OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

DOI: https://doi.org/10.20870/oeno-one.2023.57.1.5585

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Microbiome in soils of Mendoza: microbial resources for the development of agroecological management in viticulture

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Associate editor:
Stephane Compant

Received:
20 June 2022

Accepted:
6 January 2023

Published:
20 February 2023

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ABSTRACT

Rhizosphere microorganisms are considered an extension of plants, representing critical actors involved in the promotion of plant nutrient intake from the surrounding environment. Consequently, a great focus is being made on soil microorganisms since they are considered a promising source for crop resilience improvements under a global climate change scenario. To explore bacterial and fungal communities from arid soils in vineyards and their surroundings from two regions with very different climate and tillage histories, an amplicon sequencing analysis was performed. Specifically, Santa Rosa (SR) is in a region commonly known as the first zone, characterised by low altitude (607 m.a.s.l., Winkler V), while Gualtallary (G) is in the Uco Valley Zone, a region with high altitude (1245 m.a.s.l., Winkler III); both in the productive wine region of Mendoza. SR is characterised by its long cultivation history, while G is a recently cultivated region. Topsoil samples were collected and used for bacterial and fungal community profile characterisation. *Ascomycota* was the predominant phylum (38–97 %) in mycobiome composition, whereas *Proteobacteria* was the most abundant bacterial phylum (26–34 %) in both regions. Moreover, the main factor explaining microbiome differences between regions was the carbon-to-nitrogen ratio. *Anaerolineae* and *Gammaproteobacteria* were a distinctive bacterial class in SR-cultivated soils. *Azospirillales* were highly abundant in SR uncultivated soils, while *Rhizobiales* were differentially abundant in G uncultivated soils. Regarding functional analysis, soils from SR showed a higher denitrification activity of nitrifiers as well as glucose-related metabolism, while in G soils, bacterial photosynthesis activities were a differential trait. In addition, *Actinobacteria* abundance was lower in SR-cultivated soils, indicating a higher susceptibility of this phylum to grapevine crop practices. These results allow the development of hypothetical models of the local microbial resources and their contribution to grapevine nutrition, which is highly important to elaborate recommendations for grapevine management to preserve soil health in vine areas of Mendoza.

KEYWORDS: metagenomics, 16S, ITS, biological control agents, grapevine nutrition, plant growth-promoting microorganisms

INTRODUCTION

Since the green revolution, agriculture has changed towards monoculture practices which implies crop exclusion from a diverse ecological context, including microorganisms that co-evolved with the plant. In terms of resources, the plants being sessile organisms, depend on microorganisms to reach and capture nutritional resources from distant sites that plant roots cannot reach due to size constraints (Pérez-Jaramillo *et al*., 2016). Monocropping practices initially improve crop yields; however, they also lead to the loss of long-term benefits of the surrounding diversity that provides the microbial balance needed for resilience, pathogen antagonism or plant growth promotion (Vukicevich *et al*., 2016; Newton, 2016; Saleem *et al*., 2019). As time passes, under intensive farming conditions, the system is maintained only through chemical inputs of fertilisers, herbicides, insecticides, and microbicides that will even alter and, in some cases, deplete the microbial diversity in the agricultural environment and ultimately, will alter the health of the soil (Delitte *et al*., 2021). Hence, the scientific community and the agri-food industry are increasing efforts to explore soil microorganism resources to promote sustainable agriculture (Wei *et al*., 2015; Pérez-Jaramillo *et al*., 2016; Toju *et al*., 2018; Saleem *et al*., 2019). The utilisation of high throughput sequencing (HTS) technologies for the characterisation of microbiomes has revealed the existence of a huge microorganisms diversity in soils (Pérez-Jaramillo *et al*., 2016; Jansson and Hofmockel, 2018).

Previous studies on vineyard soil microbiomes indicate that microorganisms are principally determined by a biogeographic variable in the case of bacteria, while fungi communities show restructuration according to agricultural management practices (Chou *et al*., 2018; Coller *et al*., 2019). The role of the soil microbiome in the prevention of *Vitis vinifera* diseases is another important aspect currently being studied. In this regard, it has been demonstrated that pathogens causing grapevine trunk diseases are more abundant in soils from symptomatic vineyards, suggesting that pathogen inoculum remains on the soil and could reinfect the plant (Nerva *et al*., 2019). Other studies suggest that dysbiosis that favours the development of diseases could be explained by the decrease in soil bacteria (Saccà *et al*., 2019; Darriaut *et al*., 2021; Bettenfeld *et al*., 2022).

The link between grapevine nutrient uptake and soil microbiome was initially addressed by Lewis *et al*. (2018), who highlighted that bacteria rather than fungi alter plant nutrition, particularly on C, N and Mg content, which finally influences the presence of chlorotic symptoms. Furthermore, in terms of weed management, it has been shown that herbicide applications reduce mycorrhization rates and bacterial number in xylem sap, altering grapevine nutrient uptake; consequently, this imbalance could lead, over time, to the reduction of the vigour of the plant (Zaller *et al*., 2018).

Recently, characterisation of the microbial activity in arid soils of Argentina used for grapevine cultivation was carried out. Those studies focused on the quantification of functional groups based on soil respiration rates, physicochemical approaches as indicators of nitrifier activities, ammonifiers and cellulolytic microorganisms (Martínez *et al*., 2018; Vega-Avila *et al*., 2018; Uliarte *et al*., 2019). In another study, the bacterial microbiome was also characterised by amplicon sequencing in San Juan vineyards (Vega-Avila *et al*., 2015). In the present study, the objective was to characterise bacterial and fungal communities in grapevine-cultivated and uncultivated soils from Mendoza regions that differ in their geological origin and soil tillage management. These contrasting regions were selected to determine the relationship between the environment, climate and vineyard management with soil microbiota composition.

MATERIALS AND METHODS

1. Sample collection

Samples were collected during winter (August 2018) from two regions with different climatic conditions, according to measurements with weather station imetos 3.3 (Werksweg 107, 8160 Weiz, Austria) (Table S1). On one side, Gualtallary (G), located in Uco Valley, at the foothills of the Andes Mountains, 1245 m.a.s.l., and classified at zone III for Winkler index. The second selected region, Santa Rosa (SR), is located in the traditionally productive region in the east of Mendoza city, 607 m.a.s.l., classified as Winkler V region (Figure 1A) (Abatzoglou *et al*., 2018). Five soil samples were collected from the native shrubland surrounding a vineyard in Gualtallary (G1–G5, Figure 1B, –3340777 to 3340868; –69.22336 to –60.22537), three soil samples from native shrubland in Santa Rosa (SRU1-SRU3, Figure 1C, –3327912 to –3327985; –6815158 to –6815624) and six samples of soils cultivated with *Vitis vinifera* cv. Malbec (SRC1-SRC6, Figure 1C).

An auger was used to make six sub-samplings of topsoil in the middle of the interrow at a minimum distance of 50 cm from surrounding plants. Sub-samples were mixed and sieved to remove any plant debris, and 2 kg was collected in plastic bags for physicochemical analysis. Samples for amplicon-sequencing were collected in 50 mL conical tubes, immediately frozen with liquid nitrogen and finally stored at -80 °C.

2. DNA extraction and sequencing

DNA extraction was performed using a Macherey Nagel Nucleospin soil DNA kit following the manufacturer's instructions. DNA integrity was evaluated in 1 % agarose gel electrophoresis, and minimal quality of 260/280 absorbance ratio > 1.8 was quantified in Thermo Scientific™ NanoDrop™ 2000. Library preparation and sequencing were performed at the Integrated Microbiome Resource sequencing facility at Dalhousie University (Halifax, Canada). Briefly, for prokaryotic microorganisms, the hypervariable regions V4-V5 were amplified using primers 515FB (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R (5'-CCGYCAATTYMTTTRAGTTT-3'). In the case of Fungi, the Internal Transcribed Spacer (ITS) regions, located between the 5.8S and 28S genes of ribosomal

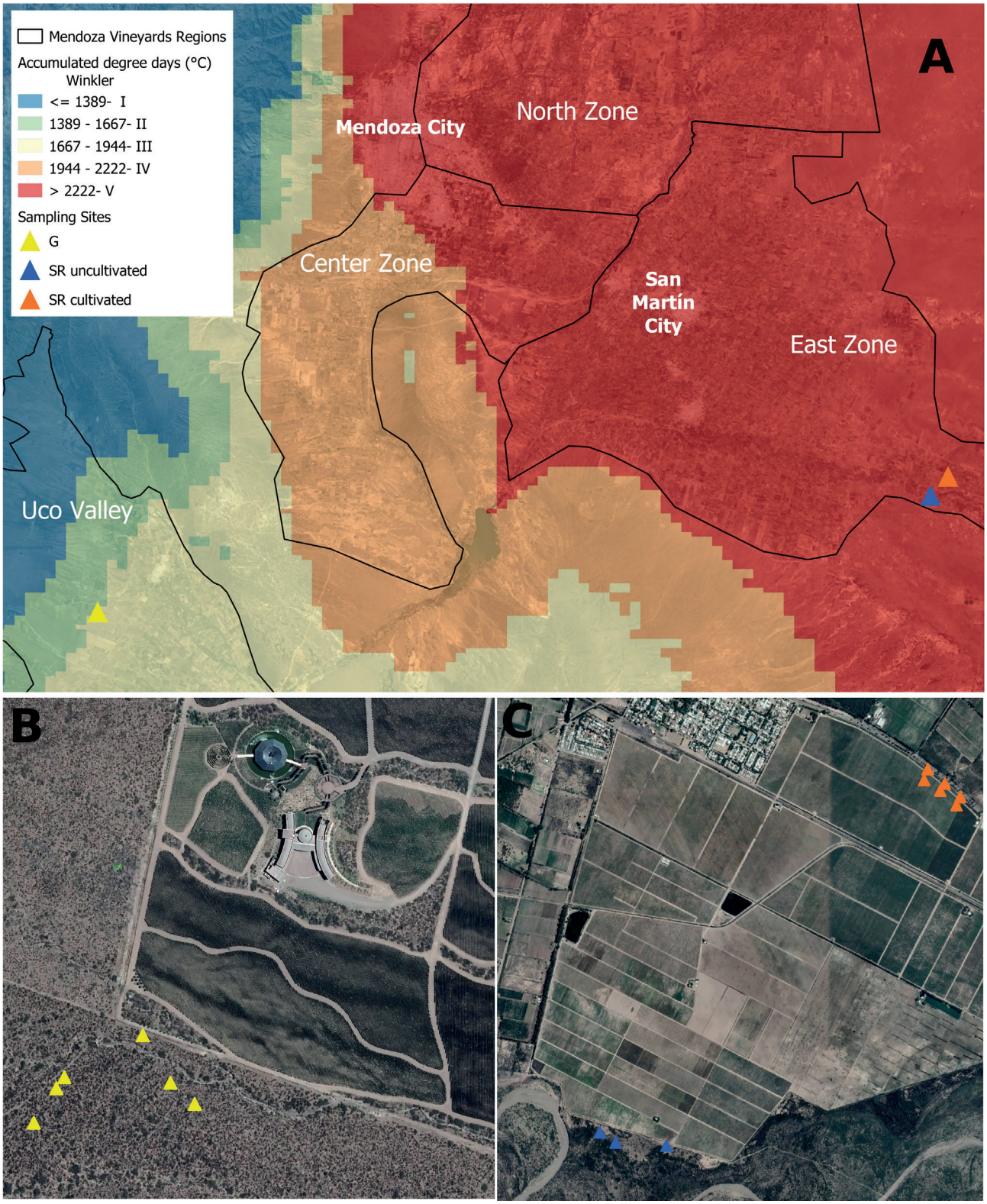


FIGURE 1. Geographic location of sample collection sites.

(A) Mendoza vineyard regions classified according to the Winkler index. (B) Zoom in on the Gualtallary map (Winkler Index III, orange marks) and (C) zoom in Santa Rosa map. SR samples were collected from grapevine-cultivated soils (Winkler Index V, blue marks) and uncultivated soil surrounding the vineyard (Winkler Index V, green marks).

ARN (ITS2), were amplified using primers ITS86F (5'-GTGAATCATCGAATCTTTGAA-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3'). Sequencing was carried out in Illumina MiSeq™ paired-end 2 × 300bp format. The resulting raw reads were deposited at BioProject in NIH-NCBI (PRJNA833442).

3. Soil fertility analysis

Organic matter (OM) in soil samples was determined according to the Walkley–Black method. Total nitrogen (N) was measured using the Kjeldahl method. Available phosphorus (P) was quantified through Bray's method. Exchangeable potassium (K) was determined based on the ammonium acetate method. All methods were performed as previously described by Page *et al*. (1982).

4. Determinations of normalised difference in vegetation index (NDVI)

In shrublands surrounding vineyards, NDVI was calculated as a quantitative estimation of vegetation growth through the QGIS software (QGIS Development Team, 2021) from a Sentinal-2 image. Only the red band (665 ηm ± 15 ηm) and the Near-Infrared (NIR) band (842 ηm ± 15 ηm) were used to compute the classical NDVI index as (NIR-red)/(NIR+red) (Rouse Jr *et al*., 1974).

5. Bioinformatic analysis

Quality filtration, merging of paired-end reads, chimaeras remotion, clustering in Operational Taxonomic Units (OTUs) and the taxonomic assignment was performed with mothur v1.45.0 (Schloss *et al*., 2009) hosted in High-Performance Computer at CICESE (Ensenada, Mexico) and based on the protocol designed by Biocore-Center for Genomic Regulation, Barcelona (https://github.com/biocorecrg/microbiome_procedures/).

The main criteria for quality filtering of 16S reads was to preserve only reads with no ambiguous bases in the merging region of paired reads and with a length in the range of 409-416 bp. Uchime was used for chimaera identification and remotion (Edgar *et al*., 2011). In the 'screen.steps' function in mothur, the homopolymers higher than 8 bp were removed; subsequently, remaining reads were grouped on OTUs based on 97 % similarity, and once assigned the taxonomy to OTUs, those classified as "Chloroplast", "Mitochondrion" or "unknown" were removed. The reference used for the taxonomic assignment was SILVA SSU132 (Quast *et al*., 2013).

The quality filtering criteria for ITS2 reads was to preserve only those with nonambiguous bases in merging regions of paired reads and having a maximal length of 350 bp. Vsearch was used for chimaera identification and remotion. The OTUs were generated with abundance-based greedy clustering with sequences sharing 95 % identity (Rognes *et al*., 2016). The dynamic version of UNITE 'Species Hypothesis' database (UNITE community, 2019) was used for taxonomic assignment to OTUs. All the OTUs assigned to the "unknown" and/or "Protista" taxon were removed. Statistics test and graphs were obtained with phyloseq v1.36.0 (McMurdie *et al*., 2013) and phylosmith v1.0.6 (Smith, 2019).

To identify the genus indicator for each group of samples (G, SR, SRC or SRU), a linear discriminant analysis was carried out with lefser v1.2.0 R package (Khleborodova, 2021). Functional predictions were obtained with picrust2 v2.4.2 (Douglas *et al*., 2020). Specifically, 16S copy numbers of the more phylogenetically related bacteria having their genome sequenced were considered for functional predictions. The information on genes in the related bacteria and the OTU counts were employed to predict the abundance profile of metabolic pathways based on the MetaCyc database (https://metacyc.org/). Aldex2 v1.24.0 (Fernandes *et al*., 2014) R package was used to identify the statistically different pathways when comparing groups. FunGuild v1.0 was used to parse fungal OTUs into guilds based on their taxonomic assignments using the FunGuild database that groups taxa based on the bibliographic information of the experimentally validated functions for fungi (Nguyen *et al*., 2016).

RESULTS

1. Soil fertility

Microorganisms are the main drivers of biogeochemical cycles in soil which is critical for plant nutrition (Rousk and Bengtson, 2014). With the aim of linking microorganisms' abundance with the availability of nutrients for plants, the content of macronutrients, OM and C:N ratio were determined on soil samples. All variables, except for phosphorous, were in higher abundance in SRU soils than in SRC and G soils (Table 1). OM was different in all the collection sites (ANOVA p-value < 0.01), reaching the lowest values in soils of Gualtallary and intermediate values in cultivated soils of Santa Rosa, whereas the richest contents were observed in uncultivated soils from Santa Rosa.

2. Operational taxonomic units, alpha and beta diversity

The output from sequencing was 899,391 pair end (PE) reads for prokaryotes and 792,396 PE reads for fungi (Table S2). After quality filtering, 468,974 and 603,649 reads for prokaryotes and fungi, respectively, were used for clustering in operational taxonomic units (OTUs). Of 1065 defined bacterial OTUs (at 97 % similarity threshold), 28.3 % were unable to be classified at any taxonomic level (Table S2). The OTUs defined for fungi were 2820 (at 95 % similarity threshold), and 78 % could not be classified at any taxonomic level (Table S2).

TABLE 1. Chemical indicators of soil fertility in sites of collection

Collection sites	Nitrogen (mg/kg)	Phosphorous (mg/kg)	Potassium (mk/kg)	Organic matter (% g/g)	C/N ratio
Gualtallary uncultivated soils (G)	458 ± 34^{b}	9.1 ± 1,5^{a}	207 ± 31^{b}	0.2 ± 0.02^{c}	2.6 ± 0.3^{b}
Santa Rosa cultivated soils (SRC)	580 ± 57^{b}	12.5 ± 1.9^{a}	303 ± 35^{b}	0.82 ± 0.13 ^{b}	8.1 ± 1.0^{a}
Santa Rosa uncultivated soils (SRU)	1255 ± 314^{a}	18 ± 7.7^{a}	1554 ± 289^{a}	1.93 ± 0.49 ^{a}	9.1 ± 0.8^{a}

Different letters indicate statistical differences (ANOVA p-value < 0.01)

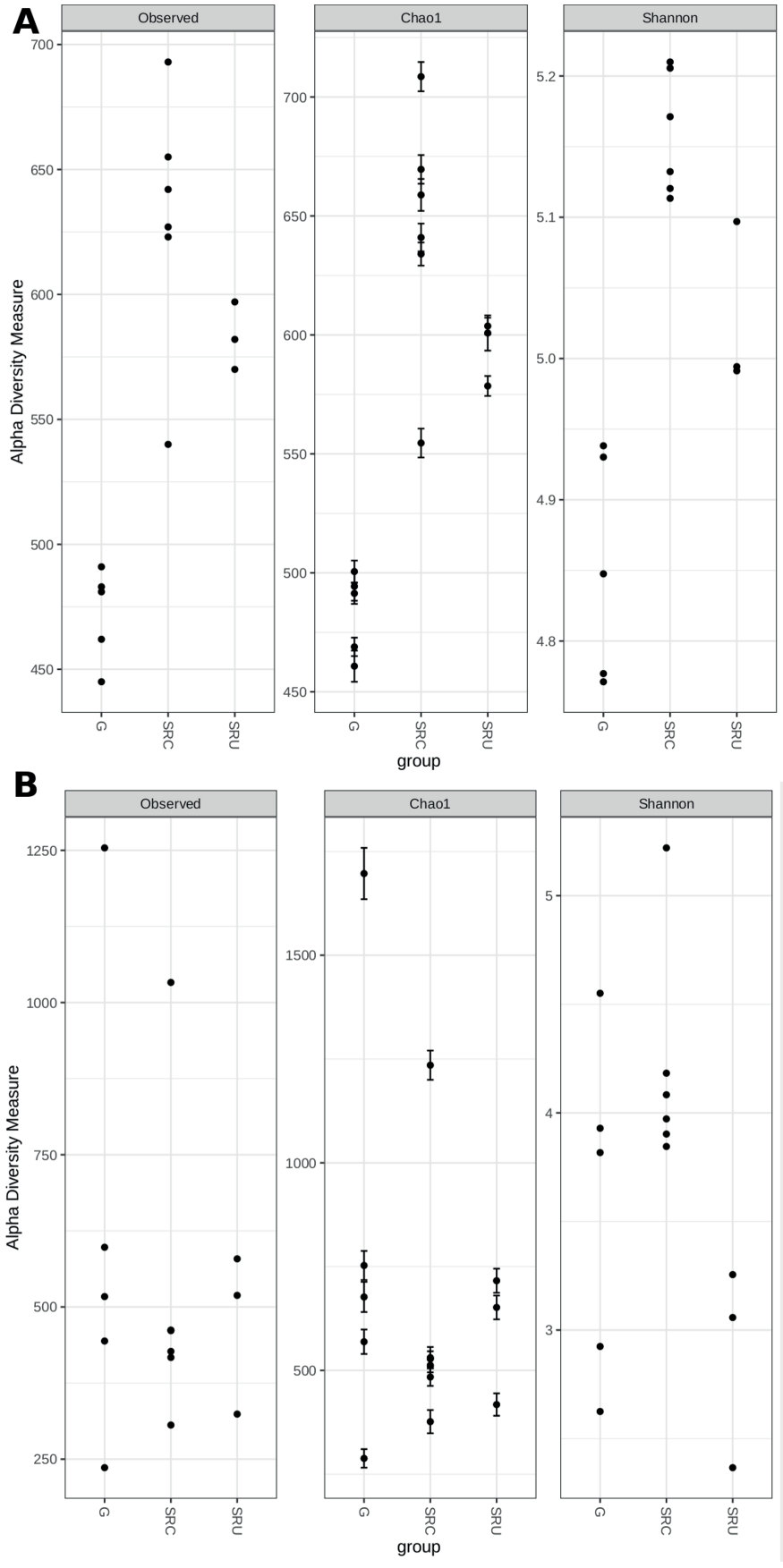


FIGURE 2. Microbial alpha-diversity indicated as observed OTUs, Chao1 and Shannon index.

Bacterial (A) and fungal (B) diversity in each group of samples from Gualtallary (G) and grapevine cultivated (SRC) and uncultivated (SRU) soils from Santa Rosa.

Regarding bacterial alpha-diversity, SR samples showed a higher number of species, Chao1 and Shannon values than G samples. Moreover, samples from SRC soils showed the highest Shannon alpha diversity (Figure 2A). In contrast, fungal alpha diversity was not different between groups (Figure 2B).

Microbial beta-diversity was evaluated using Bray–Curtis dissimilarities and plotted in a Principal Coordinates Analysis (PCoA). Bacterial composition is mainly differentiated by the geographic location, being SR soils separated from G soils in the PCoA1 axes, which explains the 49.9 % of variation (Figure 3A). Fungal composition is differentiated in SR from G in the PCoA1, explaining 28.8 % of data variation, but also samples from SRC are differentiated from SRU in the PCoA2, which explains 13.5 % of data variation (Figure 3B).

3. Composition of bacterial microbiome

Regarding bacterial microbiome composition, the most representative phyla detected were *Proteobacteria* (26–34 %), *Actinobacteria* (10–25 %), *Acidobacteria* (11–20 %), *Planctomycetes* (8-14 %), *Chloroflexi* (5–8 %) and *Bacteroidetes* (3–8 %) (Figure S1). *Actinobacteria* phylum particularly showed a higher variation in its representation in the microbiome in the different soils and management evaluated (G: 17–25 %, SRC: 10–11 %, SRU: 15–17 %, Figure S1). At the class level, the major differences among collection sites were observed in *Thermoleophilia* (G: 6–9 %, SRC: 2–3 %, SRU: 3–5 %) and *Actinobacteria* (G: 5–12 %, SRC: 3–5 %, SRU: 5–7 %). In SR, both in cultivated and uncultivated soils, a higher representation of *Proteobacteria* phylum compared to G soils (G: 26–32 %, SRC: 30–34 %, SRU: 28–31 %) was observed; specifically, in *Gammaproteobacteria* class (G: 5–7 %, SRC: 12–14 %, SRU 9–10 %) and *Anaerolineae* (G: 0.3–0.5 %, SRC: 3–4 %, SRU: 2–3 %, Figure 4 and Figure S1). In contrast, *Alphaproteobacteria* (G: 16–21 %, SRC: 12–16 %, SRU: 15–17 %) and *Acetobacteraceae* (G: 2–3 %, SRC: 0.09–2 %, SRU: 0.3–0.6 %) classes were detected in higher proportions in G soils than in SR soils. The genera members of the *Beijerinckiaceae* family, *Microvirga* (G: 2– 4 %, SRC: 0.3–0.6 %, SRU: 0.9–1 %) and *Psychroglaciecola* (G: 0.7–1 %, SRC: 0.04–0.1 %, SRU: 0.04–0.15 %; Figure S1) were the bacterial genera that better explained the differences between collection sites.

Considering the PCoA results (Figure 3), a linear discriminant analysis test was employed to determine which bacterial taxa explained the differences between G and SR soils. Several members of the *Beijerinckiaceae* family, including *Microvirga*, *Psychroglaciecola*, and other unclassified genera, were highly represented in G soils (lda threshold > 2, Kruskal threshold < 0.05 and Wilcox threshold < 0.05; Figure S3). *Segetibacter*, a genus identified as 64-14; a *Pyrinomonadaceae* (*Acidobacteria*) identified as RB41 and *Bryobacter* (both belonging to *Acidobacteria*); a *Tepidisphaeraceae* identified as WD2101; *Rubellimicrobium*; a *Chloroflexia* identified as AKIW781; an *Acetobacteraceae* not classified at genus level and *Belnapia*; a Nitrosococcaceae (ammonia-oxidising bacteria) identified as wb1-P19 were differentially abundant in G soils (lda threshold > 2, Kruskal threshold < 0.05 and Wilcox threshold < 0.05; Figure S3).

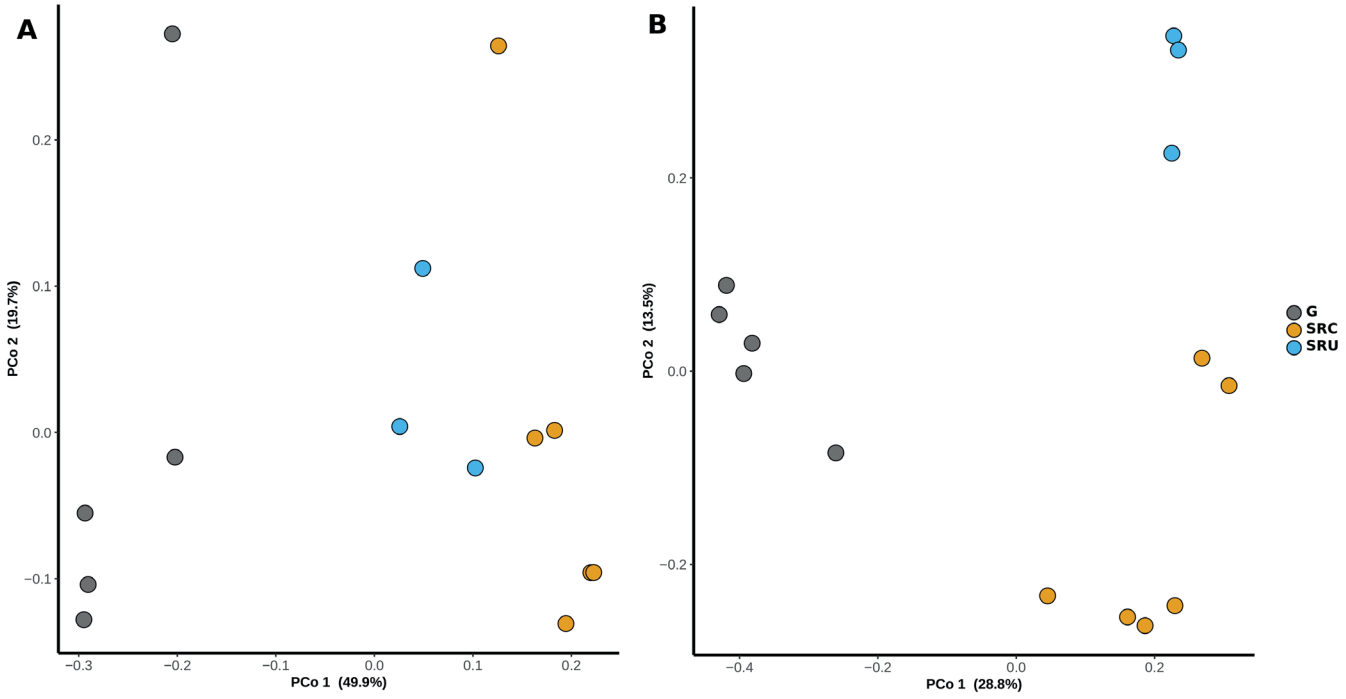


FIGURE 3. Microbial Beta-diversity.

Bray–Curtis dissimilarity metrics for bacterial microbiome (A) and fungal microbiome (B) from Gualtallary (G) and grapevine cultivated (SRC) and uncultivated (SRU) soils from Santa Rosa.

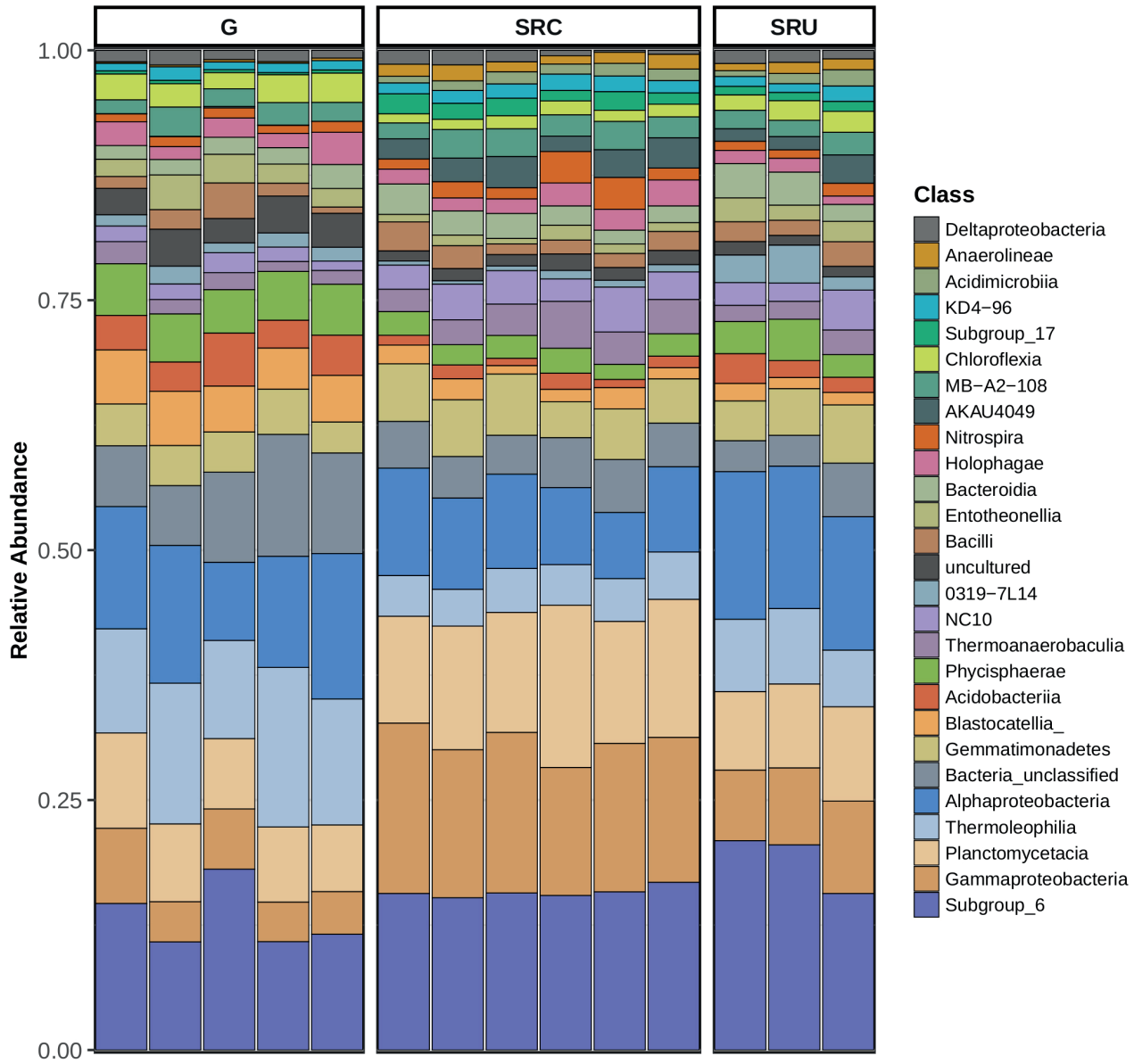


FIGURE 4. Bacterial microbiome composition.

Relative abundance of the most representative bacterial class (relative abundance > 0.005).

Acidobacteria subgroups 6, 10 and 17; *Nitrosomonadaceae* MND1; *Pirellulaceae* Pir4 lineage; *Gemmatimonadetes* AKAU4049; a genus of *Rokubacteriales*; a *Gammaproteobacteria Polycyclovorans*, *Acidibacteria* identified as PLTA13; *Anaerolineae* A4b and *Planctomycetes* OM190 were highly abundant in SR soils (lda threshold > 2, Kruskal threshold < 0.05 and Wilcox threshold < 0.05; Figure S3A).

4. Fungal community composition

Considering fungal microbiota, *Ascomycota* (38–97 %) was the most represented fungal phylum in soil samples analysed, followed by *Basidiomycota* (1–59 %) and specifically in SRC, the third more abundant phylum was *Zygomycota* inc. sed. (G: 0–1 %, SRC: 5–12 %, SRU: 0.8–1 %). On the other hand, in G soils, the third most abundant phylum was *Chytridiomycota* (G: 0.4–4 %, SRC: 0.02–1 %, SRU: 0.1–2 %). *Glomeromycota* was the fourth most represented phylum (G: 0.4–4 %, SRC: 0.02–1 %, SRU: 0.1–2 %) in uncultivated soils from Gualtallary (Figure S2).

When analysing at the order level, a higher proportion of *Hypocreales* was observed in SR compared to G soils (G:0.8–3%,SRC:16–31%,SRU:23–67%).*Botryosphaeriales* was more represented in SR soils (G: 0–0.06 %, SRC: 0.07–0.5 %, SRU: 0.3–0.5 %), whereas *Mortierellales* (G: 0–1 %, SRC: 5–12 %, SRU: 0.8–1 %) and *Tremellales* (G: 0.08–0.8 %, SRC: 0.6–6 %, SRU: 0.2–0.35 %) were specifically more represented in SRC soils. In contrast, *Coniochaetales* (G: 0.2–6 %, SRC: 0.02–0.2 %, SRU:0) and *Filobasidiales* (G: 0.08–4 %, SRC: 0.07–0.5 %, SRU: 0.04–1 %) were particularly abundant in G soils (Figure 5 and Figure S2).

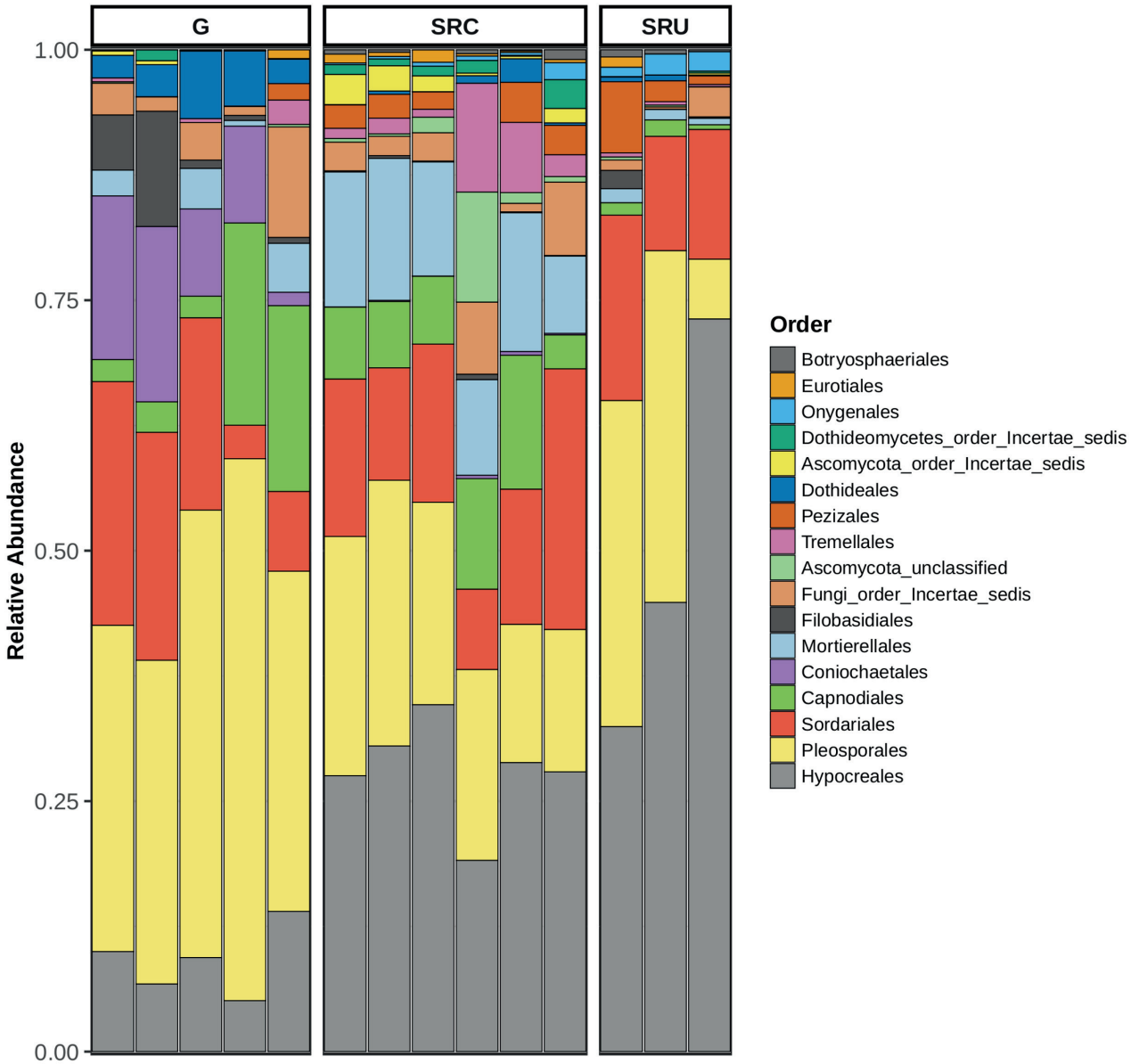


FIGURE 5. Fungal microbiome composition.

Relative abundance ranges of the most representative fungal orders (relative abundance > 0.001).

When comparing the abundance of fungal genera among collections sites, *Mortierella*, *Alternaria*, *Fusarium*, *Coprinellus*, *Metarhizium*, *Retroconis*, *Arthrographis* and *Acremonium* were more abundant in SR soils (lda threshold > 2, Kruskal threshold < 0.05 and Wilcox threshold < 0.05; Figure S3B). In contrast, *Umbilicaria* (lichen-forming fungus), *Lophiostoma*, *Rhizophlyctis*, *Phaeococcomyces*, *Coniochaeta*, *Aureobasidium* and *Deniquelata* fungal genera were more abundant in G soils (lda threshold > 2, Kruskal threshold < 0.05 and Wilcox threshold < 0.05; Figure S3B). On the other hand, when comparing SRC with SRU, *Mortierella* and several other unclassified genera were particularly abundant in SRC soils (lda threshold > 2, Kruskal threshold < 0.05 and Wilcox threshold < 0.05; Figure S3C), while *Neophaeosphaeria* and *Chrysosporium* were highly abundant in SRU soils (lda threshold > 2, Kruskal threshold < 0.05 and Wilcox threshold < 0.05; Figure S3C).

5. Functional prediction based on microbial taxa composition

Relating taxa with functions is important to understand the functional potential of soils. Aldex2 T-test was used to compare the predicted metabolic pathways in G and SR samples (explaining 49.9 % of differences in the PcoA plot, Figure 3). Biosynthesis of pyridoxal, acetylhomosamine biosynthesis, nitrifier denitrification, and glucose degradation were the differential pathways in SR (Figure 6). In contrast, the degradation of aromatic biogenic amine and amino acid, propanediol, salicylate, glycogen, catechol, glutamate, nicotinate and inositol were differential pathways in G. Moreover, the biosynthesis of ergothioneine, isopropanol, peptidoglycan, adenosylcobalamin (vitamin B12), phospholipases, and kdo2 lipid A were the biosynthetic pathways that differentiated the G soils (Figure 6).

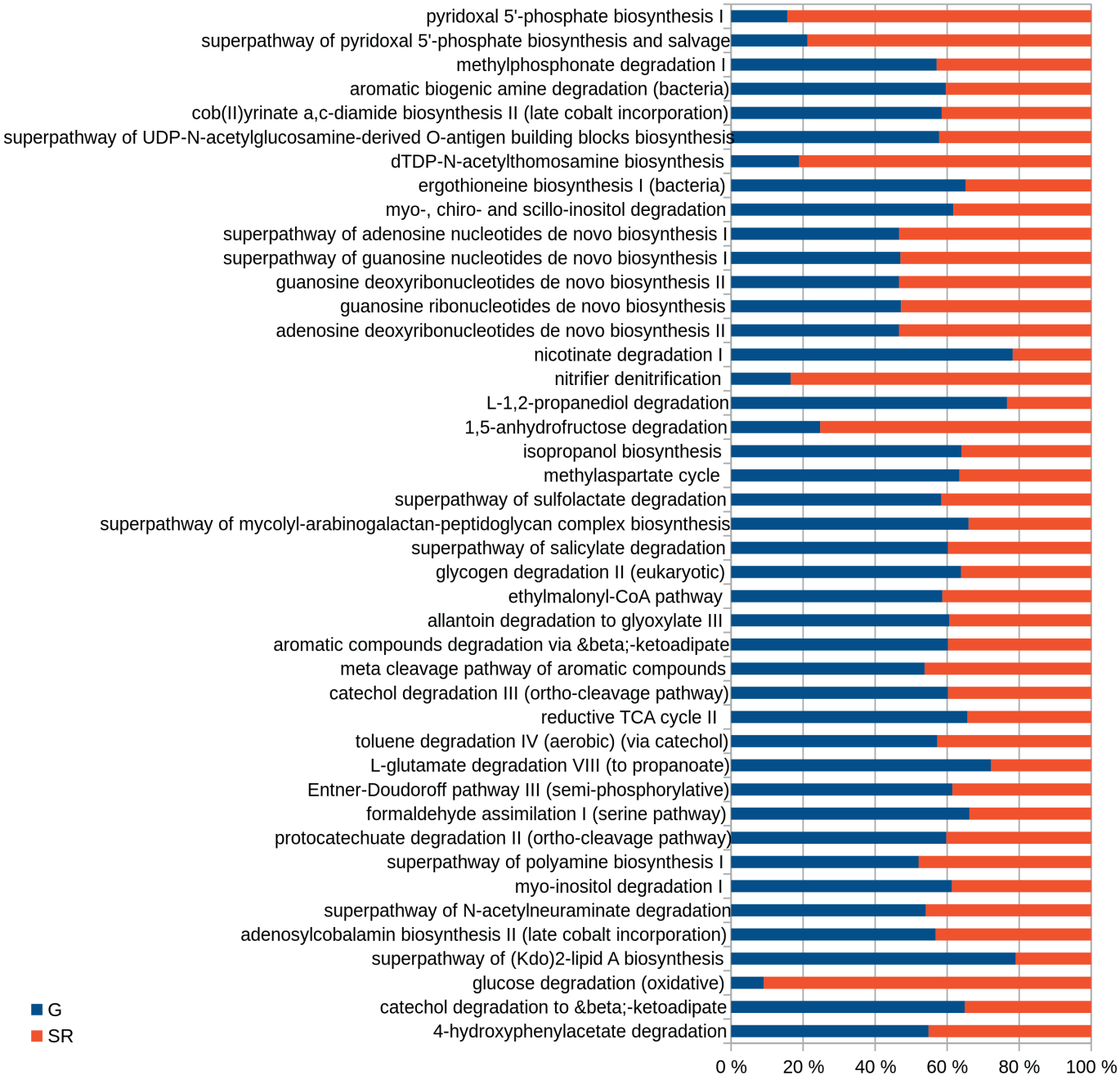


FIGURE 6. Bacterial functional predictions based on MetaCyc metabolic pathways.

The prokaryotic ecological function was predicted using picrust2. Significant differential Metacyc pathways are shown, comparing Gualtallary (G) and Santa Rosa (SR) samples (aldex2 T-test, BH *p*-value < 0.01).

The fungal community in SR samples was dominated by saprotrophs, while in G soils, a larger diversity of ecological roles was characteristic, emphasising the presence of fungus with the ability to form lichens (Table S3).

6. Relationship between soil nutrients and microbial composition

A multidimensional plot that incorporates both the microbiome composition (Bray–Curtis dissimilarity) and soil nutrient parameters was constructed to determine the interaction between these variables. The groups were differentiated mainly by collection site (CAP1 axis explains 41 % for bacterial composition and 24.5 % for fungal composition in Figure 7). Moreover, SRC differentiates from SRU in the CAP2 (11.1 % and 11.7 % variation explained for bacteria and fungi, respectively). The C:N ratio is the environmental factor that better explained the differences in microbial composition observed between locations, while N, P, K and OM better explained the differences between SRU and SRC soils (Figure 7).

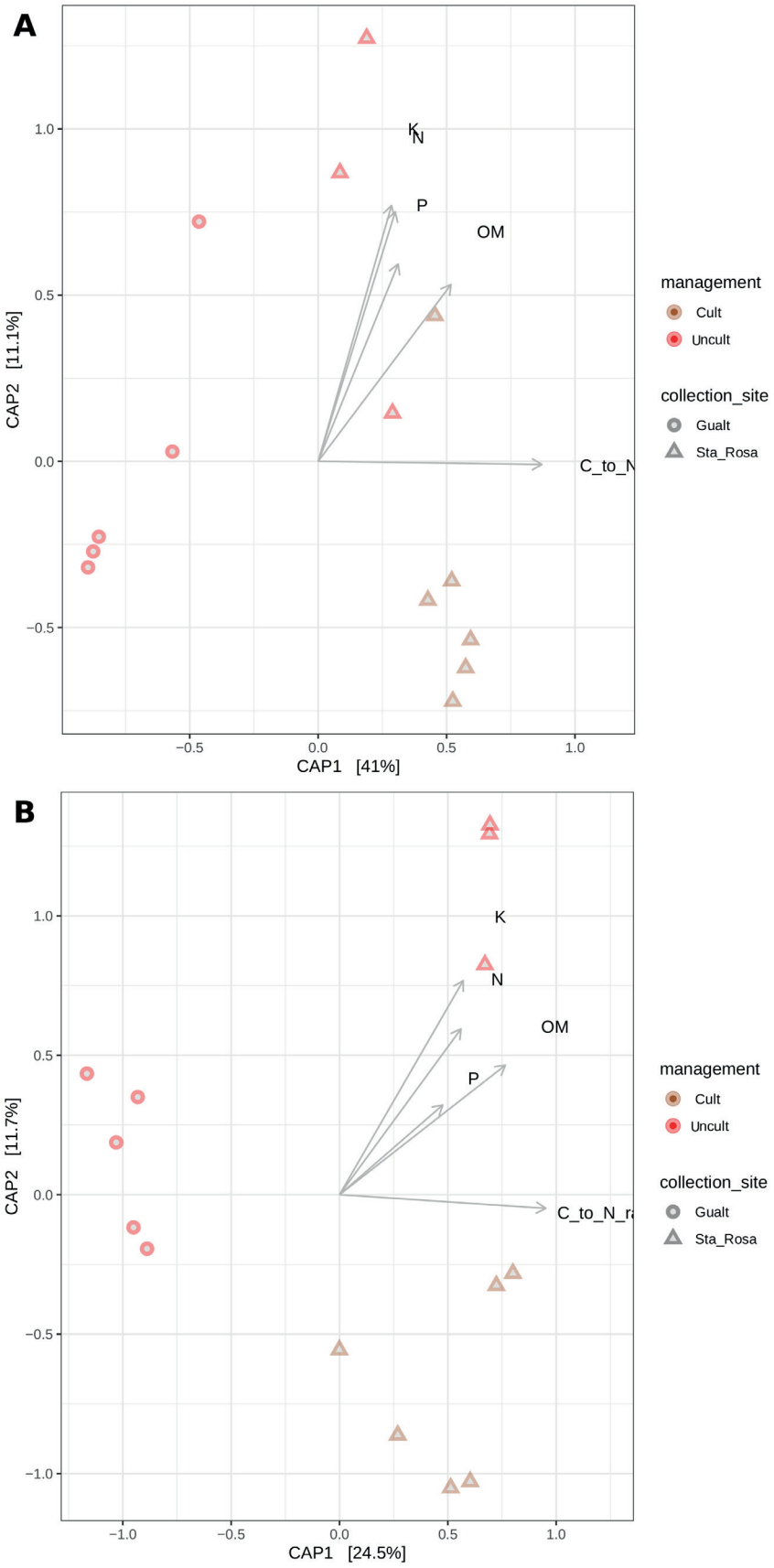


FIGURE 7. Nutritional variables and microbial composition.

Constrained graphs from Canonical Analysis of Principal Components (CAP) were employed to understand the relationship between quantified soil nutrients such as total nitrogen (N), soluble phosphorous (P), potassium (K), organic matter (OM) and C:N ratio with bacterial composition (A) and fungal composition (B).

DISCUSSION

The present study evaluates the composition of the soil microbiome of two sites located in geographically different viticultural regions of Mendoza, Gualtallary and Santa Rosa, to understand the relationship of climate, nutrients and microbial composition. Furthermore, the comparison of cultivated and non-cultivated soils in Santa Rosa allows us to better understand the effect of soil management on the microbiome. The selected sites showed great differences in terms of nutrient and organic matter content (Table 1), where non-cultivated soils from Santa Rosa were highly richer than cultivated soils. This was an unexpected result considering the irrigation and fertilisation regime of grapevine-cultivated areas. Similar observations have been previously reported in a similar study carried out on soils of San Juan (Vega-Avila *et al*., 2018) and were attributed to the presence of "nurse plants" whose shade promotes the growth of annual plants during wet periods and then die during dry seasons. Consequently, dead plant material provides resources for microbial growth and, thus, favours nutrient availability. Although the canopy of the vine also generates shade, it does not seem to behave in the same way as shrubs for the nursery of other plants. This could be possibly attributed to the lack of balance between annual plants and microbial degraders due to vine management, such as the addition of chemical inputs or soil compaction. On the other hand, Gualtallary cultivated soils showed higher nutrient content than non-cultivated soils (data not shown). Non-cultivated sites in G had lower NDVI than SR (Table S4); hence, higher exposure to UV radiation in G could limit the growth of annual plant growth under shrubs.

Soil microbial resources could be a promising alternative to address several important concerns in viticulture, such as poor nitrogen availability, water use efficiency, abiotic stress, soil nutrient deficiencies due to phosphate erosion and the presence of nematodes. In the present discussion, we focus on microorganisms with a well-documented ecological role detected in sufficient abundance in our data and that might have the potential to be used in promoting soil health or at least mitigating the erosion caused by grapevine cultivation. A schematic model to summarise the available microbial resources in the soils analysed in this study is depicted in Figure 8. The Oxalobacteraceae *Massilia* sp., a member of the Burkholderiaceae family, can solubilise P and have a positive interaction with arbuscular mycorrhizal fungi (AMF) (Scheublin *et al*., 2010). This bacterium was found with greater abundance in non-cultivated soils (G and SRU), suggesting that these soils never exposed to tillage could have a greater P solubilisation ability. Furthermore, the fungus *Mortierella* sp., also known for its positive interaction with AMF and P-solubilisation capacity (Zhang *et al*., 2011; Ozimek and Hanaka, 2020), was more represented in the microbiome of SRC soils. This indicates that different microorganisms with the ability to interact with AMF and solubilise P are represented, possibly influenced by plant species present on soil surfaces. This could be explained by the interaction of the root system of different plants (grapevine instead of native shrubs) with different AMF and, therefore, also with different bacteria or fungi associated with such AMF. Furthermore, *Proteobacteria*, *Gammaproteobacteria* and *Anaerolineae* showed higher representation in SR soils. Similar results were obtained in a study carried out in soils from China under long-term N fertilisation (Zhou *et al*., 2017).

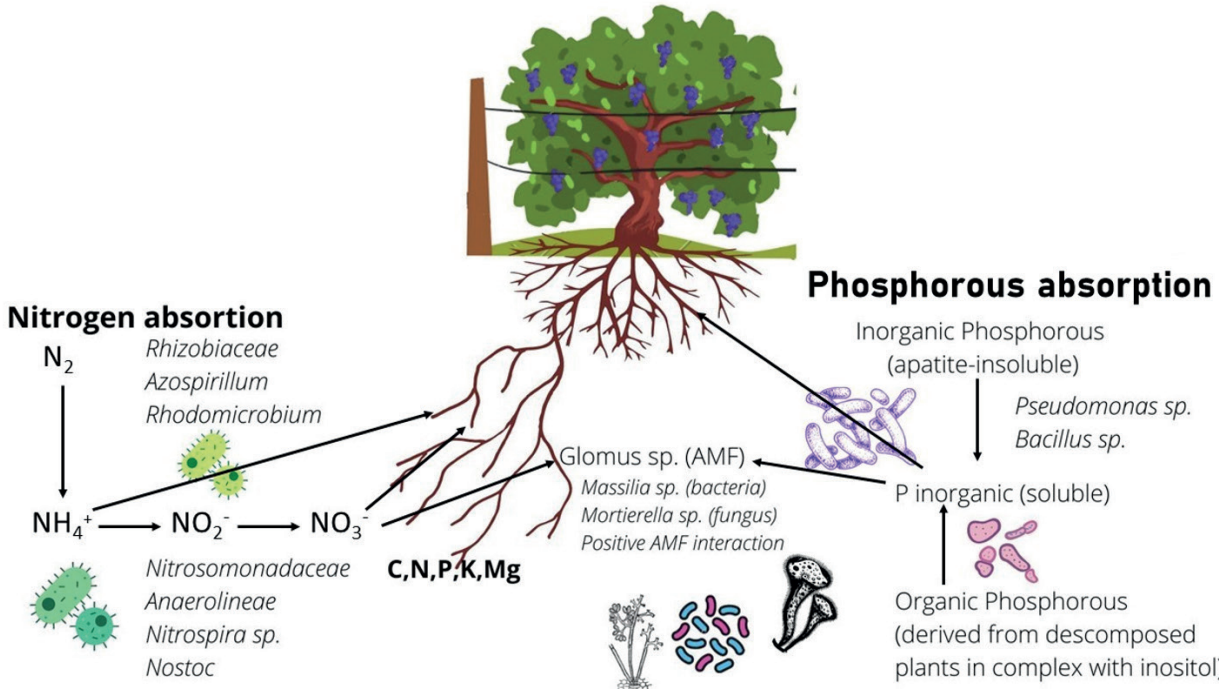


FIGURE 8. Microorganisms and grapevine nutrition.

Schematic model of the microorganisms identified in this study and their contribution to biogeochemical cycles of macronutrients that could contribute to improving nitrogen and phosphorous acquisition by the grapevine.

Anaerolineae is a bacterial genus that has been associated with the N cycle (Jimenez-Bueno *et al*., 2016) due to its ability to carry out the transformation of nitrite to nitrate. On the other hand, in SRC soils, the application of N could perturb the biogeochemical cycle of C and N, promoting the growth of more generalist microorganisms such as *Gemmatimonadetes*, and denitrifiers such as *Anaerolineae* (Jimenez-Bueno *et al*., 2016). *Anaerolineae* has also been associated with an anaerobic process induced by soil compaction (Hartmann *et al*., 2014). The high representation of *Anaerolineae* in SRU was unexpected and difficult to explain. Nonetheless, a possible interpretation could be the lower altitude of collection sites with respect to the vineyard, which could lead to nitrogen drain from cultivation sites to surrounding wild sites, benefiting from external N input and, therefore, altering the microbial composition. Similarly, in a previous study of the soil microbiome of the Pampean region of Argentina, it was described that prolonged conventional tillage favours *Gammaproteobacteria* growth (Carbonetto *et al*., 2014).

Moreover, several diazotrophic microorganisms were also identified, which can convert nitrogen from the air to NH4+ forms assimilable by plants, an important function in soils with low N levels, such as the soils of Mendoza. The main genera of diazotrophic bacteria that were in higher abundance in G soils were members of the *Beijerenckiaceae* family, *Microvirga* and *Psychroglaciecola*, which are described as symbionts of bryophytes (Cao *et al*., 2020), indicating that they could contribute to Biological Soil Crust (BSC) formation. Encouraging BSC formation is an interesting strategy to improve crop productivity in semi-arid soils due to long-term nutrient accumulation (Nevins *et al*., 2022; Wang *et al*., 2022). The effect of BSC on grapevine cultivation has not been studied yet; thus, it is interesting to address this topic in future research.

When comparing cultivated vs non-cultivated soils, the representation of the *Actinobacteria* phylum was more abundant in soils without tillage. This result agrees with a previous study in Argentina (Carbonetto *et al*., 2014) that suggests a greater sensitivity of *Actinobacteria* to structural disturbation of the soil due to tillage activity. *Streptomyces* sp. belongs to the *Actinobacteria* phylum and is a very important plant growth-promoting bacteria (PGPB) as it can solubilise phosphates in alkaline soils, although its abundance is affected by long-term N fertilisation (Zheng *et al*., 2017). The reduced level of *Actinobacteria* and the lower abundance of *Streptomyces* in SR soils could be due to the history of higher N fertilisers input in that region.

Interestingly, the differences in bacterial composition were strongly supported by the geographic variable rather than crop management, which is in line with a previous work that assigns a very important role for geological origin in the configuration of the prokaryotic composition in the soil (Reith *et al*., 2015). Regarding the fungal microbiome, the alpha and beta diversity indicate that, unlike our observations on bacterial composition, fungi are more influenced by local variations, such as grapevine crop management in Santa Rosa. This result is in agreement with several recent studies (Chou *et al*., 2018; Coller *et al*., 2019; Wipf *et al*., 2021). The greater sensitivity of fungi to soil tillage, compared to bacteria, could be due to the filamentous growth of fungi. This type of growth is more likely to be interrupted by mechanical effects than the unicellular type of bacterial growth. This hypothesis is also consistent with the decrease in abundance observed for *Actinobacteria* in SRC. This bacterial phylum is particularly able to grow in a filamentous manner and, therefore, could be more susceptible to soil tillage. Another possible explanation is that compaction in soils under tillage management reduces the oxygen level, specifically affecting fungi as eukaryotic organisms are more sensitive to low oxygen pressures (Hartmann *et al*., 2014). Further studies are needed to confirm these.

Regarding soil chemical composition, our results suggest that the carbon-to-nitrogen (C:N) ratio was the main factor driving the differences observed in bacterial and fungal composition between the two evaluated regions. In the case of bacteria, the denitrification activity in SR could lead to the loss of fixed N (as N_2O is emitted into the atmosphere), leading to an increase in the C:N ratio (Wrage *et al*., 2001). In the case of fungi, the observed separation between SRC and SRU groups (Figure 7B) suggests that N, P, K and OM shape the fungal composition. This result agrees with a previous study which found that fungal abundances positively correlate with soil fertility (Delgado-Baquerizo *et al*., 2017).

Moreover, this study also aimed to focus on functional aspects based on the estimation of gene abundance and metabolic pathways by crossing data from taxa genomes with taxa abundance. Nicotinate degradation is a differentially abundant pathway in G soils (aldex2 t-test, BH corrected p-value < 0.01). This metabolism is related to the use of alternative carbon sources, such as sugars derived from the degradation of polysaccharides, which can support microbial survival in conditions of low OM, such as those of the G soils, highlighting the recycling capacity of nutrients in soils of Gualtallary. Phospholipase activity was an important feature in G soils (aldex t-test, BH corrected p-value < 0.05, Table S4). Since these soils are characterised by a low phosphorous content, the recycling of phospholipids could play an important role in overcoming this nutritional deficit (Park et al., 2022). On the other hand, the biosynthetic activity of chlorophyllide, which is a precursor of chlorophyll, is a distinctive feature of G soils (aldex t-test, BH corrected p-value < 0.05, Table S4). A possible interpretation of this feature is that photo dependence (protection or photosynthesis) is more important in G soils with a higher UV radiation exposure than those from the SR soils. Chlorophyll biosynthesis occurs under both aerobic and anaerobic conditions. Recently, the importance of phototrophic microorganisms in arid soils for the development of biological crusts has become evident (Tang *et al*., 2018; Bay *et al*., 2021). Therefore, in G soils, the primary production of C and N would be mainly sustained by photoautotrophs.

CONCLUSIONS

The relationship between microbial and biogeochemical cycles is underexplored in arid soils. In the present study, we evaluated the microbial composition of different Winkler-classified sites as well as grapevine-cultivated and uncultivated soils. Based on our results, a model of the microorganisms' roles in ecological and functional contexts was proposed. The identification of nitrifier denitrification microbial activity in SR grapevine cultivated soils suggest that ammonium input promotes the growth of such taxa, and this activity could favour nitrous oxide release, impacting the environment and reducing the efficiency of fertilisation. The identification of chlorophyll synthesis activity in G soils highlights the importance of phototrophs (possibly forming or promoting the formation of BSC) in those unperturbed soils and heavily exposed to UV radiation. All the hypotheses proposed here regarding the functional microbial's roles naturally need to be validated with specific experiments to document the presence and importance of the microorganisms in the soil. The associations of agronomic handling in the vineyard with the changes in microbiome structure and functional profiles would contribute to select crop practices that preserve and promotes microbial diversity and ecological equilibrium. Microorganisms with a putative beneficial impact on ecological and agricultural interest were also described in this study. This information will be useful to develop future bioinoculants that exploit the biologically well-adapted microbial resources from the particularities of the viticultural soils of Mendoza.

ACKNOWLEDGEMENTS

The authors acknowledge Clara Elizabeth Galindo-Sanchez for kindly sharing the CICESE HPC resources and Sylvia Camacho Lara for all the informatics administration support required for this work. Authors acknowledge Luciana García for sharing Nanodrop2000 at Instituto Nacional de Vitivinicultura (INV). The authors thank Gabriela Ruiz for editing the image of the schematic model. MP acknowledges the postdoctoral fellowship from Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Argentina). The authors acknowledge the helpful comments of anonymous reviewers.

Author's contribution

GA contributes with map design, NDVI determinations and altimetry. LM carried out the physicochemical analyses of the soil. MP performed the DNA extraction from the soil and all the bioinformatics analysis. MP, GA, MB, CL and LAM wrote the draft of the manuscript. All authors reviewed, edited, and approved the final version of the manuscript.

Funding

This work was possible due to an STE contract between INTA and Grupo Peñaflor S.A. and funding by Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (Argentina) under PICT-STARTUP-2018-0217.

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