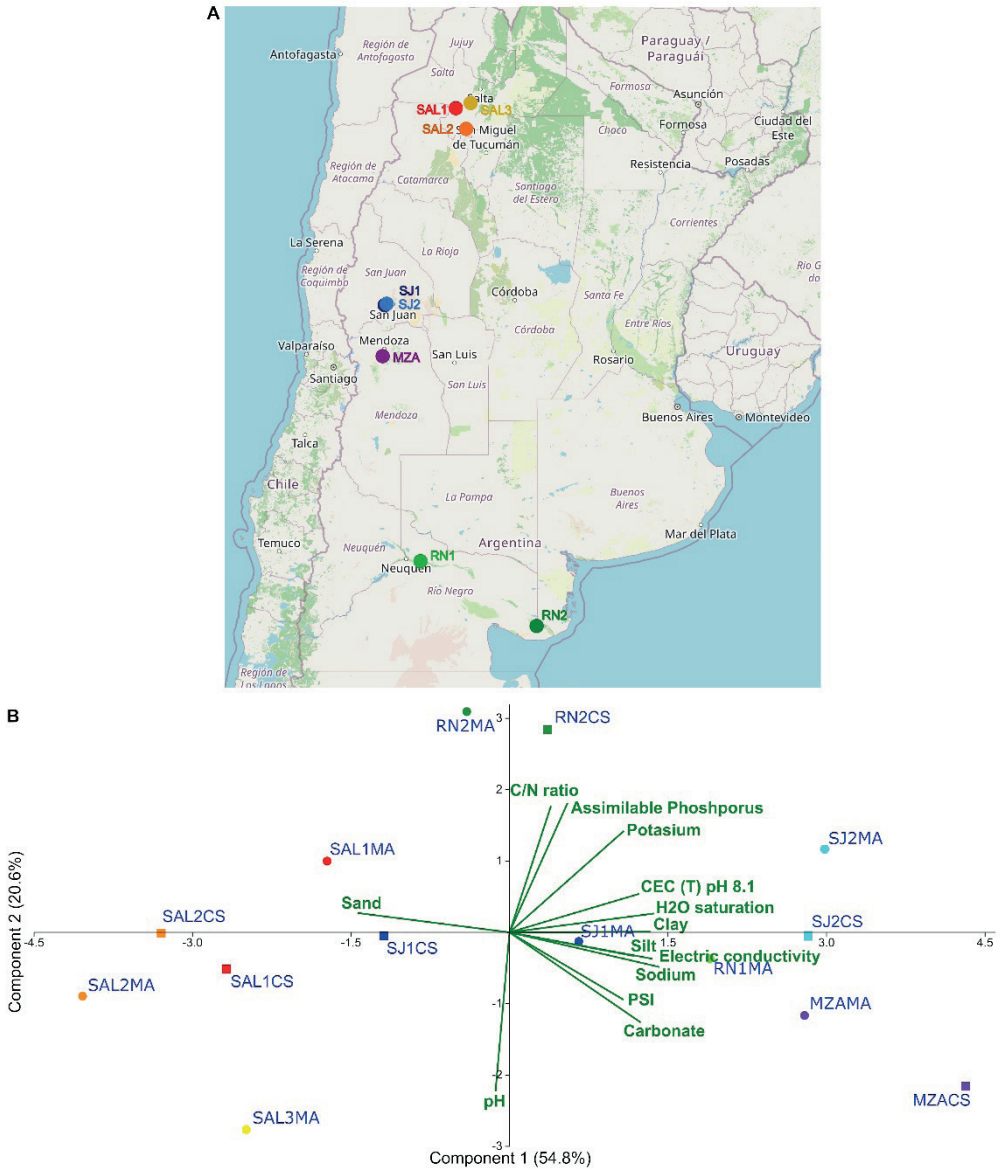


FIGURE 1. Soil physicochemical characteristics of sampled vineyards.

A) Map of Argentina indicating sampled sites. This map was created using OpenStreetMap (openstreetmap.org/copyright). The data is available under the Open Database License. B) PCA analysis of soil physicochemical characteristics. Samples from Río Negro (Green), San Juan (Blue), Mendoza (Purple) and Salta (Red, Orange, and Yellow). Two first dimensions are shown. Dots and squares represent Malbec and Cabernet-Sauvignon cultivars respectively.

Further information regarding location, geographical characteristics, grapevine age, type of crop management (organic or conventional) and inter-row-management (bare ground or presence of other plants) is available in Oyuela Aguilar *et al*. (2021). Soil and root samples were collected from each vineyard at a depth of 30 cm and a distance of 20–30 cm from the vine trunks of nine vines per plot, covering an area of 49 m². Each plot was situated at least 7 m from the edge of the area. Samples were placed in sterile containers, transported on ice, and stored at −20 °C until analysis. The nine samples of each vineyard were combined from which three biological replicates were formed.

2. Soil physicochemical characteristics analysis

Soil samples were obtained by carefully removing soil from the roots sampled in the vineyards. It was rubbed off gently into a bag, using a scalpel when needed. Rhizosphere soil samples were sieved (pore size 0.5 mm) to eliminate the remaining plant debris. Soil samples were analysed at the Centro Nacional de Investigaciones Agropecuarias (CNIA-INTA) (Buenos Aires, Argentina). Samples were processed as previously described by Oyuela Aguilar *et al*. (2020). The pH was determined using the potentiometric method and a 1:2.5 distilled water ratio (ISO 10694-Soil quality determination of pH norms). A strong oxidising microscale mixture was used to assess organic carbon (Walkley & Black, 1934), and the mass loss calcination method and the Kjeldahl method were used to assess organic material (Ball, 1964) and organic nitrogen (Bremner, 1960) respectively. Soil phosphorus content was determined using the Bray & Kurtz method (Bray & Kurtz, 1945). Soil texture was analysed according to Kilmer *et al*. (1949). USDA soil texture diagram was used to determine the soil type in each vineyard, acquired lime, sand and clay content**.**

3. DNA extraction, library preparation and sequencing

DNA extractions were performed using the FastDNA Spin Kit for Soil (MP Biomedicals, LLC, Solon, OH, USA) from 0.4 g of rhizosphere soil following the supplier's instructions. The extracted DNA was quantified using a Qubit1 2.0 Fluorometer (Thermo Scientific). DNA purity at 260/280 nm and 260/230 nm absorbance ratios were assessed using a Nanodrop spectrophotometer.

The bacterial diversity was analysed by amplifying the hypervariable V3-V4 region of the 16S rRNA gene using 341F (CCTACGGGNGGCWGCAG) and 806R (GACTACHVGGGTATCTAATCC) primers. A 16S rRNA gene double PCR-step approach was used for Illumina sequencing library preparations (Gobbi *et al.,* 2020). Sequencing was performed on Illumina's MiSeq platform using the V2 500 cycles reagent kit.

The double amplification was performed as follows. For the initial PCR, a final volume of 25 µL contained a mixture of 12 µL of AccuPrime™ SuperMix II (Thermo Scientific™), 0.5 µL of bovine serum albumin (BSA; final concentration, 0.025 mg/mL), 0.5 µL each of forward and reverse primers (10 µM stock), 1.5 µL of sterile water, and 5 µL of template DNA. This reaction mixture underwent pre-incubation at 95 °C for 2 min, followed by 33 cycles of 95 °C for 15 s, 55 °C for 15 s and 68 °C for 40 s, followed by a final extension at 68 °C for 4 min. Total DNA was quantified using a Qubit® 2.0 fluorometer (Thermo Scientific™).

The second PCR aimed to add indices to each amplicon from the first PCR. A final reaction volume of 28 µL was used, containing 12 µL of AccuPrime™ SuperMix II (Thermo Scientific™), 2 µL of primers with index sequences and Illumina P7 (CAAGCAGAAGACGGCATACGAGAT) and P5 (AATGATACGGCGACCACCGA) adapters, 7 µL of sterile water, and 5 µL of amplicons from the initial PCR. Cycling conditions included denaturation at 98 °C for 1 min, followed by 13 cycles of 98 °C for 10 s, 55 °C for 20 s and 68 °C for 40 s, followed by a final extension at 68 °C for 5 min. In both instances, PCR products were verified via 1.5 % agarose gel electrophoresis.

4. Bioinformatics and statistical analysis

Illumina reads were demultiplexed using bcl2fastq V.2.17.1.14 (Illumina). Adapters were trimmed with Trim Galore v0.4 (https://github.com/FelixKrueger/TrimGalore.git) running cutadapt v1.8.3 (Martin, 2011) and primer sequences were deleted from the 5' ends of each read using the custom script (https://github.com/padbr/asat/blob/master/strip_degen_primer.py). Raw sequencing data associated with this work was uploaded to the SRA under the BioProject accession number PRJNA1087980.

Data analysis was performed using Shaman platform (https://shaman.pasteur.fr/), following the pipeline described by Volant and collaborators (Volant *et al.,* 2020). Read and operational taxonomic unit (OTU) processing parameters were set with strict quality and annotation thresholds to ensure high-fidelity analyses. For read processing, a Phred quality score cutoff of 20 was applied to trim low-quality ends, with a minimum requirement of 80 % correctly called nucleotides per read and a minimum read length of 50 nucleotides. In the OTU processing stage, reads were dereplicated with a prefix setting, no upper limit on OTU length (maximum OTU length set to 0) and a minimum OTU length of 50 nucleotides. Only sequences with a minimum abundance of 4 were retained at dereplication, and clustering was performed on both strands with a 0.97 similarity threshold. For OTU annotation, both strands were used, and identity thresholds were progressively more stringent across taxonomic levels: a minimum of 0.75 for Kingdom, 0.75–0.785 for Phylum, 0.785–0.82 for Class, 0.82–0.865 for Order, 0.865–0.945 for Family, 0.945–0.98 for Genus, and 0.98–100 for Species annotations. These parameters ensured accurate read trimming, clustering and taxonomic assignment throughout the analysis.

Taxonomic annotation of OTUs was performed using SILVA database (Pruesse *et al.,* 2007) version 138. A weighted non-null normalisation by site and a filtering step for a threshold of a minimal number of 8 samples and 2.4 on the total abundance (in log) was perform prior to data analysis. Richness, Shannon and Inverse Simpson indexes, principal

coordinate analysis (PCoA), rarefaction plots, scatterplots, families' barplot and heatmaps were analysed and plotted using the on-line Shaman platform.

Principal component analysis (PCA), clustering analysis using Euclidean similarity index and Canonical Correspondence Analysis (CCA) were performed using Past software (Hammer *et al.,* 2001). Data were normalised by Standard score ((X-μ)/σ) before analysis (Han *et al.,* 2012).

Linear Discriminant Analysis Effect Size (LEfSe) (Chang *et al.,* 2022) was performed to identify overrepresented OTUs in soil samples. Alpha values for the factorial Kruskal-Walli's test among classes and for the pairwise Wilcoxon test between subclasses used were 0.05. Taxonomic units above a logarithmic LDA score of 4 were considered as overrepresented and are shown in the graph. LEfSe was run within the Galaxy Metabiome environment (http://mbac.gmu.edu/mbac_wp).

PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Douglas *et al.,* 2020)–run within the Galaxy Metabiome environment–was used to predict metabolic pathways in 16sRNA NGS sequencing data. The presence of differential pathways was inferred using LEfSe.

Microbial networks were constructed using the SparCC algorithm (Friedman & Alm, 2012) in the Integrated Network Analysis Pipeline 2.0 (iNAP 2.0. https://inap.denglab.org.cn) (Peng *et al.,* 2024). Network graphs were generated with Cytoscape v3.10.3 (Shannon *et al.,* 2003). The SparCC algorithm was employed to infer microbial associations from compositional data. Pseudo p-values were calculated through 100 permutations, with 20 inference iterations averaged per calculation. To reduce the influence of strongly correlated pairs, 10 exclusion iterations were applied, using a correlation strength exclusion threshold of 0.7. This approach ensured robust estimation of microbial associations. A SparCC correlation matrix was generated and filtered based on a threshold of 0.6. Correlation coefficients with absolute values below this threshold were excluded. Additionally, the SparCC pseudo p-value matrix, derived from the 100 permutations, was filtered at a p-value threshold of 0.05 to retain statistically significant correlations. Network modularisation was performed using the Greedy modularity optimisation method, as implemented in the Galaxy tool (Version 1.0.0). The network matrix, pre-filtered after applying correlation and p-value cutoffs, was analysed to identify modular structures and key hub nodes. The modularity analysis also incorporated Z-P scores to classify module hubs and assess their significance within the microbial association network.

The figures were created using the Inkscape (Inkscape Project, 2020. https://inkscape.org) and GIMP (The GIMP Development Team, 2019. https://www.gimp.org) software packages.

RESULTS

1. Sample collection and soil characterization

Soil physicochemical properties were determined for the fourteen sampled sites (Table S1). Like in any edaphic study on soils, and as a starting point for visualising any variation in the soil composition of different sites, we performed a principal component analysis (PCA). This two-dimensional PCA plot (Figure 1B) clearly distinguished the samples, showing a clear difference between locations. Component 1 differentiates SAL soils from the rest of the sampled sites, while Component 2 discriminates RN2. This analysis showed a clear grouping of SAL and RN2, while samples from the western region (SJ and MZA) are dispersed in quadrants 1 and 4. In contrast to RN2, RN1 is positioned between SJ and MZA, showing a clear distinction between the Patagonian samples.

2. Sequencing results

After performing the 16S rRNA sequencing, we obtained a total of 903,462 amplicons, with a remaining 668,603 after dereplication. Followed by the sequence removal of singletons and chimeras, 14,469 sequences remained for classification into OTUs, resulting in a total of 2,945. Further description of the sequenced samples can be found in Table S2. Although three composite samples were collected for each vine, one sample from the SAL3MA set could not be successfully sequenced (Table 1). Additionally, because the rarefaction curve for sample run.MO.A31_S31_L001.fastq - which belonged to one sequence from the SJ2CS - did not reach saturation, it was excluded from the results (Figure S1), leaving a total of 40 sequences for the final analysis. Samples were normalised per site prior to the statistical analysis.

3. Diversity

Three estimators of α-diversity were used: species (OTUs) richness, Shannon diversity index and the inverse Simpson index. As there were no observed differences between MA and CS indexes (Figure S2), we decided to pool samples in terms of diversity according to the sites.

To evaluate the effect of location on rhizosphere microbial composition, diversity indexes were plotted accordingly (Figure 2). Although the species richness comparison using the non-parametric Kruskal-Walli's test showed no significant differences in bacterial communities, pairwise-comparisons through a post hoc Mann-Whitney Bonferroni adjusted test showed differences for RN1 with respect to SAL1 and RN2 (Figure 2A). While global comparison given by the Shannon index of Kruskal-Walli's test showed no significant differences, pair to pair comparisons showed statistically significant differences between RN1 with respect to SJ1, SJ2, MZA, SAL1 and RN2 (Figure 2B). Similar results were obtained for the inverse Simpson index (Figure 2C).

A PCoA was performed to investigate clustering according to regions (Figure 3 and Figure S3A). We found a clear distinction between SAL and the rest of the regions. Samples from MZA, SJ and RN form a group that behaves as a

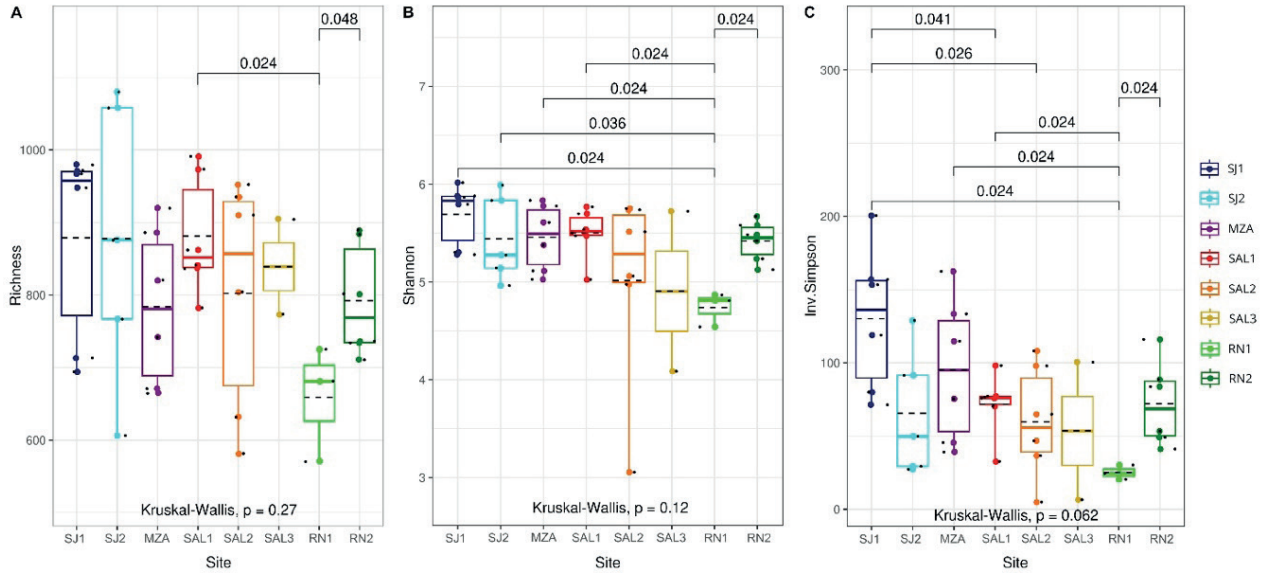


FIGURE 2. Boxplot of microbial species Richness (S), Shannon (H') and Inverse Simpson (1/D) Index values.

Boxplots (A), (B) and (C) represent the prokaryotic species α-diversity indexes. The statistical analysis was performed using a Kruskal Walli's test. The identified pairwise-comparisons results were obtained through a post hoc Mann–Whitney Bonferroni adjusted test.

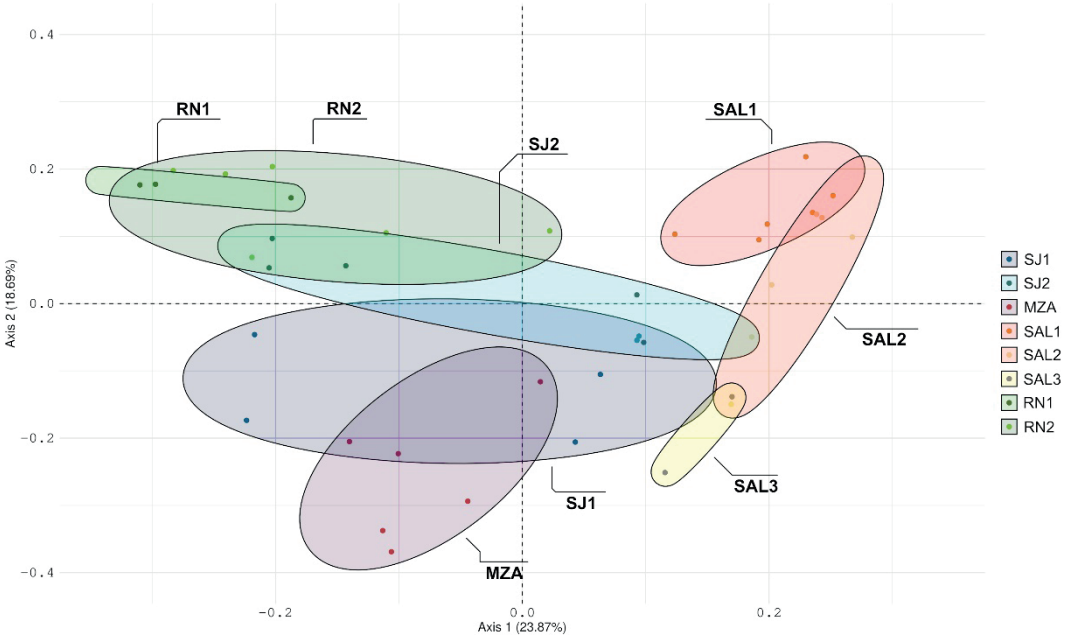


FIGURE 3. PCoA of prokaryotic OTUs.

PCoA summarizes the differences between rhizospheric bacterial 16S rRNA gene sequence analysed. The two first axis are shown. Permanova test was performed for the analysis of variance using distance matrices. p-value = 0.001.

gradient across Axis 2, with positive values for RN1 and RN2 and negatives for SJ1 and MZA, while SJ2 remained in intermediate values. No differences were found for MA and CS (Figure S3B).

4. Taxonomic identification of 16S rRNA sequencing results

As previously mentioned, 2,945 OTUs were obtained after sequencing. All these OTUs were annotated using SILVA (Pruesse *et al.,* 2007) at the Kingdom level, of which 2,934 (99.63 %) were assigned to the phylum level, 2,864 (97.25 %) to the class level, 2,584 (87.74 %) to the order level, 2,113 (71.54 %) to the family level and 1,273 (43.23 %) to the genus level. Only 514 (17.45 %) of the OTUs could be assigned to the species level.

Samples from the different sites were compared to find the most abundant soil microbiota at family level (Figure 4A). Two prokaryotes domains are shown in our results. Regarding the Bacteria Domain, the most abundant Gram-negative microorganisms belonged to the *Microscillaceae, Chitinophagaceae, Xanthobacteraceae, Pseudomonadaceae, Sphingomonadaceae, Rhizobiaceae, Vicinamibacteraceae, Steroidobacteraceae, Pedosphaeraceae*,

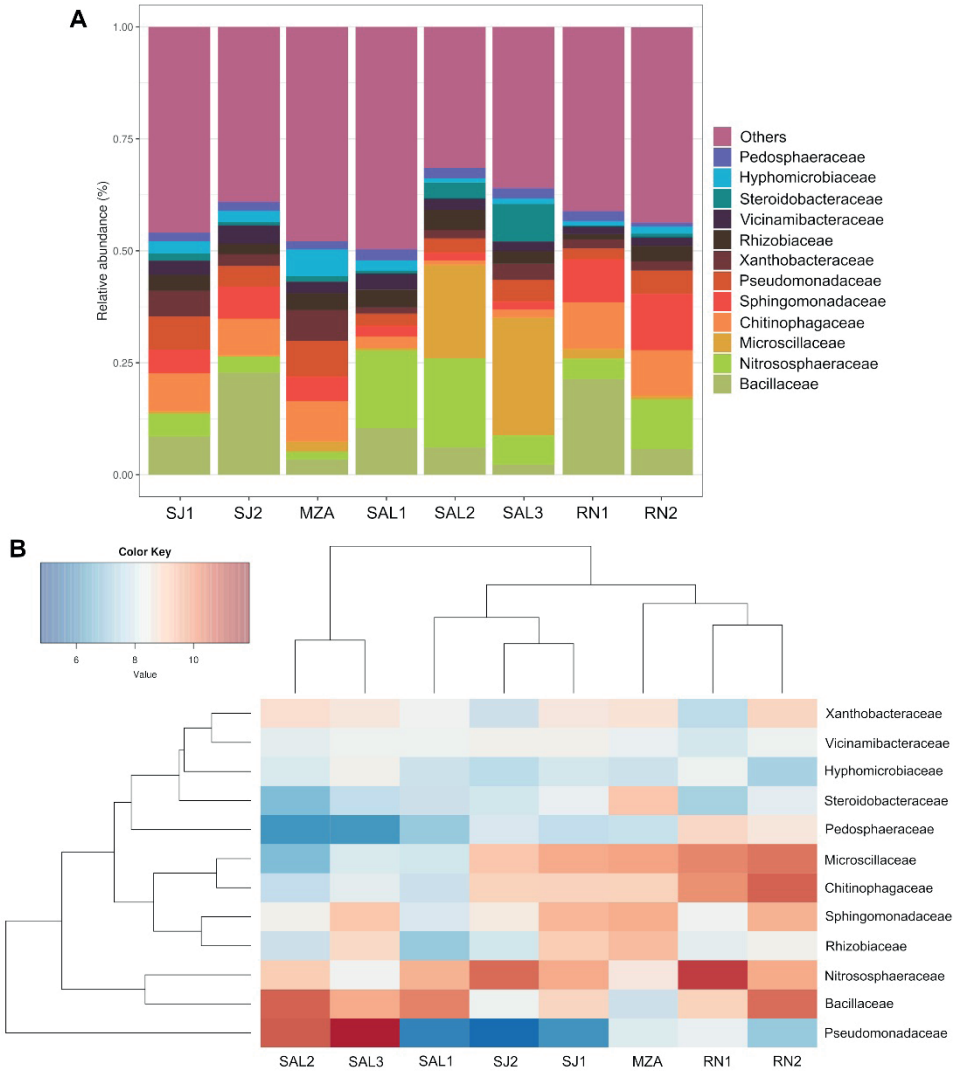


FIGURE 4. Taxonomic analysis of soil samples.

A- Distribution of the bacterial families identified by 16S rRNA gene sequencing in the site sampled. Families above 1% are shown individually. B- Heatmap for differential families of bacteria identified by 16S rRNA barcoding.

and *Hyphomicrobiaceae* families, while the *Bacillaceae* family was the most frequently encountered Gram-positive bacteria. Regarding Archaea, the most abundant family was *Nitrososphaeraceae*.

The *Pseudomonadaceae* and *Bacillaceae* families were the most abundant clades in SAL2 and SAL3 (Figure 4B), forming a taxonomical cluster that could be clearly distinguished from the rest of the sites. In contrast, although *Bacillaceae* was highly represented in SAL1 and RN2, *Pseudomonadaceae* levels were low in these locations.

Even though it was possible to determine the main families present in each sampled site, this approach was not useful for determining the organisms differentially found in each soil. To assess any significant shifts in soil microbiological composition, the LEfSe differential abundance method was used to perform class comparisons among samples from different locations (Segata *et al.,* 2011).

Although 256 differential OTUs were determined, only the most significantly shifted (LDA > 4.0) were analysed (Table 2 and Figure S4). Surprisingly, all significantly shifted organisms in RN2 belonged to kingdom Archaea, four of them corresponding to phylum *Parvarchaeota*. All remaining differential OTUs were assigned to organisms of the kingdom Bacteria. By contrast, RN1 is enriched in *Deltaproteobacterias* of order *Caldilineales* and family *Cystobacteraceae*, *Gammaproteobacterias* of order *Pseudomonadales* and Gram-positive bacteria of family *Paenibacillaceae*.

SAL1 showed a prevalence of individuals of the phylum *Firmicutes*. In addition to *Bacillus badius* (also of phylum *Firmicutes*), SAL3 showed actinobacteria of the family *Microccocaceae* and a particularly well-represented OTU, which could only be assigned to the kingdom Bacteria. No significantly differential OTUs were identified in SAL2.

Regarding the Cuyo region, SJ1 was found to be enriched in bacteria of the order *Chromatinales*, the genus *Chthoniobacter* and the families *Rubrobacteraceae* and *Chitinophageceae*. SJ2 was enriched in *Deltaproteobacteria*, mostly of the order

TABLE 2. OTUs identified by LEfSe.

Site	OTU taxonomic assignment	LDA score (log10)
SJ1	o_Chromatiales	4.34
	f_Chromatiaceae	4.33
	f_Rubrobacteraceae	4.32
	f_Chromatiaceae	4.32
	g_*Chthoniobacter*	4.05
	g_*Chthoniobacter*	4.05
	f_Chitinophagaceae	4.05
SJ2	p_Proteobacteria	5.13
	c_Deltaproteobacteria	4.75
	o_Desulfuromonadales	4.60
	f_Pelobacteraceae	4.60
	f_Pelobacteraceae	4.59
	o_Rhodocyclales	4.11
	f_Rhodocyclaceae	4.11
MZA	Bacteria.Chloroflexi	4.51
	o_Rhizobiales	4.46
	c_Betaproteobacteria	4.31
	g_*OR_59*	4.17
	g_*OR_59*	4.17
	Bacteria.Chloroflexi.	4.14
	f_Phyllobacteriaceae	4.06
	s_*Bacillus endophyticus*	4.04
	f_Phyllobacteriaceae	4.03
SAL1	p_Firmicutes	4.79
	c_Bacilli	4.75
	o_Bacillales	4.74
	f_*Bacillaceae*	4.63
	g_*Bacillus*	4.61
	s_*Bacillus flexus*	4.56
	f_Planococcaceae	4.10
	f_Caulobacteraceae	4.04
	f_Caulobacterales	4.04
	s_*Sporosarcina spp.*	4.00
	s_*Sporosarcina spp.*	4.00
SAL3	k_Bacteria	5.40
	f_Micrococcaceae	4.27
	g_*Arthrobacter*	4.27
	s_*Arthrobacter crystallopoietes*	4.24
	s_*Bacillus badius*	4.15
RN1	o_Myxococcales	4.29
	o_Pseudomonadales	4.18
	c_SC3	4.16
	f_Cystobacteraceae	4.16
	f_Caldilineaceae	4.14
	c_SC3	4.14
	f_Cystobacteraceae	4.13
	p_*TM7*	4.11
	f_Caldilineales	4.09
	g_*Ammoniphilus*	4.04
	g_*Ammoniphilus.*	4.04
	f_Streptosporangiaceae	4.03
	f_*Paenibacillaceae*	4.02
RN2	k_Archaea	4.18
	g_*WCHD3_30*	4.08
	c_Parvarchaea	4.08
	g_*WCHD3_30*	4.08
	p_Parvarchaeota	4.08

Desulfomonadales, and *Betaproteobacteria* of the order *Rhodocyclales*. On the other hand, MZA showed a prevalence of individual bacteria of the phylum *Chloroflexi*, the order *Rhizobiales* and the family *Chthoniobacteraceae,* as well as Gram-positive bacteria, such as *Bacillus endophyticus*.

PICRUSt2 analysis was carried out to examine any metabolic pathways that were overrepresented in particular locations (Table S3). Pathways that were enriched in SJ2 included PWY_3781, P108_PWY, PWY_6167, PWY_7332 and PWY_6122. SAL3 showed enrichment in pathways such as PWY_7237, PWY_7094, the_ortho_cleavage_PWY, PWY0_1061, HOMOSER_METSYN_PWY, ORNDEG_PWY, PWY_5022, and PWY_6628. Meanwhile, SAL2 was enriched in PWY_1861 and PWY_2941, SAL1 in PWY_6895, RN1 in THISYN_PWY, DENOVOPURINE2_PWY, FASYN_INITIAL_PWY, and P23_PWY, and MZA in PWY-5971.

Interestingly, SJ2 and SAL3 showed an increase in polyamine biosynthesis (P108_PWY and PWY_6628 respectively). Such compounds (e.g., putrescine and spermidine) significantly influence plants by acting as signalling and modulatory molecules in growth and stress responses (Dunn & Becerra-Rivera, 2023). Furthermore, SAL3 showed pathways involved in the degradation of aromatic compounds through meta-cleavage, phenylacetate degradation and ortho cleavage (PWY_7237, PWY_7094, and the_ortho_cleavage_PWY), facilitating the breakdown of complex compounds.

5. Microbial network analysis

Network analysis can identify disproportionately influential species, or keystones, within communities that contribute to the system's resilience to external disturbances. A SparCC-based network was therefore constructed to explore the interactions between the OTUs in the analysed samples. This network was compared to random networks generated using Greedy modularity optimisation and Short random walks. In the comparison of the empirical network with both randomised networks, the values of the Clustering Coefficient, Average Path Distance, Geodesic Efficiency, Centralisation of Stress Centrality, Modularity, Transitivity, Hierarchy and Lubness validated the network generated with our data

(Table S4). The empirical network has a significantly higher clustering coefficient than the random networks and larger average distances between nodes. Nodes in the empirical network show high stress centrality, implying that certain species are crucial for connectivity and may act as "hotspots" within the network. The higher transitivity observed in the empirical network suggests a greater tendency for a node's neighbours to also be connected to each other, which is typical in organised networks and less common in random networks.

The obtained network consisted of 214 nodes (OTUs) grouped into 13 modules (Figure 5A): module 1 with 55 nodes, module 2 with 36 nodes, module 3 with 41 nodes, module 4 with 13 nodes, module 5 with 25 nodes, module 6 with 9 nodes, module 7 with 15 nodes, module 8 with 9 nodes, modules 9 and 10 with 4 nodes each, and modules 11, 12, and 13 with 2 nodes each. The five most abundant modules (1, 2, 3, 5 and 7) represented 80.4 % of the total nodes in the network. These 214 OTUs were assigned to 53 families, with *Cytophagaceae, Chitinophagaceae, Bacillaceae* and *Hyphomicrobiaceae* representing 35.5 % of the total taxa. OTU_003 (family *Cytophagaceae*) was highlighted for its centrality, as it holds the highest values for degree, stress, eigenvector and closeness centrality (Table S5). Z-P analysis showed that the network is composed of 195 peripheral species, 5 module hubs, 13 connector hubs, and only one network hub corresponding to OTU_145 (class *Acidobacteria*) (Figure 5B and Figure S5A).

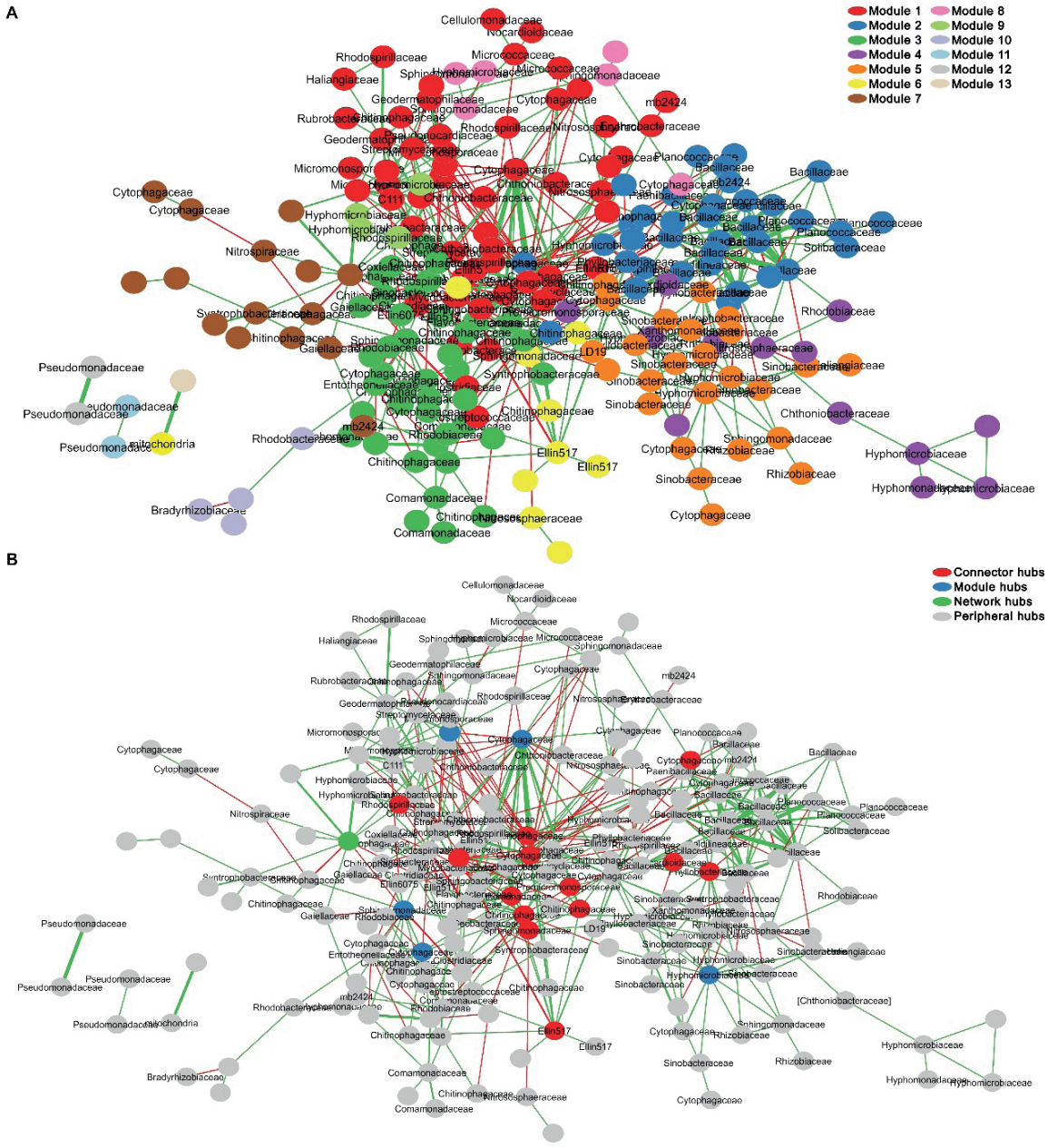


FIGURE 5. SparCC OTUs' network.

A. Colours show modules that comprise the OTUs' network. B. Colour code indicates connector, module, network and peripheral hubs.

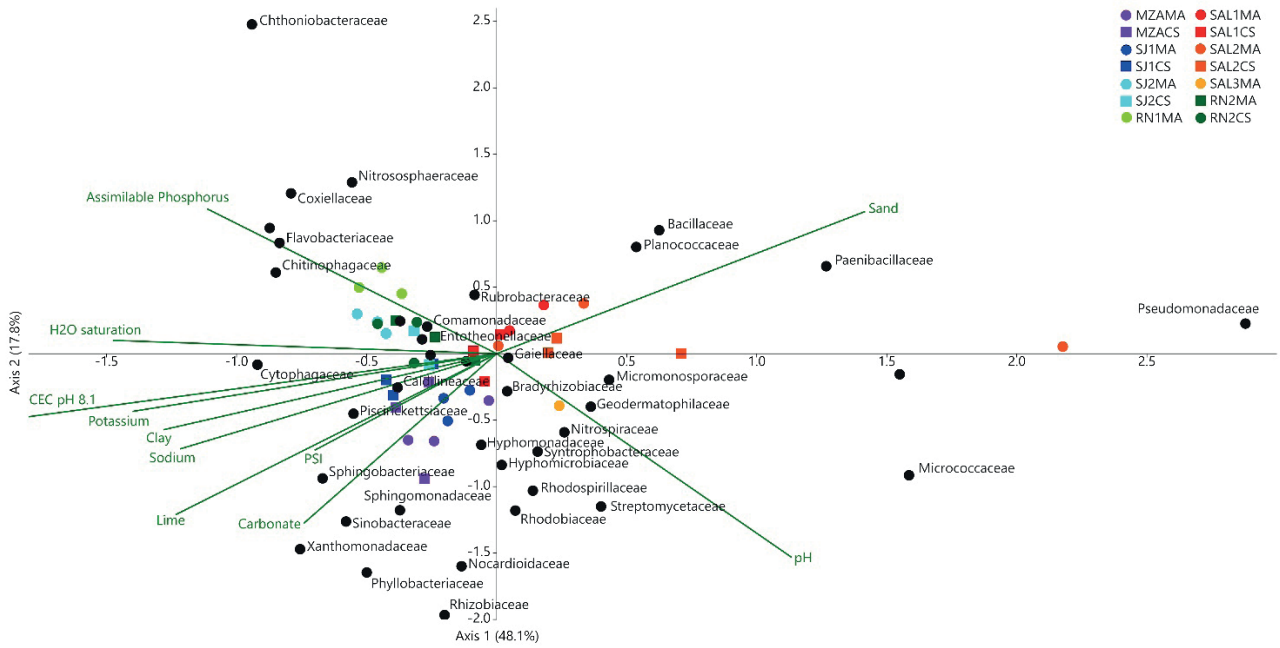


FIGURE 6. Canonical Correspondence Analysis (CCA) of bacterial communities.

CCA analysis of the most abundant bacterial families and their relationship with the physicochemical features of soil. Only families representing more than 0.5% of total counts are shown. Dots and squares represent Malbec and Cabernet-Sauvignon cultivars respectively. Perm (n = 999) Trace = 0.6385, p = 0.001.

6. The soil physicochemical characteristics influences its microbiota

The results of various metagenomics studies have supported the connection between the grape microbiome and factors such as vineyard location, climatic conditions, and other vineyard-related variables (Mezzasalma *et al.,* 2018; Oyuela Aguilar *et al.,* 2020; Zarraonaindia *et al.,* 2015). To assess the possible existence of patterns linking the physicochemical characteristics of the soil and the families of bacteria found, a canonical correspondence analysis (CCA) was performed (Figure 6). Quadrant 1 is characterised by high sand contents, with a high number of bacteria from families such as *Pseudomonadaceae, Bacillaceae* and *Paenibacillaceae*. The opposite quadrant (Quadrant 3) is characterised by soils rich in lime, clay, sodium, potassium and carbonate and in bacteria such as *Rhizobiaceae, Sphingomonadaceae* and *Xanthomonadaceae*, among others.

This therefore indicates a clear positive correlation between the families of Gram-positive bacteria *Bacillaceae* and *Paenibacillaceae* and soils with high sand content (Figure S6A and S6B). Consistent with the results of the CCA, these Gram-positive bacterial families negatively correlated with *Xanthomonadaceae* (Figure S6C and S6D) and *Steroidobacteraceae* (Figure S6E and S6F). Thus, the results regarding the *Xanthomonadaceae* and *Steroidobacteraceae* families are related to the amount of clay in the soil (Figure S6G and S6H), reinforcing the hypothesis that a soil's physicochemical characteristics condition its microbiota.

DISCUSSION

Viticulture is a crucial part of Argentina's socioeconomic landscape, evidenced by its substantial wine market and tourism industry (Straffelini *et al.,* 2023). Nonetheless, the sector faces pressing challenges due to climate change and associated extreme weather occurrences. Argentina's vineyards span a lattitude of 22° to 45° south, being primarily situated along the foothills of the Andes Mountain range. Ninety-two percent of the country's vineyards are notably dedicated to grape varieties suitable for wine and must production. While Mendoza's MA has long been recognised as an iconic Argentine wine, several other regions have also gained prominence for their high-quality soils and the distinctive characteristics of their wines. Topography, climatic conditions and soil features are considered factors that define the microbial communities associated with vines. Together, these physicochemical and biological factors contribute to the development of organoleptic properties of wine produced in each location, defining the regional terroir (Belda *et al.,* 2017; Remenyik *et al.,* 2024; Zarraonaindia *et al.,* 2015). Being a large, geographically and climatologically diverse country, every region in Argentina has its own set of features that imparts special characteristics to its wines. In the present work we studied the prokaryotic microbiota associated with soils in vineyards of four Argentinian wine-producing provinces: Mendoza, San Juan, Salta and Río Negro.

When soil rhizosphere microbiota was analysed through 16S sequences, we observed that the north western samples (SAL1, SAL2 and SAL3) were clearly differentiated from the rest of the sites, probably due to their high sand and low sodium contents, which is distinctive of these soils. In addition, the genus *Bacillus* was overrepresented in the north

western region and clearly correlated with the sand content. The study of α-diversity revealed that RN1 had significantly lower Richness, Shannon and Inverse Simpson index values, which, while clearly a particular feature of this location, could not justify, whether physicochemically or climatologically, the diversity patterns present here. LEfSe analysis allowed us to identify the taxonomical signature of each site. The most distinctive profile was the prevalence of Archaea as a feature that distinguishes RN2 from the rest of the sites, which could be related to its closer proximity to the sea. Compared to the work of Remenyik *et al*. (2024), we identified a higher number of differential taxa in each studied site, which may be due to the fact that our approach took into account vineyards in different regions (Remenyik *et al.,* 2024).

To study the relationships between OTUs, SparCC-based networks were constructed and validated by comparison with stochastic associations. The obtained network has a strong tendency to form densely connected groups or communities. The geodesic efficiency of the empirical network is lower than that of random networks, indicating that the exchange of information or interactions within the network is more localised. Nodes in the empirical network show high stress centrality, which may mean that certain species are crucial for connectivity and act as "hotspots" within the network. However, the entire network is centred around a particular node: OTU_003 (family *Cytophagaceae*, Table S5). Such an OTU could represent a keystone species, which are highly influential and significant members of the microbial community. These species play a pivotal role in preserving the community's structure and function, having a greater impact on the ecosystem than other taxa (Banerjee *et al.,* 2018; Röttjers & Faust, 2019).

Regarding the soil's physicochemical properties, the sand and clay content proved to be a key factor shaping the microbiota. A subset of bacterial families, such as *Bacillaceae* and *Paenibacillaceae,* is prone to growing in sandy soils, while *Xanthomonadaceae* and *Steroidobacteraceae* families, among others, are more likely to be found in clay-rich soils. The correlation between *Bacillus* and sand has already been observed: in a study on the influence of abiotic factors on the abundance of microorganism populations in soil, Santos *et al.* (2022) showed that sand content positively correlated with *Bacillus thuringiensis* (Santos *et al.,* 2022); similarly, Vu *et al*. (2022) found that, in one of the studied soils, the genus *Bacillus* (among others) was well-adapted to sand dune ecosystems and could colonise the roots of various plant species growing in different sand dune areas (Vu *et al.,* 2022). Therefore, the negative correlation between sand- and clay-trophic organisms conditions the soil's microbiota, thereby defining the regional terroir.

The overrepresentation of beneficial bacteria, such as *Bacillaceae*, *Pseudomonadaceae* and *Rhizobiales*, may have positive effects on grapevine health and wine production. Several species within the genus *Bacillus* have been extensively characterised as beneficial bacteria, functioning as biofertilizers due to their ability to transform plant-unavailable nutrient forms into plant-available ones (commonly referred to as plant growth-promoting rhizobacteria, PGPR). They also suppress the development of plant pathogens and help plants resist both abiotic and biotic stresses, among other benefits (Borriss, 2020; Hashem *et al.,* 2019; Wang *et al.,* 2021). Consistent with this, the network analysis showed that OTUs assigned to the *Bacillaceae* and *Paenibacillaceae* families were closely related, with all these OTUs being part of Module 3 (Figure S5B), supporting the hypothesis that certain environmental conditions influence the presence of these families. Moreover, *Bacillus* was identified as a shared microbial taxon, forming part of a "global core microbiome" that has microbial signatures common to vineyard soils, irrespective of geographical and environmental variability (Gobbi *et al.,* 2022).

Previous work from our group isolated fourteen *Bacillus spp*. strains: seven from SAL2, two from MZA, four from SJ2 and one from SJ1. These strains were capable of inhibiting phytopathogenic fungi, such as *Botrytis cinerea, Alternaria alternata,* Fusarium *oxysporum, Fusarium gramineraum* or *Penicillium oxalicum,* and of solubilising phosphorus due to their being siderophore producers, and, together with *Paenibacillus AMCV14* isolated from SAL, they showed protease, amylase and pectinase activity (Oyuela Aguilar *et al.,* 2021). The fact that this genus was differentially found in such locations in the present study is consistent with the frequency of *Bacillus spp*. isolations in the SAL region found by Oyuela Aguilar *et al*. (2021). In addition to the *Bacillaceae* family, RN1 was found to have a high number of a strain of the family *Paenibacillaceae. Pseudomonadaceae* was also found in SAL2 and SAL3, but its presence was not relevant in SAL3 and RN2. Meanwhile, the bacteria characteristic of MZA comprised strains of the *Rhizobiales* order. Together with *Bacillus*, species of the genus *Paenibacillus, Pseudomonas* and *Rhizobium* have also been characterised as PGPRs and are able to inhibit plant pathogen growth (Dorjey *et al.,* 2017; Goswami *et al.,* 2016; Hussain *et al.,* 2020; Shailendra Singh, 2015; Wang *et al.,* 2021). Our research group isolated 3 PGPR strains of *Rhizobium spp*–two from RN1 and one from SAL2–and 59 strains of *Pseudomonas spp*. capable of solubilising phosphate and producing siderophores, and which showed protease, amylase, cellulase and/or pectinase activity (Oyuela Aguilar *et al.,* 2021). The high proportion of *Pseudomonas spp*. isolated by culture techniques can be explained by the ubiquitous presence of this genus (despite the region analysed 16S barcoding), which is characteristic of this bacterial group (Compant *et al.,* 2011; David *et al.,* 2018; Lyng & Kovács, 2023).

The findings reported here can be used to help develop agricultural practices that reduce reliance on chemical inputs while promoting greater efficiency and sustainability, thus improving plant health and soil quality (de Andrade *et al.,* 2023). By inducing systemic resistance, rhizobacteria can activate biological or chemical responses in host plants, enhancing their protection from pathogens (Vacheron *et al.,* 2013). This protection is further supported by competition for space and the secretion of lytic enzymes, such as chitinases, glucanases and proteases,

which are the primary hydrolytic enzymes that degrade fungal cell walls (Tariq *et al.,* 2017). Given the numerous benefits that bacteria confer to plants, inoculation with PGPRs has become increasingly popular to improve crop productivity and is now widely recognised as an essential tool for managing plant diseases within the framework of sustainable agriculture (Mohanty *et al.,* 2021). In the light of climate change and the increasing prevalence of crop diseases, modern viticulture faces significant challenges: maintaining the high productivity, quality and environmental sustainability of its products, while ensuring the safety of consumers and workers alike. There is thus a demand for sustainable strategies to reduce fungicide usage and mitigate soil health risks, like copper accumulation. (Pii *et al.,* 2024). Understanding how microbial communities are established and exploring strategies to engineer them represents a promising and forward-thinking approach to optimising the plant microbiome for desired functions.

CONCLUSIONS

This study provides valuable insights into the prokaryotic diversity of Argentine vineyard soils, with a particular focus on the rhizosphere microbiota of two grapevine cultivars, MA and CS, across four key wine-producing provinces: Mendoza, San Juan, Río Negro, and Salta. The physicochemical characteristics of the soils, including the sand-to-clay ratio and concentrations of sodium, potassium and carbonates, play a pivotal role in defining regional groups and shaping microbial diversity. Taxonomic assignment and LEfSe analysis identified a unique taxonomic signature for each site, highlighting the prevalence of specific microbial families associated with particular regions. These patterns likely reflect variations in environmental factors, soil properties and vineyard management practices. While the microbial communities associated with the different grapevine regions displayed distinct compositions, a subset of beneficial bacteria–including *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Rhizobium*–was consistently identified across all studied locations. These bacteria, recognised as plant growth-promoting rhizobacteria (PGPR), may enhance nutrient availability, confer pathogen resistance and promote overall vine health. The findings underscore the potential of microbial communities to support grapevine health and contribute to wine quality. This comprehensive analysis sheds light on the intricate relationship between soil characteristics, microbial diversity and the concept of terroir in Argentine vineyards. Understanding these interactions is essential for optimising vineyard management practices and ultimately influencing the organoleptic properties of wines produced in different regions.

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REFERENCES

Bacon, C. W., & White, J. F. (2016). Functions, mechanisms and regulation of endophytic and epiphytic microbial communities of plants. *Symbiosis*, *68*(1–3), 87–98. https://doi.org/10.1007/s13199-015-0350-2

Ball, D. F. (1964). Loss□On□Ignition as An Estimate of Organic Matter and Organic Carbon in Non□Calcareous Soils. *Journal of Soil Science*, *15*(1), 84–92. https://doi.org/10.1111/j.1365-2389.1964.tb00247.x

Banerjee, S., Schlaeppi, K., & van der Heijden, M. G. A. (2018). Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology*, *16*(9), 567–576. https://doi.org/10.1038/s41579-018-0024-1

Belda, I., Navascués, E., Marquina, D., Santos, A., Calderón, F., & Benito, S. (2016). Outlining the influence of non-conventional yeasts in wine ageing over lees. *Yeast (Chichester, England)*, *33*(7), 329–338. https://doi.org/10.1002/yea.3165

Belda, I., Zarraonaindia, I., Perisin, M., Palacios, A., & Acedo, A. (2017). From Vineyard Soil to Wine Fermentation: Microbiome Approximations to Explain the "terroir" Concept. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00821

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome*, *8*(1), 103. https://doi.org/10.1186/s40168-020-00875-0

Bokulich, N. A., Collins, T. S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S. E., & Mills, D. A. (2016). Associations among Wine Grape Microbiome, Metabolome, and Fermentation Behavior Suggest Microbial Contribution to Regional Wine Characteristics. *MBio*, *7*(3), e00631-16. https://doi.org/10.1128/mBio.00631-16

Borneman, A. R., Schmidt, S. A., & Pretorius, I. S. (2013). At the cutting-edge of grape and wine biotechnology. *Trends in Genetics*, *29*(4), 263–271. https://doi.org/10.1016/j.tig.2012.10.014

Borriss, R. (2020). *Bacillus*. *Beneficial Microbes in Agro-Ecology: Bacteria and Fungi*, 107–132. https://doi.org/10.1016/B978-0-12-823414-3.00007-1

Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, *59*(1), 39–46. https://doi.org/10.1097/00010694-194501000-00006

Bremner, J. M. (1960). Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science*, *55*(1), 11–33. https://doi.org/10.1017/S0021859600021572

Burns, K. N., Kluepfel, D. A., Strauss, S. L., Bokulich, N. A., Cantu, D., Steenwerth, K. L., 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*, *91*, 337–348. https://doi.org/10.1016/j.soilbio.2016.09.007

Chang, F., He, S., & Dang, C. (2022). Assisted Selection of Biomarkers by Linear Discriminant Analysis Effect Size (LEfSe) in Microbiome Data. *Journal of Visualized Experiments*, *183*. https://doi.org/10.3791/61715

Compant, S., Mitter, B., Colli-Mull, J. G., Gangl, H., & Sessitsch, A. (2011). Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb Ecol*, *62*(1), 188–197. https://doi.org/10.1007/s00248-011-9883-y

Darriaut, R., Antonielli, L., Martins, G., Ballestra, P., Vivin, P., Marguerit, E., Mitter, B., Masneuf-Pomarède, I., Compant, S., Ollat, N., & Lauvergeat, V. (2022). Soil composition and rootstock genotype drive the root associated microbial communities in young grapevines. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.1031064

David, B. V., Chandrasehar, G., & Selvam, P. N. (2018). *Pseudomonas fluorescens*: A Plant-Growth-Promoting Rhizobacterium (PGPR) With Potential Role in Biocontrol of Pests of Crops. In *Crop Improvement Through Microbial Biotechnology* (pp. 221–243). Elsevier. https://doi.org/10.1016/B978-0-444-63987-5.00010-4

de Andrade, L. A., Santos, C. H. B., Frezarin, E. T., Sales, L. R., & Rigobelo, E. C. (2023). Plant Growth-Promoting Rhizobacteria for Sustainable Agricultural Production. *Microorganisms*, *11*(4), 1088. https://doi.org/10.3390/microorganisms11041088

Dorjey, S., Dolkar, D., & Sharma, R. (2017). Plant Growth Promoting Rhizobacteria Pseudomonas: A Review. *International Journal of Current Microbiology and Applied Sciences*, *6*(7), 1335–1344. https://doi.org/10.20546/ijcmas.2017.607.160

Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C., & Langille, M. G. I. (2020). PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology*, *38*(6), 685–688. https://doi.org/10.1038/s41587-020-0548-6

Dunn, M. F., & Becerra-Rivera, V. A. (2023). The Biosynthesis and Functions of Polyamines in the Interaction of Plant Growth-Promoting Rhizobacteria with Plants. *Plants*, *12*(14), 2671. https://doi.org/10.3390/plants12142671

Friedman, J., & Alm, E. J. (2012). Inferring Correlation Networks from Genomic Survey Data. *PLoS Computational Biology*, *8*(9), e1002687. https://doi.org/10.1371/journal.pcbi.1002687

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*(1), 241. https://doi.org/10.1038/s42003-022-03202-5

Gobbi, A., Kyrkou, I., Filippi, E., Ellegaard-Jensen, L., & Hansen, L. H. (2020). Seasonal epiphytic microbial dynamics on grapevine leaves under biocontrol and copper fungicide treatments. *Scientific Reports*, *10*(1), 681. https://doi.org/10.1038/s41598-019-56741-z

Goswami, D., Dhandhukia, P., & Thakker, J. N. (2016). Expanding the Horizons for the Use of Paeni*bacillus* Species as PGPR for Sustainable Agriculture. In *Bacilli and Agrobiotechnology* (pp. 281–307). Springer International Publishing. https://doi.org/10.1007/978-3-319-44409-3_12

Griggs, R. G., Steenwerth, K. L., Mills, D. A., Cantu, D., & Bokulich, N. A. (2021). Sources and Assembly of Microbial Communities in Vineyards as a Functional Component of Winegrowing. *Frontiers in Microbiology*, *12*(April). https://doi.org/10.3389/fmicb.2021.673810

Hammer, Ø., Harper, D., & Ryan, P. (2001). Past: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica, 4*(1), 5–7. https://palaeo-electronica.org/2001_1/past/issue1_01.htm

Han, J., Kamber, M., & Pei, J. (2012). Data Preprocessing. In *Data Mining* (pp. 83–124). Elsevier. https://doi.org/10.1016/B978-0-12-381479-1.00003-4

Hashem, A., Tabassum, B., & Fathi Abd_Allah, E. (2019). *Bacillus* subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, *26*(6), 1291–1297. https://doi.org/10.1016/j.sjbs.2019.05.004

Hussain, A., Ahmad, M., Nafees, M., Iqbal, Z., Luqman, M., Jamil, M., Maqsood, A., Mora-Poblete, F., Ahmar, S., Chen, J.-T., Alyemeni, M. N., & Ahmad, P. (2020). Plant-growth-promoting *Bacillus* and Paeni*bacillus* species improve the nutritional status of Triticum aestivum L. *PLOS ONE*, *15*(12), e0241130. https://doi.org/10.1371/journal.pone.0241130

Kilmer, V. J., & Alexander, L. T. (1949). Methods of making mechanical analyses of soils. *Soil Science*, *68*(1), 15–24. https://doi.org/10.1097/00010694-194907000-00003

Knight, S., Klaere, S., Fedrizzi, B., & Goddard, M. R. (2015). Regional microbial signatures positively correlate with differential wine phenotypes: evidence for a microbial aspect to terroir. *Scientific Reports*, *5*, 14233. Https://doi.org/10.1038/srep14233

Kogel, K.-H., Franken, P., & Hückelhoven, R. (2006). Endophyte or parasite – what decides? *Current Opinion in Plant Biology*, *9*(4), 358–363. Https://doi.org/http://dx.doi.org/10.1016/j.pbi.2006.05.001

Liu, D., 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*. Https://doi.org/10.3389/fmicb.2019.02679

Lyng, M., & Kovács, Á. T. (2023). Frenemies of the soil: *Bacillus* and *Pseudomonas* interspecies interactions. *Trends in Microbiology*, *31*(8), 845–857. https://doi.org/10.1016/j.tim.2023.02.003

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

Mezzasalma, V., Sandionigi, A., Guzzetti, L., Galimberti, A., Grando, M. S., Tardaguila, J., & Labra, M. (2018). Geographical and Cultivar Features Differentiate Grape Microbiota in Northern Italy and Spain Vineyards. *Front Microbiol*, *9*, 946. https://doi.org/10.3389/fmicb.2018.00946

Mohanty, P., Singh, P. K., Chakraborty, D., Mishra, S., & Pattnaik, R. (2021). Insight Into the Role of PGPR in Sustainable Agriculture and Environment. *Frontiers in Sustainable Food Systems*, *5*. https://doi.org/10.3389/fsufs.2021.667150

Mueller, U. G., & Sachs, J. L. (2015). Engineering Microbiomes to Improve Plant and Animal Health. *Trends in Microbiology*, *23*(10), 606–617. https://doi.org/10.1016/j.tim.2015.07.009

National Institute of Viticulture. (2023). *Annual report on viticulture in Argentina*.

Newton, A. C., Fitt, B. D. L., Atkins, S. D., Walters, D. R., & Daniell, T. J. (2010). Pathogenesis, parasitism and mutualism in the trophic space of microbe–plant interactions. *Trends in Microbiology*, *18*(8), 365–373. https://doi.org/10.1016/j.tim.2010.06.002

Oyuela Aguilar, M., Álvarez, F., Medeot, D., Jofré, E., Semorile, L., & Pistorio, M. (2021). Screening of epiphytic rhizosphere-associated bacteria in Argentinian Malbec and Cabernet-Sauvignon vineyards for potential use as biological fertilisers and pathogen-control agents. *OENO One*, *55*(4), 145–157. https://doi.org/10.20870/oeno-one.2021.55.4.4655

Oyuela Aguilar, M., Gobbi, A., Browne, P. D., Ellegaard-jensen, L., Hansen, L. H., Semorile, L., & Pistorio, M. (2020). Influence of vintage, geographic location and cultivar on the structure of microbial communities associated with the grapevine rhizosphere in vineyards of San Juan Province. *PLoS ONE*, 1–19. https://doi.org/10.1371/journal.pone.0243848

Paolinelli, M., Martinez, L. E., García-Lampasona, S., Diaz-Quirós, C., Belmonte, M., Ahumada, G., Pirrone, M. A., Farber, M. D., Escoriaza, G., Longone, V., González, M., Lerena, C., Combina, M., & Mercado, L. A. (2023). Microbiome in soils of Mendoza: microbial resources for the development of agroecological management in viticulture. *OENO One*, *57*(1), 191–205. https://doi.org/10.20870/oeno-one.2023.57.1.5585

Peng, X., Feng, K., Yang, X., He, Q., Zhao, B., Li, T., Wang, S., & Deng, Y. (2024). iNAP 2.0: Harnessing metabolic complementarity in microbial network analysis. *IMeta*, *3*(5). https://doi.org/10.1002/imt2.235

Pieterse, C. M. J., de Jonge, R., & Berendsen, R. L. (2016). The Soil-Borne Supremacy. *Trends in Plant Science*, *21*(3), 171–173. https://doi.org/10.1016/j.tplants.2016.01.018

Pii, Y., Orzes, G., Mazzetto, F., Sambo, P., & Cesco, S. (2024). Advances in viticulture via smart phenotyping: current progress and future directions in tackling soil copper accumulation. *Frontiers in Plant Science*, *15*. https://doi.org/10.3389/fpls.2024.1459670

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glockner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, *35*(21), 7188–7196. https://doi.org/10.1093/nar/gkm864

Remenyik, J., Csige, L., Dávid, P., Fauszt, P., Szilágyi-Rácz, A. A., Szőllősi, E., Bacsó, Z. R., Szepsy Jnr, I., Molnár, K., Rácz, C., Fidler, G., Kállai, Z., Stündl, L., Dobos, A. C., & Paholcsek, M. (2024). Exploring the interplay between the core microbiota, physicochemical factors, agrobiochemical cycles in the soil of the historic tokaj mád wine region. *PLOS ONE*, *19*(4), e0300563. https://doi.org/10.1371/journal.pone.0300563

Rivas, G. A., Guillade, A. C., Semorile, L. C., & Delfederico, L. (2021). Influence of Climate on Soil and Wine Bacterial Diversity on a Vineyard in a Non-traditional Wine Region in Argentina. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.726384

Rivas, G. A., Semorile, L., & Delfederico, L. (2022). Microbial diversity of the soil, rhizosphere and wine from an emerging wine-producing region of Argentina. *LWT*, *153*, 112429. https://doi.org/10.1016/j.lwt.2021.112429

Röttjers, L., & Faust, K. (2019). Can we predict keystones? *Nature Reviews Microbiology*, *17*(3), 193–193. https://doi.org/10.1038/s41579-018-0132-y

Sanchez, C., Iannino, F., Deakin, W. J., Ugalde, R. A., & Lepek, V. C. (2009). Characterization of the mesorhizobium loti MAFF303099 type-three protein secretion system. *Molecular Plant-Microbe Interactions*, *22*(5), 519–528. http://www.scopus.com/inward/record.url?eid=2-s2.0-66149084890&partnerID=40&md5=3b98f20544cd12a84f4957478dca5bf8

Santos, S. R. N., Silva, J. S., Souza, M. O., Souza, H. A., & Pinheiro, V. C. S. (2022). Relations between soil attributes and the abundance of *Bacillus thurigiensis* in the Cerrado of Maranhão state, Brazil. *Brazilian Journal of Biology*, *82*. https://doi.org/10.1590/1519-6984.261840

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60

Shailendra Singh, G. G. (2015). Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. *Journal of Microbial & Biochemical Technology*, *07*(02). https://doi.org/10.4172/1948-5948.1000188

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*, *13*(11), 2498–2504. https://doi.org/10.1101/gr.1239303

Singh, P., Santoni, S., This, P., Peros, J. P., & Péros, J.-P. (2018). Genotype-Environment Interaction Shapes the Microbial Assemblage in Grapevine's Phyllosphere and Carposphere: An NGS Approach. *Microorganisms*, *6*(4), 96. https://doi.org/10.3390/microorganisms6040096

Straffelini, E., Carrillo, N., Schilardi, C., Aguilera, R., Estrella Orrego, M. J., & Tarolli, P. (2023). Viticulture in Argentina under extreme weather scenarios: Actual challenges, future perspectives. *Geography and Sustainability*, *4*(2), 161–169. https://doi.org/10.1016/j.geosus.2023.03.003

Tariq, M., Noman, M., Ahmed, T., Manzoor, N., & Zafar, M. (2017). Antagonistic features displayed by Plant Growth Promoting Rhizobacteria (PGPR): A Review. *Journal of Plant Science and Phytopathology*, *1*(1), 38–43. https://doi.org/10.29328/journal.jpsp.1001004

Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moenne-Loccoz, Y., Muller, D., Legendre, L., Wisniewski-Dye, F., & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Front Plant Sci*, *4*, 356. https://doi.org/10.3389/fpls.2013.00356

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

Volant, S., Lechat, P., Woringer, P., Motreff, L., Campagne, P., Malabat, C., Kennedy, S., & Ghozlane, A. (2020). SHAMAN: a user-friendly website for metataxonomic analysis from raw reads to statistical analysis. *BMC Bioinformatics*, *21*(1), 345. https://doi.org/10.1186/s12859-020-03666-4

Vu, M. T., Geraldi, A., Do, H. D. K., Luqman, A., Nguyen, H. D., Fauzia, F. N., Amalludin, F. I., Sadila, A. Y., Wijaya, N. H., Santoso, H., Manuhara, Y. S. W., Bui, L. M., Hariyanto, S., & Wibowo, A. T. (2022). Soil Mineral Composition and Salinity Are the Main Factors Regulating the Bacterial Community Associated with the Roots of Coastal Sand Dune Halophytes. *Biology*, *11*(5), 695. https://doi.org/10.3390/biology11050695

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, *37*(1), 29–38. https://doi.org/10.1097/00010694-193401000-00003

Wang, H., Liu, R., You, M. P., Barbetti, M. J., & Chen, Y. (2021). Pathogen Biocontrol Using Plant Growth-Promoting Bacteria (PGPR): Role of Bacterial Diversity. *Microorganisms*, *9*(9), 1988. https://doi.org/10.3390/microorganisms9091988

Wang, X., Chi, Y., & Song, S. (2024). Important soil microbiota's effects on plants and soils: a comprehensive 30-year systematic literature review. *Frontiers in Microbiology*, *15*. https://doi.org/10.3389/fmicb.2024.1347745

Xie, M., Jia, J., Luan, D., Li, R., Zhang, J., & Zhang, J. (2024). Processes of assembly of endophytic prokaryotic and rhizosphere fungal communities in table grape are preponderantly deterministic. *Applied Soil Ecology*, *203*, 105660. https://doi.org/10.1016/j.apsoil.2024.105660

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