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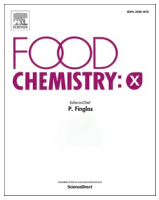

From microbial communities to aroma profiles: A comparative study of spontaneous fermentation in merlot and cabernet sauvignon wines

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ABSTRACT

This study aimed to compare the microbial community composition and aroma characteristics during the fermentation of different grape cultivars, Merlot and Cabernet Sauvignon. Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and Odor Activity Value (OAV) screening identified 15 distinct active compounds. The sensory evaluation indicated that Merlot wine exhibited a more intense fruity aroma and received higher overall scores than Cabernet Sauvignon wine. High-throughput sequencing (HTS) results revealed that the microbial diversity in Merlot was higher than in Cabernet Sauvignon wine. *Lachancea*, *Acremonium*, *Fructobacillus*, and *Lactiplantibacillus* were unique to the Merlot wine, whereas *Penicillium*, *Wickerhamomyces*, *Gluconobacter*, and *Klebsiella* were exclusive to Cabernet Sauvignon wine. *Saccharomyces* and *Tatumella* were identified as the dominant microorganisms during the fermentation of both Merlot and Cabernet Sauvignon wines. Correlation analysis demonstrated a significant positive association among the dominant microbial communities, which played a crucial role in determining the formation of volatile compounds.

1. Introduction

Wine is one of the three major alcoholic beverages globally, and its production primarily relies on microbial activity. During fermentation process, yeasts, bacteria, and molds metabolize sugars into various volatile compounds and carbon dioxide through complex biochemical reactions, playing a crucial role in the sensory properties of wine (Barata et al., 2012; Beckner Whitener et al., 2016). Previous studies have shown that *Lachancea thermotolerans*, *Pichia kluyveri*, *Rhodotorula mucilaginosa*, and *Metschnikowia* spp. exhibit distinct enzymatic mechanisms, allowing for the introduction of unique aromas in wines (García-Izquierdo et al., 2024). These microbial communities primarily originate from grapevines, air, soil, and the grape surfaces, entering the fermentation medium during spontaneous, where they are better adapted to the environmental conditions of natural fermentation (Ma et al., 2023).

During fermentation process, yeasts produce a variety of secondary metabolites, including higher alcohols, esters, ketones, phenols, fatty acid, and sulfur compounds. To date, over 1300 volatile compounds have been identified (Fariña et al., 2015). In industrial wine production, commercial *Saccharomyces cerevisiae* is typically inoculated to better control the fermentation process and ensure consistent product quality stability (Liang et al., 2023). However, this approach often leads to homogenization of wine styles. In contrast, the active role of indigenous microorganisms in spontaneous fermentation contributes to unique and complex aromas that consumers find appealing. This style is gaining increasing attention, and the complexity of flavors produced during natural fermentation may be a key factor in differentiating wine quality. Therefore, a more detailed evaluation of the complex flavors arising from spontaneous fermentation is necessary (Tronchoni et al., 2022; Wei, Ding, et al., 2022).

From a biogeographical perspective, the activity and distribution of winemaking microorganisms are influenced by specific ecological conditions, leading to significant spatial differences in microbial communities across regions (García-Izquierdo et al., 2024). For example, Tronchoni et al. (2022) demonstrated that the composition of microbial communities varies significantly among vineyards in different parts of the world. Further research has shown that the volatile compounds of young wines are not only affected by topography but also significantly

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influenced by vineyard location and management practices, which in turn impact wine quality (Jiang et al., 2013; Wei, Ding, et al., 2022). Additionally, researchers have increasingly recognized intra-vineyard variability as a crucial factor, with grape cultivar being one of the most distinguishing parameters within a single vineyard, significantly affecting microbial communities (Tronchoni et al., 2022). To date, several grape cultivars, such as Italian Riesling (Wang, Zhong, et al., 2024), Pinot Noir (Ohwofasa et al., 2024), Sousão, Touriga Nacional, and Viosinho (Martins et al., 2024), have been explored concerning microbial diversity and its correlation with volatile compounds during the spontaneous fermentation. Recent studies suggest that the influence of grape cultivars on microbial communities is often overshadowed by geographical factors and vineyard management, leaving a gap in the understanding of the relationship between indigenous microbial communities and metabolite profiles in different grape cultivars (Martins et al., 2023).

The Hexi Corridor wine region is located between 36° and 40° north latitude, one of the world's prime areas for wine grape cultivation. The region is characterized by an arid climate, significant diurnal temperature variation, which facilitate the accumulation of sugars and volatile compounds. The soil is predominantly sandy and rich in minerals. It is one of the earliest regions in China for grape cultivation and winemaking. Among the grape cultivars, Cabernet Sauvignon (*Vitis vinifera* L.) and Merlot (*Vitis vinifera* L.) are the dominant red wine grapes in this region due to their strong adaptability and excellent enological properties, making them ideal raw materials for spontaneous fermentation. This study employed headspace solid-phase microextraction gas chromatography coupled with mass spectrometry (HS-SPME-GC–MS) to investigate the composition and dynamic changes of volatile aroma compounds in Merlot and Cabernet Sauvignon under controlled fermentation temperature and stringent sanitation measures within the same vineyard. Additionally, high-throughput sequencing (HTS) was employed to investigate the microbial community composition and its dynamic succession. Furthermore, this study systematically explored the regulatory impact of physicochemical parameters on microbial community dynamics and explored the relationship between microorganisms and the formation of volatile compounds. By elucidating the co-evolutionary mechanisms of microorganisms and volatile compounds from multiple perspectives, this study underscores the potential of indigenous microorganisms in enhancing the complexity and diversity of volatile compounds in wine.

2. Materials and methods

2.1. Sample collection and winemaking

Merlot (XM, rootstock SO4, 10 years old, clone 1103P) and Cabernet Sauvignon (XC, rootstock 169, 10 years old, clone 343) grapes were harvested in mid-September 2023 from the Xiabolan Vineyard (Hexi Corridor wine region, Gansu Province, China). Grape samples were collected from multiple locations in the vineyard. After harvest, the Merlot and Cabernet Sauvignon grapes were destemmed and crushed on-site, then immediately transferred into sterile fermentation tanks and transported to the laboratory for spontaneous fermentation (without the addition of sulfur dioxide). The fermentation progress was monitored based on changes in total sugar, and sample collection times were adjusted according following the method of Liang et al. (2023). The specific sampling points were as follows: the pre-fermentation stage (F0, immediately after destemming and crushing), the early fermentation stage (F1, after a one-third reduction in reducing sugars), the mid-fermentation stage (F2, following a two-thirds decrease in reducing sugars), and the end of fermentation (F3, when residual sugar fell below 4 g/L). Three fermentation batches were conducted for each grape cultivar, and each sample was analyzed in triplicates ($n = 3$).

2.2. Determination of physicochemical parameter

We referenced the approach outlined by Liang et al. (2023) and Ma et al. (2023), the physicochemical parameters of the fermentation samples, including total sugar, ethanol (*v*/v), pH, total acidity (in tartaric acid), and organic acids (including malic acid, citric acid, and tartaric acid), were measured using a FOSS Wine Scan® multi-parameter wine analyzer (Denmark). Yeast assimilable nitrogen (YAN) levels were determined with a Y15 multiparametric analyzer (BioSystem), we referenced the approach outlined by Luzzini et al. (2021).

2.3. Determination of volatile compounds

The volatile compounds in the wine were both qualitatively and quantitatively analyzed using HS-SPME-GC–MS (Liang et al., 2023). An 8 mL wine sample, 2.5 g NaCl, 10 μL of an internal standard (2-octanol, 820.7 mg/L concentration, diluted with anhydrous ethanol (≥99.9 %); Sigma-Aldrich, Milwaukee, WI, USA), and a magnetic stirring bar were sealed in a sample vial. The sealed vial was placed on a thermostatic magnetic stirrer (Bonna Technology, China) for water bath equilibration at 40 °C for 30 min, with continuous stirring at 400 rpm. A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (length 1 cm, Supelco, Bellefonte, PA, United States) was exposed to the headspace of the vial for 30 min while maintaining the sample at 40 °C under constant stirring at 400 rpm. After headspace aroma sampling, the SPME fiber was immediately introduced into the gas chromatograph (GC) inlet at 240 °C, and thermal desorption was performed for 5 min in splitless mode, allowing the target compounds to be released into the Thermo Fisher Trace 1310/ISQ GC–MS system (San Jose, CA, United States). The analysis was conducted using a TG-WAX column (60 m × 0.25 mm × 0.5 μm). The temperature program began at 40 °C and was held for 5 min, followed by a ramp of 3.5 °C/min to 180 °C, with a final hold for 15 min. Helium was used as the carrier gas at a 1 mL/min flow rate. The ion source operated in electron impact (EI) mode with 70 eV energy. The transfer line temperature was set at 180 °C, the ion trap was maintained at 250 °C, and the full scan range was set from 50 to 350 *m/z*.

Volatile compounds were identified by comparing the mass spectra obtained through GC–MS with those in the NIST 14 and Wiley databases (Thermo Fisher Scientific) and by calculating the retention indices (RI) using n-alkane mixtures (C6-C22) under the same conditions. The identified volatiles were quantified based on their peak areas in the samples against their respective standards. Detailed quantitation of the calibration curves for the pure standards and purity information are shown in Supplementary Table S1. Compounds without a standard curve were semi-quantitatively analyzed using 2-octanol as an internal standard.

2.4. Sensory evaluation

The evaluation panel consisted of 16 assessors (8 females and 8 males) from the College of Food Science and Engineering, Gansu Agricultural University, all of whom had extensive experience in wine sensory evaluation. A 30 mL wine sample was presented to the assessors in wine glasses. The indicators used to assess wine quality included appearance (color), aroma (floral, fruity, overall aroma intensity, and peculiar odor), mouthfeel (acidity and astringency), and overall quality (aftertaste, typicality, and overall score). A 9-point scale (1 = extremely low, 5 = moderate intensity, 9 = extremely high) was used to rate the intensity of the wine's sensory attributes (Liang et al., 2023). The assessors evaluated the wine samples in isolated booths.

2.5. DNA extraction and polymerase chain reaction (PCR) amplification

Microbial genomic DNA was extracted from the samples using the Mag-Bind® Soil DNA Kits (MP Biomedicals, Santa Ana, USA). Specific

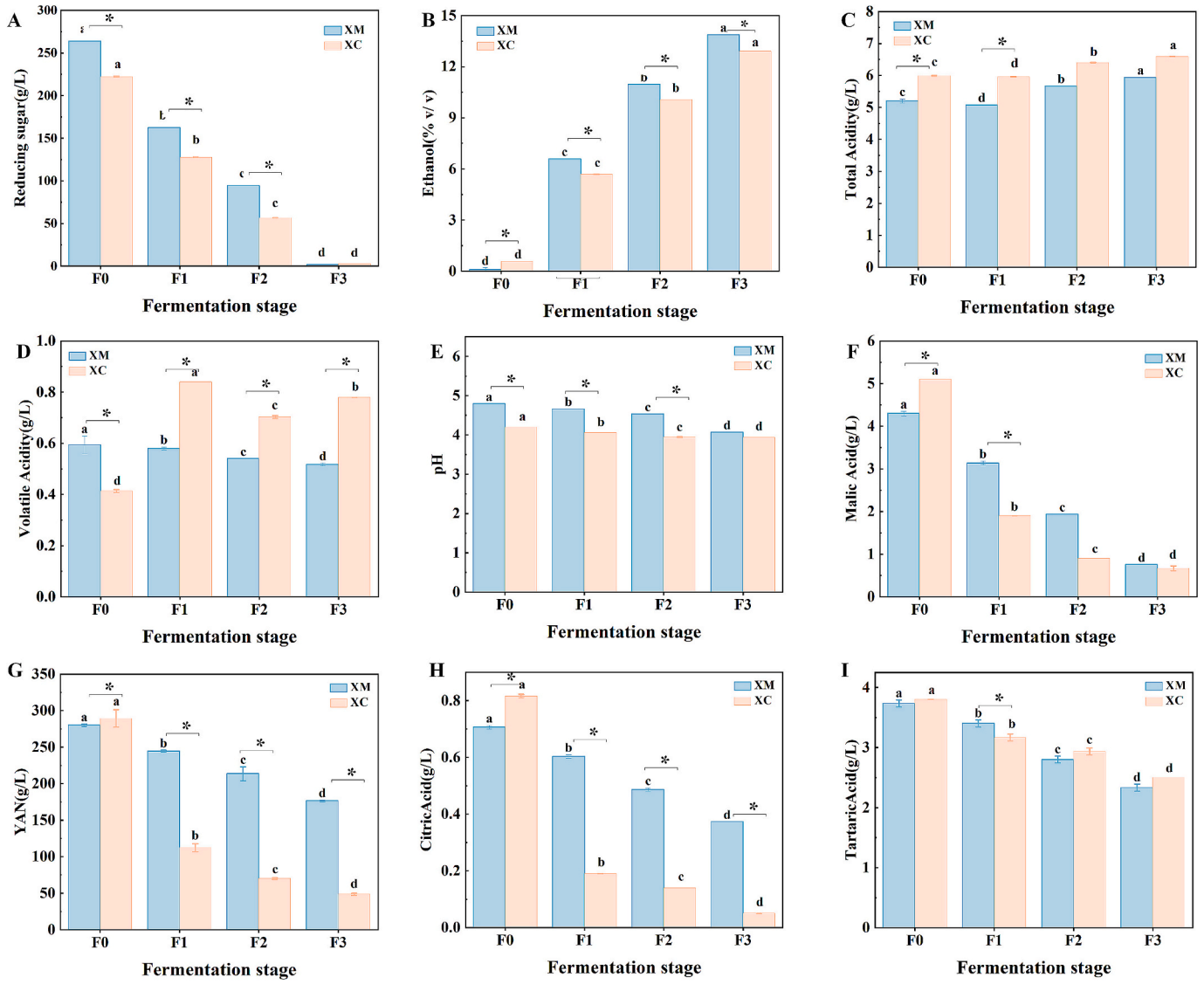

Fig. 1. Physicochemical changes during the natural fermentation processes of Merlot (XM) and Cabernet Sauvignon (XC): (A) Total sugar, (B) Ethanol content, (C) Total acidity, (D) Volatile acidity, (E) pH, (F) Malic acid, (G) YAN (yeast assimilable nitrogen), (H) Citric acid, and (I) Tartaric acid. F0: Pre-fermentation (destemmed and crushed grape juice). F1: Early fermentation stage (approximately one-third of total sugar consumed). F2: Mid-fermentation stage (approximately two-thirds of total sugar consumed). F3: End of fermentation stage (total sugar <4 g/L). a-d: Different letters indicate significant differences ($P < 0.05$).

primers were used for genomic DNA amplification: the internal transcribed spacer (ITS) region of fungi was amplified using the forward primer ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3′) and the reverse primer ITS2 (5'-GCTGCGTTCTTCATCGATGC-3′). The hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified using the forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3′).

The PCR amplification process was carried out as follows: an initial denaturation step at 98 °C for 5 min to fully denature the template DNA, followed by 25 amplification cycles consisting of denaturation at 98 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 45 s. A final extension step was performed at 72 °C for 5 min to ensure complete product elongation. After amplification, paired-end sequencing (PE250) was conducted on the Illumina NovaSeq platform. All sequencing procedures were conducted by Shanghai Paisenuo Biotechnology Co., Ltd. (Shanghai, China).

2.6. Bioinformatics analysis

All experimental results were presented as mean ± standard deviation. ANOVA was performed using SPSS (version 26, SPSS Inc., Chicago, IL, USA). Graphs were generated using Origin 2021 and https://www.genescloud.cn/chart/ChartOverview. Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) were performed using SIMCA 14.1 software (Umetrics, Umeå, Sweden). α-diversity analysis (QIIME2, version 2019.4), principal coordinate analysis (PCoA), and linear discriminant analysis effect size (LEfSe) were conducted on the Paisenuo cloud platform. Figures were created using the Lianchuan Biological Cloud Platform. The relationships between dominant microbial communities and enological parameters, as well as between microorganisms and characteristic volatile compounds were analyzed based on the Spearman coefficient.

3. Results and discussion

3.1. Physicochemical parameters during fermentation

The dynamic changes in physicochemical parameters during natural fermentation reflect the state and progression of fermentation and play a pivotal role in influencing microbial activity and the development of

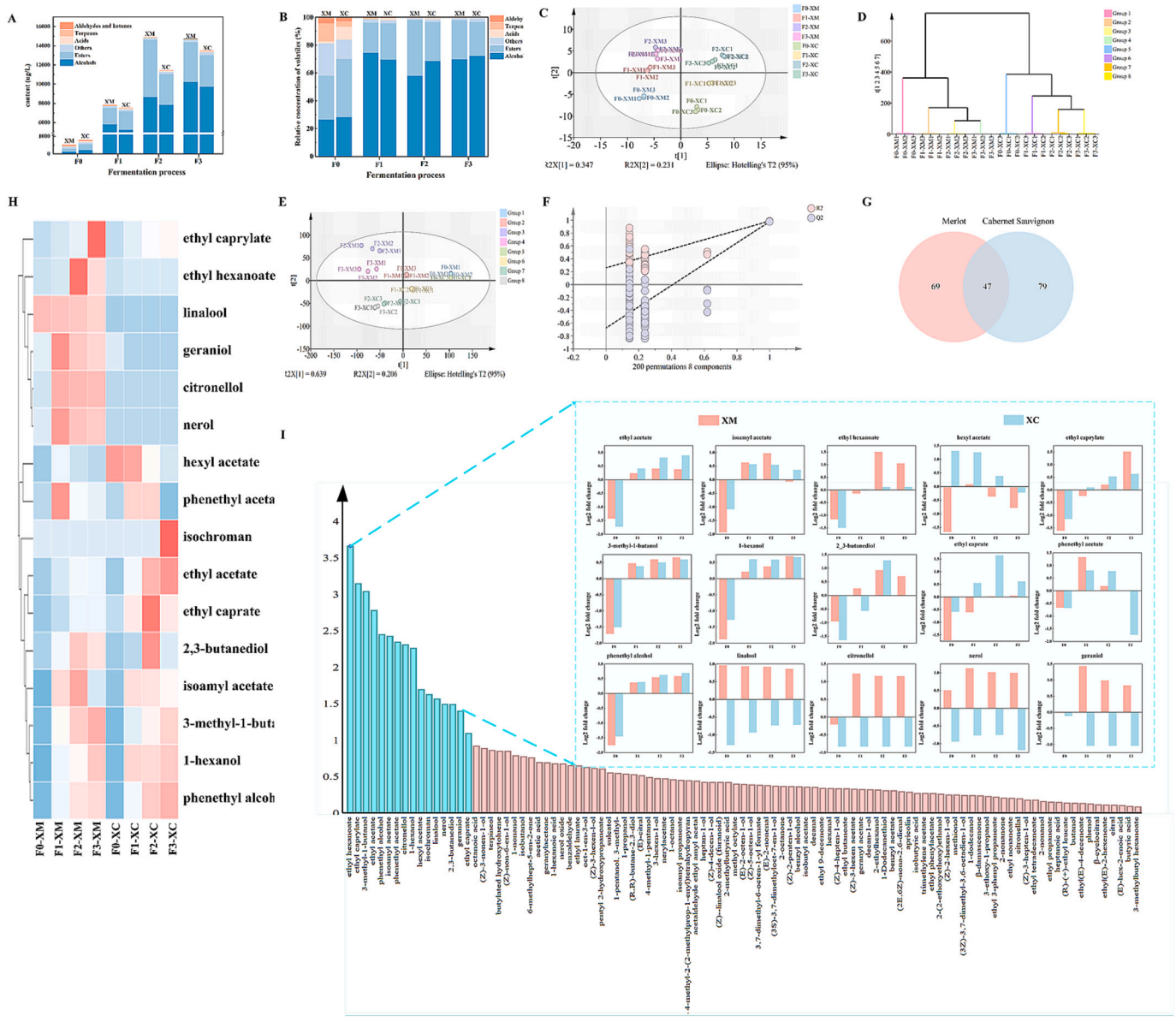

Fig. 2. Dynamic changes in volatile compound contents during the natural fermentation of Merlot and Cabernet Sauvignon: (A) Changes in the concentration of different types of volatile compounds; (B) Changes in the percentage composition of volatile compounds; (C) Principal component analysis (PCA) score plot; (D) Hierarchical cluster analysis (HCA); (E) Partial least squares discriminant analysis (PLS-DA) score plot; (F) Results of 200-time permutation tests for the PLS-DA model ($R^2 = 0.259$, $Q^2 = -0.675$); (G) Venn diagram showing the number of shared and unique volatile compounds in Merlot and Cabernet Sauvignon, with overlapping regions indicating the number of shared compounds; (H) Heatmap of 16 differential compounds (VIP > 0.1, $P < 0.05$); (I) Variable importance in projection (VIP) scores of differential volatile compounds between grape cultivars calculated via PLS-DA and the Log2 fold change of 15 differential volatile flavor compounds during the natural fermentation of Merlot and Cabernet Sauvignon.

wine aromas (Ma et al., 2023). Specifically, before fermentation, the total sugar content of Merlot samples (264.1 g/L) was significantly higher than that of Cabernet Sauvignon samples (222.4 g/L). As fermentation advanced, total sugar levels declined, ultimately dropped to 1.7 g/L and 2.5 g/L, respectively, in the final wines, meeting the standard for dry wines (<4 g/L) (Fig. 1A). The ethanol content of the final Merlot wine was 13.87 %, 0.97 % higher than the 12.9 % observed in Cabernet Sauvignon (Fig. 1B). During fermentation, the total acid content increased significantly, reaching 5.94 g/L and 6.6 g/L in the final Merlot and Cabernet Sauvignon wines (Fig. 1C). This increase in acidity corresponded to a decline in pH levels, with Merlot consistently showing a higher pH values than Cabernet Sauvignon at all sampling points (Fig. 1E). In terms of volatile acidity, the final concentrations were 0.52 g/L for Merlot and 0.78 g/L for Cabernet Sauvignon (Fig. 1D), both of which fall within the national standard limit of ≤1.2 g/L as specified in GB15037–2006 Wine. These levels of volatile acidity may contribute to the complexity of the wine's flavor through interactions with other volatile compounds.

YAN is a critical indicator of the fermentation potential wine quality (Martínez-Gil et al., 2012). The YAN content in Merlot and Cabernet Sauvignon samples exhibited significant differences, likely due to the distinct free amino acid profiles of the two grape cultivars (Verdenal et al., 2021). As fermentation progressed, declining trends were observed for malic acid, citric acid, tartaric acid, and YAN (Figs. 1F-I), a pattern consistent with previous findings on the natural fermentation of Merlot under both microvinification conditions (Liang et al., 2023) and industrial-scale production (Ma et al., 2023). Notably, the decrease in YAN content occurred more rapidly in Cabernet Sauvignon than in

Merlot, likely due to the higher nitrogen demand by microorganisms during fermentation, reflecting their greater metabolic activity. Organic acids, including malic, citric, and tartaric acids, contribute to the smoothness of wine mouthfeel, and their consumption is influenced by microbial composition. Their consumption decline may be attributed to esterification reactions between acids and alcohols, forming esters such as ethyl lactate, ethyl acetate, and diethyl succinate, which are key aroma precursors (Wei et al., 2020). Thus, changes in physicochemical parameters may be key factors influencing the microbial community structure during fermentation.

3.2. Dynamic analysis of volatile compounds in merlot and cabernet sauvignon wines

Volatile compounds are key determinants of wine quality, significantly influencing its overall sensory profile (Liu et al., 2023). In this study, HS-SPME-GC–MS was used to monitor the dynamic evolution of volatile compounds during the natural fermentation of different grape cultivars. As shown in Table S2, 69 volatile compounds were identified in Merlot samples and 79 in Cabernet Sauvignon samples, with 47 compounds common to both cultivars (Fig. 2G). Throughout fermentation, the total content of volatile compounds gradually increased (Fig. 2A). Alcohols were the most abundant class of volatile compounds, with their concentrations increasing steadily during fermentation, followed by esters (Fig. 2B). The differences in alcohol and ester concentrations between Merlot and Cabernet Sauvignon samples are pivotal in shaping the distinctive sensory characteristics and contributing to the distinctive styles of the resulting wines (Fariña et al., 2015). By the end of fermentation process, the total alcohol content in Merlot samples (10,266.87 μg/L) was significantly higher than that in Cabernet Sauvignon samples (9753.43 μg/L). This disparity is closely related to differences in the concentrations of corresponding amino acid precursors between the two grape cultivars and is further influenced by variations in their YAN levels (Liu et al., 2023).

PCA and PLS-DA were employed to identify key volatile compounds that exhibited significant changes during spontaneous fermentation between Merlot and Cabernet Sauvignon wines (Wang et al., 2025). The PCA score plot (Fig. 2C) clearly distinguished wine samples based on grape cultivars and fermentation stages. Hierarchical clustering analysis (HCA) further grouped Merlot and Cabernet Sauvignon samples at different fermentation stages into eight clusters (Fig. 2D), highlighting the substantial differences in volatile profiles between cultivars and fermentation phases. Supervised PLS-DA was applied to further identify significantly different volatile compounds. The PLS-DA score plot demonstrated a progressive shift leftward as fermentation advanced, with clear distinctions observed between samples according on both grape cultivars and fermentation stages (Fig. 2E). Notably, the differences between unfermented and early fermentation samples were greater than those between grape cultivars at the same stage. However, during the mid- and late-fermentation stages, differences between Merlot and Cabernet Sauvignon cultivars became more pronounced than the effects of the fermentation stage (Fig. 2E). The robustness of the PLS-DA model was confirmed through 200 permutation tests, yielding R^2 and Q^2 intercepts of 0.259 and − 0.675, respectively (Fig. 2F). Based on Variable Importance in Projection (VIP) criteria (VIP $\geq$ 1.0 and $P <$ 0.05), 16 differential volatile compounds were identified (Fig. 2H). Incorporating recent findings on Odor Activity Vlues (OAVs), it was determined that compounds with OAV values between 0.1 and 1, as well as those exceeding 1, may contribute to wine aroma through cumulative or synergistic effects (Liang et al., 2023). Consequently, 15 aroma-active differential volatile compounds were identified (Fig. 2I), underscoring their crucial role in defining the sensory characteristics of wine.

Ester compounds are primarily formed through the condensation of acids with alcohols. These biochemical reactions play a key role in the aroma formation of fruit wine (He et al., 2023). During the fermentation of Merlot, five esters with OAV > 0.1 were identified, namely ethyl butanoate (banana, pineapple, and strawberry); isoamyl acetate (sweet and fruity), ethyl hexanoate (banana, apple), ethyl caprylate (pineapple, pear), and phenethyl acetate (rose, sweet honey, and raspberry). In contrast, nine esters with OAV > 0.1 were detected in Cabernet Sauvignon, including ethyl acetate (fruity, solvent), isobutyl acetate (fruity), ethyl butanoate (banana, pineapple, strawberry), isoamyl acetate (sweet fruity), ethyl hexanoate (banana, apple), hexyl acetate (pear), ethyl caprylate (pineapple, pear), ethyl caprate (wax, fruity), and phenethyl acetate (rose, sweet honey, raspberry) (Table S2). These ethyl esters fatty acid and acetate esters are enzymatically produced, and prior research has demonstrated that their concentrations are significantly influenced by factors such as yeast strain, fermentation temperature, aeration, and sugar content (Fariña et al., 2015). Notably, seven esters exhibited significant differences in concentration between Merlot and Cabernet Sauvignon, specifically ethyl acetate (fruity, solvent), isoamyl acetate (sweet, fruity), ethyl hexanoate (banana, apple), hexyl acetate (pear), ethyl caprylate (pineapple, pear), ethyl caprate (wax, fruity), and phenethyl acetate (rose, sweet honey, raspberry) (Fig. 2I). By the conclusion of fermentation, the concentration of these esters reached 4071.63 μg/L in Merlot, compared to 3260.39 μg/L in Cabernet Sauvignon, thereby significantly enhancing the floral and fruity profiles of Merlot wines. Additionally, the concentration of isoamyl acetate declined slightly during the later stages of fermentation, likely due to its involvement in complex reactions that produce other volatile compounds (Chen et al., 2023).

The synthesis pathway for higher alcohols involves the formation of pyruvate from glucose through glycolysis, contributing significantly to the spicy, sweet, and fruity aroma characteristics of wines (Chen et al., 2023). In this study, the primary alcohols detected during Merlot fermentation included 3-methyl-1-butanol (cheese, whisky, ripe fruit), oct-1-en-3-ol (mushroom), 2-ethylhexanol (sweet, lightly floral), and phenethyl alcohol (roses, honey). For Cabernet Sauvignon, the predominant alcohols comprised 3-methyl-1-butanol (cheese, whisky, ripe fruit), 1-octanol (citrus, rose), and phenethyl alcohol (roses, honey) (Table S2). Notably, oct-1-en-3-ol imparted a distinct mushroom aroma to Merlot wines, while 1-octanol contributed citrus and rose-like notes to Cabernet Sauvignon. To enhance the sensory profile of Merlot wines, it may be advantageous to control the expression of mushroom aromas by reducing the formation of precursor compounds (Tian et al., 2024). Additionally, 3-methyl-1-butanol (cheese, whisky, ripe fruit), 1-hexanol (green), 2,3-butanediol (butter, cheese, red berries), and phenethyl alcohol (roses, honey) were identified as the primary contributors to the variation in alcohol-associated aromas between Merlot and Cabernet Sauvignon (Fig. 2I). At the end of fermentation, the cumulative alcohol concentration reached 10,074.26 μg/L in Merlot samples, compared to 9534.30 μg/L in Cabernet Sauvignon. Among these, phenethyl alcohol, isoamyl alcohol, and 2,3-butanediol are key volatile compounds predominantly synthesized through microbial metabolism of glucose to valine and isoleucine via the pyruvate pathway. Notably, 2,3-butanediol, with its creamy and buttery aroma, is one of the few polyols exhibiting aroma activity and serves as an essential precursor for the functional compound tetramethylpyrazine (Chen et al., 2023). Similarly, 1-hexanol, formed through the biodegradation of unsaturated fatty acids, acts as a precursor in the synthesis of long-chain esters and is a major contributor to certain green, plant-like aromas (Liu et al., 2024). Conversely, trans-3-hexen-1-ol and (Z)-2-hexen-1-ol are associated with less desirable grassy and herbal notes (Fariña et al., 2015).

Medium-chain fatty acids (MCFAs, C6-C12) are produced through triglyceride hydrolysis, lipid oxidation, or the transformation of aldehydes and ketones. Although their relatively high olfactory thresholds (200–5000 μg/kg) limit their direct contribution to wine aroma, MCFAs play a critical role in enhancing wine's sensory complexity and serve as indispensable substrates for ester synthesis during fermentation (He et al., 2023). At the conclusion of fermentation, acetic acid was the most abundant acid, formed primarily through the oxidation of acetaldehyde during fermentation (Liu et al., 2023). Notably, the acetic acid content in

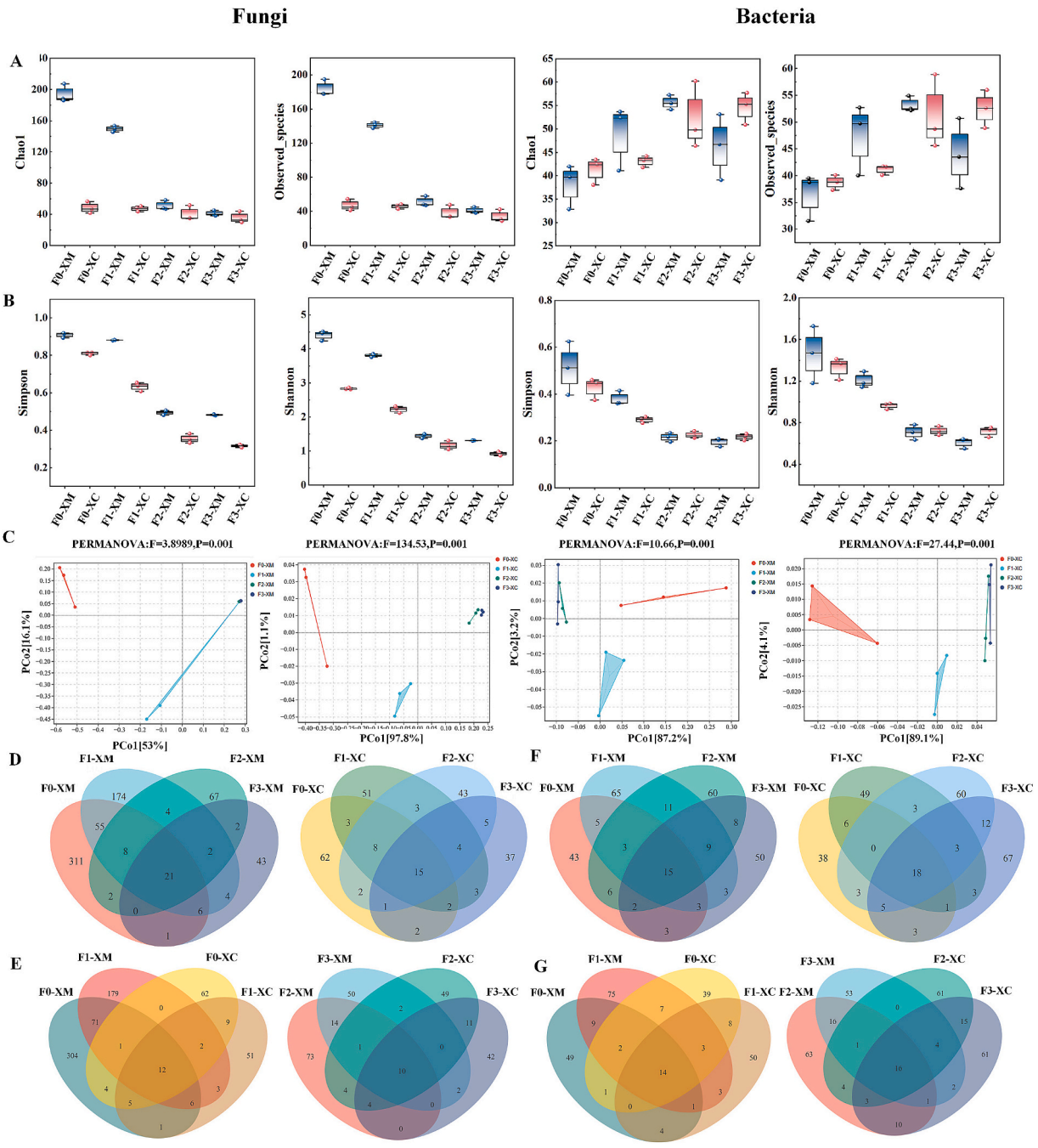

Fig. 3. Microbial diversity analysis during the natural fermentation of Merlot (XM) and Cabernet Sauvignon (XC): (A) Richness indices for Merlot and Cabernet Sauvignon: fungal Chao1 and Observed-species indices, bacterial Chao1 and Observed_species indices; (B) Diversity indices for Merlot and Cabernet Sauvignon: fungal Simpson and Shannon indices, bacterial Simpson and Shannon indices; (C) Principal coordinate analysis (PCoA) of fungal community Bray-Curtis distances and bacterial community Unweighted UniFrac distances for Merlot and Cabernet Sauvignon. (D, E) Shared fungal OTUs during the natural fermentation of Merlot and Cabernet Sauvignon; (F, G) Shared bacterial OTUs during the natural fermentation of Merlot and Cabernet Sauvignon.

Cabernet Sauvignon was significantly higher than in Merlot, suggesting that yeast cells in Cabernet Sauvignon underwent greater osmotic stress during fermentation. This stress likely triggered the upregulation of the ALD3 gene in yeast, enhancing aldehyde dehydrogenase activity and promoting increased acetic acid production. Additionally, this phenomenon may also be related to differences in fatty acid metabolism and bacterial activity (Liu et al., 2023). Interestingly, certain acids—including isobutyric acid, 2-methylbutyric acid, 1-hexanoic acid, heptanoic acid, and (*E*)-hex-2-enoic acid-were exclusively identified in the natural fermentation of Cabernet Sauvignon. This observation suggests that these acids may be associated with specific microorganisms specific to this grape cultivar.

As key volatile compounds in wine, aldehydes are generated through the oxidation of fatty acids and the degradation of amino acids, contributing substantially to the complexity and balance of wine aromas (Ma et al., 2023). In this study, the total concentration of aldehydes and ketones exhibited a progressive decline during fermentation, aligning with observations from hawthorn wine fermentation, where these compounds were metabolized as fermentation progressed (Tian et al., 2024). During the fermentation of Merlot, the primary aldehydes and ketones identified were 2-octanone and decanal, which are associated with undesirable grassy or soapy notes. In contrast, the key aldehydes

and ketones detected in Cabernet Sauvignon included hexanal, decanal, and (E)-2-nonenal (He et al., 2023). While these compounds contributed fruity aromas, they also introduced less desirable attributes, such as fatty and soapy odors, underscoring their dual role in shaping the sensory profile of the wine (He et al., 2023).

Terpenes are regarded as the principal carriers of cultivar-specific aroma in wine, playing a critical role in defining the unique characteristics of each grape cultivar. During Merlot fermentation, the most prominent terpenes identified were linalool (rose, citrus, fruity), citronellol (grass, lemon, rose), β-damascenone (bark, apple, plums), geraniol (lemon, peach, rose), and geranylacetone (sweet, rose). In Cabernet Sauvignon, the key terpenes included linalool (rose, citrus, fruity), β-damascenone (bark, apple, plums), geraniol (lemon, peach, rose), and geranylacetone (sweet, rose). These terpenes are notable for their exceptionally low odor detection thresholds, making them highly influential in shaping wine aroma (Ma et al., 2023). Interestingly, terpineol was exclusively detected during Merlot fermentation and exhibited the highest concentration among the identified terpenes, corroborating findings previously reported by Fariña et al. (2015). Additionally, citronellal, terpineol, geranyl acetate, and geranylacetone were found solely in Merlot must, which may contribute to its distinctive cultivar-specific aroma. Notably, linalool (rose, citrus, fruity), citronellol (grass, lemon, rose), nerol (flower, fragrant), and geraniol (lemon, peach, rose) emerged as the primary terpenes responsible for the aromatic differences observed between Merlot and Cabernet Sauvignon.

Thiols constitute another crucial class of aroma compounds in wine (Verdenal et al., 2021). For example, methionol can negatively affect wine flavor (Liang et al., 2023). In summary, these aroma-active compounds are synthesized through pathways such as glycolysis, amino acid metabolism, and enzymatic reactions. Their diversity is intricately linked to the microbial composition and specific strains involved in fermentation (Liang et al., 2023). Therefore, studying the microbial dynamics during natural fermentation and the relationship with flavor development is essential.

3.3. Sensory evaluation of wines

To better evaluate the sensory characteristics of Merlot and Cabernet Sauvignon wines, sensory evaluation was conducted on wine samples at the end of fermentation (Fig. S1). The overall score of Merlot wine (7.89) was higher than that of Cabernet Sauvignon wine (7.33). Merlot wine exhibited intense floral and fruity aromas with higher overall aroma intensity, which may be closely related to its higher ester content. Cabernet Sauvignon wine had lower peculiar odor and a better mouthfeel. The differences in sensory results between Merlot and Cabernet Sauvignon wines were strongly influenced by indigenous microorganisms.

3.4. Changes in microbial diversity during natural fermentation

The diversity of fungal and bacterial communities was assessed using high-throughput sequencing targeting the ITS region and the 16S rRNA variable region. Rarefaction curves plateaued as sequencing depth increased (Fig. S2), with coverage rates ranging from 99.96 % to 100.00 %, demonstrating the representativeness of the samples' diversity and the reliability of taxonomic classification. After removing singletons, the fungal and bacterial sequence counts totaled 2,519,861 and 1,777,370 reads, respectively (Table S3). All sequences were assigned to their respective OTUs at a 97 % similarity level.

To differentiate the microbial diversity among grape cultivars within the same vineyard, this study utilized α-diversity indices for comparative analysis. The F0 samples were collected immediately after the fermentation tanks were filled with freshly pressed juice, reflecting the microbial communities residing on the grape skin and pulp. These samples were used to estimate the core microbiota associated with different grape cultivars (Onetto et al., 2024). Based on the Chao1 index and observed-species index, fungal richness in unfermented Merlot samples was significantly higher than in Cabernet Sauvignon samples, whereas bacterial richness showed the opposite trend (Fig. 3A). Additionally, the Shannon and Simpson indices revealed that both fungal and bacterial diversity in Merlot samples were significantly higher than in Cabernet Sauvignon samples (Fig. 3B). Consistent with previous studies, the microbial composition on wine grapes was shown to be significantly influenced by grape cultivar (Martins et al., 2023). As fermentation progressed, diversity indices declined. By the end of fermentation, fungal diversity in Merlot samples remained significantly higher than in Cabernet Sauvignon samples, while bacterial diversity showed the opposite trend (Fig. 3B). Notably, although unfermented Merlot samples exhibited greater microbial richness compared to Cabernet Sauvignon, this does not necessarily imply that Merlot supports stronger microbial ecosystem functions than Cabernet Sauvignon (Boynton & Greig, 2016).

β-diversity captures the similarities and differences in microbial communities during the natural fermentation of Merlot and Cabernet Sauvignon. PerMANOVA analysis revealed significant differences in microbial community composition across samples during fermentation (Fig. 3C; $P < 0.001$). Microbial communities were distinctly separated at different fermentation stages. As fermentation progressed, the Bray-Curtis distance for fungi and the Unweighted UniFrac distance for bacteria decreased, suggesting a tendency for microbial communities to become more similar during the mid-and late fermentation stages. Consistent with previous studies on the dynamic evolution of microbial communities in naturally fermented wines (Wei, Ding, et al., 2022), unfermented and early fermentation samples exhibited considerable dispersion, reflecting the instability of microbial communities during the initial stages of fermentation. In contrast, the increased overlap observed during the mid-and late fermentation stages suggested enhanced microbial stability as fermentation advanced (Onetto et al., 2024). Furthermore, during the spontaneous fermentation of Merlot and Cabernet Sauvignon wines, the absence of SO_2 likely contributed to the higher initial microbial diversity observed in our samples, which may have affected wine quality and stability. Therefore, strict fermentation temperature control and rigorous sanitation measures were implemented to mitigate potential risks.

As illustrated in Figs. 3D-G, significant differences in fungal and bacterial OTUs were observed during the natural fermentation of Merlot and Cabernet Sauvignon, with trends aligning closely to those of α-diversity (Fig. 3A and B). During fermentation, 21 fungal OTUs and 15 bacterial OTUs were shared in Merlot samples, while 15 fungal OTUs and 18 bacterial OTUs were shared in Cabernet Sauvignon samples (Fig. 3D and F). In the natural fermentation process, the fungal OTUs were highest in the unfermented samples, with Merlot (404) significantly higher than Cabernet Sauvignon (95). Bacterial OTUs counts were lowest, with Merlot and Cabernet Sauvignon samples containing 80 and 74 OTUs, respectively. As fermentation progressed, fungal OTUs decreased in both Merlot and Cabernet Sauvignon, while bacterial OTUs did not follow a consistent trend (Fig. 3D and F). For fungi, the number of shared OTUs between Merlot and Cabernet Sauvignon decreased progressively from the unfermented stage to the end of fermentation, with 22, 23, 19, and 12 OTUs shared, respectively (Fig. 3D and E). In contrast, with 17, 21, 24, and 23 OTUs were shared across the same stages (Fig. 3F and G). These shared OTUs are likely representative of the vineyard's core microbial community structure. The higher proportion of core fungal OTUs suggests a stronger association between fungal communities and the vineyard environment compared to bacterial communities. This finding aligns with studies conducted in the DOC Vinhos Verdes wine region and underscores the need for further investigation (Martins et al., 2021, 2023).

3.5. Distribution patterns and dynamic succession of microbial communities in merlot and cabernet sauvignon wines during fermentation

Monitoring the predominant microbial taxa (Top 10) throughout the

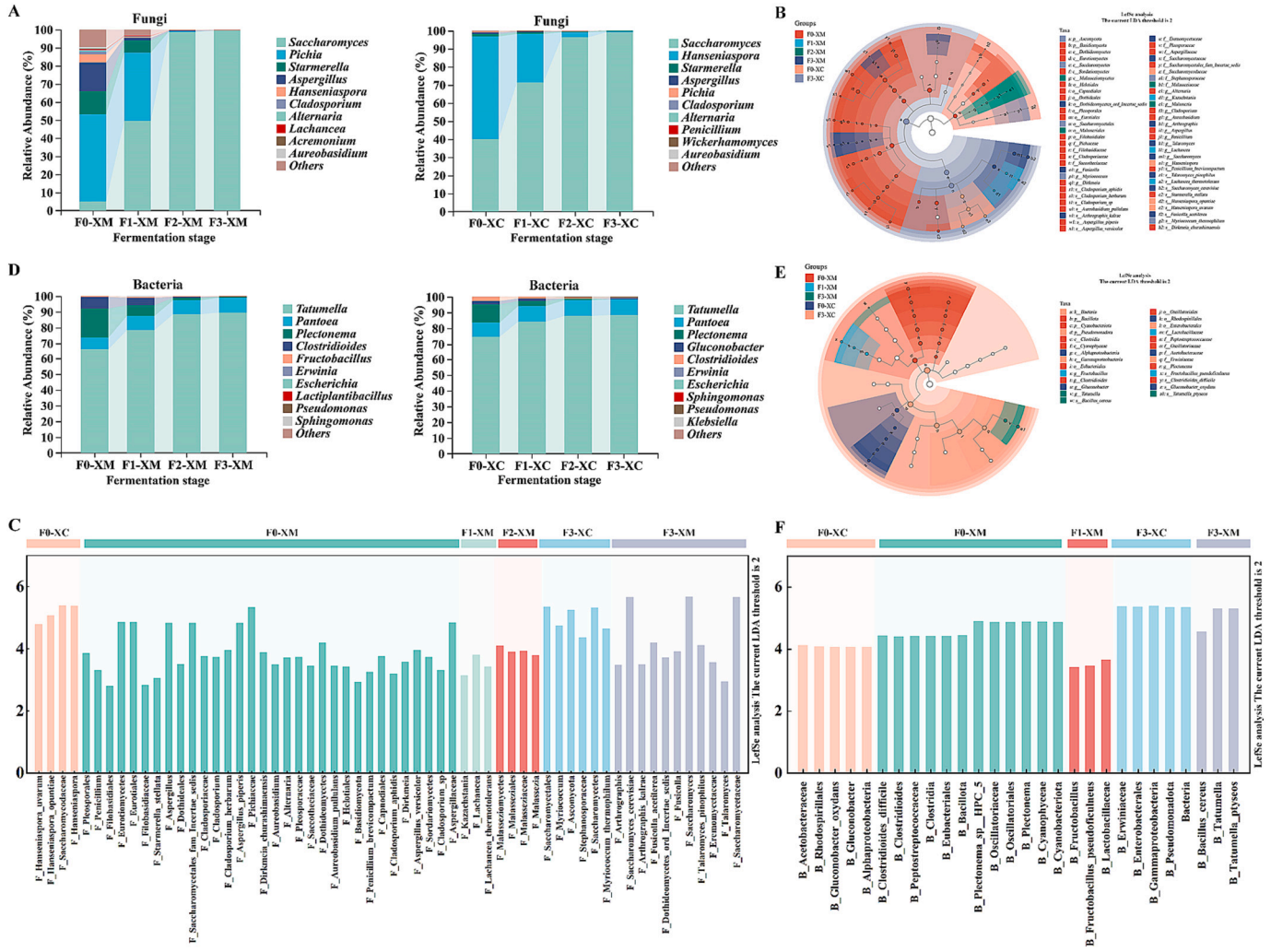

Fig. 4. Microbial community distribution patterns during the natural fermentation of Merlot (XM) and Cabernet Sauvignon (XC): (A) Stacked bar chart showing changes in the relative abundance of dominant fungal taxa during the fermentation processes of Merlot and Cabernet Sauvignon; (D) Stacked bar chart showing changes in the relative abundance of dominant bacterial taxa during the fermentation processes of Merlot and Cabernet Sauvignon; (B) Phylogenetic tree illustrating the taxonomic relationships of major fungal taxa during the natural fermentation of Merlot and Cabernet Sauvignon.
(E) Phylogenetic tree illustrating the taxonomic relationships of major bacterial taxa during the natural fermentation of Merlot and Cabernet Sauvignon; (C) Bar chart showing fungal taxa with significant differences during the natural fermentation of Merlot and Cabernet Sauvignon; (F) Bar chart showing bacterial taxa with significant differences during the natural fermentation of Merlot and Cabernet Sauvignon.

natural fermentation process provided insight into the dynamics and succession of both fungal and bacterial communities. At the phylum level, *Ascomycota* emerged as the dominant fungal phylum before fermentation in both Merlot and Cabernet Sauvignon samples, representing 95.52 % and 99.83 % of the total sequences, respectively. Among the bacterial phyla, *Pseudomonadota* was the most abundant, accounting for 73.69 % and 85.76 % of the bacterial sequences in the Merlot and Cabernet Sauvignon samples, respectively (Table S4). At the genus level, the fungal communities in both Merlot and Cabernet Sauvignon included filamentous fungi (*Aspergillus*, *Cladosporium*, *Alternaria*, and *Aureobasidium*) and fermentative yeasts (*Saccharomyces*, *Hanseniaspora*, *Pichia*, and *Starmerella*). The bacterial communities comprised spoilage bacteria (*Tatumella*, *Pantoea*, *Erwinia*, *Escherichia*, *Pseudomonas*, and *Sphingomonas*), anaerobic bacteria (*Clostridioides*), and *Plectonema* (Fig. 4). However, the relative abundances of shared microbes varied significantly among grape cultivars. In unfermented Cabernet Sauvignon samples, the dominant fungi were *Saccharomyces* and *Hanseniaspora*, with relative abundances of 40.00 % and 56.99 %, respectively (Fig. 4A). *Hanseniaspora*, the predominant non-*Saccharomyces* in the spontaneous wine fermentation, has been widely isolated from various environments and used as a candidate fermenting agent in alcoholic beverage production. For example, *Hanseniaspora uvarum* plays a key role in the synthesis of aromatic esters, with the ethyl acetate produced during fermentation being 9 times higher than that produced by *Saccharomyces cerevisiae* (Wang, Zhao, et al., 2024). In this study, the ethyl acetate production at the end of fermentation in Cabernet Sauvignon was three times higher than in Merlot. This result may be associated with specific species within the *Hanseniaspora*. Additionally, the unfermented samples also contained low abundances of *Starmerella*, *Pichia*, and *Wickerhamomyces*. These are notable non-*Saccharomyces* yeasts. Among them, *Starmerella bacillaris*, a common species of *Starmerella*, is known for its preference for fructose and its competition with *S. cerevisiae*, which has been shown to benefit both yeasts (Borren & Tian, 2020). Furthermore, *Starmerella bacillaris* produces large amounts of glycerol and can reduce volatile acidity (Englezos et al., 2022). This may explain the significantly higher volatile acidity observed in the early fermentation stages of Cabernet Sauvignon compared to Merlot (Fig. 1D). However, *Starmerella*, *Pichia*, and *Wickerhamomyces* exhibit limited ethanol tolerance and are unable to complete alcoholic fermentation independently (Liu et al., 2023). In Merlot samples, the

dominant fungi were *Pichia*, *Starmerella*, *Aspergillus*, and *Hanseniaspora*, with relative abundances of 48.00 %, 12.68 %, 16.14 %, and 4.57 %, respectively (Fig. 4B). Additionally, lower abundances of *Lachancea* were also detected. *Lachancea* spp., known for its lactic acid production, is often used to acidify low-acid wines in warm regions (Liu et al., 2023). Notably, the relative abundance of *Pichia* in the Merlot samples was significantly higher than in the Cabernet Sauvignon samples. *Pichia kluyveri*, a common species within this genus, has a strong esterase production ability, which may explain the significantly higher levels of phenylethyl acetate and ethyl caprylate in the Merlot samples at the end of fermentation compared to Cabernet Sauvignon. In addition, this is also related to the concentration of volatile thiols (Wang, Zhao, et al., 2024).

The presence of specific non-fermentative taxa in unfermented samples, such as *Aspergillus*, *Cladosporium*, *Alternaria*, *Penicillium*, and *Aureobasidium* in Cabernet Sauvignon (combined relative abundance: 1.29 %) and *Aspergillus*, *Cladosporium*, *Alternaria*, *Acremonium*, and *Aureobasidium* in Merlot (combined relative abundance: 19.83 %), raises concerns about the quality of naturally fermented wines, particularly given the higher abundance and persistence of these genera in Merlot samples (Fig. 4A and D). These microorganisms are commonly associated with spoilage. Filamentous fungi, such as *Aspergillus* and *Penicillium*, can significantly affect the sanitary and sensory quality of wine by producing mycotoxins (e.g., aflatoxins, ochratoxin A) or off-flavors (e.g., earthy aromas) (García-Izquierdo et al., 2024). the high abundance of Aspergillus in Merlot samples may indicate the presence of black mold disease before harvest (Liu et al., 2023). Interestingly, *Aspergillus* may also contribute to fermentation by producing acidic proteases and carboxypeptidases, which break down proteins into peptides and amino acids, providing yeast with a nitrogen source (Chen et al., 2024). *Aspergillus Niger*, a species within the *Aspergillus* genus, is one of the most effective producers of *β*-glucosidase and significantly catalyzes the hydrolysis of grape aroma glycosides (Liu et al., 2023). In summary, it seems that the production of natural wines could benefit from practices similar to those in organic winemaking, such as biological control. For example, using species like *Lysobacter Capsici*, *Trichoderma* spp., and *Aureobasidium pullulans*, which have antifungal effects against these pathogens, may play a significant role in inhibiting undesirable microorganisms, thus offering potential benefits for natural fermentation wines (García-Izquierdo et al., 2024).

After the onset of fermentation, *Saccharomyces* gradually became dominant, primarily due to the early growth of non-*Saccharomyces*, which consumed amino acids and vitamins during the initial stages of fermentation, thereby limiting the subsequent growth of *Saccharomyces* (Ciani & Comitini, 2015). By the end of fermentation, *Saccharomyces* had become the predominant genus in both Merlot and Cabernet Sauvignon samples, with relative abundances of 99.48 % and 99.26 %, respectively. This result is consistent with previous studies. Meanwhile, the relative abundance of some non-*Saccharomyces* and plant pathogens progressively declined after fermentation began, falling below 1 % by the end of fermentation (Fig. 4A and D). The primary cause of this decline was the increasingly hostile microbial growth environment, characterized by elevated alcohol levels and reduced nitrogen sources, which are major factors in the reduction of species richness in winemaking environments (Boynton & Greig, 2016; Liu et al., 2020). Bokulich et al. (2014) demonstrated that filamentous fungi constituted approximately 70 % of the grape juice microbiota, yet their survival was severely restricted by the low pH, high alcohol content, and anaerobic conditions of fermentation. Despite their low abundance, their metabolic activity can still influence wine (MacLean & Gudelj, 2006). During fermentation, a small number of microbial taxa in the community can gradually outcompete others, reducing the overall functionality of the community. The most notable example of this phenomenon is the dominance of *Saccharomyces*. One key reason for this dominance is *Saccharomyces*' ability to produce large amounts of ethanol, which suppresses or inhibits ethanol-sensitive microorganisms (Wei, Chen, et al., 2022). Additionally, *Saccharomyces* secretes toxic proteins or glycoproteins (killer toxins), short-chain fatty acids, and sulfur dioxide, all of which have toxic effects on other microorganisms (MacLean & Gudelj, 2006). Direct cell-to-cell contact is another mechanism by which *Saccharomyces* inhibits other yeasts (MacLean & Gudelj, 2006). Finally, the rapid growth rate of *Saccharomyces* accelerates nutrient depletion, limiting the growth of slower-growing microorganisms (MacLean & Gudelj, 2006). As a result, the dominance of *Saccharomyces* during natural fermentation reduces the differences in microbial community differences in Merlot and Cabernet Sauvignon wines (Li et al., 2021).

Linear discriminant analysis (LDA) effect size (LEfSe) analysis further confirmed the significant correlation between fungal taxa and grape cultivars (Wilcoxon test, $\alpha < 0.05$) (Fig. 4B). Specifically, in Merlot samples, *Alternaria*, *Cladosporium*, and *Aureobasidium* served as genus-level biomarkers during the unfermented stage; *Kazachstania* and *Lachancea* were significantly enriched after the onset of fermentation; *Malassezia* was significantly enriched during mid-fermentation; and *Arthrographis*, *Talaromyces*, and *Saccharomyces* were significantly enriched by the end of fermentation (Fig. 4C). In Cabernet Sauvignon samples, *Hanseniaspora* was the genus-level biomarker during the unfermented stage, while *Saccharomyces*, *Talaromyces*, and *Arthrographis* were significantly enriched by the end of fermentation (Fig. 4C).

The composition of unfermented samples was similar for bacterial genera dominant in different grape cultivars. In both Merlot and Cabernet Sauvignon, *Tatumella*, *Pantoea*, *Plectonema*, and *Clostridioides* collectively accounted for over 90 % of bacterial abundance. As fermentation progressed, these four dominant bacterial genera exhibited similar trends. By the end of fermentation, the relative abundance of *Tatumella* reached 89.50 % in Merlot and 88.51 % in Cabernet Sauvignon, consistent with previous observations (Bubeck et al., 2020; Wei, Chen, et al., 2022). Some Gram-negative bacteria were exclusively present during the unfermented stage and declined to undetectable levels within the first few days of fermentation, likely due to their intolerance to acidic conditions (Wei, Chen, et al., 2022).

LEfSe analysis further confirmed a significant correlation between bacterial taxa and grape cultivars (Wilcoxon test, $\alpha < 0.05$) (Fig. 4E). Specifically, during the natural fermentation of Merlot samples, *Plectonema* and *Clostridioides* were identified as genus-level biomarkers in the unfermented stage, *Fructobacillus* showed higher abundance after the onset of fermentation, and *Tatumella* was significantly enriched by the end of fermentation. In Cabernet Sauvignon samples, *Gluconobacter* served as the genus-level biomarker during the unfermented stage (Fig. 4F).

In summary, differences in microbial community composition were observed between grape cultivars collected from Xiabolan Vineyard (Fig. 4A and D). Among the studied Merlot and Cabernet Sauvignon samples, the differences in the abundance of many shared fungal OTUs substantially contributed to inter-cultivar differentiation. These included fungal genera such as *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Starmerella*, *Alternaria*, *Aureobasidium*, *Cladosporium*, and *Aspergillus*, which are widely detected in vineyards globally (Martins et al., 2021, 2023; Xu et al., 2022). Similarly, bacterial genera such as *Tatumella*, *Pseudomonas*, *Clostridioides*, *Plectonema*, *Pantoea*, *Erwinia*, *Escherichia*, and *Sphingomonas* were also found to play a comparable role in cultivated diversity in other regions (Huang et al., 2023). In the unfermented samples, *Lachancea*, *Acremonium*, *Fructobacillus*, and *Lactiplantibacillus* were unique to Merlot, whereas *Penicillium*, *Wickerhamomyces*, *Gluconobacter*, and *Klebsiella* were specific to Cabernet Sauvignon. The observed differences under identical management conditions may be attributed to the selective influence of grape cultivars on microbial communities. However, the differences between grape cultivars within the same vineyard were less pronounced compared to differences between vineyards. This is likely due to the consistency in climate, management systems, and microbial interactions within the same region, which contribute to certain similarities in microbial community composition (Wang et al., 2022).

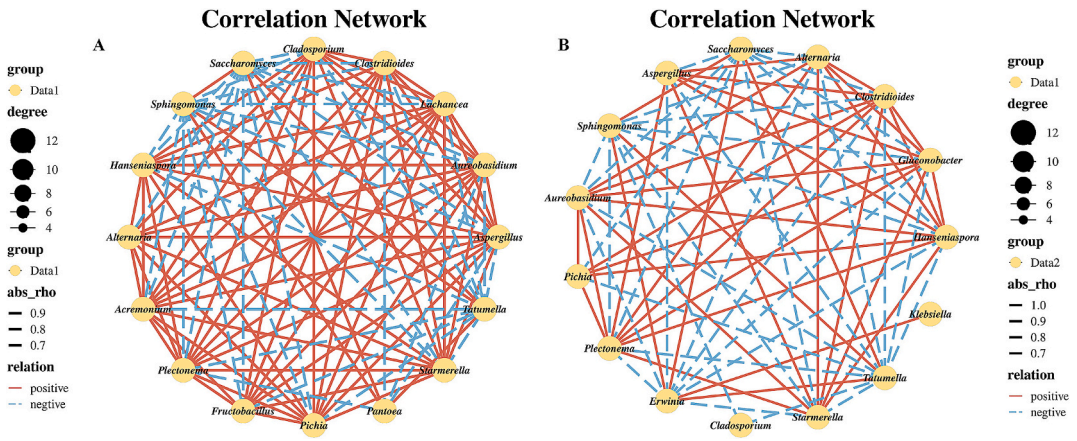

Fig. 5. Network diagrams illustrating the co-occurrence and exclusion relationships among microbial taxa at the genus level during the natural fermentation of Merlot (A) and Cabernet Sauvignon (B) ($|r| > 0.6$, $P < 0.05$): Oval nodes represent microbial taxa.
The color of the lines indicates the type of relationship: red lines represent positive correlations, while blue lines represent negative correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

During the fermentation process, the grape must exhibit a clear selective bias, favoring species with fermentation capabilities and strong ethanol tolerance (Ciani & Comitini, 2015). Both α- and β-diversity assessments revealed a reduction in diversity and richness (Fig. 3E and G), accompanied by a rapid shift in microbial community composition toward species such as *Saccharomyces* and *Tatumella*. This observation aligns with the findings of numerous previous studies (Onetto et al., 2024), including those on industrial fermentations (Ma et al., 2023), where these microorganisms potentially influence the dynamics of other fermentative microbes (Onetto et al., 2024). In summary, microbial specificity differentiated the grape cultivars under the same microclimatic conditions, consistent with the results of a previous study, which demonstrated the effect of grape cultivar on microbial communities (Martins et al., 2023).

3.6. Microbial interactions as drivers of community dynamics

The biochemical activities, growth, survival, and death dynamics of microorganisms during natural fermentation result from both intra-community interactions and interactions with the fermentation environment. These interactions are crucial for the success and safety of wine fermentation, as well as for achieving the desired sensory and chemical properties (Wang et al., 2022). To investigate the interactions among dominant microbial genera during fermentation in different grape cultivars, co-occurrence network analysis based on Spearman correlation coefficients was performed (Fig. 5). The Merlot co-occurrence network comprised 16 nodes and 107 edges, whereas the Cabernet Sauvignon network contained 15 nodes and 74 edges, indicating stronger microbial associations in Merlot compared to Cabernet Sauvignon. Notably, species-rich fungal communities likely involved more complex microbial interactions, whether competitive or facilitative (Liu et al., 2017) (Fig. 3A and B). In Merlot samples, *Saccharomyces*, which gradually became dominant during fermentation, showed the highest connectivity (15), with significant positive correlations to *Tatumella* and *Pantoea* ($P < 0.05$). It showed negative correlations with *Hanseniaspora*, *Lachancea*, *Pichia*, *Starmerella*, and plant pathogens such as *Alternaria* and *Aspergillus*, providing a plausible explanation for their lower relative abundance at the end of fermentation. This phenomenon is likely due to the inhibitory effects of *Saccharomyces* on other microbes through various metabolic mechanisms (Liang et al., 2023). Connectivity values for *Hanseniaspora*, *Lachancea*, *Pichia*, and *Starmerella* were 13, 13, 13, and 14, respectively. Except for the non-significant correlation between *Lachancea* and *Pichia*, these genera showed significant positive correlations with one another, suggesting potential horizontal gene transfer or exchange of extracellular DNA, which may confer mutual benefits. Recent studies suggest that bioactive compounds in wine, such as proteins, fatty acids, and cyclic higher alcohols, play a crucial role in yeast-yeast interactions (Ciani & Comitini, 2015). Englezos et al. (2022) noted that microbial interactions in the wine ecosystem are driven by nutrient demands and metabolites production, which can influence other populations either as nutrient sources or through the generation of inhibitory compounds. Furthermore, the synergistic relationships between dominant fungi and bacteria during fermentation warrant further investigation. The complexity of fungal-bacterial interactions is proportional to the microbial diversity of each cultivar, a trend consistent with previous reports (Martins et al., 2023). This study provides insights into microbial dynamics in winemaking, offering foundational knowledge for winemakers to control and fine-tune microbial population dynamics to achieve specific product outcomes. Moreover, microbial metabolite profiles can vary significantly depending on community composition, underscoring the need for further research into these interrelationships.

3.7. Correlation analysis of dominant microbial genera with key aroma compounds in merlot and cabernet sauvignon wines

The volatile compounds of wine are determined by the microbial communities involved in fermentation, which are influenced by the physicochemical properties of the fermentation process, including pH, ethanol content, and YAN. To investigate the relationship between fermentation physicochemical parameters and the indigenous microbiome, as well as the microbiome's relationship to flavor compounds, a correlation analysis was performed on dominant microbes, potential characteristic volatile compounds, and physicochemical properties during the spontaneous fermentation of Merlot and Cabernet Sauvignon. In Merlot samples, *Alternaria* exhibited the highest connectivity (18), despite not being the most abundant taxon. Among the predominant non-*Saccharomyces* in unfermented samples, *Pichia* and *Starmerella*, had connectivities of 14 and 15, respectively. Meanwhile, the dominant microorganisms during fermentation, *Saccharomyces* and *Tatumella* had connectivity values of 17 and 16, respectively. In the Cabernet Sauvignon samples, *Sphingomonas* exhibited the highest connectivity (21), whereas the dominant non-*Saccharomyces* in unfermented samples, *Hanseniaspora*, and *Saccharomyces*, both had connectivity values of 19. The dominant microorganism during fermentation, *Tatumella*, had a connectivity value of 20.

Both Merlot and Cabernet Sauvignon samples revealed a negative correlation between ethanol and non-*Saccharomyces* (*Pichia*, *Starmerella*,

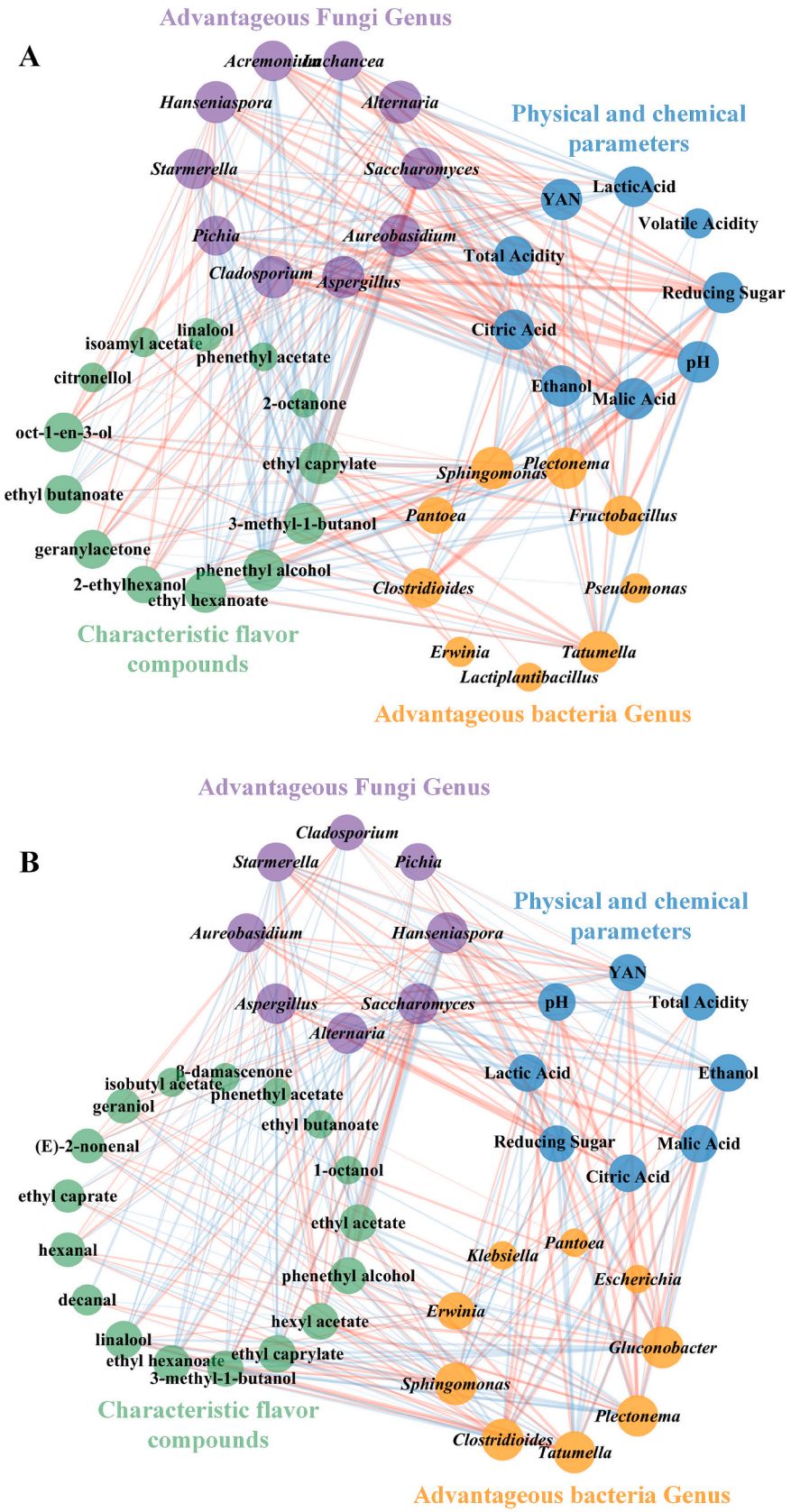

Fig. 6. Correlation analysis between dominant microbial genera and key volatile compounds in Merlot (A) and Cabernet Sauvignon (B) ($P < 0.05$): Lines connecting nodes represent correlations; Red lines between circles indicate positive correlations, while blue lines indicate negative correlations; The size of the circles represents the number of associated variables; larger circles indicate more associated variables; The thickness of the lines represents the strength of the correlation; thicker lines indicate stronger correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Hanseniaspora, *Lachancea*), while showing a positive correlation with *Saccharomyces* (Fig. 6). This finding is consistent with previous studies on spontaneous wine fermentation (Liang et al., 2023). The combined results of microbial interaction suggest that the fermentation environment and microbial competition are the key driving forces shifting the microbial community toward a *Saccharomyces*-dominated fermentation profile (Liu et al., 2017). Additionally, This also confirms the dominance of ethanol-tolerant yeast strains, which facilitates the fermentation process (Liu et al., 2017). Additionally, *Saccharomyces* and *Tatumella* exhibited significantly positively correlated with ethanol, total acidity, and lactic acid, while showing significantly negative correlations with reducing sugar, pH, malic acid, citric acid, and YAN in both Merlot and Cabernet Sauvignon samples. Surprisingly, the dominant bacterial genus *Pantoea*, which played a major role in Cabernet Sauvignon fermentation, showed no significant correlation with physicochemical properties. However, in Cabernet Sauvignon, *Pantoea* was positively correlated with reducing sugar, ethanol, pH, malic acid, and YAN. The following factors can explain the observed differences in the fermentation processes of the Merlot and Cabernet Sauvignon: the composition of the wine, the richness of microbial species, and the interactions among microbial populations, which may be the cause of this phenomenon (Liu et al., 2017). Both Merlot and Cabernet Sauvignon samples indicated that volatile acidity had no significant impact on the dominant fungal and bacterial genera. While controlling environmental factors that influence microbial communities remains challenging, direct regulation of physicochemical parameters during fermentation can effectively control the wine fermentation microbiota (Ma et al., 2023).

The correlation analysis of dominant microbial genera and characteristic aroma compounds showed that in Merlot samples, *Saccharomyces* and *Tatumella* were significantly positively correlated ($r > 0.6$; $P < 0.05$) with 5 aroma compounds, including ethyl butanoate, ethyl hexanoate, ethyl caprylate, 3-methyl-1-butanol, and phenethyl alcohol. They were significantly negatively correlated with geranylacetone, 2-ethylhexanol, and oct-1-en-3-ol ($r > 0.6$; $P < 0.05$). Furthermore, *Saccharomyces* was also significantly negatively correlated with linalool. For Cabernet Sauvignon, *Saccharomyces* was significantly positively correlated with 7 volatile compounds, including ethyl acetate, ethyl hexanoate, ethyl caprylate, ethyl caprate, 3-methyl-1-butanol, phenethyl alcohol, and linalool, while *Tatumella* exhibited an additional significant positive correlation with decanal ($r > 0.6$; $P < 0.05$) compared to *Saccharomyces*.

Non-*Saccharomyces* and phytopathogenic were predominantly negatively correlated with most volatile compounds. Nonetheless, non-*Saccharomyces* species present in unfermented grape must and at the onset of fermentation significantly enhanced the aromatic profile of the wine. This observation aligns with recent findings, which describe the extracellular enzymatic activity of non-*Saccharomyces*, such as pectinase, protease, and β-glucanase, contributing positively to flavor complexity (Liu et al., 2023). For Cabernet Sauvignon, *Hanseniaspora* and *Saccharomyces* were identified as the predominant fermentative species. *Hanseniaspora uvarum*, which exhibits high β-glucosidase and esterase activity, facilitates the formation of acetates esters and aromatic compounds during wine fermentation (Huang et al., 2023). This study revealed significant positive correlations between *H. uvarum* and the concentrations of hexyl acetate, hexanal, (E)-2-nonenal, and geraniol. The similar correlation patterns observed for *Saccharomyces* and *Tatumella* during fermentation are likely related to their synergistic interactions throughout the fermentation process (Fig. 6). Thus, microbial terroir further promotes the expression of wine terroir through metabolic activity during fermentation (Liu et al., 2020). However, the specific contributions of these microorganisms to wine aroma require further investigation.

4. Conclusion

This study compared the composition and dynamic changes of volatile compounds and microbial communities during the spontaneous fermentation of two different grape cultivars, Merlot and Cabernet Sauvignon. This study compared the composition and dynamic changes of volatile compounds and microbial communities during the spontaneous fermentation of two different grape cultivars, Merlot and Cabernet Sauvignon. The results showed significant flavor differences between Merlot and Cabernet Sauvignon samples, with Merlot exhibiting a strong fruity aroma along with a mild mushroom-like note, while Cabernet Sauvignon wine displayed sweet and green aromas. To investigate the causes of these flavor differences, we examined the influence of microorganisms. As expected, the initial microbial composition demonstrated cultivar specificity. Furthermore, differences in the abundance of many shared fungal OTUs significantly contributed to inter-cultivar differentiation, while the fermentation process established the ecological dominance of *Saccharomyces*. Correlation analyses revealed that the synergistic interactions between *Saccharomyces* and *Tatumella* were positively associated with most characteristic volatile compounds. However, to leverage the microbiome for producing wines with terroir-specific characteristics, significant advancements are required. This includes a comprehensive characterization of fermentation-associated microbial communities and their functions, a thorough analysis of genome-level regulatory factors for each microorganism, and the integration of technologies such as electronic nose, HS-SPME-GC–MS, and Headspace Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS) to develop a systematic method for tracking and detecting wine volatile compounds.

CRediT authorship contribution statement

Qinqin Liu: Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. **Nan Hao:** Writing – review & editing, Investigation. **Lan Mi:** Writing – review & editing, Investigation. **Shuai Peng:** Writing – original draft, Visualization, Validation. **Akumawah Kyen Marie-Colette:** Writing – review & editing. **Xuefang Zhao:** Writing – review & editing. **Jing Wang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

All authors ensure the absence of known conflicts of interest or personal relationships that could bias the research work presented 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.fochx.2025.102317.

Data availability

Data will be made available on request.

References

Barata, A., Malfeito-Ferreira, M., & Loureiro, V. (2012). The microbial ecology of wine grape berries. *International Journal of Food Microbiology, 153*(3), 243–259. https://doi.org/10.1016/j.ijfoodmicro.2011.11.025

Beckner Whitener, M. E., Stanstrup, J., Panzeri, V., Carlin, S., Divol, B., Du Toit, M., & Vrhovsek, U. (2016). Untangling the wine metabolome by combining untargeted SPME–GCxGC-TOF-MS and sensory analysis to profile sauvignon blanc co-fermented

with seven different yeasts. *Metabolomics, 12*(3), 53. https://doi.org/10.1007/s11306-016-0962-4
Bokulich, N. A., Thorngate, J. H., Richardson, P. M., & Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences, 111*(1). https://doi.org/10.1073/pnas.1317377110
Borren, E., & Tian, B. (2020). The Important Contribution of Non-Saccharomyces Yeasts to the Aroma Complexity of Wine: A Review. *Foods, 10*(1), 13. https://doi.org/10.3390/foods10010013
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), Article 13828. https://doi.org/10.1038/s41598-020-70535-8
Chen, T., Wang, H., Su, W., Mu, Y., & Tian, Y. (2023). Analysis of the formation mechanism of volatile and non-volatile flavor substances in corn wine fermentation based on high-throughput sequencing and metabolomics. *Food Research International, 165*, Article 112350. https://doi.org/10.1016/j.foodres.2022.112350
Chen, X., Song, C., Zhao, J., Xiong, Z., Peng, L., Zou, L., Shen, C., & Li, Q. (2024). Application of strain selection Technology in Alcoholic Beverages: A review. *Foods, 13*, 1396. https://doi.org/10.3390/foods13091396
Ciani, M., & Comitini, F. (2015). Yeast interactions in multi-starter wine fermentation. *Current Opinion in Food Science, 1*, 1–6. https://doi.org/10.1016/j.cofs.2014.07.001
Englezos, V., Jolly, N. P., Di Gianvito, P., Rantsiou, K., & Cocolin, L. (2022). Microbial interactions in winemaking: Ecological aspects and effect on wine quality. *Trends in Food Science & Technology, 127*, 99–113. https://doi.org/10.1016/j.tifs.2022.06.015
Fariña, L., Villar, V., Ares, G., Carrau, F., Dellacassa, E., & Boido, E. (2015). Volatile composition and aroma profile of Uruguayan Tannat wines. *Food Research International, 69*, 244–255. https://doi.org/10.1016/j.foodres.2014.12.029
García-Izquierdo, I., Colino-Rabanal, V. J., Tamame, M., & Rodríguez-López, F. (2024). Microbiota ecosystem Services in Vineyards and Wine: A review. *Agronomy, 14*(1), 131. https://doi.org/10.3390/agronomy14010131
He, Y., Wang, X., Li, P., Lv, Y., Nan, H., Wen, L., & Wang, Z. (2023). Research progress of wine aroma components: A critical review. *Food Chemistry, 402*, Article 134491. https://doi.org/10.1016/j.foodchem.2022.134491
Huang, R., Jiang, J., Su, Y., Yu, H., Shen, L., Liu, Y., Qin, Y., & Song, Y. (2023). Microbial community succession and volatile compounds changes during spontaneous fermentation of cabernet sauvignon (*Vitis vinifera* L.) under rain-shelter cultivation. *Food Chemistry, X, 17*, Article 100618. https://doi.org/10.1016/j.fochx.2023.100618
Jiang, B., Xi, Z., Luo, M., & Zhang, Z. (2013). Comparison on aroma compounds in cabernet sauvignon and merlot wines from four wine grape-growing regions in China. *Food Research International, 51*(2), 482–489. https://doi.org/10.1016/j.foodres.2013.01.001
Li, R., Lin, M., Guo, S., Yang, S., Han, X., Ren, M., Song, Y., Du, L., You, Y., Zhan, J., & Huang, W. (2021). A fundamental landscape of fungal biogeographical patterns across the main Chinese wine-producing regions and the dominating shaping factors. *Food Research International, 150*, Article 110736. https://doi.org/10.1016/j.foodres.2021.110736
Liang, L., Ma, Y., Jiang, Z., Sam, F. E., Peng, S., Li, M., & Wang, J. (2023). Dynamic analysis of microbial communities and flavor properties in merlot wines produced from inoculation and spontaneous fermentation. *Food Research International, 164*, Article 112379. https://doi.org/10.1016/j.foodres.2022.112379
Liu, D., Legras, J.-L., Zhang, P., Chen, D., & Howell, K. (2020). Diversity and dynamics of fungi during spontaneous fermentations and association with unique aroma profiles in wine. *International Journal of Food Microbiology, 338*, Article 108983. https://doi.org/10.1016/j.ijfoodmicro.2020.108983
Liu, M., Deng, N., Hou, X., Zhang, B., Li, H., & Wang, J. (2024). Characterisation of flavour profiles and microbial communities of fermented peppers with different fermentation years by combining flavouromics and metagenomics. *Food Chemistry, 443*, Article 138550. https://doi.org/10.1016/j.foodchem.2024.138550
Liu, S., Lou, Y., Li, Y., Zhao, Y., Laaksonen, O., Li, P., Zhang, J., Battino, M., Yang, B., & Gu, Q. (2023). Aroma characteristics of volatile compounds brought by variations in microbes in winemaking. *Food Chemistry, 420*, Article 136075. https://doi.org/10.1016/j.foodchem.2023.136075
Liu, Y., Rousseaux, S., Tourdot-Maréchal, R., Sadoudi, M., Gougeon, R., Schmitt-Kopplin, P., & Alexandre, H. (2017). Wine microbiome: A dynamic world of microbial interactions. *Critical Reviews in Food Science and Nutrition, 57*(4), 856–873. https://doi.org/10.1080/10408398.2014.983591
Luzzini, G., Slaghenaufi, D., Pasetto, F., & Ugliano, M. (2021). Influence of grape composition and origin, yeast strain and spontaneous fermentation on aroma profile of Corvina and Corvinone wines. *LWT-Food Science and Technology, 143*, 111120. https://doi.org/10.1016/j.lwt.2021.111120
Ma, Y., Peng, S., Mi, L., Li, M., Jiang, Z., & Wang, J. (2023). Correlation between fungi and volatile compounds during different fermentation modes at the industrial scale of merlot wines. *Food Research International, 174*, Article 113638. https://doi.org/10.1016/j.foodres.2023.113638
MacLean, R. C., & Gudelj, I. (2006). Resource competition and social conflict in experimental populations of yeast. *Nature, 441*(7092), 498–501. https://doi.org/10.1038/nature04624
Martínez-Gil, A. M., Garde-Cerdán, T., Lorenzo, C., Félix Lara, J., Pardo, F., & Rosario Salinas, M. (2012). Volatile compounds formation in alcoholic fermentation from grapes collected at 2 maturation stages: Influence of nitrogen compounds and grape variety. *Journal of Food Science, 77*(1), C71–C79. https://doi.org/10.1111/j.1750-3841.2011.02441.x
Martins, V., López, R., Teixeira, A., & Gerós, H. (2024). Combined omics expose microbial niches of fungi and bacteria correlating with wine volatile profiles in Douro wine region. *LWT- Food Science and Technology, 193*, Article 115769. https://doi.org/10.1016/j.lwt.2024.115769
Martins, V., Szakiel, A., Pączkowski, C., Teixeira, A., & Gerós, H. (2021). The restructuring of grape berry waxes by calcium changes the surface microbiota. *Food Research International, 150*, Article 110812. https://doi.org/10.1016/j.foodres.2021.110812
Martins, V., Szakiel, A., Teixeira, A., Abdallah, C., Moreira, C., Pączkowski, C., Lanoue, A., & Gerós, H. (2023). Combined omics approaches expose metabolite-microbiota correlations in grape berries of three cultivars of Douro wine region. *Food Chemistry, 429*, Article 136859. https://doi.org/10.1016/j.foodchem.2023.136859
Ohwofasa, A., Tian, B., Torrico, D., Dhami, M., Winefield, C., & On, S. L. W. (2024). Differences in organic pinot noir wine production systems correlated with microbiome analysis, sensory characteristics and volatile composition. *Food Bioscience, 61*, Article 104562. https://doi.org/10.1016/j.fbio.2024.104562
Onetto, C. A., Ward, C. M., Van Den Heuvel, S., Hale, L., Cuijvers, K., & Borneman, A. R. (2024). *Temporal and spatial dynamics within the fungal microbiome of* grape fermentation. *Environmental Microbiology, 26*(6), Article 574562. https://doi.org/10.1101/2024.01.15.574562
Tian, H., Xiong, J., Sun, J., Du, F., Xu, G., Yu, H., Chen, C., & Lou, X. (2024). Dynamic transformation in flavor during hawthorn wine fermentation: Sensory properties and profiles of nonvolatile and volatile aroma compounds coupled with multivariate analysis. *Food Chemistry, 456*, Article 139982. https://doi.org/10.1016/j.foodchem.2024.139982
Tronchoni, J., Setati, M. E., Fracassetti, D., Valdetara, F., Maghradze, D., Foschino, R., … Bauer, F. F. (2022). Identifying the Main drivers in microbial diversity for cabernet sauvignon cultivars from Europe to South Africa: Evidence for a cultivar-specific microbial fingerprint. *Journal of Fungi, 8*(10), 1034. https://doi.org/10.3390/jof8101034
Verdenal, T., Dienes-Nagy, Á., Spangenberg, J. E., Zufferey, V., Spring, J.-L., Viret, O., … Van Leeuwen, C. (2021). Understanding and managing nitrogen nutrition in grapevine: A review. *OENO one, 55*(1), 1–43. https://doi.org/10.20870/oeno-one.2021.55.1.3866
Wang, D., Chen, G., Tang, Y., Li, J., Huang, R., Ye, M., Ming, J., Wu, Y., Xu, F., Lai, X., Zhang, Q., & Zhang, W. (2022). Correlation between autochthonous microbial communities and flavor profiles during the fermentation of mustard green paocai (*Brassica juncea* Coss.), a typical industrial-scaled salted fermented vegetable. *LWT-Food Science and Technology, 172*, Article 114212. https://doi.org/10.1016/j.lwt.2022.114212
Wang, F., Zhao, P., Du, G., Zhai, J., Guo, Y., & Wang, X. (2024). Advancements and challenges for brewing aroma-enhancement fruit wines: Microbial metabolizing and brewing techniques. *Food Chemistry, 456*, Article 139981. https://doi.org/10.1016/j.foodchem.2024.139981
Wang, L., Liu, Q., Li, Y., Shi, C., Zhang, Y., Wang, P., Zhang, H., Wang, R., Zhang, W., & Wen, P. (2025). Revealing the impact of organic selenium-enriched *Lactiplantibacillus plantarum* NML21 on yogurt quality through volatile flavor compounds and untargeted metabolomics. *Food Chemistry, 474*, Article 143223. https://doi.org/10.1016/j.foodchem.2025.143223
Wang, L., Zhong, X., Ding, Y., Shao, X., Zhang, Z., Yin, H., Zhang, L., Wang, H., & Li, H. (2024). Multi-omics co-analysis of the differences in microbial species composition and function between spontaneous and inoculated wine fermentation must. *LWT-Food Science and Technology, 200*, Article 116181. https://doi.org/10.1016/j.lwt.2024.116181
Wei, J., Zhang, Y., Qiu, Y., Guo, H., Ju, H., Wang, Y., Yuan, Y., & Yue, T. (2020). Chemical composition, sensorial properties, and aroma-active compounds of ciders fermented with *Hanseniaspora osmophila* and *Torulaspora quercuum* in co- and sequential fermentations. *Food Chemistry, 306*, Article 125623. https://doi.org/10.1016/j.foodchem.2019.125623
Wei, R., Chen, N., Ding, Y., Wang, L., Liu, Y., Gao, F., Zhang, L., Li, H., & Wang, H. (2022). Correlations between microbiota with physicochemical properties and volatile compounds during the spontaneous fermentation of cabernet sauvignon (*Vitis vinifera* L.) wine. *LWT- Food Science and Technology, 163*, Article 113529. https://doi.org/10.1016/j.lwt.2022.113529
Wei, R., Ding, Y., Chen, N., Wang, L., Gao, F., Zhang, L., Song, R., Liu, Y., Li, H., & Wang, H. (2022). Diversity and dynamics of microbial communities during spontaneous fermentation of cabernet sauvignon (*Vitis vinifera* L.) from different regions of China and their relationship with the volatile components in the wine. *Food Research International, 156*, Article 111372. https://doi.org/10.1016/j.foodres.2022.111372
Xu, X., Miao, Y., Wang, H., Du, J., Wang, C., Shi, X., & Wang, B. (2022). Analysis of microbial community diversity on the epidermis of wine grapes in Manasi's vineyard, Xinjiang. *Foods, 11*(20), 3174. https://doi.org/10.3390/foods11203174