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Research article

Metabarcoding analysis reveals an interaction among distinct groups of bacteria associated with three different varietals of grapes used for wine production in Brazil

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A R T I C L E I N F O

MSC:
Soil microbiota
nutrients
grape varieties
and regions are closely related and could contribute to an important characteristic of wines known as *terroir*

Keywords:
Grape
Microbiota
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A B S T R A C T

Grapes are globally popular with wine production being one of the most well-known uses of grapes worldwide. Brazil has a growing wine industry, and the Serra Gaúcha region is a significant contributor to the country's wine production. Nonetheless, other states are increasing their relevance in this segment. Environmental factors and the soil microbiome (bacteria and fungi) heavily influence grape quality, shaping the crucial "*terroir*" for wines. Here, soil quality was assessed through nutrient analysis and bacteria microbial diversity, which could significantly impact grape health and final wine attributes. Soil samples from São Paulo's vineyards, focusing on Syrah, Malbec, and Cabernet Sauvignon, underwent chemical and microbial analysis via 16S rRNA metabarcoding and highlighted significant differences in soil composition between vineyards. Statistical analyses including PCA and CAP showcased region-based separation and intricate associations between microbiota, region, and grape variety. Correlation analysis pinpointed microbial genera linked to specific soil nutrients. Random Forest analysis identified abundant bacterial genera per grape variety and the Network analysis revealed varied co-occurrence patterns, with Cabernet Sauvignon exhibiting complex microbial interactions. This study unveils complex relationships between soil microbiota, nutrients, and diverse grape varieties in distinct vineyard regions. Understanding how these specific microorganisms are associated with grapes can improve vineyard management, grape quality, and wine production. It can also potentially

Abbreviations: (PCA), Principal Component Analysis; (CAP), Canonical Analysis of Principal Coordinates; (ST), Syrah in Terrassos vineyard; (MPL), Malbec in Portal da Luz vineyard; (CSPL), Cabernet sauvignon in Portal da Luz vineyard; (SPL), Syrah in Portal da Luz vineyard; (SOM), Soil Organic Matter; (CEC), Cation Exchange Capacity.

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optimize soil health, bolster grapevine resilience against pests and diseases, and contribute to the unique character of wines known as *terroir*.

1. Introduction

Grapes are one of the most consumed fruits worldwide in the form of raisins, juice, sweets and mainly as wine [1]. Wine production has been growing worldwide due to the spread of grapevine cultivation. The major wine producers are Italy, France, Spain, USA and Australia [2,3]. Brazil is the 14th world wine producer and the fifth largest consumer with 90 % of the production coming from Serra Gaúcha, in South Brazil [4,5]. Recently, there has been an increase in the number of wine-growers in other states of Brazil like São Paulo, Minas Gerais and in the Vale do São Francisco, located in the north-east of Brazil, in the states of Bahia and Pernambuco [4]. The climate and environmental factors changes, from planting to harvest, are of paramount importance to the quality of the grape and directly related to growing conditions, genetic factors, and plant development [6].

Chemical and microbiological soil characteristics, climatic elements and the type of vineyard together with the management of the crop are directly involved in the phenotype of grapes, the so-called "*terroir*". Wineries use *terroir* to correlate with the quality of grapes and, consequently, with the typicity and quality of the wine [7,8]. Undoubtedly, soil quality is one of the most important factors linked to the health of vines and the quality of the fruits. This quality can be measured by the micro and macronutrients and by the diversity of microorganisms [9]. Generally, the diversity of microorganisms is greater in the soil's rhizosphere where there is a strict interaction between the microorganisms and the roots [10,11]. Furthermore, many microorganisms could be intricately associated with other plant organs including the fruit. Thus, their presence exerts a discernible impact on the organoleptic attributes, thereby influencing the end product [12]. Although the interactions between plants and microorganisms are considered complex and elusively understood, microorganisms certainly play a major role in several aspects of plant health [7,13].

Several studies point out an interaction of microorganisms in the promotion of plant growth, tolerance to abiotic stress, nutrition, productivity, and with the manifestation or combat of diseases [10,11,13–15]. In grapes, most studies have focused on plant genome and transcriptome. Recently, several studies have aimed at understanding the interactions among vines/microorganisms to assess the effects on the quality of the plant as a whole. These studies have shown that the microbiome is modulated when comparing different organs, variety, regions and soils [7,16]. Agricultural management is also described as a modulator of the grape's microbiota and therefore contributes to the presence of these microorganisms during wine fermentation [16–18].

The interaction between the microbiome and vines has been extensively documented, and its significance on a global scale has been

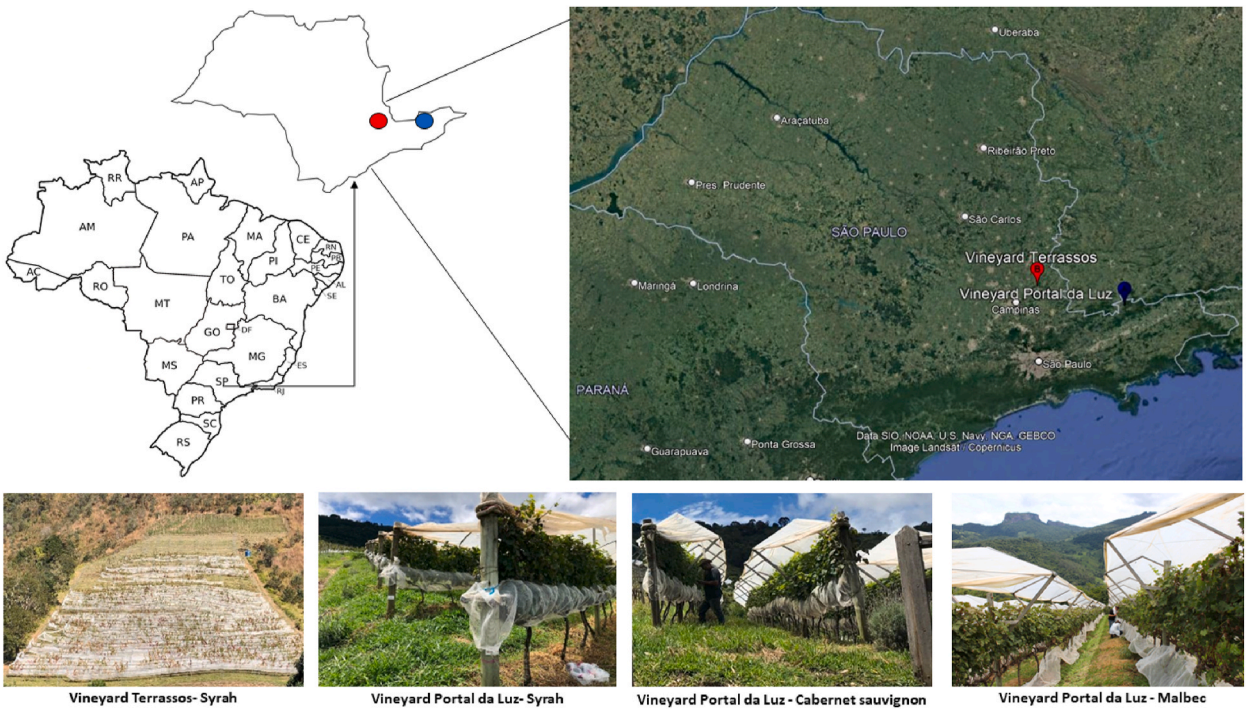

Fig. 1. Geographic location of the vineyards with an indication of the sampling sites in the city of Amparo- Terrassos (22°39′58.76″S 46°47′32.45″W) (red) and in the city of São Bento do Sapucaí – Fazenda Portal da Luz per vineyard Portal da luz (22°42′48.54″S 45°39′20.26″W) (blue) in the state of São Paulo. In each site, two samples (in summer and in winter) were collected over two years (2020 and 2021). In the Portal da Luz farm, soil was collected from Syrah, Cabernet sauvignon and Malbec varieties and in Terrassos farm, only from Syrah.

established. However, research focused on this topic in Brazil is scarce. By using the 16S rRNA metabarcoding technique, the purpose of this study was to evaluate and analyze the relationship between the profiling of microbial communities and nutrient composition of the soil in two different vineyard sites and three different grape varietals (Syrah, Cabernet Sauvignon, and Malbec).

2. Material and methods

2.1. Soil sampling

Soil samples were collected at the state of São Paulo. One vineyard was in the region of the city of Amparo- Fazenda Terrassos per vineyard Terrassos (22º39′58.76″S 46º47′32.45″W) and another in the region of the city of São Bento do Sapucaí – Fazenda Portal da Luz per vineyard vineyard Portal da luz (22º42′48.54″S 45º39′20.26″W), 200 km away from each other (Fig. 1). Soil sampling was collected for chemical analysis at a depth of 30–40 cm after the removal of dust, roots, leaves and other surface residues. Around 0.5 kg of soil was collected per sample and placed in labeled plastic bags. For chemical analysis the triplicates were mixed and evaluate in 12 samples. All samples for metabarcoding analysis were also collected at a depth of 30 - 40 cm. They were homogenized and 0.25g was used for DNA extraction. The varieties of grape collected in the different vineyards are described Table 1.

All the samples were collected in triplicate (3 soils from 3 different plants of each variety) totalizing 12 samples per season (summer and winter), over two years (2020 and 2021). In total, we evaluated metabarcoding in 48 samples.

2.2. Soil nutrient analysis

Soil nutrient analyses were carried out using several protocols. The analysis for pH $CaCl_2$, P, K, Ca, Mg and Al followed the Manual of soil analysis methods – EMBRAPA [19]. The quantification of nutrients H + Al and SOM (Soil Organic Matter) was done according to Soil Chemical Analysis and Quality Control Manual [20].

2.3. DNA extraction

Total DNA from each rhizosphere soil sample was extracted using the PowerSoil® DNA Isolation Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. For extraction, approximately 0.25 g of rhizospheric soil was used. The integrity of the extracted DNA was assessed using agarose gel electrophoresis (0.7 % w/v) at (3 V.cm-1) in 1X TAE buffer (2.42 g Tris; 0.57 ml of acetic acid; 1 ml 0.5 M EDTA; pH 8.0) stained with ethidium bromide. Then, the DNA was quantified by fluorescence in a Quantus spectrophotometer (Promega). The extracted DNA was kept at −20 °C for construction and sequencing of metagenomic libraries.

2.4. Library preparation and sequencing

DNA libraries for each sample were prepared following Illumina guidelines (16S Metagenomic Sequencing Library Preparation, Part #15044223 Rev. B). The bacterial V3 and V4 regions (bold in the sequence) of the 16S rRNA gene were amplified using the 16S Amplicon PCR Forward Primer 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCTACGGGNGGCWGCAG** and 16S Amplicon PCR Reverse Primer 5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GACTACHVGGGTATCTAATCC** with the addition of the Illumina overhang adapter sequences. The sequencing of partial 16S ribosomal RNA was performed by next-generation sequencing using the Illumina MiSeq platform that produced thousands of 300 bp paired-end reads (2 × 300 bp) for each library.

2.5. Microbial composition and community structure analysis

For the bacterial community analysis, the quality of raw reads was accessed initially by FASTQC software (v. 0.11.9). Adapters and primer were removed from the sequences using the "atropos" software (v.1.1.21), and The FASTP software (v. 0.23.2) [21] was used for quality control process (–disable_adapter_trimming, –average_qual 20, –cut_right, –cut_right_window_size 5, –cut_right_mean_quality 20). The sequence pairs filtered were merged using FLASH (v. 1.2.11) [22]. Pre-processed data was analyzed using the DADA2 pipeline [23] using the R software (v.4.1.2; R Core Team). Initially, the quality-filter and trimming were carried using the DADA2 function "filterAndTrim()", with options maxEE = 2, truncQ = 2, truncLen = 400. Error rate models were fitted using the DADA2 function "learnErrors()" and reads were replicated using function "derepFastq", and the filtered data was inferred to amplicon sequence variants (ASVs) using the "dada()" function. Chimeric sequences were removed using the "removeBimeraDenovo()" function and the taxonomy was then assigned using the RDP Naïve Bayesian Classifier [24] implemented in the "assign Taxonomy()" function

Table 1
Varieties of each grapes collected in each sample sites.

Vineyard	Varieties of grapes
Terrassos	Syrah (ST)
Portal da Luz	Syrah (SPL)
	Cabernet sauvignon (CSPL)
	Malbec (MPL)

using SILVA database (v. 138.1) [25]. The resulting ASV table was converted into a phyloseq object [26] for downstream analysis (Table S1). The full data sequence has been registered at NCBI BioProject, under the bioproject number PRJNA1053291.

2.6. Statistical analysis

Principal Component Analysis (PCA) was used to visualize differences in soil characteristics between vine varieties according to the distribution of their soil chemical variables. PCA was conducted using 'factoextra' (v.1.0.7) R package [27]. The analysis of soil nutrients between wineries was done through permutation using "a posteriori" test. To identify the magnitude of the influence of soil variables on soil bacterial community structure, a constrained Canonical Analysis of Principal Coordinates (CAP) was performed using the Bray–Curtis dissimilarity index as a measurement of beta diversity. Subsequently, the factors were compared through permutational analysis of variance (PERMANOVA, 999 permutations). A Random Forest analysis was used to classify the most important genera based on differential abundance in each treatment evaluated and the outputs were also used for correlation analysis with the soil variables.

This step ensures that the model can be evaluated on unseen data, which helps in assessing its performance and generalizability. Here, the response variable is the difference between grape varieties in the two studied locations. The RandomForest package identified the most informative microbial features that contribute most to differentiating between samples. The MeanDecreaseGini metric was used to assess feature importance. This metric evaluates how much a particular feature contributes to the overall decrease in Gini impurity within the trees. Gini impurity is a measure of randomness or disorder in the data; thus, a feature that significantly decreases Gini impurity is considered highly informative. Features with higher MeanDecreaseGini values are deemed more influential in predicting the microbial abundance patterns. The use of RF analysis through the 'microeco' package provides a robust approach to discerning key microbial features that differentiate grape varieties across different locations. This process not only highlights the power of machine learning in ecological and biological studies but also underscores the importance of selecting and validating the most informative features to achieve accurate and meaningful predictions.

The correlation-based network was used to identify whether the pattern of connectivity between the microbiomes from the different bacterial soil communities varies depending on the influence of the rhizosphere of each vine variety. Only correlations above 0.75 and p-value <0.001 were used for the construction of the network. Graphical analysis and the generation of topologies were carried out using the open-access software Gephi v. 0.9.2. Analyses were carried out in R environment, mainly supported by 'phyloseq' v.1.30.0 [26], 'vegan' (v.2.5–6) [28], 'ampvis2' (v.2.5.5) [29], and 'microeco' (v.0.14.0) packages and dependencies. All p-values were corrected by the false discovery rate method [30] to avoid the inflation of Type-I error due to multiple tests.

3. Results

3.1. Microbial community diversity and composition

Sequencing of the bacterial community present in grapevine soils resulted in a total of 9,823,516 reads. After quality control, filtering process and removal of chimeras and contaminants, a total of 3,741,879 reads remained. The rarefaction curves in Fig. S1 indicate that all samples have been sufficiently sequenced to cover bacterial diversity in soil samples. The microbial taxonomic

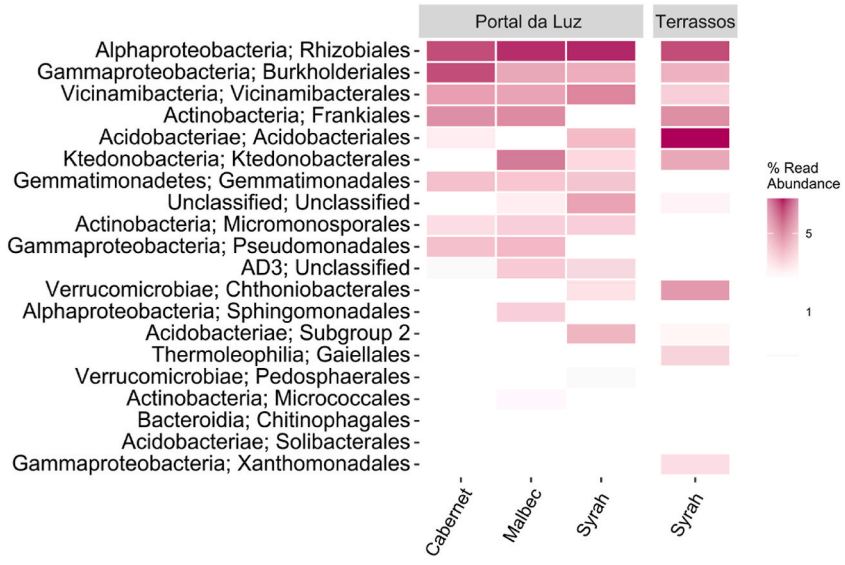

Fig. 2. Relative abundance of soil microbiota by grape variety and location, ranked by Order and their related Classes. The relative abundance of the top 20 groups are presented.

composition of soil samples, using only classified ASV, encompasses a total of 46 phyla, 124 classes, 297 orders, 393 families, 1151 generas, and 500 species (Table S2).

The bacterial communities were dominated by *Proteobacteria* (26 %), *Actinobacteria* (20 %), *Acidobacteria* (19 %) phylum (Table S2). The most abundant orders of bacteria in all sampling locations and cultivars were *Burkholderiales*, *Rhizobiales* and *Vicinamibacterales* (Fig. 2).

3.2. Microbial community structure

Principal component analysis was able to capture the multivariate dispersion of soil chemical variables in each evaluated system, highlighting the formation of clusters by location based on soil nutritional characteristics (Fig. 3A). Samples from Portal da Luz were clustered according to the high concentration of most nutrients in the soil while the samples from Terrassos were grouped with low concentration of all of nutrients, except to K^{+}. In the permutational analysis, the soil from Syrah Terrassos (ST) differed from Syrah Portal da Luz (SPL) ($p = 0.02$ $T = 2.62$) and Cabernet Sauvignon Portal da Luz (CSPL) ($p = 0.02$ $T = 2.41$), showing a strong tendency to differ with MPL ($p = 0.05$ $T = 1.67$). Soil samples from the same locality did not differ significantly (data not shown). Likewise, when analyzed the different seasons (summer and winter), there were no significant differences (data not shown). To further explore the influence of soil components on the structure of the bacteriome, a canonical analysis of principal coordinates using Bray-Curtis distance (Fig. 3B and Fig. S2). The joint evaluation of soil chemical variables and bacterial community structure showed that the type of grape variety was more important than soil variables for the varieties in Portal da Luz. However, when comparing the bacteriome of Syrahs among locations (Terrassos and Portal da Luz), we observed that soil chemical composition had a significant influence on the structuring of global bacterial communities, especially regarding the higher soil fertility in Portal da Luz (Table 2). This does not indicate that there is no presence of an intrinsic core microbiome associated with the rhizosphere of this grape variety.

An effect variation partition analysis was performed to detect the percentage of explanation of the following factors on the variation in the structure of the microbial community: Location, Grape variety, Time/Collection and Soil chemistry (Fig. S3). Interaction between collection time and soil characteristics showed to be the effect with the highest explained percentage (11%) .The second most important microbiome influencing factor was the soil chemistry with 9 %.

Correlation between microbial community and soil characteristics.

The correlation between chemical soil properties and the microbiome based on Pearson's correlation analysis (Fig. 4). The genera *Oikopleura, Actinorhabdospora, Enteractinococcus, Paenisporosarcina, Thiobacillus, Ornithinicoccus, Oleiagrimonas, Membranicola, Dietzia, Chryseomicrobium, Chelativorans, Candidatus, Caldatribacterium, Anseongella, Bhargavaea, Tepidimicrobium, Globicatella, Planococcus, OLB17, Xenophilus, Demequina, Dinghuibacter, Mitsuaria* and *Pedosphaera* had a significant positive correlation with K^{+} in the soil.

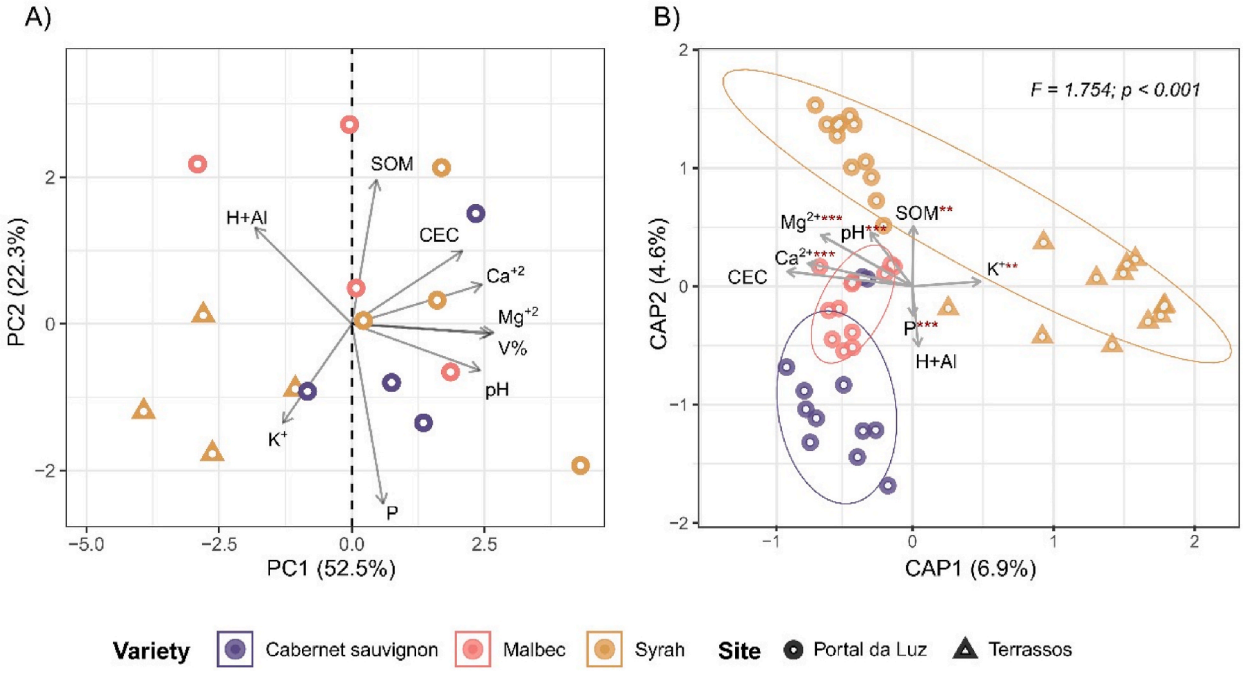

Fig. 3. (A) Principal component analysis (PCA) based on soil chemical characteristics (composite soil samples in each sampling area, 4 samples per treatment). Beta diversity indices were fitted as factors with significance <0.05 onto the ordination. **(B)** Canonical analysis of principal coordinates (CAP) for evaluating the effects of soil properties on bacterial communities (non-composite soil samples, 12 samples sequenced by treatment). SOM= Soil organic matter; P= Phosphorus; K^{+}= Potassium; Ca^{2+}= Calcium; Mg^{2+} = Magnesium; H + Al^{3+}= Acidity potential; CEC= Cation Exchange capacity; V% = Base saturation."

Table 2
PERMANOVA analysis comparing the structure of the microbial community between grape varieties and locations. All comparisons were generated after 999 permutations.

Comparison	F Model	R^2	P-value
Cabernet x Malbec	1.7991	0.0756	<0.001
Cabernet x Syrah PL	2.4928	0.1017	<0.001
Cabernet x Syrah T	3.2857	0.1299	<0.001
Malbec x Syrah PL	1.7665	0.0743	<0.001
Syrah PL x Syrah T	3.3915	0.1335	<0.001

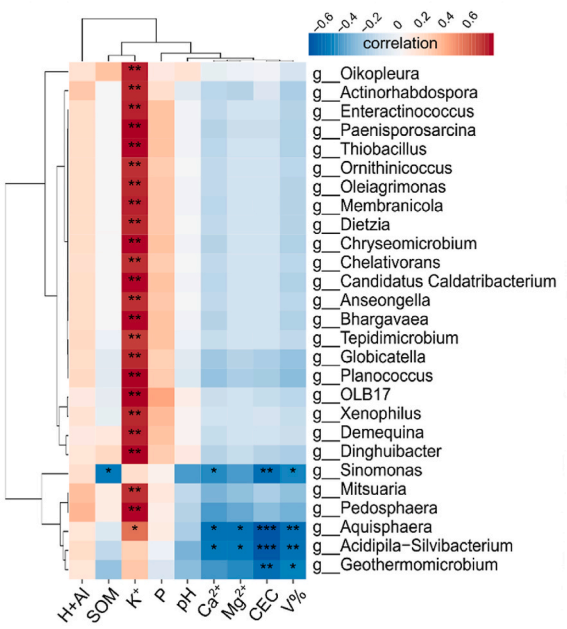

Fig. 4. Pearson correlation analysis organized by clustering, between soil nutrients and bacterial genera. Asterisks indicate statistical significance: (***) $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$.

Regarding the CEC and V%, the genera *Sinomonas*, *Aquisphaera*, *Acidipila-Silvibacterium* and *Geothermomicrobium* were the least negatively correlated. In addition to the negative correlation with CEC and V%, the genus *Sinomonas* also presented the same correlation with SOM and Ca^{2+}. The genera *Aquisphaera* and *Acidipila-Silvibacterium* were negatively correlated with Ca^{2+} and Mg^{2+}.

3.3. Bacterial diversity and correlation with varieties

The MeanDecreaseGini coefficient from Random Forest analysis was performed to show which bacterial genera have more influential role in predicting microbial abundance patterns in each variety and location. Our results ranked the most important bacterial genera in each system (grape variety/location). Six different genera were found as most important in the ranking of genera for the Cabernet soil system (CSPL), 10 for MPL, four for SPL and 10 for ST (left side of Fig. 5). The relative abundance of the selected genera in each evaluated system was shown in the right side of Fig. 5 in order to demonstrate the magnitude of these differences. The genus *Puia* is the most important in ranking genera for the Cabernet soil system. For MPL, SPL and ST were *Aridibacter*, *Candidatus_Xiphinematobacter and Nitrosospira* respectively (Fig. 5).

3.4. Complexity of microbiome communities associated with the soil in different Grape varieties

Co-occurrence network analysis including the class of bacteria showed that the network complexity (here defined by average changes in network properties, focusing particularly on nodes and edges) was different among grape varieties. The Malbec variety showed the least complex interactions (104 nodes; 123 edges; 27 communities; Fig. 6B), while the Cabernet Sauvignon presented the most complex interaction (151 nodes; 371 edges; 26 communities; Fig. 6A). The Syrah variety of both vineyards (Portal da Luz and Terrassos) had the same result (161 nodes; 224 edges; 30 communities; Fig. 6C and D). Patterns in topological measures, such as the graph density and average weighted degree, were similar to the patterns observed for the edge numbers (Table S4).

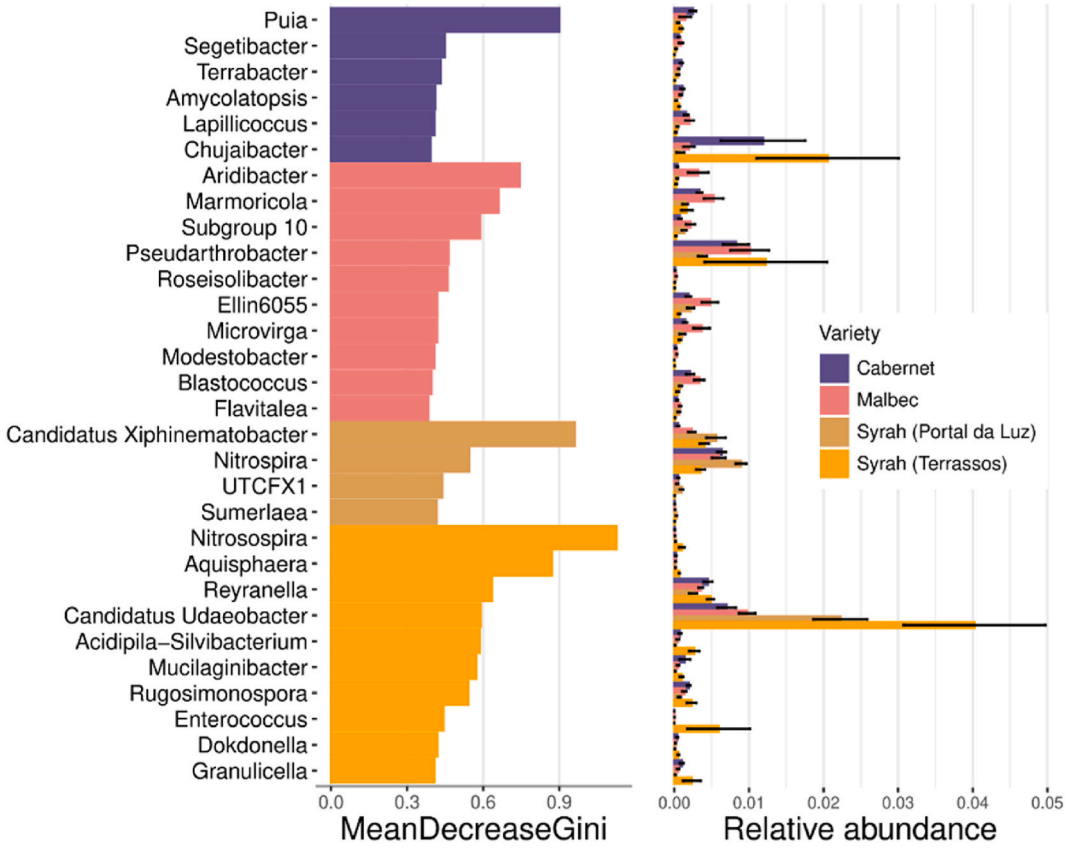

Fig. 5. The 30 most important bacterial genera, classified based on random forest analysis for each grape variety (left barplot), along with the mean ± standard deviation of the relative abundance of each of these genera in each environment (right barplot).

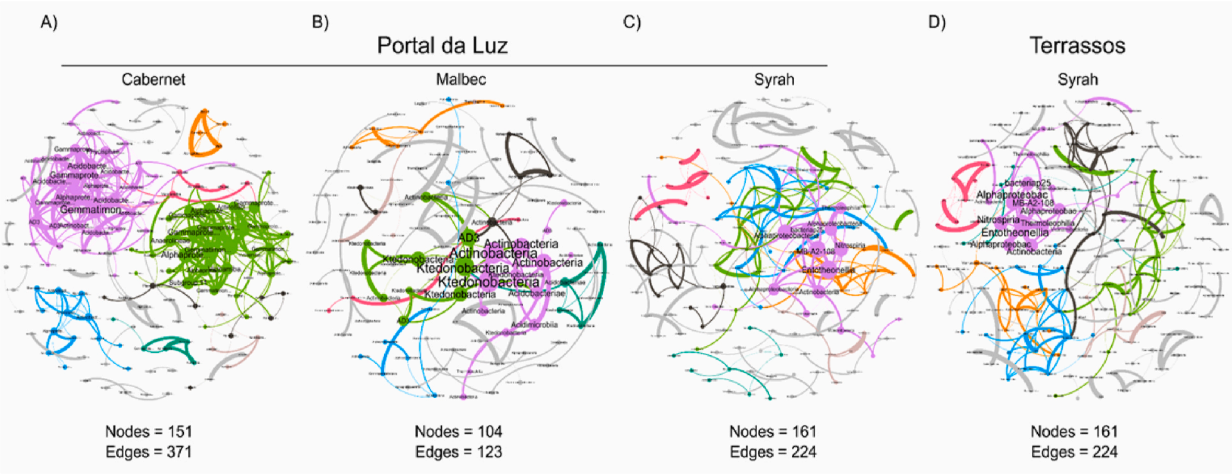

Fig. 6. Network analysis using relative abundance by class of bacteria. Co-occurrence of bacterial groups associated with each variety. Number of the total nodes and edges are shown at the bottom of each network.

4. Discussion

Worldwide, there are few studies on the effects of physical (climate, soil, nutrients), biological (microbiota, grape variety, and fauna), and geographical factors on vineyard soil and grape quality [31,32]. Since in Brazil these association still very poorly explored we aim to evaluate as the first time a deeply study comparing the bacteria soil diversity in different locations and with different

varieties.

Our results showed that at a taxonomic level, the phyla *Proteobacteria*, *Actinobacteria* and *Acidobacteria*, *Chloroflexi* were the most prevalent in all samples analyzed (Table S2). In a similar study carried out in northern China, in which different grape varieties grown in the same region, *Actinobacteria* was significantly enriched in Syrah compared to other varieties, and *Proteobacteria* was significantly enriched in Cabernet Sauvignon [33]. In another study in vineyard soil of the Pinot Noir variety, the most common phyla were *Actinobacteria*, *Proteobacteria*, *Gemmatimonadetes* and *Bacteroidetes*, similar to our results [34]. Mezzasalma et al. (2018) also found *Proteobacteria*, *Actinobacteria* and *Acidobacteria* as predominant phyla in vineyard soils in Italy and Spain [16].

The *Acidobacteria* group is widely associated with vineyard soil worldwide [7]. *Proteobacteria* and *Actinobacteria* group predominance are thought to correlate with several processes such as biological nitrogen fixation, phosphate solubilization, degradation of organic matter and nutrient cycling that promote plant growth [35,36]. Therefore, our results indicate that Brazilian vines exhibit the same prevailing phyla in the soil, suggesting ecological homogeneity.

Multiple studies have demonstrated that both *Burkholderiales* and *Rhizobiales* play a crucial role in the denitrification process of paddy soil [37]. The former was abundant in the CSPL samples while the latter was present in all samples from this study (Fig. 2). According to Nerva et al. (2019), the orders of bacteria most present in vineyard soil were similar to those found in rice paddies, supporting our results [38]. For rice crops, a bacterium belonging to the order *Burkholderiales* plays a main role in the decrease of nitrate in the soil [39]. Rivas et al. (2021) described *Rhizobiales* as playing an important role in nitrogen fixation, and may also be involved in promoting plant growth [40]. *Vicinabacterales* was the third most abundant order in our study, and this group indicates phosphate solubilization in the soil [41]. Is widely described that these microorganisms can also be associated with the health of the plant facilitating the nutrient uptake and utilization, stabilization of soil structure, reduction of disease and competing with pathogens [7,42].

Another important factor for grapevine cultivation is the pH. The optimum soil pH for grapevine is around 6.0. Acidic pH (considered lower than 5.5), reduce the roots' ability to uptake phosphorus, potassium, magnesium while increasing the concentration of aluminum and manganese. An excess of aluminum decreases the intercellular transport of micronutrient ions and attenuated elongation of roots and excessive manganese concentration oxidized phenolic compounds that can result in the chlorosis and necrosis of leaves [43–45].

The main factors associated with soil nutrients in a study by Zhao et al. were planting area and plant age, and there were no significant differences between varieties [46]. In the PCA analysis (Fig. 3A) in which only soil nutrients were interpolated with farms, a clear separation between the locations was observed. Nevertheless, when nutrients and microbiota were interpolated (Fig. 3B and Fig. S3), the varieties clustered showing an integration among the microbiota with the region and variety. Geographical location, management and varieties were independently mentioned by several authors as determining factors in the predominance of a specific microbiome [47–49]. The soil composition and the season of the year also had an important influence in the microbiome, however the cultivars seem to possess a specific microbiota association (Fig. 3B). When our results were compared to that by Gobbi et al. (2022) we can conclude in the same way that spatial distance is the main variable explaining beta-diversity for bacteria communities [50]. Authors also showed that the same pattern was observed for fungi. These authors demonstrated that the climate factors were only associated in fungal alpha diversity but not with prokaryotic alpha diversity, reinforcing our results indicating that seasons do not influence in the bacterial alpha diversity. Additionally, our results also found a specific association of groups of bacteria with the different varieties of grape and correlated with specific specie of plant. In the results described on Fig. 6 we found a very similar results when Syrah of 2 different regions were compared and a significant discrepancy when others varieties were compared. This means that different groups of bacteria were associated specifically with a variety of grapes as well described in the literature for coffee [51]. For alpha diversity, no statistical difference was found, corroborating the results found for Lijun et al. (2022) that performed the same analysis in different varieties [33].

Some genera of bacteria were found to be correlated with some soil nutrients according to our Pearson correlation analysis (Fig. 4). This is the first study that observed a positive correlation of soil potassium and the genera *Oikopleura*, *Actinorhabdospora*, *Enteractinococcus*, *Paenisporosarcina*, *Thiobacillus*, *Ornithinicoccus*, *Oleiagrimonas*, *Candidatus Caldatribacterium*, *Anseongella*, *Bhargavaea*, *Globicatella*, *Planococcus*, *OLB17*, *Xenophilus* and *Mitsuaria*. These groups of bacteria were largely found in soils of cultures other than grapevines, although no correlation analysis with nutrients have been previously shown [52–62]. *Dietzia* and *Xenophilus* have also been found in other studies on grapevine soil [63,64]. However, our results first describe a positive correlation with K+ content.

The genera *Membranicola*, *Chryseomicrobium*, *Chelativorans*, *Tepidimicrobium*, *Demequina*, *Dinghuibacter* and *Pedosphaera* are related with process involved in plant growth promotion and development such a as carbon and nitrogen cycle, phosphorus absorption, solubilization of phosphate and degradation of organic matter, according to the literature. As far as we know no confirmation with potassium has been found so far, differing from our results [62,65–70].

This is the first report describing the genus *Sinomonas* negatively correlated with SOM, Ca^{2+}, CEC and V%. Although no correlation was found in the literature for these components, this group possibly plays an important role in nitrogen fixation, biodegradation process and is involved in plant growth [71,72].

In a recent study using rice rizhospheric soil, the genus *Aquisphaera* was one of the most abundant and its presence negatively correlated with the macronutrients P, K^+, Mg^{2+} and Ca^{2+} in the rice soil [73]. Another study using the same culture found that this bacteria has a negative correlation with N_2 and K^+[74]. Here, we found a negative correlation with CEC, Mg^{2+}, Ca^{2+} and V%. Our data reinforce the results from the literature and further suggest that the presence of *Aquisphaera* has a negative relationship with several macronutrients in the rizhospheric soil.

A negative correlation between CTC, V%, Ca2+, Mg2+ and *Acidipila-Silvibacterium* was found in our results. In the study by Zhou et al., *Acidipila-Silvibacterium* presented a positive and negative presentation with K, P and N. Although in our study we did not evaluate

P and N, the result for nutrient K corroborated this, indicating that this group of bacteria has a potential relationship with soil nutrients [75]. Regarding the negative correlation of CEC and V% with the genus *Geothermomicrobium*, a genus broadly found in soils, our study is the first that shows the correlation between this bacterium and the macro and micronutrients present in the soil of grapevines [76].

In the Random Forest analysis (Fig. 5), the 30 most abundant bacterial genera were identified for each grape variety. For Cabernet Sauvignon, six highly abundant genera were found (*Puia*, *Segetibacter*, *Terrabacter*, *Amycolatopsis*, *Lapilicoccus* and *Chujaibacter*). For Syrah, we found four genera (*Candidatus Xiphinematobacter*, *Nitrospira*, *UTCFX1*, *Sumerlaea*) for the SPL location and ten (*Nitrosospira*, *Aquisphaera*, *Reyranella*, *Candidatus Udaeobacter*, *Acidipila-Silvibacterium*, *Mucilaginibacter*, *Rugosimonospora*, *Enterococcus*, *Dokdonella* and *Granulicella*) for the ST location. To our knowledge, this is the first time that several of these genera are associated specifically with a grape variety. Our results suggest that the abundance of certain generas associated with specific grape varieties. This association could be involved in a complex and intricate interrelationship between grape variety and soil microbiota.

Similar co-occurrence patterns with microorganisms in different varieties and regions were also found. Our results suggest that distinct communities have an intricate correlation associated with each variety of grape and that the microbiota is similar for the same variety in different regions. Regarding overall network topology, the results showed that the bacterial community network for the Cabernet Sauvignon is more connected than the other varieties (Fig. 6 and Table S4), which indicates that it presents a more complex and integrated microbiota. In contrast, both networks for the Syrah variety were intermediate and had the highest modularity (Fig. 6 and Table S4). This implies that the bacterial community occurring in both Syrah in different locations is probably the most dynamic community with the largest communities number with common co-occurrence of bacteria between different sites planted on the same variety.

Extensively studies have been demonstrated that vineyard soil is a vital reservoir of fungi, bacteria and nutrients intimately connected to the vine. These factors play critical roles in a variety of processes, from nutritional functions and plant development to grape formation [8,77]. It has also been demonstrated that the yeasts and bacteria present in vineyard soil are dependent on the management practices and can significantly influence the efficiency of spontaneous wine fermentation contributing to the quality of wines [78].

5. Conclusions

Our study evaluated as the first time the interaction of soil microbiota with different soil nutrients, correlating the results with grapevine varieties in two distinct regions of Brazil. We could observe that soil composition varies significantly between vineyards, affecting microbial communities, with specific genera showing notable correlation with certain soil components (Figs. 3 and 4). The varieties of grapevines also had a great influence in bacterial communities, since the results showed a distinct microbial pattern associated with each grape variety (Fig. 5). Interactions among bacterial groups were specific to each grapevine variety, as shown for Syrah cultivated in different regions with similar interaction patterns for this microorganism (Fig. 6). Deeply understand these results can revolutionize approaches to optimize soil health, fortify grapevine resilience, and enhance the unique *terroir* of wines.

Additional files: The following supporting information can be downloaded at: https://redu.unicamp.br/dataset.xhtml?persistentId=doi:10.25824/redu/XCR8XR. The subtitle of Suplementary materials are described below:

Figure S1. Rarefaction curves based on the V3–V4 region of the 16S rRNA gene obtained from rhizospheric grape soil samples; Fig. S2. Statistical analysis using PERMANOVA for nutrients of the soil; Fig. S3. Variation partitioning analysis (VPA). Table S1. ASV_count_table; TableS2. Phylogenetic classification of ASVs; Table S3. Soil nutrients analysis of the grape varieties in different seasons. ST= Syrah of Terrassos; SPL= Syrah of Portal da Luz; MPL = Malbec of Portal da Luz; CSPL= Cabernet sauvignon of Portal da Luz; 1 = Collection in summer 2020; 2 = Collection in winter 2020; 3 = Collection in summer 2021; 4 = Collection in winter 2021; SOM= Soil organic matter; P= Phosphorus; K+= Potassium; Ca2+= Calcium; Mg2+ = Magnesium; H + Al3+= Acidity potential; CEC= Cation Exchange capacity; V% = Base saturation. Table S4. Network analysis to assess complexity and integrity of bacterial groups at the class level by grape variety.

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Data availability statement

The raw sequence reads generated in this study are available at the NCBI Sequence Read Archive (SRA) database under BioProject-PRJNA1053291. Supplementary manual is available under DOI https://doi.org/10.25824/redu/XCR8XR.

CRediT authorship contribution statement

G.S. Rezende: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **F.I. Rocha:** Software, Methodology, Formal analysis, Data curation. **M.I.G. Funnicelli:** Software, Methodology, Formal analysis, Data curation. **I. Malavazi:** Visualization, Supervision. **S. Crauwels:** Software, Methodology, Formal analysis, Data curation. **M.M. Brandao:** Software, Formal analysis, Data curation. **A.F. Cunha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32283.

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