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Intra-vineyard heterogeneity of bacterial and fungal communities on cultivated *Vitis vinifera* grapes in the Atacama Desert

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ABSTRACT

Understanding the microbial diversity associated with grape berries is essential for improving vineyard management and wine production, particularly in extreme environments such as the Atacama Desert. This study examined the intra-vineyard heterogeneity of bacterial and fungal communities colonising *Vitis vinifera* cv. Ahmeur bou Ahmeur grapes cultivated under a hyper-arid environment. Grape samples were collected from individual vines, and microbial assemblages were characterised using high-throughput sequencing of the 16S rRNA and ITS regions. Results revealed marked heterogeneity among vines within the same vineyard, with fungal communities showing greater variability than bacterial ones. Fungal assemblages were dominated by *Starmerella*, *Hanseniaspora*, *Malassezia*, and *Cladosporium*, whereas bacterial communities were mainly composed of *Gluconobacter*, *Tatumella*, and *Asaia*. Alpha and beta diversity analyses demonstrated significant differences in microbial composition among vines, indicating strong plant-specific signatures. These results provide new insights into the fine-scale heterogeneity of grape-associated microbiota in desert viticulture and highlight the importance of considering individual plants as ecological units shaping the microbial dimension of terroir.

KEYWORDS: Ahmeur bou Ahmeur, *Vitis vinifera*, grape microbiome, Atacama Desert, *microbial terroir*, wine microbiology, intra-vineyard heterogeneity

INTRODUCTION

Microbial communities inhabiting the surface of grape berries form intricate assemblages of bacteria and fungi that play critical roles in viticulture and enology (Bokulich *et al*., 2014). These microorganisms play key roles in grapevine health (Brischetto *et al*., 2024; Jumbam *et al*., 2025) and contribute to the sensory attributes of wine by shaping the dynamics of spontaneous fermentations (Barata *et al*., 2012; Belda *et al*., 2017; Bokulich *et al*., 2016). The concept of *microbial terroir* has emerged to capture the idea that microbial diversity, alongside soil and climate, constitutes an integral component of regional wine identity (Gilbert *et al*., 2014; Knight *et al*., 2015). Studies across diverse viticultural regions have shown that geography (Bokulich *et al*., 2014), grape cultivar (Mezzasalma *et al*., 2018), vineyard management practices (Vitulo *et al*., 2019), and climatic conditions (Bokulich *et al*., 2014) exert strong influences on grape-associated microbiota, leading to distinct microbial signatures at regional and vineyard scales (Morrison-Whittle & Goddard, 2018; Liu *et al*., 2019).

Despite important advances in vineyard microbiome research, variability at finer spatial scales remains understudied. Factors such as plant age (Berlanas *et al*., 2019; Cui *et al*., 2024), microclimatic heterogeneity (Kraus *et al*., 2018), canopy structure (Austin *et al*., 2011; Bokulich *et al*., 2014), and berry physiology (Barata *et al*., 2012; Wei *et al*., 2022a) can generate variability in the grapevine-associated microbiome. Intra-vineyard heterogeneity may have important consequences: sampling only a single plant or pooling berries from multiple vines can mask the unique microbial signatures of individual plants and lead to biased interpretations of vineyard-wide microbial diversity (Bokulich *et al*., 2016). Recent work by Zhang *et al*. (2019) showed that even clones – genetically distinct lines within the same variety – sustain distinct bacterial assemblages on berry surfaces, emphasising that subtle genetic differences can influence microbial colonisation. Building on this perspective, it becomes essential to ask whether individual grapevines of the same cultivar sustain distinct microbial signatures that may arise from microenvironmental variability. Such effects could be especially pronounced in extreme environments, where aridity, radiation, and large diurnal temperature shifts may magnify fine-scale ecological divergence among plants and their associated microbiota.

The Atacama Desert in northern Chile is the driest non-polar desert on Earth (Bull *et al*., 2016) and lies at one of the geographic limits of grapevine cultivation (Gobbi *et al*., 2022). Viticulture in the Pampa del Tamarugal relies on scarce water resources and is characterised by intense solar radiation, large diurnal temperature fluctuations, and minimal precipitation (Lanino & Poblete, 2022). The Atacama Desert's environmental constraints create a unique natural laboratory for studying how microbial communities are assembled and maintained under extreme abiotic stress (Araya *et al*., 2024). The local cultivar *Vitis vinifera* cv. Ahmeur bou Ahmeur, traditionally cultivated in this region, provides an exceptional model to examine intra-vineyard variability in microbial communities at the limits of viticulture (Poblete *et al*., 2011). This heritage cultivar, now being revalorised in the new viticultural cycle of the Tarapacá Region, offers a unique resource for understanding how extreme desert conditions shape the grape-associated microbiomes.

Although vineyard-level studies have demonstrated that grape-associated microbiomes are shaped by cultivar, climate, and geography (Bokulich *et al*., 2014; Knight *et al*., 2015; Belda *et al*., 2017), the extent of intra-vineyard variability remains less clear. Previous work suggests that fungal assemblages may exhibit stronger plant-specific signatures than bacterial communities, reflecting differences in dispersal capacity, ecological niches, and interactions with berry physiology (Morrison-Whittle & Goddard, 2015; Liu *et al*., 2019). We therefore hypothesised that fungal communities on *V. vinifera* cv. Ahmeur bou Ahmeur would be more strongly structured by plant identity, while bacterial assemblages would remain relatively homogeneous across vines. Testing this hypothesis in the Atacama Desert – a viticultural frontier where abiotic stress is extreme – provides a novel opportunity to understand how fine-scale variability contributes to the microbial dimension of terroir and to inform sampling strategies in vineyard microbiome research.

MATERIALS AND METHODS

1. Sampling site

Berry replicates of *Vitis vinifera* cv. Ahmeur bou Ahmeur were collected from three individual vines located in a varietal garden (~ 2000 m^2) in the Pampa del Tamarugal, Atacama Desert (Tarapacá Region, northern Chile; approximately 20.4° S, 69.6° W). The vines are trained in a simple spur-pruned vertical shoot-positioned trellis system, spaced at 1.5 × 2 m, and irrigated via drip irrigation with two emitters per plant. Sampling was conducted in March 2024, at the ripening stage, under the extreme climatic conditions of the site (annual precipitation < 2 mm, intense solar radiation, and large diurnal temperature variations). From each vine, one sun-exposed cluster was selected. For each cluster, three independent replicates were obtained, each consisting of three healthy berries at comparable phenological stages. Each berry was individually swabbed using a sterile flocked nylon swab. The three swabs were pooled into a single tube prior to DNA extraction. All manipulations were carried out aseptically using sterile gloves and forceps, and samples were placed in sterile containers and transported on ice to the laboratory for DNA extraction and sequencing (Figure 1).

2. DNA extraction and metabarcoding sequencing

The epiphytic microbiota was sampled by swabbing the surface of grape berries using sterile flocked nylon swabs. For each replicate, three berries were individually swabbed using separate sterile swabs to collect surface-associated microorganisms. DNA was extracted from swabs using

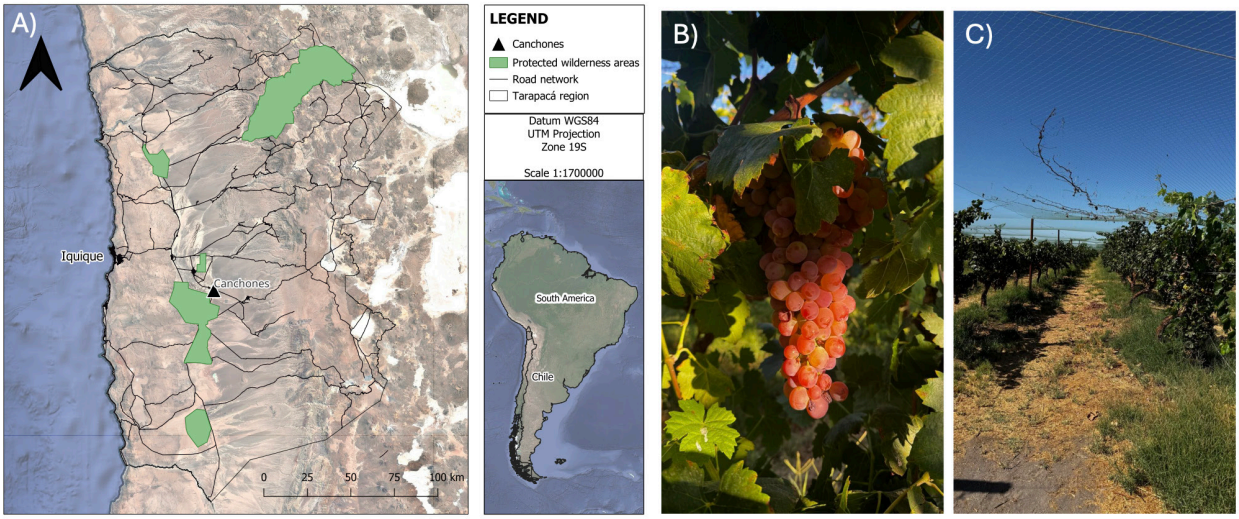


FIGURE 1. Study site and sampling context for *Vitis vinifera* cv. Ahmeur bou Ahmeur cultivated in the Atacama Desert vineyard (Tarapacá Region, Chile).

(A) Geographic location of Canchones within Chile. (B) Grape cluster of Vitis vinifera cv. Ahmeur bou Ahmeur. (C) Varietal garden at the Canchones Experimental Station (~ 2000 m^2, Tarapacá Region, Chile).

the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's instructions with minor modifications. Swabs were suspended in PBS, followed by incubation at 60 °C for 10 minutes, centrifuged, and the resulting pellets were lysed mechanically in a FastPrep instrument (MP Biomedicals) with two 30-second cycles. DNA binding, washing, and elution were performed with the kit's spin column system, and DNA concentrations were quantified using a Qubit 4.0 fluorometer and the dsDNA HS Assay Kit. Negative extraction controls were included in all runs. Amplicons of the bacterial 16S rRNA V4 region and the fungal ITS1–ITS2 region were generated using a high-fidelity polymerase and Illumina-adapted primers. For the 16S V4 region, primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) were used, while the ITS1–ITS2 region was amplified with primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). PCR products were visualised by agarose gel electrophoresis, purified with magnetic beads, and quantified fluorometrically.

Amplicon libraries were prepared following the Illumina 16S Metagenomic Sequencing Library Preparation protocol. The V4 region of the 16S rRNA gene and the ITS1 region were amplified using KAPA HiFi HotStart ReadyMix (Roche). PCR conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min.

Sequencing libraries were prepared by pooling equimolar amounts of amplicons, including PCR negative controls (NTC). A second PCR (8 cycles) was performed to add Illumina P5/P7 adapters and Nextera XT indices under the following conditions: 95 °C for 3 min; 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. Libraries were purified using magnetic beads, eluted in 22 µL of 10 mM Tris buffer, and assessed by 1.5 % agarose gel electrophoresis and fragment size analysis using a Fragment Analyzer. Libraries were purified, quantified, and pooled equimolarly, including negative controls. Negative controls were inspected after sequencing to confirm the absence of contamination. Sequencing was performed on an Illumina NextSeq 1000 platform with a 600-cycle kit, generating paired-end reads. All raw reads (16S rRNA and ITS amplicons) have been deposited in the European Nucleotide Archive (ENA) under the Study Accession Number PRJEB97168.

3. Data analysis

For fungi and bacteria, amplicon sequence variants (ASVs) were inferred using the DADA2 pipeline (Callahan *et al.*, 2016). Raw reads were quality filtered and trimmed prior to denoising. Forward and reverse reads were truncated to 270 bp and 200 bp, respectively. Reads containing ambiguous bases were discarded, and reads with more than two expected errors were removed for both forward and reverse reads. A quality truncation threshold of 2 was applied, and PhiX reads were removed. Chimeric sequences were identified and removed using the consensus method implemented in DADA2. For bacteria, taxonomic assignments were obtained against the curated SILVA v138.2 16S reference database (Callahan *et al.*, 2024), whereas fungal ASVs were assigned using the UNITE sh-general-release-dynamic-19.02.2025 database train set (Abarenkov *et al.*, 2025).

All analyses were conducted in R using the phyloseq package (McMurdie & Holmes, 2013). Raw sequences were quality-filtered, chimeras removed, and ASVs with zero counts across all samples were discarded. For bacterial datasets, sequences assigned to chloroplasts or mitochondria were also removed to avoid interference from non-bacterial signals. Abundances were transformed to relative values prior to downstream analyses.

Alpha diversity was estimated for both datasets using observed richness (number of ASVs per sample), Shannon index, and Inverse Simpson index, calculated with the vegan package (Oksanen *et al*., 2019). Differences among samples were tested using non-parametric Kruskal-Wallis tests. Rarefaction curves were generated from unrarefied counts with the vegan package to evaluate sequencing depth and completeness, standardised to the minimum library size among samples.

Beta diversity was assessed by calculating Bray-Curtis dissimilarities. These dissimilarities were visualised using non-metric multidimensional scaling (NMDS) with two dimensions and 200 random starts. PERMANOVA (999 permutations) was applied to test for differences among samples and homogeneity of dispersion was examined using the betadisper function (Anderson, 2001). Pairwise Bray-Curtis distances were further compared with Wilcoxon rank-sum tests to evaluate whether within-plant replicates were more similar than between-plant comparisons.

To explore the contribution of individual taxa to community differences, similarity percentage (SIMPER) analysis was performed on Bray-Curtis dissimilarities (Clarke, 1993). The top taxa explaining dissimilarities were summarised and visualised with a barplot in ggplot2, collapsed to the lowest available taxonomic rank. Presence/absence matrices were also used to construct Venn diagrams with the ggvenn package, highlighting shared and unique ASVs among plants.

RESULTS

1. Amplicon quantification and sequencing output

The sequencing effort yielded successful amplification and variable concentrations of both 16S and ITS markers across all samples. For 16S, raw reads ranged from 110,397 to 387,923 per sample, while ITS libraries produced between 208,572 and 424,992 reads. The number of detected ASVs also varied, with 16S ranging from 153 to 381 ASVs and ITS from 37 to 92 ASVs. This variability was observed among replicates within the same plant as well as between plants, reflecting a combination of biological differences and technical factors such as variation in amplicon yield, sequencing depth, and ASV recovery across the dataset (Table S1).

2. Distinct composition and diversity patterns of fungal and bacterial communities on desert-grown grapes

The analysis of microbial communities associated with *Vitis vinifera* berries in the Atacama Desert vineyard revealed distinct patterns of dominance and variability between fungi and bacteria (Figure 2). Fungal communities were largely dominated by *Starmerella*, with intra-plant variability reflected in the relative contribution of other families, which were less consistently represented among berries from the same plant. In contrast, bacterial communities showed a strong prevalence of *Gluconobacter* across all plants, while other taxa such as *Pseudomonadaceae*, *Enterobacteriaceae*, and *Micrococcaceae* appeared more sporadically and at lower relative abundances. These results suggest that *Starmerella* and *Gluconobacter* may represent dominant taxa on grape surfaces in this extreme desert environment.

Alpha diversity analyses revealed contrasting patterns between bacterial and fungal communities across grapevines. For bacteria, no significant differences were detected among plants in observed richness (χ^2 = 0.62, df = 2, p = 0.733), Shannon index (χ^2 = 1.16, df = 2, p = 0.561), or Inverse Simpson index (χ^2 = 0.62, df = 2, p = 0.733), indicating relatively homogeneous bacterial diversity across replicates. In contrast, fungal diversity showed significant differences among plants. The Shannon index (χ^2 = 7.20, df = 2, p = 0.0273) and Inverse Simpson index (χ^2 = 6.49, df = 2, p = 0.0390) both revealed significant variation, whereas observed richness did not differ significantly (χ^2 = 3.50, df = 2, p = 0.174). These results suggest that fungal assemblages are more strongly structured by plant identity, while bacterial communities maintain relatively stable diversity levels across plants (Figure 3A).

Rarefaction analyses revealed clear differences in microbial richness between bacterial and fungal communities and across plants (Figure 3B). Bacterial 16S rRNA curves showed that samples from Plant 1 and Plant 2 reached higher richness values (~ 250–300 ASVs) and approached saturation, while Plant 3 displayed lower richness (~ 150–200 ASVs) with curves that did not fully level off, suggesting incomplete sampling of bacterial diversity in this plant. Fungal ITS curves indicated lower overall richness compared to bacteria, with Plant 1 exhibiting the highest values (~ 70–80 ASVs), followed by Plant 2 (~ 50–60 ASVs) and Plant 3 (~ 40–50 ASVs). Most ITS curves tended to plateau, indicating sufficient sequencing depth to capture the majority of fungal diversity. Together, these results highlight pronounced intra-vineyard differences in microbial richness, with fungal communities being less diverse but more completely sampled than bacterial communities.

3. Intra-vineyard differentiation of fungal and bacterial communities

The non-metric multidimensional scaling (NMDS) ordinations based on Bray-Curtis dissimilarities revealed a clear separation of microbial communities among the three plants (Figure 4). For fungi, replicates from each plant clustered tightly together and were distinctly separated from those of the other plants, indicating consistent within-plant community structure. Similarly, bacterial communities showed plant-specific clustering, although with slightly more overlap among replicates compared to fungi. PERMANOVA confirmed that both fungal (F = 4.48, R^2 = 0.599, p = 0.007) and bacterial (F = 2.96, R^2 = 0.497, p = 0.004) assemblages differed significantly among plants. Stress values for the NMDS (< 0.2) indicated that the ordinations provided an adequate representation of community dissimilarities (Figure 4A). PERMANOVA based on Bray-Curtis dissimilarities revealed that both fungal (ITS) and bacterial (16S rRNA) communities differed significantly among plants

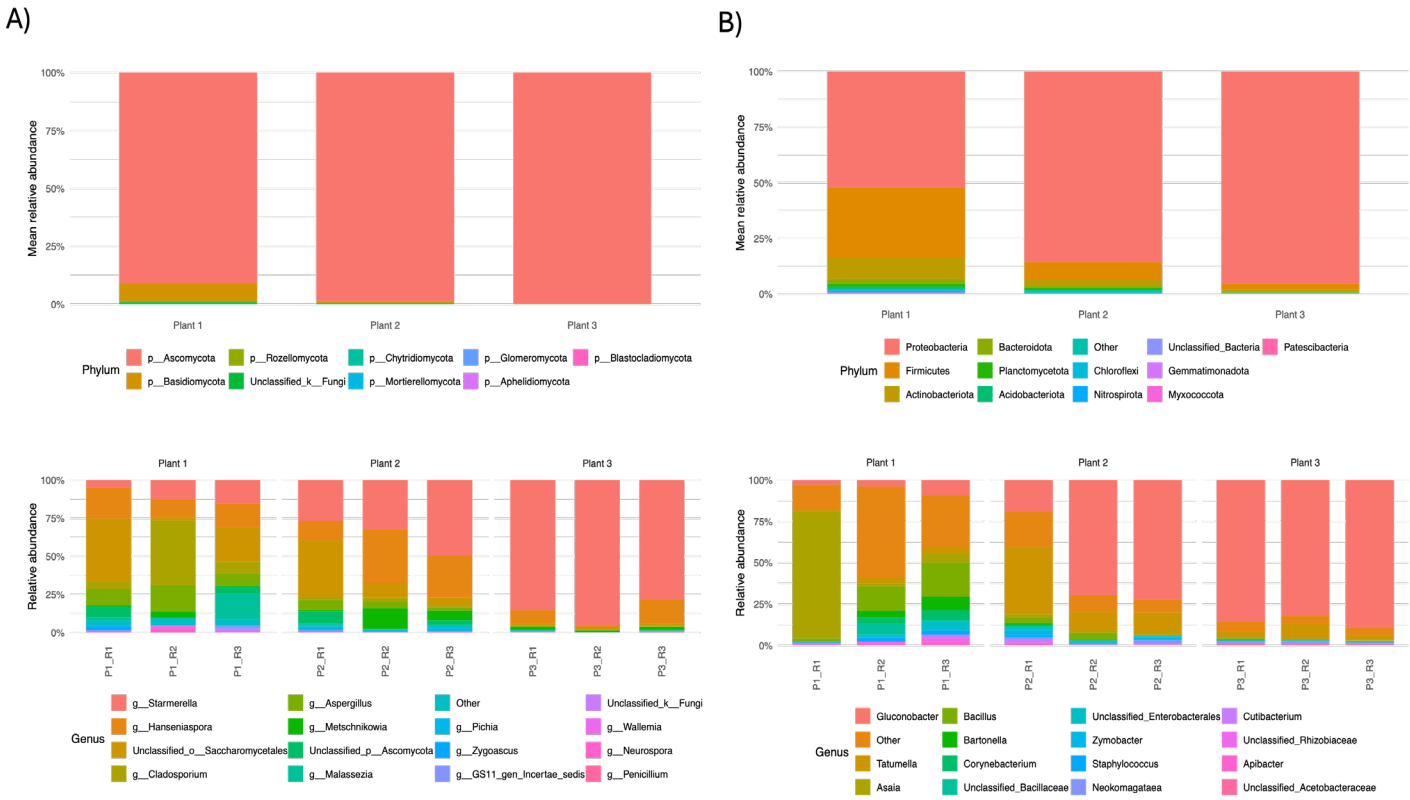


FIGURE 2. Relative composition of microbial communities associated with *Vitis vinifera* cv. Ahmeur bou Ahmeur in the Atacama Desert vineyard.

(A) Relative abundance of the most abundant fungal and (B) bacterial phyla and genera across individual berry samples from each plant. Each bar represents one sample, with colours indicating different taxa.

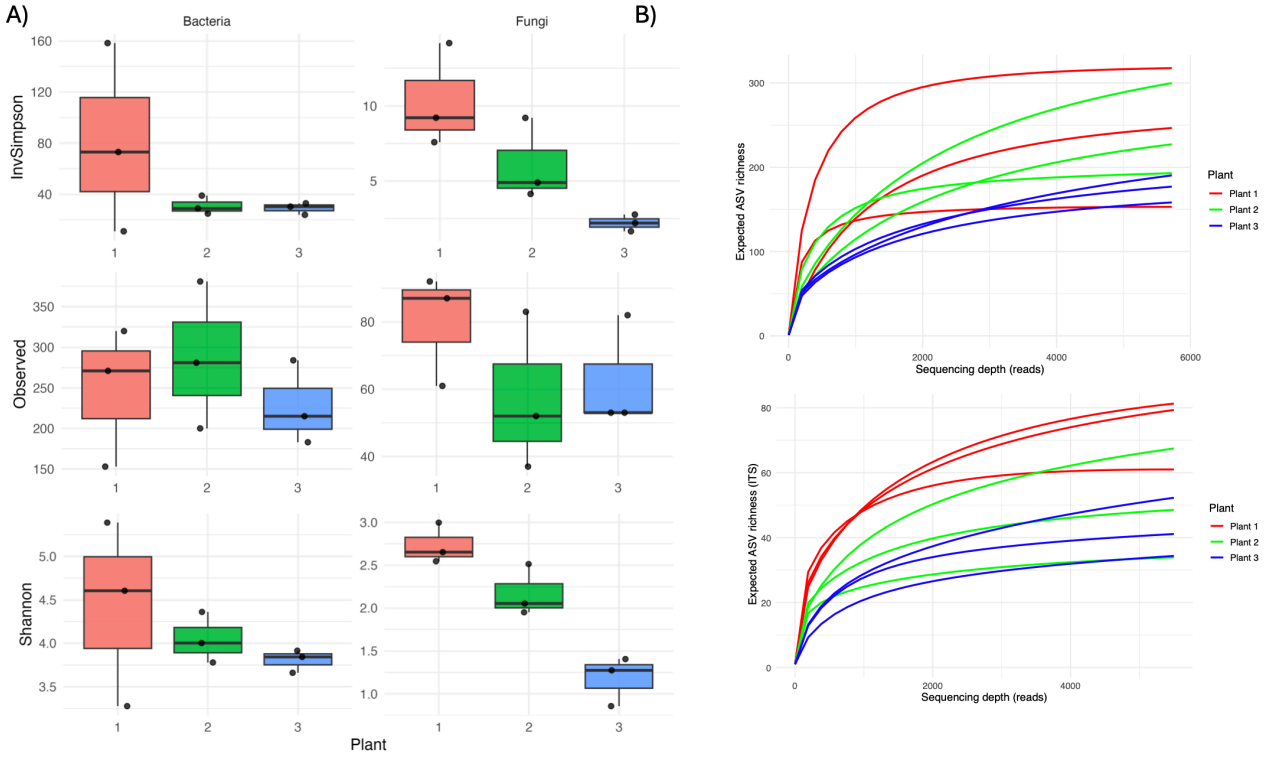


FIGURE 3. Alpha diversity and sequencing depth of microbial communities associated with *Vitis vinifera* in the Atacama Desert vineyard.

(A) Alpha diversity of bacterial (16S rRNA) and fungal (ITS) communities across three Ahmeur bou Ahmeur grape plants, measured as observed richness, Shannon index, and Inverse Simpson index. Boxplots represent the distribution of values per plant (1–3), with points showing individual replicates. (B) Rarefaction curves of bacterial and fungal communities based on 16S rRNA and ITS amplicons, respectively. Curves represent the expected ASV richness as a function of sequencing depth, standardised to the minimum library size.

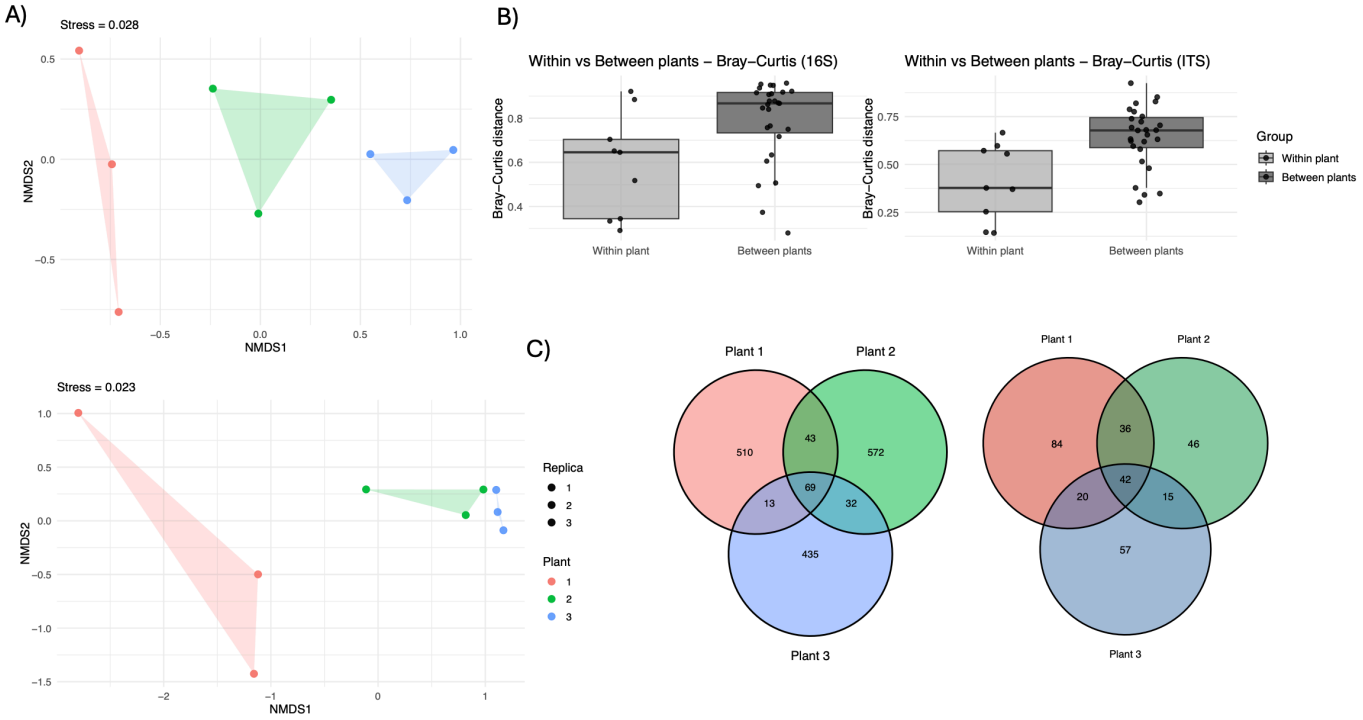


FIGURE 4. Beta diversity and community structure of microbial assemblages associated with *Vitis vinifera* cv. Ahmeur bou Ahmeur in the Atacama Desert vineyard.

(A) Non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarities showing distinct clustering of fungal (upper panel) and bacterial (lower panel) communities across the three grape plants. (B) Pairwise Bray-Curtis dissimilarities comparing within-plant and between-plant distances for both fungal and bacterial communities. Boxplots display median and interquartile ranges, with lower within-plant dissimilarities indicating higher intra-plant similarity. (C) Venn diagrams illustrating the number of unique and shared amplicon sequence variants (ASVs) among the three plants, highlighting the presence of both plant-specific and core microbial taxa. The left panel shows bacterial ASVs (16S rRNA), while the right panel shows fungal ASVs (ITS).

within the vineyard. Plant identity explained nearly 60 % of the variation in fungal community composition (R^2 = 0.599, F = 4.48, p = 0.007) and almost 50 % of the variation in bacterial communities (R^2 = 0.497, F = 2.96, p = 0.004). Homogeneity of dispersions (betadisper) was not significant for either fungi (F = 2.40, p = 0.172) or bacteria (F = 1.93, p = 0.226), confirming that the observed effects reflect true compositional differences rather than differences in within-group variability. These results indicate that plant identity exerts a strong structuring influence on both domains, with a slightly stronger effect on fungal than on bacterial communities. To assess whether microbial assemblages are more homogeneous within individual grape plants than between different plants, we compared pairwise Bray-Curtis dissimilarities among replicates from the same plant ("within plant") and among replicates from different plants ("between plants"). This analysis tests the expectation that berries from a single plant share a more similar microbial community compared to berries from neighbouring plants within the vineyard. For fungi (ITS), within-plant distances were significantly lower (median = 0.378, IQR = 0.319) than between-plant distances (median = 0.678, IQR = 0.157), as confirmed by a Wilcoxon rank-sum test (W = 39, p = 0.0014, one-sided). A similar pattern was observed for bacterial communities (16S rRNA), although the effect was less pronounced: within-plant distances had a median of 0.646 (IQR = 0.359) compared to 0.867 (IQR = 0.183) between plants (W = 63, p = 0.017, one-sided) (Figure 4B). These results demonstrate that both fungal and bacterial microbiomes retain a strong intra-plant signature, with the effect being more pronounced in fungal communities. The Venn diagrams further showed that both fungal and bacterial assemblages contained a larger number of plant-specific ASVs than those shared across all three plants: bacteria had 62 ASVs in the core versus 510, 572, and 435 unique ASVs in Plants 1, 2, and 3, respectively, while fungi had 42 ASVs in the core versus 84, 46, and 57 unique ASVs in the same plants (Figure 4C).

4. Shared fungal taxa drive intra-vineyard variability among grape plants

SIMPER analysis revealed that the separation among plants was mainly driven by fungal taxa shared across all plants, rather than by plant-specific ASVs. The barplot (Figure 5) illustrates that taxa such as *Hanseniaspora uvarum*, *Starmerella* sp., *Malassezia restricta*, and *Cladosporium* sp. were consistently present across plants but displayed large differences in relative abundance. For example, *H. uvarum* and *Starmerella* sp. were more abundant in Plant 1, while *M. restricta* was enriched in Plant 3. These shifts in the abundance of shared taxa explain much of the observed intra-vineyard variability.

DISCUSSION

The grape-associated microbiome has been extensively characterised in recent years, with several studies demonstrating that geography, climate, vintage, and grape variety strongly shape microbial community composition on berry surfaces (Bokulich *et al*., 2014; Vitulo *et al*., 2019; Wei *et al*., 2022a; Africa *et al*., 2025). However, fewer reports have focused on intra-vineyard microbial heterogeneity, despite its potential relevance to terroir and spontaneous fermentation outcomes. Understanding fine-scale variability is particularly critical in extreme viticultural environments, such as the Atacama Desert, where grapes develop under intense solar radiation, large diurnal temperature fluctuations, and severe water scarcity (Lanino & Poblete, 2022).

Our study showed that while both fungal and bacterial assemblages differed significantly in beta diversity among plants, variation was more pronounced in fungal communities. This pattern is consistent with previous observations reporting stronger differentiation in fungal assemblages compared to bacterial ones (Bokulich *et al*., 2014; Steenwerth *et al*., 2021; Papadopoulou *et al*., 2022). Large-scale vineyard microbiome surveys have similarly shown that fungal alpha diversity is more responsive to climatic and spatial variation than bacterial diversity (Gobbi *et al*., 2022), reinforcing the notion that fungal communities are more sensitive to microhabitat-level conditions. Cultivar-level studies further highlight that fungal community composition is more variable across genotypes than bacterial assemblages, suggesting a stronger dependence on host factors (Castanera *et al*., 2024). It is important to note that part of the variability observed in sequencing depth and ASV richness may also reflect technical factors inherent to amplicon-based approaches, and should therefore be interpreted cautiously when inferring biological patterns.

With respect to community composition, bacterial assemblages were dominated by members of the genera *Gluconobacter* and *Tatumella*, taxa previously reported as abundant in vineyard environments (Steenwerth *et al*., 2021; Ohwofasa *et al*., 2024). Additionally, *Asaia*, a genus primarily described in insects such as mosquitoes (Favia *et al*., 2007), was detected, reinforcing its broader ecological distribution and potential role as a vector-associated taxon in vineyards.

Fungal communities were characterised by the recurrent detection of non-*Saccharomyces* yeasts. The genus *Starmerella* was identified on grape surfaces (Martiniuk *et al*., 2023; Ohwofasa *et al*., 2024). This yeast has attracted attention in oenology due to its ability to produce moderate volatile acidity, reduced ethanol, and high glycerol levels (Lemos Junior *et al*., 2019). *Hanseniaspora* was also abundant on grape surfaces (Snyder *et al*., 2024), displaying temporal and spatial dynamics within the fungal microbiome during grape fermentation, with higher abundance at the onset of fermentation (Onetto *et al*., 2024). These findings align with evidence that vintage and fermentation stage are critical drivers of yeast succession and microbial community dynamics during wine fermentation in grapes (Liu *et al*., 2019). At later stages, these taxa were progressively replaced by unclassified ASVs affiliated with *Saccharomycetales*, consistent with the typical yeast succession described in spontaneous fermentation.

In desert viticulture, where grapes are exposed to intense solar radiation, large diurnal temperature shifts, and extreme aridity, microbial differences among plants may be amplified. Studies in arid wine-grape soils show that microbial diversity is strongly modulated by these stressors (Wang *et al*., 2024), and similar patterns have been reported in semi-arid Peruvian vineyards, where the metagenome of *Vitis vinifera* berries

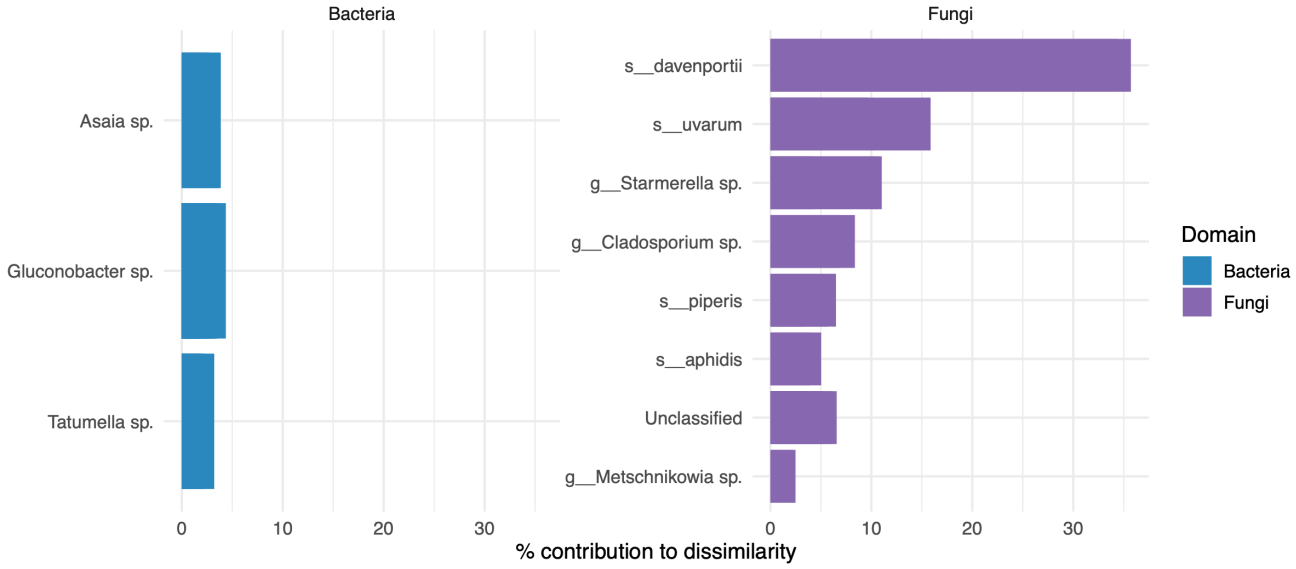


FIGURE 5. Main fungal (ITS) and bacterial (16S rRNA) taxa contributing to community differentiation among *Vitis vinifera* cv. Ahmeur bou Ahmeur grape plants, as identified by SIMPER analysis.

Bars show the relative contribution of each taxon to Bray-Curtis dissimilarities, with ASVs collapsed to the lowest available taxonomic rank.

reflects edaphoclimatic influences on the regional microbial identity (Sotomayor-Parian *et al*., 2024). Epidermal microbial communities have also been shown to correlate negatively with extremely high and low temperatures (Wei *et al*., 2022b), and a recent review underscores how climatic and soil-related factors drive divergence in vineyard microbiomes (Silva *et al*., 2025). Consistent with these environmental influences, our results show that although it may seem intuitive that microbial communities on berries from the same plant would be more similar to each other than to those from different plants, our study provides quantitative evidence supporting this assumption at an intra-vineyard scale. Similar evidence of significant heterogeneity at small spatial scales has also been reported in vineyard soils (Liang *et al*., 2019), suggesting that both edaphic and plant-level factors can contribute to fine-scale microbial differentiation. By showing that plant identity accounts for nearly 60 % of the variation in fungal community composition and that within-plant replicates are significantly more homogeneous than between-plant comparisons, we highlight the plant as a relevant ecological unit in vineyard microbiome studies.

Our findings complement and extend previous work by Zhang *et al*. (2019), who demonstrated that even genetically related clones within a single grape variety can harbour distinct bacterial assemblages on berry surfaces, highlighting the influence of fine-scale genetic variation on the grape microbiome. Although clonal identity was not verified in our vineyard, all sampled plants belong to the same *Vitis vinifera* variety (*Ahmeur bou Ahmeur*), and yet exhibited pronounced microbial differentiation – particularly in fungal assemblages. This suggests that factors other than host genotype, such as microenvironmental conditions, plant physiology, or stochastic colonisation processes, may play a dominant role in structuring grape-surface microbial communities (Minerdi & Sabbatini, 2025; Swift *et al*., 2023; Wei *et al*., 2022b). Together, these results reinforce the concept that vineyard microbiomes are heterogeneous across multiple spatial and biological scales, underscoring the need to consider within-vineyard variability when investigating the microbial dimension of terroir.

These findings have both methodological and oenological implications. Methodologically, they highlight the importance of considering plant-level variability within vineyards, as ignoring these differences may lead to biased interpretations of microbial terroir. Biologically, the presence of a shared core microbiome, together with plant-specific taxa, shows that both common and unique microorganisms shape the grape-surface microbiota. From an oenological perspective, variation in fungal community composition – especially in the relative abundance of non-*Saccharomyces* yeasts such as *Starmerella*, *Hanseniaspora*, and *Metschnikowia* – may influence fermentation dynamics and the production of aroma compounds. Recognising intra-vineyard microbial heterogeneity is therefore key to understanding the ecological and oenological dimensions of terroir in desert-grown grapes.

Although the number of plants analysed was limited (three vines with three berry replicates each), this exploratory design follows a similar rationale to previous work on microbiomes from bat guano in vineyard ecosystems (Ramírez-Fernández *et al*., 2025), where small yet carefully selected samples revealed meaningful ecological patterns. This approach proved sufficient here to detect plant-specific microbial differentiation, while future studies including more vines and vineyards will help validate the robustness of these intra-vineyard trends.

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