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Wild microbial communities shaping the spontaneous fermentation of biodynamic Malbec wine

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ABSTRACT

Prior to the industrialisation of winemaking, spontaneous fermentation was the only option available to wineries, relying exclusively on native micro-organisms from the vineyard due to the absence of commercial inoculants. While nowadays inoculation is a practical choice for industrial-scale producers, small wineries aiming to craft distinctive wines can benefit from the complexity and uniqueness of spontaneous fermentation. However, the unpredictable interactions among the diverse community of bacteria and fungi during fermentation pose significant challenges. Understanding these microbial interactions is essential for making informed winemaking decisions. In this study, we analysed the microbiome, physicochemical, and sensorial profiles of Malbec wines produced from biodynamic vineyard plots subjected to different management practices, including organic amendment incorporation and hail net placement, over three seasons. Our results revealed significant heterogeneity between individual fermentations, with no clear effects of vineyard treatments on the final wine profiles. Notably, metabarcoding analysis revealed a remarkable persistence of DNA signatures from the native fungal consortium; specifically, *Hanseniaspora nectarophila* and *Hanseniaspora guilliermondii* remained detectable until 75 % of alcoholic fermentation (AF). Along with *Saccharomyces cerevisiae*, *Schizosaccharomyces japonicus*, and *Metschnikowia pulcherrima*, these taxonomic signals were consistent throughout the vintages, though the latter two were more sensitive to seasonal climate variations. These findings underscore the critical role of locally adapted consortia in shaping the unique expression of wines.

KEYWORDS: biodynamic viticulture, non-*Saccharomyces* yeasts, *Hanseniaspora* spp., vineyard management, native microbiota, microbial terroir, organic amendment

INTRODUCTION

Most modern wine production relies on yeast inoculation, where commercial yeast strains are inoculated to control the fermentation process, rather than allowing the indigenous yeasts present in the must to initiate spontaneous fermentation (Gardner *et al.*, 2023). Inoculation with commercial yeast strains ensures predictability but can reduce microbial diversity and potentially affect sensory complexity (Capozzi *et al.*, 2015; Bartle *et al.*, 2019; Philipp *et al.*, 2021). Wine producers are constantly looking for sensorial differentiation of their wine, and a deeper understanding of microbial interactions during fermentation could help to achieve their commercial goals. However, the dynamics of different microbial populations during spontaneous fermentation and the changes influenced by vineyard location, grape sanity, and winery practices make their behaviour difficult to predict and result sometimes in languishing or stuck fermentations (Boynton & Greig, 2016). Some microorganisms produce inhibitory compounds detrimental to others, highlighting the need to study microbial interactions. This allows one to maintain the balance that ensures reliable fermentation while preserving the microbial fingerprint of the wine (Bartle *et al.*, 2019).

Given this context, studying the microbial resources present in the vineyard is essential to identify the core vineyard-winery microbiome that contributes to producing distinctive wines (Stefanini & Cavallieri, 2018).

Culture-independent techniques such as metabarcoding have expanded our understanding of microbial diversity beyond traditional taxa, revealing additional yeasts and bacterial contributors to wine composition (Bubeck *et al.*, 2020; Ohwofasa *et al.*, 2024a; Ohwofasa *et al.*, 2024b).

A recent study found that 86 % of wines in Europe contain pesticide residues due to conventional vineyard management practices aimed at controlling grape diseases, particularly *Botrytis* and black rot (European Food Safety Authority *et al.*, 2022). In the last 20 years, organic vineyard management has expanded by 600 %, with biodynamic practices contributing to this growth, mainly motivated by consumer preferences for healthy, sustainable, and terroir-expressing wines (da Rocha Oliveira Teixeira *et al.*, 2023). Minimal chemical interventions in the vineyard ensure that only minor undesirable chemical residuals are found in the wine. Recent studies showed that organic amendments such as compost and vermicompost can influence grape and wine composition, providing a sustainable nutrient source for vineyards (Palenzuela *et al.*, 2023; Rosado *et al.*, 2022).

In Mendoza, Argentina, where hailstorms pose a major risk to viticulture, anti-hail nets are widely used to protect grape yields. Beyond their mechanical function, these nets modify the vineyard microclimate—altering light, temperature, and humidity—and may influence grape composition and wine quality. However, the effects of different anti-hail net configurations on microbial populations of grape and their effect on fermentations in Mendoza's terroirs remain poorly studied.

Based on this context, this study aimed to evaluate the influence of two vineyard practices—organic amendments and hail nets—over three consecutive vintages of a biodynamically managed Malbec vineyard in Mendoza (Argentina). We combined microbial cultivation methods, metabarcoding of bacteria and fungi, physicochemical measurements, and sensory analyses to disentangle the relative effects of vineyard management and vintage on the wine microbiome and fermentation outcomes.

MATERIALS AND METHODS

1. Study design

The study was conducted in a 1.5 ha Malbec vineyard located in Ugarteche, Luján de Cuyo, Mendoza (33° 14' 01" S 68° 55' 59" W, Figure 1A). The grapevines, planted in 2008, have been consistently managed using a biodynamic approach with the applications, through foliar aspersion, of 40 g/ha of the biodynamic preparation Horn manure (500) after winter pruning and at budburst; 5 g/ha of the biodynamic preparation Horn silica (501) at veraison and 50 g/ha of Fladen (cow manure) after harvest. Phytosanitary management included the application of approximately 2 kg/ha of copper oxychloride, three times per season, and 3 kg/ha of elemental sulfur at the budburst. The soil was left untilled, while the spontaneous cover vegetation between vineyard rows was maintained using brush cutters.

The experimental design consists of 18 randomly distributed plots to evaluate the application of organic amendments in triplicate: 15 tons/ha of vermicompost (V), 15 tons/ha of compost (C), and a control without any application (D) combined with two anti-hail net configurations: Chapelle (CH) and Grembiule (G) (Table 1 and Figure 1B). In the 2021 season, only the amendments factor was evaluated (nine plots) because of a problem with anti-hail net collocations, while in 2022 and 2023, both factors were evaluated. Each plot was designated based on its treatment combination: vermicompost with Chapelle (VCH) or Grembiule (VG); compost with Chapelle (CCH) or Grembiule (CG); and untreated control with Chapelle (DCH) or Grembiule (DG).

2. Grape must fermentations

Twenty kilograms of bunches from at least six grapevine plants were harvested from each evaluated plot and transported immediately to the microbiology laboratory at EEA Mendoza INTA. Fermentations were carried out for each plot, and as the plots were replicated in triplicate, the fermentations constitute biological triplicates. Bunches were placed in sterilised plastic bags, manually destemmed and crushed, and the resulting must was transferred to an 8-litre plastic tank. Determinations of pH, Brix, total acidity, total contents of sugars, and density were recorded in the musts. Fermentation temperature was maintained in the range of 20–25 °C. Weight loss and must density were daily monitored. Must density was measured using a densitometer (Densito 30PX, Mettler Toledo Co., Columbus, OH, USA). After the completion of alcoholic fermentation (considered as a weight loss of less than 0.5 g per day), the wine was separated from grape pomace and transferred to 2-litre

TABLE 1. Factors evaluated in experimental design. Three replicates in each combination of factors during three consecutive vintages (2021: *n* = 9*, 2022: *n* = 18, and 2023: *n* = 18).

Factor 1. Amendments applications	
V: Vermicompost	Foliar spraying of amendment tea Buried in soil
C: Compost	Foliar spraying of amendment tea Buried in soil
D: Untreated control	Without the application of amendments
Factor 2. Anti-hail net system disposition	
CH: Chapelle format	
G: Grembiule format	

* During the 2021 season, all parcels were managed using the Grembiule anti-hail net system.

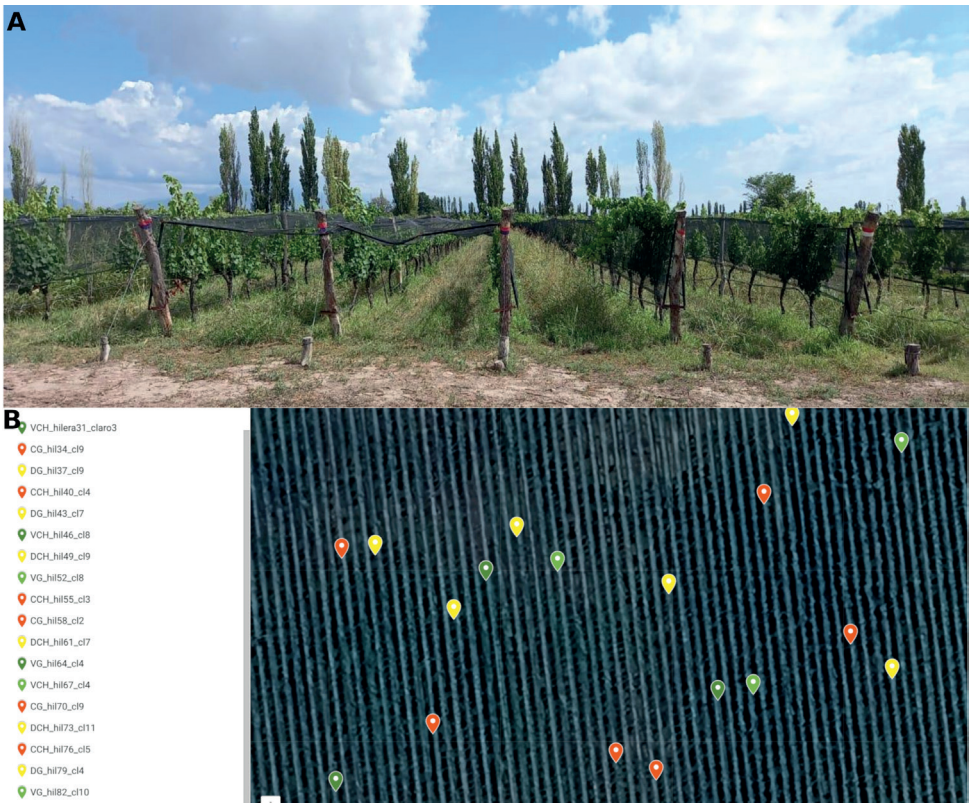


FIGURE 1. A) Vineyard under study in spring. At left: Chapelle anti-hail net configuration; at right: Grembiule configuration. B) Layout of treatments within the studied parcel: green dots correspond to vermicompost treatment (V), red dots to compost treatment (C), and yellow dots to untreated control (D).

plastic bottles and placed in a temperature-conditioned chamber (15–20 °C) to promote malolactic fermentation. After one month, wines were stabilised in a cool room for two weeks and then bottled and stored for six months until sensory profile evaluation.

The microbiological evaluation was conducted using two complementary approaches: (1) microbiome characterisation through metabarcoding, targeting both fungi and prokaryotes in an advanced stage of fermentation, and (2) the assessment of the culturable yeast population along the fermentation process.

Samples for microbiological cultivation were obtained once the must were placed in the tanks (fresh must = M), and at three additional stages: when density dropped at 3 $g.L^{-1}$ (beginning of fermentation: BF), at 30 $g.L^{-1}$ (mid-fermentation: MF), at 50 $g.L^{-1}$ (75 % F) and at 70 $g.L^{-1}$ which is considered as the end of alcoholic fermentation (FF). Samples of 75 % F from each fermentation were selected for metabarcoding analysis. Fifty millilitres of the fermentation mixture were collected in a sterilised plastic tube and immediately processed to obtain a pellet through four rounds of centrifugation at 8,000 rpm at 4 °C in Thermo Scientific Sorvall ST 16R and resuspension with NaCl 150 mM. Washed pellets were stored at –80 °C and lyophilised for 16 hours in Biobase BK-FD10P lyophilizer. Lyophilised pellets were finally kept at –20 °C until DNA extraction was performed.

3. Microbial plate cultivation

For yeast and fungi cultivation, serial dilutions of the fermentation samples were performed in peptone water 0.1 % *(w/v)*, and plated on WL agar medium (Oxoid, Basingstoke, UK) with chloramphenicol (50 µg/mL) for bacterial inhibition. After incubation for 48 hours at 28 °C, colony-forming units (CFUs) were counted from plates with 10–300 colonies of each treatment replicate, which was duplicate-seeded. Colonies were differentiated into *Saccharomyces* yeasts, non-*Saccharomyces* yeasts, or fungi based on colony morphology and colour. *Saccharomyces* colonies appeared cream to green with a creamy consistency, while non-*Saccharomyces* yeasts typically formed white, cream, grey-green, blue grey, red, cream with red hint, or intense green colonies with different consistency, as previously described (Pallmann *et al.*, 2001). It was also differentiated separately into a group among non-*Saccharomyces* yeasts, easily differentiated by colony and cellular morphology and mentioned as apiculate yeasts.

4. Physicochemical analysis

Samples of must were analysed according to OIV (2024) methods for soluble solids (Brix degrees), pH, total acidity, and samples of wine were analysed with OenoFoss wine scan for: density, alcohol, total sugars, tartaric, acetic, malic, and lactic acid.

5. Sensory analysis

The sensory technique used was QDA (Quantitative Descriptive Analysis) for 2021 wines and RATA (Rate-All-That-Apply) (Ares *et al.*, 2014) for 2022 and 2023 wines. The evaluations were carried out in the sensory analysis room of the Oenology Studies Centre (EEA Mendoza INTA). The number of panellists who participated in the evaluations was 16. An analysis of variance and a principal components analysis with 95 % confidence ellipses were performed, with the SensoMineR (Lê & Husson, 2008) and FactoMineR packages (Lê *et al.*, 2008).

6. Climatic data

Microclimate variables were monitored using an AgroSens (https://ueingenieria.com/) weather station located in the vineyard. Daily maximum and minimum temperatures, precipitation events, wind speed, solar radiation, and atmospheric pressure were recorded.

7. DNA extraction and amplicon sequencing

DNA extraction was assessed with the Inbio Highway DNA PuriPrep soil kit, using around 100 mg of the lyophilised pellet of wine. The quality and integrity of extracted DNA were evaluated with a DeNovix spectrophotometer and electrophoresis in agarose gel 1.5 %. The extracted DNA was sent to the Integral Microbiome Resources facility in Dalhousie (Canada) for amplification of the V3–V4 region of rDNA 16S gene with primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC), and amplicons were sequenced in Illumina MiSeq 2 × 300 bp. Fungal characterisation was done through the amplification of full-length ITS with primers ITS1-F_KYO2 (TAGAGGAAGTAAAAGTCGTAA) and ITS4_KYO1 (TCCTCCGCTTWTTGWTWTGC) and sequenced with PacBio Sequel II in circular consensus sequencing (CCS). The data generated were deposited in NCBI (PRJNA1229409).

8. Bioinformatic analysis

8.1. Prokaryotic metabarcoding analysis

Prokaryotic microbiome characterisation was assessed using FASTQ files containing data from V3–V4 16S reads, which were used in the QIIME 2 pipeline version 2023.7 (Bolyen *et al.*, 2019). Based on the demux summary of per-base quality, the forward reads were trimmed at 280 bp, while reverse reads were trimmed at 220 bp. After chimera remotion, amplicon sequencing variants (ASVs) were assigned through the dada2 plugin in QIIME 2. For taxonomic classification, a pre-trained classifier optimised for the V3–V4 region was obtained from https://doi.org/10.6084/m9.figshare.20430963 (Sangphukieo, 2022), which is derived from the SILVA database (Quast *et al.*, 2013). After the first classification, several ASVs were assigned as chloroplasts, therefore, a filtering step was included to eliminate "mitochondria and chloroplast" classified ASVs. The filtered ASVs were used for making plots of rarefaction curves, alpha and beta-diversity analysis in QIIME 2. Posterior analysis and plots of phylogeny profiles and Principal Coordinates Analysis (PCoA) were done with animalcules and phylosmith R packages (Smith, 2019; Zhao *et al.*, 2021). The number of raw sequenced reads and those retained after each quality filtering step are shown in Table S1.

8.2. Fungal metabarcoding analysis

The full-length ITS reads obtained from PacBio Sequel II sequencing were processed according to the standard operating procedure of the Langille Lab https://github.com/LangilleLab/microbiome_helper/wiki/PacBio-CCS-Amplicon-SOP-v1-(qiime2).

In this workflow, the PacBio reads were formatted to be used in a QIIME 2 pipeline. The FASTQ files contained reads in both 5′-3′ and 3′-5′ orientations. To standardise the orientation, the reverse complement of each read was appended to its original sequence. Subsequently, Cutadapt was used to retain only reads in the 5′-3′ direction, applying a length filter (300–900 bp) and removing the reads that did not start and end with the expected primer sequences (TAGAGGAAGTAAAAGTCGTAA…GCAWAWCAAWAAGCGGAGGA). The resulting FASTQ files were processed in QIIME 2 version 2023.7 (Bolyen *et al.*, 2019). The input was specified as 'SingleEndFastqManifestPhred33V2'. Chimera remotion and ASV identification were done using the dada2 plugin in QIIME 2. For taxonomic classification,

the ITS database was downloaded from the UNITE database version 9.0 (Abarenkov *et al.*, 2022), and after changing the lowercase to uppercase, the reference sequences and taxonomy were imported to QIIME 2 and then used to train the classifier using a naive Bayes model. The resulting file was used for taxonomic assignments to the identified ASVs. Alpha and beta-diversity analysis and taxonomic abundance bar plots were obtained with QIIME 2, animalcules and phylosmith R packages (Bolyen *et al.*, 2019; Smith, 2019; Zhao *et al.*, 2021). The number of raw sequenced reads, and those retained after each quality filtering step, are shown in Table S2.

RESULTS

1. Seasonal climatic variation

The climatic variables were monitored using data from a local climate station. Spring frost occurred only during the 2023 season. The average temperature exhibited an increasing trend, with 2023 being the warmest year (Table 2). In terms of precipitation, the 2021 and 2022 seasons were markedly wetter than the 2023 season.

2. Grape musts and wine characteristics

Musts showed homogeneous general physicochemical characteristics (Table S3). However, the season had a significant effect on total soluble solids, density, and pH, with the 2022 must showing the lowest values. The general microbial characteristics of the must were evaluated by plate seeding. Yeast counts, expressed as colony-forming units (CFU), were obtained after cultivation on the differential and selective medium described previously. In fresh must, yeast counts ranged from 10^4 to 10^5 CFU/mL (Table S3). Viable counts are informed for each treatment, and reflect variation between biological replicates, which is characteristic of this type of vineyard, known for its high heterogeneity.

Seasonal differences in microbial populations were also evident. In 2021, filamentous fungi (FF) predominated in the must samples. Conversely in 2022, apiculate (AP) and non-*Saccharomyces* (NS) yeasts became more prevalent, while fungi were nearly absent. In 2023, NS yeasts were significantly more abundant, and FF were also present (Figure 2). *Saccharomyces* yeasts were generally absent in the musts, except for a few cases in 2021.

Vintage-related differences were observed in fermentation performance, particularly in the evolution of *Saccharomyces* counts—the main fermentative yeast—and total yeast counts. In the 2021 vintage, *Saccharomyces* predominated along all fermentation stages in all treatments (Figure 3A). In contrast, during the 2022 vintage, *Saccharomyces* were either present in very low proportions or completely absent at the beginning of fermentation (BF samples). A slight increase was observed at the MF stage, and by the FF stage, three treatments showed *Saccharomyces* dominance, while in the other three, its proportion remained low (Figure 3B). The 2023 vintage followed a pattern like 2022; however, higher CFU counts for *Saccharomyces* were recorded at the MF stage, and by the FF stage, this yeast genus became completely dominant in all samples (Figure 3C). Samples that did not exhibit *Saccharomyces* dominance at the FF stage presented higher residual sugar content and lower ethanol levels (treatments DG, CCH, and VCH in the 2022 vintage; Figure 3B and Table S4).

3. Prokaryotic community profile

A total of 1,141,157 16S-V3–V4 reads were obtained, and after quality trimming, dada2 denoising, merging, and chimera removal, 164,659 reads were retained (Table S1). Rarefaction curves indicated that the sequencing depth was enough to capture most of the taxonomic diversity (Figure S1). Shannon alpha diversity was not affected by amendments applications nor by anti-hail net configurations, but in vintage 2022, diversity was significantly higher than in 2021 (Wilcoxon test, $p < 0.01$, Figure S2). Bacterial beta diversity was affected by vintages and not by treatments (PERMANOVA test, $p < 0.05$, Figures S2 and S4).

The prevalent prokaryotic community (defined as taxa with a relative abundance > 0.01) comprised 4 phyla, 5 classes, 10 orders, and 13 families. The dominant genera were: *Pantoea*, *Ralstonia*, *Gluconobacter*, and *Tatumella*, while 10.6 % of the sequences remained unidentified (Figure 4A).

No significant differences in taxon abundance were observed across field treatments in any of the three vintages evaluated (DESeq2 test, adjusted *p*-value < 0.05).

When comparing vintages, *Pantoea* was the dominant bacterium in many samples from 2021 and 2023 vintages but was completely absent in 2022 (Figure 4A). *Ralstonia* and *Gluconobacter* were present in some samples from 2021 and 2022 but not present in 2023; *Acetobacter* was only present in two samples from 2021, and *Tatumella* was present in some samples from 2022 and 2021 and only in one from 2023 (Figure 4A).

TABLE 2. Climatic variables per season.

Vintage	Late frosts in spring (November)	Average temperature (January–March)	Range of maximal temperature in summer	Seasonal precipitation (mm)
2021	–	19.81 °C	32 to 36 °C	263
2022	–	20.14 °C	33 to 34 °C	260
2023	–1.1 °C	21.80 °C	34 to 36 °C	181

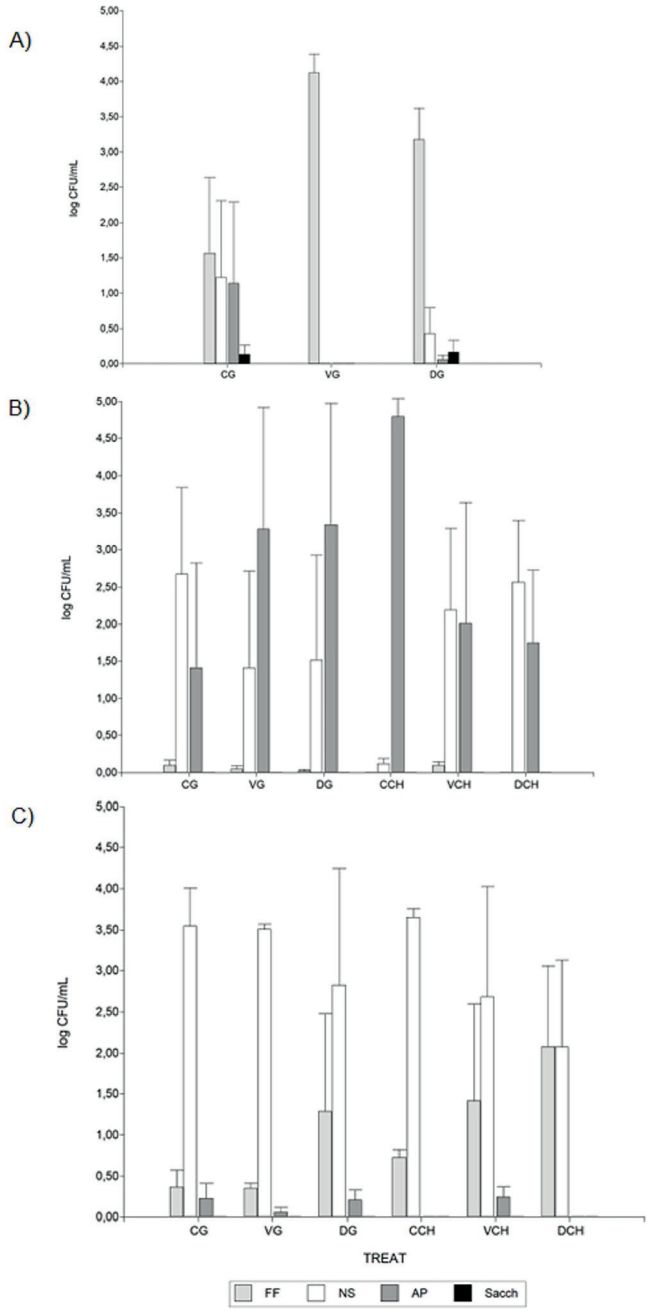


FIGURE 2. Relative abundance of colony-forming units of filamentous fungi (FF), No-*Saccharomyces* (NS), apiculate (AP), and *Saccharomyces* (Sacch) yeasts in grape must from different treatments across vintages 2021 (A), 2022 (B), and 2023 (C).

4. Fungal community profile

The full ITS sequencing using the PacBio Sequel platform generated 434,271 reads. After quality filtering, 399,716 high-quality reads were retained. Rarefaction curves showed a plateau across all samples (Figure S5), indicating sufficient sequencing depth to capture diversity. Shannon diversity was not affected by treatments in vineyards but was higher in season 2022 compared to 2021 (Wilcoxon test, $p < 0.01$, Figure S6). Fungal beta-diversity was modified according to vintages, and as an effect of anti-hail net configuration but not according to amendment applications (PERMANOVA test, $p < 0.05$, Figures S3B and S7).

Taxonomic classification of the most abundant (relative abundance > 0.001) ASVs identified 12 species. Due to the high accuracy of PacBio circular consensus sequencing (CCS) and the full-length ITS region, fungal species were identified with high confidence (error probability < 1 %). ASV clustering against the UNITE database at a 99 % similarity threshold further supported species-level classification. However, 7 % of ASVs remained unclassified at the species level, likely due to the limited representation of fungal ITS sequences in reference databases—an inherent challenge in fungal taxonomy. The species identified in high abundance (relative abundance > 0.001), and listed in descending order of abundance, were *Saccharomyces cerevisiae*,

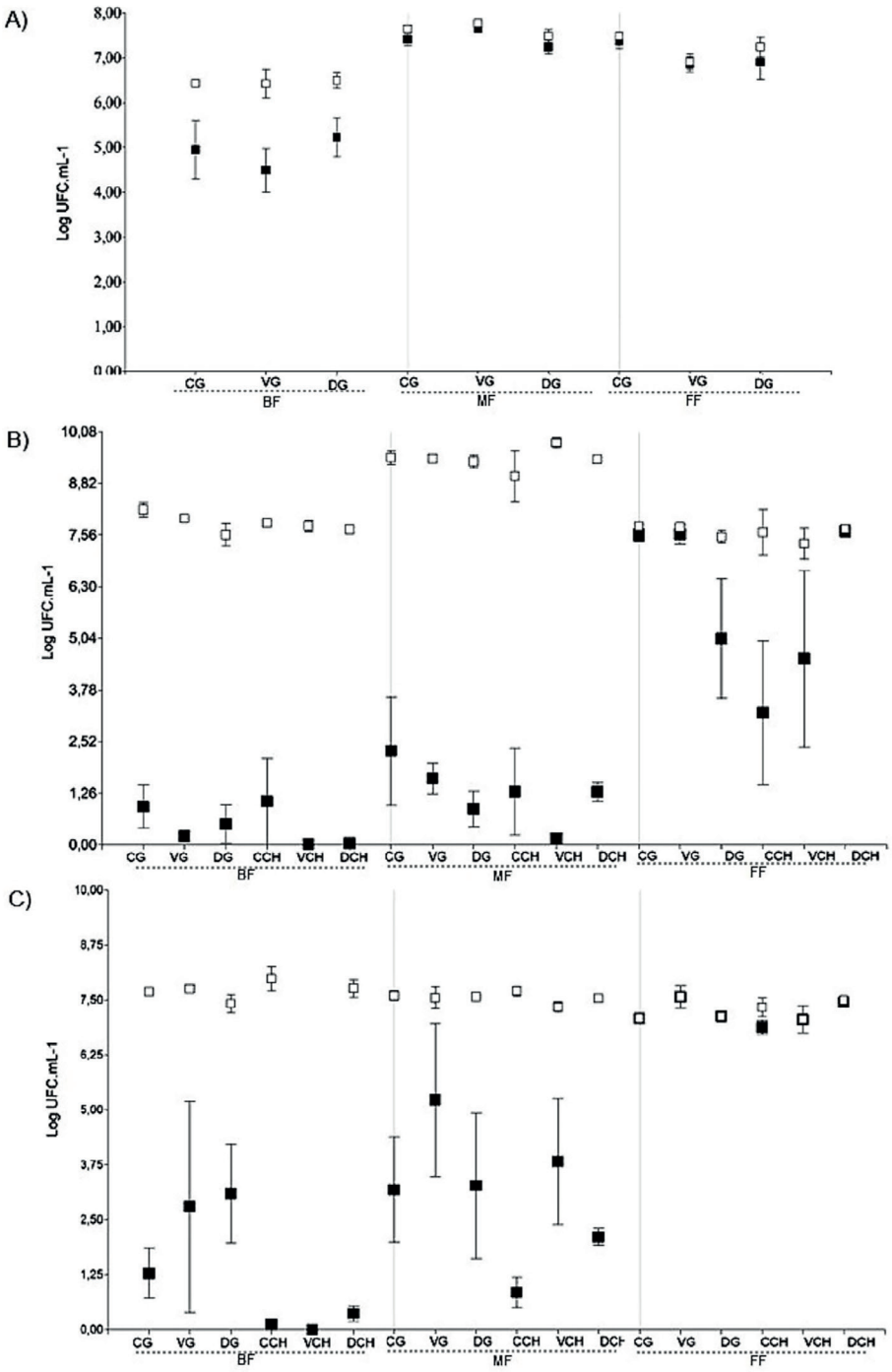


FIGURE 3. Yeast colony-forming units count at different stages of fermentation, beginning (BF), medium (MF), or final of fermentation (FF) from the 2021 (A), 2022 (B), and 2023 (C) vintages. Black squares: *Saccharomyces* counts, white squares: total yeast counts. A smooth vertical line indicates the beginning of the next stage.

Hanseniaspora nectarophila, *Hanseniaspora guilliermondii*, *Schizosaccharomyces japonicus*, *Aureobasidium pullulans*, and *Metschnikowia pulcherrima* (Figure 4B).

Within each vintage, no significant differences in wine fungal microbiome composition were observed across treatments (DESeq2 test, adjusted p-value < 0.05).

Considering the core mycobiome as the species present across all three vintages, the yeasts *S. cerevisiae*, *H. nectarophila*, and *H. guilliermondii* were consistently detected in every vintage (Figure 4B). *M. pulcherrima* was only present at high relative abundance in one sample of 2021, while *Schizosaccharomyces japonicus* was abundant in three samples of vintage 2023 (Figure 4B). *Aureobasidium pullulans,* despite in low proportions, was also present in all three seasons (Figure 4B).

5. Wine characteristics

The physicochemical characterisation of the wines was carried out upon completion of the winemaking process (Table S4), after stabilisation and before bottling.

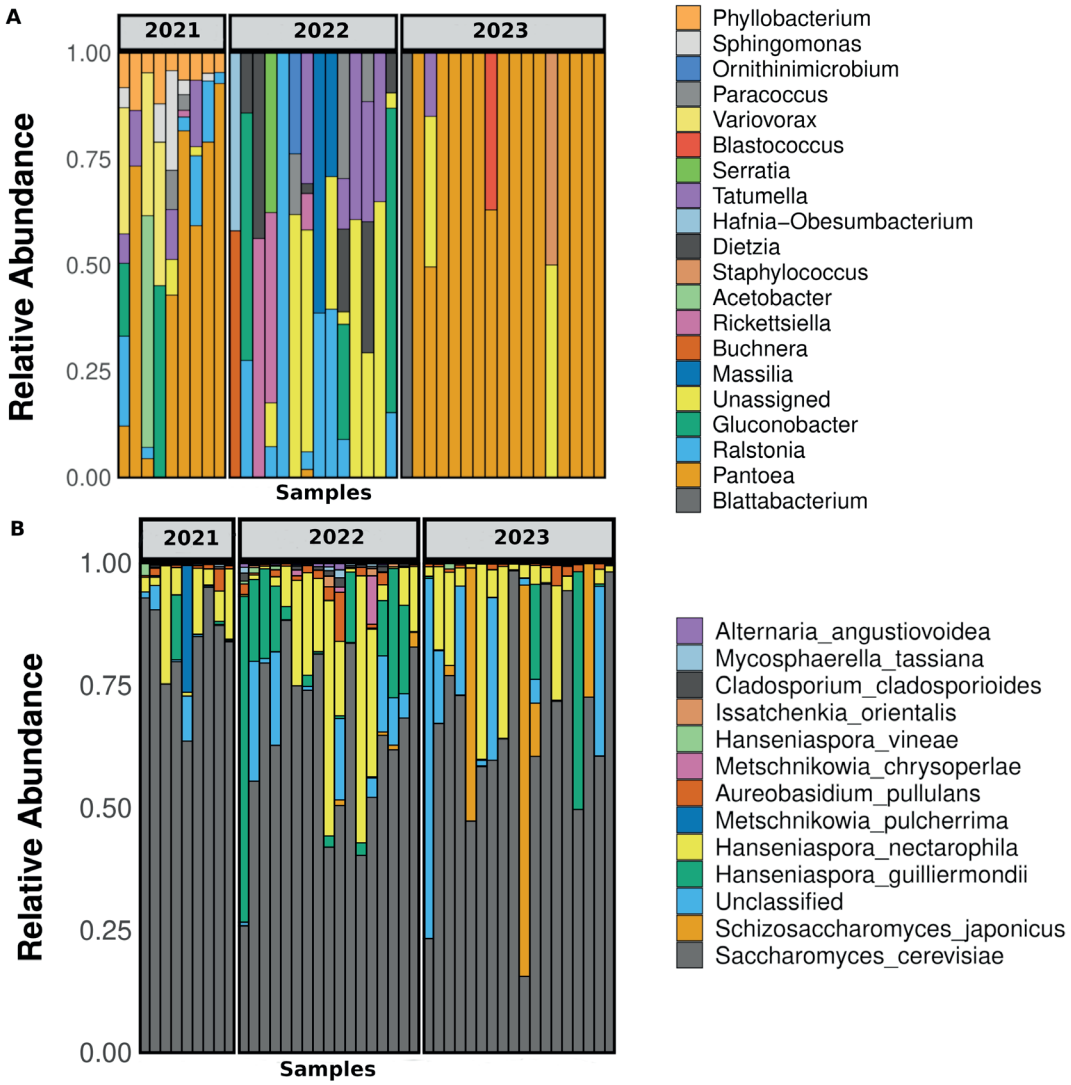


FIGURE 4. Microbial community profiles at 75 % of Malbec wine fermentation. (A) Prokaryotic genus composition after applying a minimum relative abundance threshold of 0.01. (B) Fungal species composition, filtered with a minimum relative abundance of 0.005.

6. Wine physicochemical variables

Despite variations verified every season during fermentation, the overall wine composition was relatively homogeneous, with only minor significant differences observed (Table S4). Some treatments experienced difficulties in completing alcoholic fermentation, resulting in residual sugars in the final wines. In this sense, only VG treatment allowed appropriate sugar consumption in all seasons. Such differences evidenced the different populations of yeasts in initial musts and involved in each fermentation (Figures 1 and 2) and evidence the complex microbial behaviour in biodynamic wines. These microbial differences were not evidenced in ethanol yield, suggesting that despite the complex microbial consortium involved, the biodynamic process has its own balance, allowing the production of quality wines. Malolactic fermentation (MLF) occurred spontaneously only in 2021 wines, completing 23 days after racking. This was evidenced by the near-total consumption of malic acid and the corresponding formation of lactic acid (Table S4).

7. Wine sensorial impact of treatments

The sensorial evaluation of wines enabled their characterisation based on aroma, mouthfeel, and colour descriptors, revealing seasonal variations (Figure 5) in sensorial descriptors, allowing wine differentiation. Wines obtained in 2021 were differentiated based on colour (violet hue) and basic taste traits (sweetness, astringency, bitterness). These descriptors allowed significant differences among the three biodynamic amendment treatments evaluated, being remarkable for the differentiation of VG wines as "dry" wines, without residual sugar, as previously mentioned. Wines from the 2022 vintage were also differentiated based on distinctive aroma traits, such as red fruits and vanilla, more associated with treatments CG and VG. Once again, VG treatment showed evidence of correlation between the optimum sugar consumption during fermentation and a "not sweet" perception. This season, the differentiation of the wine's taste characteristics found by the panel between each amendment treatment (V, C, D) in relation to the anti-hail net treatment (G or CH) was notable,

with high association of the G treatment and desirable aroma descriptors. Similar results were obtained in 2023 wines, being G wines associated with the chocolate aroma descriptor. It is interesting to observe that after three years of treatment, wines from CG and VG showed no clear differentiation and appeared in Figure 5 as overlapping ellipses.

DISCUSSION

Unlike controlled inoculations with selected yeast strains, spontaneous fermentation relies on naturally occurring microorganisms, including both *Saccharomyces* and non-*Saccharomyces* yeast species as well as bacteria, all of which can significantly impact wine quality, aroma, and stability. In the case of spontaneous fermentation from biodynamically managed vineyards, microbiome characterisation becomes even more crucial, as these vineyards typically harbour greater microbial diversity than conventionally managed ones (Castrillo, 2022). This higher diversity creates a more complex and competitive fermentation environment, highlighting the importance of a deeper understanding of the native microbial dynamics that shape the fermentation process and contribute to the wine's unique sensory and terroir-driven characteristics.

This study aimed to assess the impact of different vineyard management practices—specifically the use of organic amendments and anti-hail net configurations—on the yeast, bacterial, and fungal communities in wine. The fungal

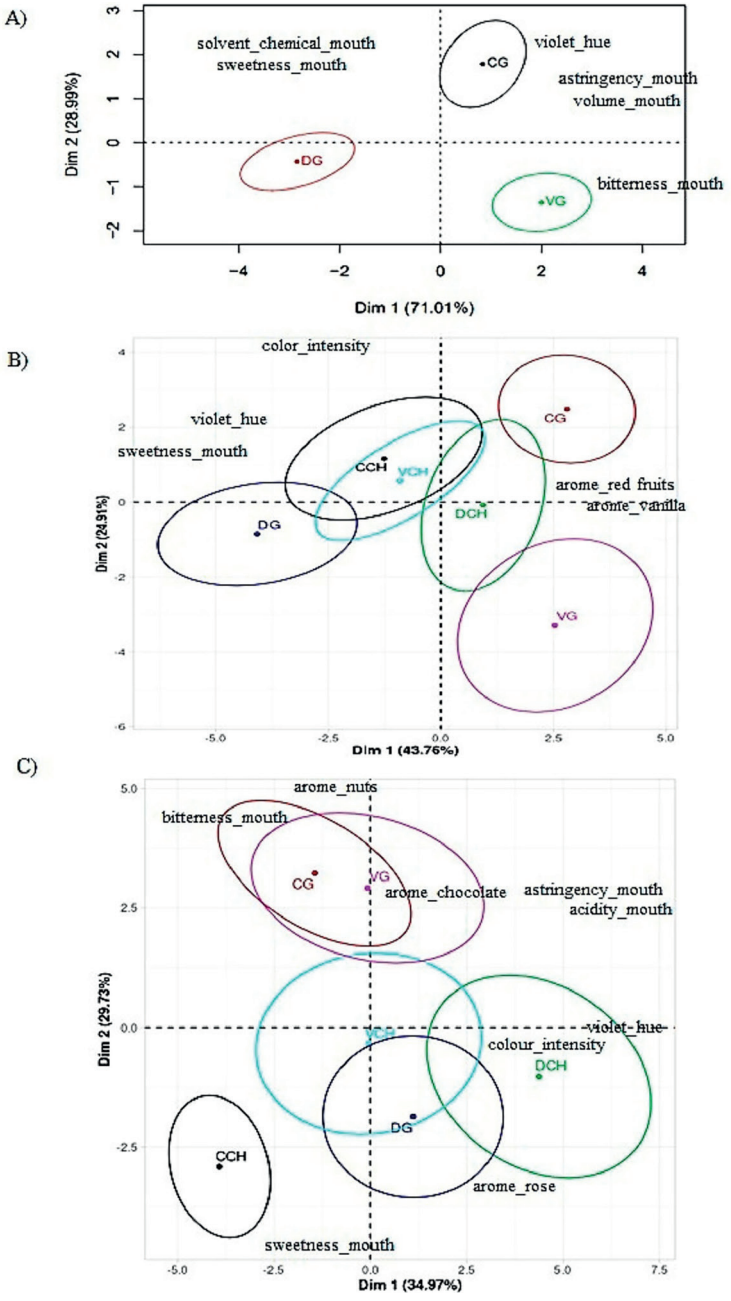


FIGURE 5. Biplot of PCA from sensory analyses of wines in 2021 (A), 2022 (B), and 2023 (C) vintages. The plots show the location of significant descriptors ($p < 0.05$), which effectively differentiate the wine profiles.

community structure (β-diversity) in wines varied according to the anti-hail net configuration. By contrast, bacterial communities were unaffected. This suggests that vineyard microclimatic modifications imposed by nets exert a stronger selective pressure on fungi, likely due to their sensitivity to changes in light and humidity. In contrast, bacterial communities appear more resilient, possibly reflecting their metabolic flexibility and adaptation to must conditions. These findings underscore the importance of fungi as bioindicators of vineyard management practices and their potential impact on wine quality.

The absence of a detectable amendment effect on bacterial and fungal α-diversity in wines may reflect the high inherent variability of vines within the vineyard block studied, combined with the stabilising influence of long-term organic management. These conditions promote a highly diverse baseline microbial community that may buffer or obscure treatment-specific impacts.

The musts presented the expected characteristics of sound mature Malbec grapes from Mendoza (Argentina) (Fanzone *et al.*, 2010). Yeast counts in the fresh must were variable but consistent with previous reports on total yeast populations present in mature healthy Malbec grapes from Mendoza (Combina *et al.*, 2005; Mercado *et al.*, 2011). Also, these viable counts varied depending on the treatment, repetition, and the vintage, reflecting the high heterogeneity of this type of vineyard (Sun *et al.*, 2024). As previously reported by Mercado *et al.* (2011), *Saccharomyces* yeasts are typically absent in fresh grapes and must—a pattern confirmed by our data, except for a few must samples.

Vintage-related differences were observed in fermentation dynamics, including the progression of CFU counts of *Saccharomyces*, the main fermentative yeast, as well as total yeast counts, and the resulting wine characteristics. These differences in populations of fermentative wine yeast associated with the year and its characteristics, also called the "vintage effect", have been previously reported (Valero *et al.*, 2007; González *et al.*, 2020). Moreover, differences in the successful completion of MLF could also be attributed to the vintage effect or to different circumstances affecting the progression of the spontaneous fermentation process. In this sense, it is well known that yeasts may affect MLF either directly or indirectly, by interfering with the growth of malolactic bacteria (Paramithiotis *et al.*, 2022).

In this study, we also aimed at understanding the relationship between seasonal variations in the wine microbiome and their influence on wine quality. Using a metabarcoding approach, we were able to identify the main microbial contributing agents for wine spontaneous fermentations from a vineyard in Ugarteche, Mendoza. Previous work in Malbec fermentation stages was conducted on a vineyard in Buenos Aires province (Rivas *et al.*, 2021). The present approach includes an extensive sampling design over three vintages to unravel the microbial agents involved in the spontaneous fermentation of Malbec wine in the main production region of this grape cultivar representative of Argentinean winemaking.

Large changes in microbiome structure were observed across each vintage, which could be related to the climatic annual fluctuations, especially in terms of temperature and precipitation (Table 1). Notably, filamentous fungi were absent in the must from the 2022 vintage, in contrast to their presence in both 2021 and 2023 (Figure 2). It is well known that filamentous fungi provide the enzymatic arsenal for the degradation of complex molecular polymers in must, thereby releasing simple sugars and assimilable nitrogen compounds that enhance nutrient availability and support a more efficient alcoholic fermentation (Viljoen, 2006). The absence of that enzymatic activity could have contributed to the poor fermentative performance, evidenced by lower *S. cerevisiae* counts in the middle and final stages of the fermentation, at vintage 2022.

In addition to the fundamental, and well-documented, role of *S. cerevisiae*, the contribution of non-*Saccharomyces* yeasts was also documented in our study, particularly *Hanseniaspora nectarophila* and *Hanseniaspora guilliermondii.* Notably, these species were well-represented in wines from all three vintages. *Hanseniaspora* yeasts are frequently detected in the early stages of spontaneous wine fermentation (Albertin *et al.*, 2016; Carrau & Henschke, 2021). In this study, these yeasts could be detected in significant abundance during 75 % of the fermentation process, suggesting a moderate tolerance to ethanol. The persistence of *Hanseniaspora* till the end of fermentation, even up to 12.5 % *v/v* ethanol, has been previously reported (Moreira *et al.*, 2011; Ohwofasa *et al.*, 2024b). Several species within the genus *Hanseniaspora* have been shown to be important as oenological agents, and *Hanseniaspora occidentalis* has also been reported to reduce malic acid levels (Ohwofasa *et al.*, 2024b). The yeast species *H. nectarophila* was first isolated from flowers of a native American plant (Čadež *et al.*, 2014) and has recently been reported as a potential terroir marker in Cyprus wines, especially due to its floral aroma's contribution (Kamilari *et al.*, 2021). An important finding of the present work, that the metabarcoding method employed allowed, was the generalised and stable presence of *H. nectarophila*, a yeast species not previously reported in local or regional research of grape and must yeast species (Combina *et al.*, 2005; Lopes *et al.*, 2007; Mestre Furlani *et al.*, 2017; Raymond Eder & Rosa, 2021; Longhi *et al.*, 2022). *H. guilliermondii* has been reported to produce higher alcohols such as 1-propanol, 2-phenylethanol, and aliphatic higher alcohol, as well as significant levels of esters such as 2-phenylethyl acetate and ethyl acetate, and high amounts of acetic acid (Moreira *et al.*, 2011). Given the persistence of *Hanseniaspora* on the studied biodynamic fermentation and its metabolic capabilities, future studies should address its potential to enhance wine complexity through co-inoculation with *S. cerevisiae.*

In the present study, *S. japonicus* and *M. pulcherrima* also contributed to Malbec wine fermentations, but their abundance varied significantly across seasons, possibly due to their higher susceptibility to climatic fluctuations. Several *Metschnikowia* species are frequently co-inoculated with *S. cerevisiae* to obtain wines with lower ethanol levels

(Hranilovic *et al.*, 2020). Additionally, *M. pulcherrima* has been reported and is being used as a bioprotectant in grape must due to its ability to control spoilage agents (Puyo *et al.*, 2023). *Metschnikowia* yeasts produce more glycerol than ethanol, and this glycerol acts as a cell membrane protectant, supporting lactic acid bacteria (LAB) survival and therefore promoting MLF (Bartle *et al.*, 2019). This is in line with the findings of this study and could explain the observed enhanced MLF performance upon the higher abundance of *M. pulcherrima* during alcoholic fermentation in 2021.

Schizosaccharomyces japonicus was particularly abundant in the 2023 vintage. This species is well known for its ability to degrade malic acid, leading to a reduction in the total acidity of wine (Romani *et al.*, 2018; Bartle *et al.*, 2019). It also promotes polysaccharide release, increasing viscosity and enhancing mouthfeel (Domizio *et al.*, 2018; Millarini *et al.*, 2020), while contributing to fruity and floral aromas (Romani *et al.*, 2018).

The presence of *A. pullulans*—a dimorphic ascomycete described as yeast-like (Kurtzman, 2011)—across all fermentations in this study confirms its status as a ubiquitous member of the grape and must microbiota, consistent with previous reports (Sternes *et al.*, 2017; Onetto *et al.*, 2020). Its universal prevalence supports the findings of Watanabe and Hashimoto (2023), who suggest that *A. pullulans* exploits plant cell wall polysaccharides and cuticular lipids to establish its ecological niche. By degrading plant cell walls, this yeast may increase the availability of fermentable sugars, potentially facilitating the establishment of *S. cerevisiae* on the grape surface and supporting spontaneous fermentation. Furthermore, while *A. pullulans* may contribute to wine colour and malic acid polymerisation (Onetto *et al.*, 2020), its constant presence across our samples suggests its primary role is ecological, acting as a foundational species in the fermentation environment.

Although sampling for metabarcoding analysis was conducted during alcoholic fermentation (AF), we hypothesise that the dominant microbial communities active during AF shape the fermentation environment, influencing the success or failure of subsequent MLF. MLF only occurred in the 2021 vintage. Although LAB such as *Pediococcus, Lactococcus*, and *Lactobacillus* were detected in a few samples, their presence was sparse and at low abundance, showing no clear association with lactic acid production and malic acid degradation verified in those wines. This could be explained by the timing of sampling at 75 % of fermentation, when, as previously observed, the LAB populations typically decrease during AF and only increase once AF is complete (Balmaseda *et al.*, 2018). In previous studies, it was observed that the LAB abundance increases with a higher bacterial diversity (Bubeck *et al.*, 2020). The newly reclassified genera of *Lactobacillus* (Zheng *et al.*, 2020) are not included in the SILVA 138.1 database used for taxonomic assignment. Therefore, we could not confidently determine whether these renamed genera were present in our metabarcoding dataset.

Interestingly, *Oenococcus oeni*, the most common LAB associated with MLF, was not detected in our study. While inhibition by non-*Saccharomyces* yeasts has been proposed as a mechanism for suppressing *O. oeni* growth (Balmaseda *et al.*, 2018), other explanations are also possible, including detection limits or sampling prior to the active MLF stage.

The acetic acid bacteria (AAB), *Gluconobacter* and *Acetobacter*, are considered potential spoilage microorganisms in winemaking. The high abundance of *Gluconobacter* in 2021 may be linked to the higher precipitation during that season, particularly near harvest (90 mm in February). In contrast, during 2023, the driest vintage, *Gluconobacter* abundance was negligible. This aligns with previous findings indicating that AAB populations tend to increase in rainy and humid vintages (Ohwofasa *et al.*, 2024b). Understanding these dynamics is crucial for spontaneous winemaking, as managing microbial composition can help minimise the risk of spoilage and undesirable flavour in the final wine.

Pantoea was found to be among the most abundant bacterial genera. Although it is commonly found in spontaneous wines or in vineyard ecosystems around the world (Walterson *et al.*, 2014; Zhang *et al.*, 2022), its role in fermentation performance remains understudied and its functional contribution to alcoholic fermentation remains unclear, as this genus is not typically associated with ethanol production. A recent study found it to be the most abundant bacteria and correlates with volatile compounds (VOCs) as higher alcohols, esters, and fatty acids (Zhang *et al.*, 2022). On the other hand, *Tatumella* was previously found as the dominant bacterial genus in spontaneous wine fermentation (Bubeck *et al.*, 2020; Ohwofasa *et al.*, 2024b). In this study, *Tatumella* was found to be highly abundant, particularly in the 2021 and 2022 vintage, but not in the 2023 vintage.

This work contributes to the characterisation of spontaneous Malbec fermentation in the particular and increasingly interesting case of biodynamic wines. It is worth noting the discovery of consistent species in each vintage: *S. cerevisiae*, *H. nectarophila*, *H. guilliermondii*, *S. japonicus*, and *A. pullulans*, which constitute the "core" microbiome of these wines. Beyond that, the "vintage effect" as a driver for variation in the context of biodynamic winemaking was verified by microbial, physico-chemical and sensorial wine characteristics evaluated.

CONCLUSION

Using a culture-independent metabarcoding approach, we identified the core microbial contributors to spontaneous wine fermentation of biodynamic Malbec wines. Vintage variation, strongly influenced by climatic differences, significantly shaped the bacterial and fungal composition. Notably, two species of *Hanseniaspora*, well-established over seasons, were highlighted for their potential role in imparting unique characteristics to wines from the parcel under study.

It is important to note that the presence of microorganisms identified through metabarcoding does not necessarily indicate their active involvement in fermentation. Therefore, culture-based methods are essential to complement and verify the activity of the microorganisms identified. Nevertheless, this study provides valuable insights for winemakers striving to understand microbial interactions and produce wines that truly reflect their terroir.

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REFERENCES

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., & Kõljalg, U. (2022). *UNITE QIIME release for Fungi*. Version 16.10.2022. UNITE Community. https://doi.org/10.15156/BIO/2483915

Albertin, W., Setati, M. E., Miot-Sertier, C., Mostert, T. T., Colonna-Ceccaldi, B., Coulon, J., … & Masneuf-Pomarede, I. (2016). *Hanseniaspora uvarum* from winemaking environments show spatial and temporal genetic clustering. *Frontiers in Microbiology*, *6*, 1569. https://doi.org/10.3389/fmicb.2015.01569

Ares, G., Bruzzone, F., Vidal, L., Cadena, R. S., Giménez, A., Pineau, B., … & Jaeger, S. R. (2014). Evaluation of a rating-based variant of check-all-that-apply questions: Rate-all-that-apply (RATA). *Food Quality and Preference*, *36*, 87–95. https://doi.org/10.1016/j.foodqual.2014.03.006

Balmaseda, A., Bordons, A., Reguant, C., & Bautista-Gallego, J. (2018). Non-*Saccharomyces* in Wine: Effect Upon *Oenococcus oeni* and Malolactic Fermentation. *Frontiers in Microbiology*, *9*, 534. https://doi.org/10.3389/fmicb.2018.00534

Bartle, L., Sumby, K., Sundstrom, J., & Jiranek, V. (2019). The microbial challenge of winemaking: yeast-bacteria compatibility. *FEMS Yeast Research*, *19*(4), foz040. https://doi.org/10.1093/femsyr/foz040

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … & Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Boynton, P. J., & Greig, D. (2016). Species richness influences wine ecosystem function through a dominant species. *Fungal Ecology*, *22*, 61-72. https://doi.org/10.1016/j.funeco.2016.04.008

Bubeck, A. M., Preiss, L., Jung, A., Dörner, E., Podlesny, D., Kulis, M., … & Fricke, W. F. (2020). Bacterial microbiota diversity and composition in red and white wines correlate with plant-derived DNA contributions and botrytis infection. *Scientific reports*, *10*(1), 13828. https://doi.org/10.1038/s41598-020-70535-8

Čadež, N., Pagnocca, F. C., Raspor, P., & Rosa, C. A. (2014). *Hanseniaspora nectarophila* sp. nov., a yeast species isolated from ephemeral flowers. *International journal of systematic and evolutionary microbiology*, *64*(Pt 7), 2364–2369. https://doi.org/10.1099/ijs.0.061499-0

Capozzi, V., Garofalo, C., Chiriatti, M. A., Grieco, F., & Spano, G. (2015). Microbial terroir and food innovation: The case of yeast biodiversity in wine. *Microbiological research*, *181*, 75-83. https://doi.org/10.1016/j.micres.2015.10.005

Carrau, F., & Henschke, P. A. (2021). *Hanseniaspora vineae* and the concept of friendly yeasts to increase autochthonous wine flavor diversity. *Frontiers in Microbiology*, *12*, 702093. https://doi.org/10.3389/fmicb.2021.702093

Castrillo, D. (2022). Organic Vineyard: Yeast Diversity, Biogeography, and Prospects for the Future. *Biodiversity Online J.*, *2*(1), BOJ.000530. https://doi.org/10.31031/BOJ.2022.02.000530

Combina, M., Mercado, L., Borgo, P., Elia, A., Jofré, V., Ganga, A., … & Catania, C. (2005). Yeasts associated to Malbec grape berries from Mendoza, Argentina. *Journal of Applied Microbiology*, *98*(5), 1055-1061. https://doi.org/10.1111/j.1365-2672.2005.02540.x

da Rocha Oliveira Teixeira, R., Arcuri, S., Cavicchi, A., Galli, F., Brunori, G., & Vergamini, D. (2023). Can alternative wine networks foster sustainable business model innovation and value creation? The case of organic and biodynamic wine in Tuscany. *Frontiers in Sustainable Food Systems*, *7*, 1241062. https://doi.org/10.3389/fsufs.2023.1241062

Domizio, P., Lencioni, L., Calamai, L., Portaro, L., & Bisson, L. F. (2018). Evaluation of the yeast *Schizosaccharomyces japonicus* for use in wine production. *American Journal of Enology and Viticulture*, *69*(3), 266-277. https://doi.org/10.5344/ajev.2018.18004

European Food Safety Authority., Carrasco Cabrera, L., & Medina Pastor, P. (2022). The 2020 European Union report on pesticide residues in food. *EFSA Journal*, *20*(3), e07215. https://doi.org/10.2903/j.efsa.2022.7215

Fanzone, M., Peña-Neira, A., Jofré, V., Assof, M., & Zamora, F. (2010). Phenolic characterization of Malbec wines from Mendoza province (Argentina). *Journal of Agricultural and Food Chemistry*, *58*(4), 2388-2397. https://doi.org/10.1021/jf903690v

Gardner, J. M., Alperstein, L., Walker, M. E., Zhang, J., & Jiranek, V. (2023). Modern yeast development: finding the balance between tradition and innovation in contemporary winemaking. *FEMS Yeast Research*, *23*, foac049. https://doi.org/10.1093/femsyr/foac049

González, M. L., Sturm, M. E., Lerena, M. C., Rojo, M. C., Chimeno, S.V., Combina, M., & Mercado, L. A. (2020). Persistence and reservoirs of *Saccharomyces cerevisiae* biodiversity in different vineyard niches. *Food Microbiology*, *86*, 103328. https://doi.org/10.1016/j.fm.2019.103328

Hranilovic, A., Gambetta, J. M., Jeffery, D. W., Grbin, P. R., & Jiranek, V. (2020). Lower-alcohol wines produced by *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* co-fermentations: The effect of sequential inoculation timing. *International Journal of Food Microbiology*, *329*, 108651. https://doi.org/10.1016/j.ijfoodmicro.2020.108651

Kamilari, E., Mina, M., Karallis, C., & Tsaltas, D. (2021). Metataxonomic analysis of grape microbiota during wine fermentation reveals the distinction of Cyprus regional *terroirs*. *Frontiers in Microbiology*, *12*, 726483. https://doi.org/10.3389/fmicb.2021.726483

Kurtzman, C., Fell, J. W., & Boekhout, T. (2011). *The yeasts: a taxonomic study*. Elsevier.

Lê, S., & Husson, F. (2008). SensoMineR: A package for sensory data analysis. *Journal of Sensory Studies*, *23*(1), 14–25. https://doi.org/10.1111/j.1745-459X.2007.00137.x

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, *25*(1), 1–18. https://doi.org/10.18637/jss.v025.i01

Longhi, S. J., Martín, M. C., Merín, M. G., & Morata de Ambrosini, V. I. (2022). Yeast Multi-Enzymatic Systems for Improving Colour Extraction, Technological Parameters and Antioxidant Activity of Wine. *Food Technology and Biotechnology*, *60*(1), 556-570. https://doi.org/10.17113/ftb.60.04.22.7777

Lopes, C. A., Rodríguez, M. E., Sangorrín, M., Querol, A., & Caballero, A. C. (2007). Patagonian wines: the selection of an indigenous yeast starter. *Journal of Industrial Microbiology and Biotechnology*, *34*(8), 539–546. https://doi.org/10.1007/s10295-007-0227-3

Mercado, L., Sturm, M. E., Rojo, M. C., Ciklic, I., Martínez, C., & Combina, M. (2011). Biodiversity of *Saccharomyces cerevisiae* populations in Malbec vineyards from the "Zona Alta del Río Mendoza" region in Argentina. *International Journal of Food Microbiology*, *151*(3), 319-326. https://doi.org/10.1016/j.ijfoodmicro.2011.09.026

Mestre Furlani, M. V., Maturano, Y. P., Combina, M., Mercado, L. A., Toro, M. E., & Vazquez, F. (2017). Selection of non-*Saccharomyces* yeasts to be used in grape musts with high alcoholic potential: a strategy to obtain wines with reduced ethanol content. *FEMS Yeast Research*, *17*(2). https://doi.org/10.1093/femsyr/fox010

Millarini, V., Ignesti, S., Cappelli, S., Ferraro, G., Adessi, A., Zanoni, B., Fratini, E., & Domizio, P. (2020). Protection of wine from protein haze using *Schizosaccharomyces japonicus* polysaccharides. *Foods*, *9*(10), 1407. https://doi.org/10.3390/foods9101407

Moreira, N., Pina, C., Mendes, F., Couto, J. A., Hogg, T., & Vasconcelos, I. (2011). Volatile compounds contribution of *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum* during red wine vinifications. *Food Control*, *22*(5), 662-667. https://doi.org/10.1016/j.foodcont.2010.07.025

Ohwofasa, A., Dhami, M., Zhang, J., Tian, B., Winefield, C., & On, S. L. W. (2024b). Influence of climatic variation on microbial communities during organic Pinot noir wine production. *PLoS ONE, 19*(2): e0296859. https://doi.org/10.1371/journal.pone.0296859

Ohwofasa, A., Tian, B., Torrico, D., Dhami, M., Winefield, C., & On, S. L. (2024a). Differences in organic Pinot Noir wine production systems correlated with microbiome analysis, sensory characteristics and volatile composition. *Food Bioscience*, 104562. https://doi.org/10.1016/j.fbio.2024.104562

OIV. (2024). *Compendium of International Methods of Wine and Must Analysis*. International organisation of vine and wine. https://www.oiv.int/standards/compendium-of-international-methods-of-wine-and-must-analysis

Onetto, C. A., Borneman, A. R., & Schmidt, S. A. (2020). Investigating the effects of *Aureobasidium pullulans* on grape juice composition and fermentation. *Food Microbiology*, *90*:103451. https://doi.org/10.1016/j.fm.2020.103451

Palenzuela, M. D. V., López de Lerma, N., Sánchez-Suárez, F., Martínez-García, R., Peinado, R. A., & Rosal, A. (2023). Aroma composition of wines produced from grapes treated with organic amendments. *Applied Sciences*, *13*(14), 8001. https://doi.org/10.3390/app13148001

Pallmann, C. L., Brown, J. A., Olineka, T. L., Cocolin, L., Mills, D. A., & Bisson, L. F. (2001). Use of WL medium to profile native flora fermentations. *American Journal of Enology and Viticulture*, *52*(3), 198-203. https://doi.org/10.5344/ajev.2001.52.3.198

Paramithiotis, S., Stasinou, V., Tzamourani, A., Kotseridis, Y., & Dimopoulou, M. (2022). Malolactic fermentation—theoretical advances and practical considerations. *Fermentation*, *8*(10), 521. https://doi.org/10.3390/fermentation8100521

Philipp, C., Bagheri, B., Horacek, M., Eder, P., Bauer, F. F., & Setati, M. E. (2021). Inoculation of grape musts with single strains of *Saccharomyces cerevisiae* yeast reduces the diversity of chemical profiles of wines. *PLoS ONE*, *16*(7), e0254919. https://doi.org/10.1371/journal.pone.0254919

Puyo, M., Simonin, S., Bach, B., Klein, G., Alexandre, H., & Tourdot-Maréchal, R. (2023). Bio-protection in oenology by *Metschnikowia pulcherrima*: from field results to scientific inquiry. *Frontiers in Microbiology*, *14*, 1252973. https://doi.org/10.3389/fmicb.2023.1252973

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1): D590-D596. https://doi.org/10.1093/nar/gks1219

Raymond Eder, M. L., & Rosa, A. L. (2021). Growth Fitness of Indigenous Wine Yeasts in Grape Musts from Different *Vitis* Species. *Acta Scientific Nutritional Health*, *5*(4), 82-89. https://doi.org/10.31080/ASNH.2020.05.0851

Rivas, G. A., Guillade, A. C., Semorile, L. C., & Delfederico, L. (2021). Influence of climate on soil and wine bacterial diversity on a vineyard in a non-traditional wine region in Argentina. *Frontiers in Microbiology*, *12*, 726384. https://doi.org/10.3389/fmicb.2021.726384

Romani, C., Lencioni, L., Gobbi, M., Mannazzu, I., Ciani, M., & Domizio, P. (2018). *Schizosaccharomyces japonicus*: A Polysaccharide-Overproducing Yeast to Be Used in Winemaking. *Fermentation*, *4*(1), 14. https://doi.org/10.3390/fermentation4010014

Rosado, D., Ramos-Tapia, I., Crandall, K. A., Pérez-Losada, M., & Domínguez, J. (2022). Grapevine treatment with bagasse vermicompost changes the microbiome of Albariño must and wine and improves wine quality. *OENO One*, *56*(3), 219–230. https://doi.org/10.20870/oeno-one.2022.56.3.5560

Sangphukieo, A. (2022). *Metadata and silva classifier*. Figshare, dataset. https://doi.org/10.6084/m9.figshare.20430963.v5

Smith, S. (2019). Phylosmith: an R-package for reproducible and efficient microbiome analysis with phyloseq-objects. *Journal of Open Source Software*, *4*(38), 1442. https://doi.org/10.21105/joss.01442

Stefanini, I., & Cavalieri, D. (2018). Metagenomic approaches to investigate the contribution of the vineyard environment to the quality of wine fermentation: potentials and difficulties. *Frontiers in microbiology*, *9*, 991. https://doi.org/10.3389/fmicb.2018.00991

Sternes, P. R., Lee, D., Kutyna, D. R., & Borneman, A. R. (2017). A combined meta-barcoding and shotgun metagenomic analysis of spontaneous wine fermentation. *GigaScience*, *6*(7), gix040. https://doi.org/10.1093/gigascience/gix040

Sun, Y., Zhang, X., Zhang, F., & Zhang, J. (2024). Impact of biodynamic and organic vineyard management on the microorganism community and aroma characteristics of Cabernet Sauvignon wine. *Journal of Cleaner Production*, *467*, 142929. https://doi.org/10.1016/j.jclepro.2024.142929

Valero, E., Cambon, B., Schuller, D., Casal, M., & Dequin, S. (2007). Biodiversity of *Saccharomyces* yeast strains from grape berries of wine-producing areas using starter commercial yeasts. *FEMS yeast research*, *7*(2), 317-329. https://doi.org/10.1111/j.1567-1364.2006.00161.x

Viljoen, B. C. (2006). Yeast ecological interactions. Yeast-Yeast, Yeast-Bacteria, Yeast-Fungi interactions and yeasts as biocontrol agents. In: *Yeasts in food and beverages*. Berlin, Heidelberg: Springer Berlin Heidelberg, p. 83-110. https://doi.org/10.1007/978-3-540-28398-0_4

Walterson, A. M., Smith, D. D. N., & Stavrinides, J. (2014). Identification of a *Pantoea* Biosynthetic Cluster That Directs the Synthesis of an Antimicrobial Natural Product. *PLoS ONE*, *9*(5): e96208. https://doi.org/10.1371/journal.pone.0096208

Watanabe, D., & Hashimoto, W. (2023). Adaptation of yeast *Saccharomyces cerevisiae* to grape-skin environment. *Scientific Report*, *13*, 9279. https://doi.org/10.1038/s41598-023-35734-z

Zhang, Z., Zhang, Q., Yang, H., Sun, L., Xia, H., Sun, W., Wang, Z., & Zhang, J. (2022). Bacterial Communities Related to Aroma Formation during Spontaneous Fermentation of 'Cabernet Sauvignon' Wine in Ningxia, China. *Foods*, *11*(18), 2775. https://doi.org/10.3390/foods11182775

Zhao, Y., Federico, A., Faits, T., Manimaran, S., Segrè, D., Monti, S., & Johnson, W. E. (2021). *Animalcules*: interactive microbiome analytics and visualization in R. *Microbiome*, *9*, 76. https://doi.org/10.1186/s40168-021-01013-0

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H. M., Mattarelli, P., … & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International journal of systematic and evolutionary microbiology*, *70*(4), 2782-2858. https://doi.org/10.1099/ijsem.0.004107