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Differences in organic Pinot Noir wine production systems correlated with microbiome analysis, sensory characteristics and volatile composition

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ABSTRACT

The action of microorganisms on grape must during the fermentation process contributes significantly to the organoleptic properties of wine. The influence of the environment on microbial growth and metabolism is also well recognized. Organic winemakers rely on indigenous yeasts to drive their fermentation processes, however there are few studies that examine the possible influence of environmental factors on fermentation, and on sensory attributes of the finished product. We previously used a community metabarcoding approach to analyse the microbiome associated with organic wine produced in two differing environmental systems; outdoors (vineyard) and indoors (winery). The resultant wine from both systems were then assessed for aroma composition using GC-MS, and sensory attributes by a group of wine experts. Possible correlations between the identified microbial populations and sensory attributes were investigated to determine potential drivers. The results confirm the crucial role of the yeast, *Saccharomyces* in the modification of wine aroma and flavour. Moreover, analysis of the output of differential gene expression analysis (DESeq2) showed that the genus *Gluconobacter* might influence the 'Mouth feel' (astringency/tannin) and taste (bitterness) attributes of wines. Some volatile compounds were uniquely associated with a single wine. This suggests that measured differences in microbial community composition might play roles in their synthesis. Collectively, these results contribute to understanding the interplay of the complex microbial community matrix present in 'wild' ferments in terms of sensory and chemical characteristics of wine.

1. Introduction

Soil and climate characteristics, the microflora of grapes, as well as the winemaking techniques applied, all have an influence on the sensory and chemical characteristics of wine (Pinto et al., 2015; Rivas et al., 2022; Roullier-Gall et al., 2014; Tonkin et al., 2015). Together their contributions to wine quality, flavour and aroma are captured in the concept of *terroir*. *Terroir* is a principal aspect of identity, consumer acceptance, and economic appreciation of wine production (Bokulich et al., 2016). Amongst these factors, the composition of microbial communities can be highly variable and is greatly influenced by environmental conditions (Li et al., 2016). Various studies have highlighted the relationship between microbial dynamics and environmental parameters in different ecosystems such as soils (Žifčáková et al., 2016) and marine environments (Tinta et al., 2015). Recently, Ohwofasa et al. (2023) described how environmental conditions influenced the microbial communities associated with the spontaneous fermentation of Pinot Noir.

Fermentation of grapes in wine making, as well as the production of any other fermented food and products, depends on the central role played by microorganisms (Tamang et al., 2016). In wine, complex microbial interactions between bacteria, fungi, and yeasts result in the production of alcohol and myriad other compounds (Belda, Ruiz, Esteban-Fernández, et al., 2017) that influence the individuality and subtlety of flavour responses (Feng et al., 2018; Xu et al., 2017). In specific terms, Swiegers et al. (2005) described how fermentative yeasts, mainly lactic acid bacteria (LAB, primarily *Oenococcus Oeni*) and *Saccharomyces cerevisiae* present in must modifies the aroma and flavour of wine. Modulation of wine flavour and aroma is not limited to these two species. Other microorganisms could alter the fermentation process or

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chemical environment by releasing metabolites thereby affecting wine characteristics (Bokulich et al., 2016; Swiegers et al., 2005). Therefore, the sensory properties of wine are heavily influenced by the microbial species that proliferate during the process of fermentation (Patrignani et al., 2016).

Different grape varieties have distinct sensory characteristics with reference to wine aroma perception. Even so, some of these differences are only perceptible after fermentation (Belda, Ruiz, Esteban-Fernández, et al., 2017). Pinot Noir has been described as an "elegant" red wine due to its fine sensory qualities (Robinson et al., 2013; Serni et al., 2020). Hence, it is no surprise that wines made from Pinot Noir grapes have received sustained interest from the research community. (Mawdsley et al., 2019; Pedri et al., 2019; Serni et al., 2020; Sherman et al., 2020; Zhu et al., 2022). In New Zealand, several studies involving the sensory properties of Pinot Noir wines have been carried out (Araujo et al., 2021; Parr et al., 2020; Valentin et al., 2016). One such study reported that wines produced from high and moderate fruit maturities had similar sensory properties. On the other hand, low fruit maturity resulted in far less complex wine (Pineau et al., 2017). Other Pinot Noir studies establish links between the chemical matrix or aroma profile of wine and the variation in vineyard sites (Schueuermann et al., 2017, 2018). All of this highlights the numerous attempts that have been made to draw correlations between wine production systems and sensory outcomes (Longo et al., 2021).

Generally, wine fermentations are usually carried out in an indoor environment. In a bid to explore a novel fermentation approach, in collaboration with an established commercial wine maker, we previously analysed the microbiome of a spontaneous Pinot Noir wine fermentation carried out in an outdoor (vineyard) environment, and compared this with the microbiome of a spontaneous wine fermentation done more conventionally, in an indoors winery (Ohwofasa et al., 2023). Here, we extend that study by carrying out a sensory and chemical analysis of the two Pinot Noir wines produced from each of those environments and correlate these results with our existing microbiome data using a statistical and mathematical modelling approach.

2. Materials and methods

2.1. Microbial components of the wine products

A metabarcoding approach, alongside high throughput sequencing was adopted to establish an accurate representation of all bacteria and fungi that must have contributed to shaping of the flavour and aroma profile of the final wine product. The methods used for DNA extraction, metabarcoding and annotation of bacterial and fungal species have been outlined (Ohwofasa et al., 2023).

Grapes (Pinot noir; 21.8 ∘Brix) utilized in this study were sourced from Greystone vineyard on the March 12, 2021. Care was taken to ensure grape samples were only harvested from the same block of vineyard. Eighty percent of bunches were destemmed while twenty percent were whole clusters. Two environmental systems were (a) Winery. This was an indoor environment (b) Vineyard. This represents a natural scenery without temperature control. This is located less than 1 km away from the winery. In all systems, grapes were homogenized and fermenting grape juice samples were taken from Day 1 (at harvest) till the end of fermentation (after press from grape skins). Note that the same cap management system was utilized in both tanks. Alcoholic fermentation went for a period of two weeks, and this was further left on skins for another two before pressing. As for sampling, this study was done in year 2021 and due to COVID, the harvest for this vintage was particularly low. Hence, the harvested grapes could serve only one tank per location.

2.2. Wine and sensory analysis

Upon completion of fermentation in both systems, using the Rebelein method, the wines were tested for residual sugar to ensure dryness. These were then pressed from the skins, racked into barrels where malolactic fermentation took place for a period of 8 months. After maturation, the pH of the samples were evaluated before they were bottled and stored at 21 °C in preparation for the sensory evaluations. The sensory panel consisted of ten wine professionals based in Canterbury, New Zealand. They were highly experienced in the testing and production processes of Pinot Noir wine. They were considered wine experts according to Parr et al. (2002) and they frequently participate in wine sensory evaluation studies. The panel comprised of eight female and two male participants. Panellists conducted the sensory evaluation without training in this study. The project was reviewed and approved by the Lincoln University Human Ethics Committee (HEC approved, 2019–68).

The sensory test consisted of two 30 min sessions with a break between samples and was conducted in the controlled sensory facility of Lincoln University (Eggert & Zook, 1986). Prior to the evaluation, all participants were given an information sheet which contained useful information including a copy of the Human Ethics Committee approval and participants' rights before, during, and after the evaluation session. The session involved a rating task where attributes that typify Pinot Noir wine were rated on an unstructured 10-point scale, with 0 meaning the absence of that attribute and 10 signifying its peak presence. Included was a total of 37 descriptors for appearance, taste, aroma, flavour and mouth feel. The literature was surveyed to generate the attributes used (Araujo et al., 2021; Valentin et al., 2016). Supplementary Table 1 outlines the descriptors and the anchors utilized.

For evaluation, a new wine bottle of each sample was opened before the participants, and 30 ml were served into specialized wine-tasting glasses (ISO, 1977) placed on clear white sheets. A full tasting that involved palate judgement, retro-nasal, and ortho-nasal was employed with clear instructions given to all participants to expectorate all wine samples. Before moving on to the next sample, evaluation sheets were withdrawn, and a short break was taken where unsalted crackers and water was provided for palate cleansing.

2.3. Determination of the volatile organic compounds (VOC) in wine samples

The approach utilized by Tomasino et al. (2015) using the automated Headspace Solid-Phase Micro-Extraction Gas Chromatograph Mass Spectrometry (HS-SPME GC-MS) technique was followed. Here we used a qualitative approach through a simplified mass spectral scan mode to determine volatile organic compounds (VOCs) found in the wine samples. The same sample dilution in tartaric acid buffer was utilized to obtain a consistent HS-SPME extraction between sample treatments and adequate chromatographic conditions for the VOC's detected. Samples were prepared by pipetting 0.9 ml of wine into 20 ml SPME sample vials. To this, we added 8.1 ml of tartaric acid buffer (5 g L^{-1}, pH 3.5) and 4.5 g of crystalline sodium chloride. Extraction was done by incubating samples for 60 min at 60 °C with their enclosed headspace exposed to a 2 cm long DVB/CAR/PDMS combination SPME fibre (p/n 57348-U, 50/30 μm thickness, 24 gauge, Supelco Bellefonte, PA, USA, through Sigma- Aldrich, Australia). This exposure period ensures the headspace volatiles were absorbed into the fibre. Volatiles were desorbed when the SPME fibre was inserted into the GC injection port for a period of 10 min at 270 °C. Actual GC-MS analysis was done on a Shimadzu GC-MS-QP2010 gas chromatograph–mass spectrometer (Shimadzu Scientific Instruments Inc, Japan). This is equipped with a Combi-Pal autosampler (CTC analytics AG, Switzerland) ready for automated SPME.

The data acquisition software used was the GCMSsolutions (version 4.45). A dual GC column setup was employed to perform the chromatography. The setup was made up of an Rtx-Wax 60.0 m x 0.25 mm ID x 0.50 μm film thickness (Polyethylene Glycol - Restek, Bellefonte, PA, USA) connected to an Rxi-1ms 15 m × 0.25 mm ID x 0.50 μm film

thickness (100% Dimethyl Polysiloxane - Restek, Bellefonte, PA, USA) in series. The carrier gas was Helium, and the GC-MS was set to a constant linear velocity of 33.5 cm s^{-1}. We operated the injector in splitless mode for 5 min and thereafter it was switched to a 20.5:1 split ratio. Note that during desorption of SPME fibre, the column oven was held at 35 °C for 3 min. It was then heated up to 250 °C at 4 °C min^{-1} and held at this temperature for 10 min. The total run time was 66.75 min. The interface and MS source temperatures were set at 250 °C and 200 °C respectively. Using an ionization energy of 70eV, we operated the MS in an electron impact mode (EI).

With all VOC peaks integrated for qualitative analysis, all analytes were evaluated in a full scan mode. This was done by comparing the mass spectral patterns to those of commercial libraries and looking for a high percentage match. Thus, no standards were run at the same time as the samples The NIST 14 (National Institute of Standards and Technology) and Wiley 10 (John Wiley & Sons Inc) mass spectral libraries were used for identification of VOC compounds. Published retention indices sourced from website chemspider.com for wax (polyethylene glycol) GC columns was used to double check our identified peaks.

2.4. Data analysis

To determine attributes that were significantly different in both wines, a one-way (ANOVA) with environmental condition (treatment) as the main factor was done using XLSTAT (2018.1.1.62926). Fisher's LSD means comparison was employed as a *post*-hoc test to establish where treatment brought about differences in the attributes. Using the matrix of the descriptive ratings provided, Pearson correlation (XLSTAT - Version: 2018.1.1.62926) was done to establish attributes that were positively or negatively correlated. For better visualization, we constructed correlation plots using the "corrplot" package (Wei et al., 2021) in R (V4.1.0).

Analysis of microbial diversity was accomplished using the Phyloseq package (McMurdie & Holmes, 2013) and many other packages (Supplementary file 1 and 2). One phyloseq object each was created for fungal and bacterial data. Dissimilarities in library sizes were accounted for by transforming the ASV abundances into their relative abundance. The core microbiome associated with each wine must was obtained by retrieving the taxa present in 70% of the samples with an abundance $\geq$0.0001. Non–metric multidimensional scaling plot (NMDS) was constructed using the Bray-Curtis distance matrix ordination. We then proceeded to fit our sensory data (Supplementary Table 2) with our microbiome data using the "envfit" function in vegan (Oksanen et al., 2015). To enumerate ASVs that differed significantly in both wine musts, we applied DESeq2 (Love et al., 2014). The within and between group similarities were tested via Analysis of similarities (ANOSIM) using 999 permutations (Clarke, 1993).

3. Results

3.1. pH and sensory evaluation of Pinot noir wines

The pH values of both wines were identical. The outdoor wine had a pH of 3.59 while the indoor wine had a pH of 3.58. The mean value for ratings of sensory attributes is shown in Supplementary Fig. 1 (A-C). Results from the analysis of variance (ANOVA) revealed no significant differences (Supplementary Table 3). This means that though both wines did not have the same sensory attributes, they showed no statistically significant differences with regards the environmental location. On average, the outdoor-fermented wine had a higher flavour (overall intensity, herbaceous, and spice) (Supplementary Fig. 1 A-C). The indoor-fermented wine on the other hand was high in aroma (overall intensity and floral). In terms of colour, both wines were judged to have a ruby colour.

3.2. Flavour and aroma attributes were closely related in both wines

Though no sensory attributes were significantly different in either of the wines as stated above, several significant ($p < .05$) correlations were found amongst these sensory attributes, for example, flavour and aroma versions of similar attributes. Specifically, for the indoor-fermented wine, flavour (oak characters) positively correlated with mouth feel (astringency/tannin) and mouth feel (warmth/alcohol). Flavour (stone fruit) positively correlated with taste (sweetness), and flavour (tropical fruit). Others include the strong positive correlation noted between taste (acidity) and taste (bitterness) (Fig. 1A). Notable negative correlations were observed with appearance (colour density) opposing flavour (spice), mouth feel (astringency/tannin), and aroma (yeast characteristics). Flavour (tropical fruit) was also negatively correlated with mouth feel (finish).

For the outdoor-fermented wine, flavour (citrus fruit) positively correlated with taste (sweetness) while it correlated negatively with mouth feel (body). Aroma (Herbaceous) correlated positively with mouth feel (finish). Aroma (herbal) was also negatively correlated with mouth feel (astringency/tannin) (Fig. 1B). The entire correlation matrix for both wines is shown in Supplementary Table 4.

3.3. Dominant bacterial communities in both wine fermentation musts were different but fungal diversity were similar

Microbial differences as outlined by Ohwofasa et al. (2023) are summarized here. The bacterial community of the indoor-fermented wine musts was richer and more diverse (Supplementary Fig. 2). We observed 48.6% of the bacterial population in the outdoor-fermented (vineyard) wine musts consisted of the genus *Tatumella.* Others were *Bacillus* (15.3%), *Gluconobacter* (13.2%), *Sphingomonas* (3.3%), and *Hyphomicrobium* (2.75%). Others such as *Caulobacter*, and *Leuconostoc,* were also present but in limited amounts (Supplementary Table 6). The indoor (winery) wine musts on the other hand had an evenly distributed community. These were *Tatumella* (33.4%), *Bacillus* (21.3%), *Sphingomonas* (8.9%), *Hyphomicrobium* (5.8%), *Fructobacillus* (7.6%) and *Lactococcus* (10.57%) (Supplementary Table 6). ANOSIM showed a significant difference based on the composition of bacterial species ($R_{ANOSIM} = 0.57$; $p = 0.001$) present in both wine musts. Lastly, the above was supported with the analysis of DESeq2 which highlights that the abundance of *Fructobacillus* (*p*.adj = 1.18E-20) and *Lactococcus* (*p*.adj = 1.39E-11) were statistically significant and abundant in the indoor-fermented samples. The outdoor-fermented samples had more of *Gluconobacter* (*p*.adj = 1.98E-05), *Leuconostoc* (0.00368) and *Tatumella* (*p*.adj = 9.18E-24) (Supplementary Table 5).

When compared with the bacterial community, the fungi populations present in both samples were near identical. Specific percentages show that the genus *Saccharomyces* made up 44.5% in the outdoor (vineyard) and 52.5% of the indoor (winery) (Supplementary Table 7). Despite the similarities observed, ANOSIM revealed significant compositional differences ($R_{ANOSIM} = 0.60$; $p = 0.001$). DESeq2 also reported *T.delbrueckii* (9.9E-05) to be more abundant in the winery musts than it was in the vineyard (Supplementary Table 5). All these are summarized in Fig. 2 (A-L).

3.4. Sensory attributes correlated with bacterial and fungal communities of wine

Correlating the bacterial and fungal communities with the sensory attributes revealed that some attributes align and are potentially driven by the microbial communities of that wine. Interestingly, the bacterial and fungal resulted in a similar pattern (Fig. 3), although with a higher correlation in the bacteria ($r^2 = 0.36$) as compared to the fungal community ($r^2 = 0.26$). The indoor -fermented wine correlated with 14 attributes. This includes Appearance (colour density) - App-CD, Taste (Sweetness) – Tas-S, Taste (acidity) – Tas-A, Aroma (Overall intensity) –

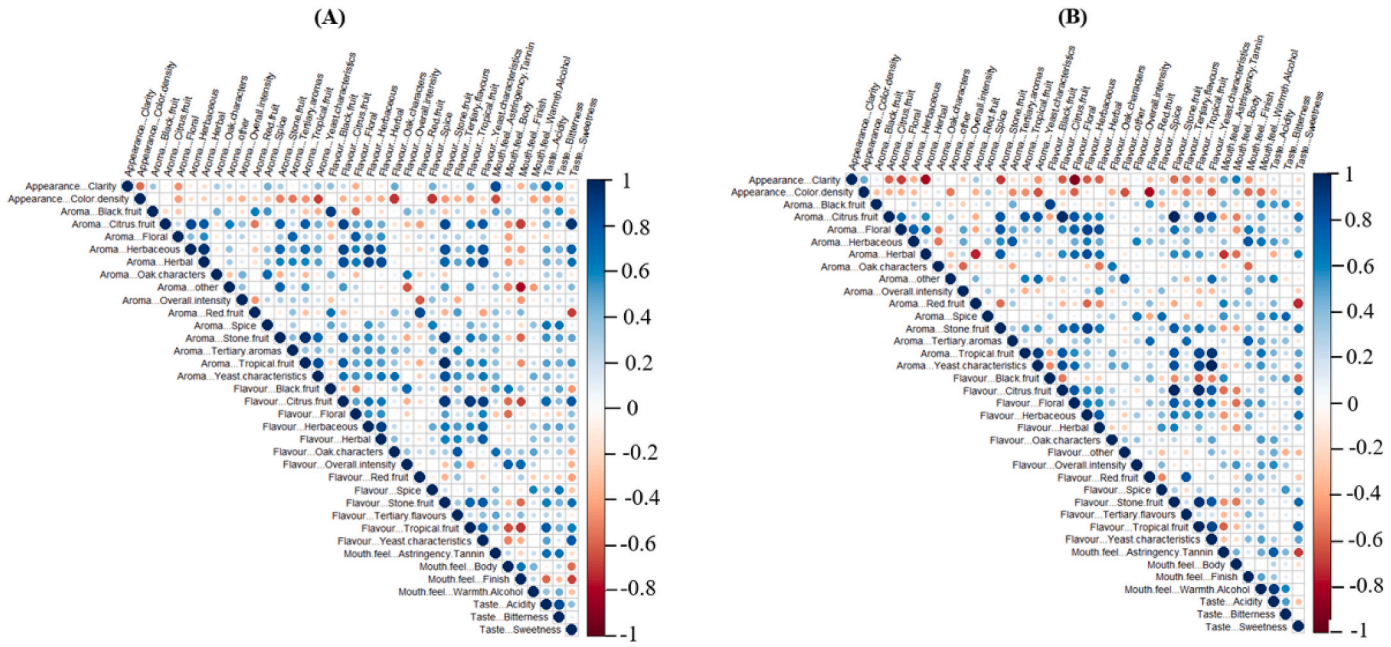

Fig. 1. Correlation plot showing the attributes of (A) the indoor (winery) wine; (B) the outdoor (vineyard) wine. Only the upper triangular matrix is shown to improve readability. Alphabetical ordering of all variables was used to make clusters. Entire correlation table is shown in Supplementary Table 4.

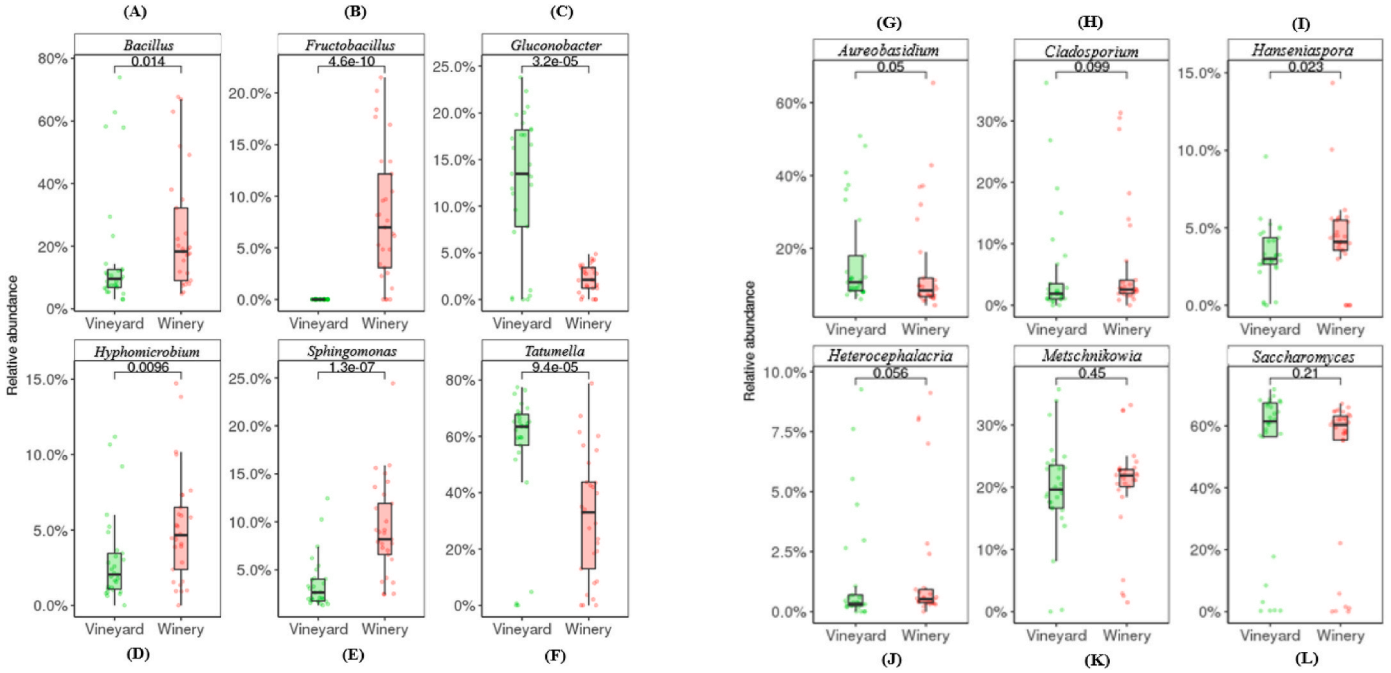

Fig. 2. Relative abundance of the most abundant bacteria (A–F) and fungal genera (G–L) in the vineyard and winery environments.

Aro-Oi, Aroma (Floral) – Aro-F, Aroma (Citrus fruit) – Aro - Cf, Aroma (Herbal) – Aro-HI, Flavour (Floral) – Fla-F, Flavour (Citrus fruit) – Fla-Cf, Flavour (Stone fruit) – Fla-Sf, Flavour (Yeast characters) – Fla-YC, Flavour (Red fruit) – Fla-Rf, Flavour (Tertiary flavour) – Fla-T.f, and Mouthfeel (Warmth-Alcohol) – MF-WA.

The Outdoor (vineyard) correlated with more variables. They are; Appearance (clarity) - App-C, Taste (Bitterness) – Tas-B, Aroma (Stone fruit) – Aro-Sf, Aroma (Tertiary flavour) – Aro-T.f, Aroma (Black fruit) – Aro-Bf, Aroma (Herbaceous) – Aro-H, Aroma (Spice) – Aro-S, Aroma (Yeast characteristics) – Aro-YC, Aroma (Oak characteristics) – Aro-Ok, Aroma (Tertiary aroma) – Aro-Ta, Aroma (Other) – Aro-O, Flavour (Overall intensity) – Fla-Oi, Flavour (Tropical fruit) – Fla-T.f, Flavour (Black fruit) – Fla-Bf, Flavour (Herbaceous) – Fla-H, Flavour (Herbal) – Fla-Hl, Flavour (Spice) – Fla-S, Flavour (Oak characteristics) – Fla-Ok, Flavour (Other) – Fla-O, Mouthfeel (Body) – MF-BD, Mouthfeel (Astringency-Tannin) – MF-As, Mouthfeel (Finish) – MF-F. Supplementary file 3 shows the list of all variables, correlation coefficient and significance level for both bacterial and fungal.

3.5. Volatile organic compounds analysis

All organic compounds, retention time, peak area, as well as the

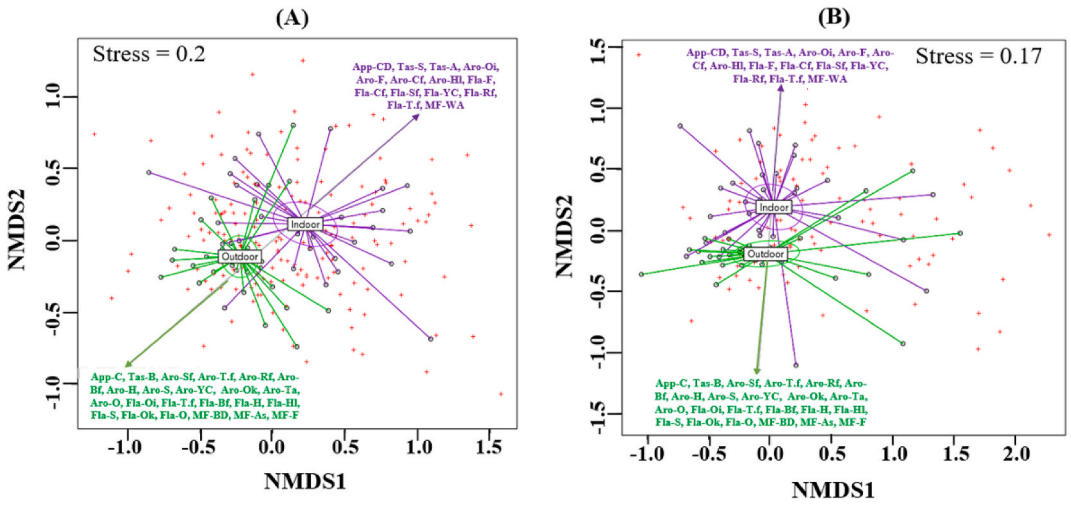

Fig. 3. NMDS ordination for (A) Bacterial and (B) Fungal community associated with outdoor (vineyard) wine musts (Green) and the indoor (winery) wine musts (Purple). Fitting with sensory data to see variables that are potentially driven by the microbial community shows that the outdoor (vineyard) wine correlated with more variables (23) as compared to the indoor wine (14) (supplementary file 3). This indicates that these variables are possibly influenced by the microbial community associated with its wine musts. Circular points represent samples and are connected with an arrow to its centroid. Red markings depict the amplicon sequence variants (ASVs). Ellipses shown represent 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

percentage of each peak area derived from both wines, are enumerated in Supplementary Table 9. Percentage here refers to the area a certain compound occupies out of all organic compounds detected. The compound with the largest peak area in both wines was 3-Methylbutan-1-ol with an area of 26.51% in the outdoor wine and 26.56% in the indoor wine. This was followed by Benzene ethanol, which appeared higher in the outdoor-fermented wine (19.20%) as compared to the indoor-fermented wine (16.81%). Other compounds found in both wines include Ethyl Acetate (outdoor wine - 2.20%; indoor wine 2.09%), Ethanol (outdoor wine – 9.56%; indoor wine 9.06%), Ethyl caproate (outdoor wine – 6.77%; indoor wine 6.56%) and many others (Supplementary Table 9). Some volatile compounds were found in one wine and not the other. The outdoor-fermented (vineyard) wine had 1-propanol, 3-Octanol, 2-Hexen-1-ol, (Z)-, 4-Methylbenzaldehyde, 3-Phenylpropanol, and Neric acid; volatiles only found in the indoor-fermented (winery) wine included Isobutyl acetate, Triphenylphosphine oxide, 3-Tolualdehyde, and Ethyl 3-hydroxytridecanoate. Supplementary Table 10 shows volatile compounds that had the largest peak areas.

4. Discussion

The impact of environmental conditions on the microbial community associated with different fermented food and beverages is well established (Hao et al., 2021; Kirchmayr et al., 2017; Li et al., 2016; Ohwofasa et al., 2023). Here, we seek to explore if microbial differences associated with organic wine production using spontaneous fermentation techniques as earlier reported (Ohwofasa et al., 2023) can be correlated with perceptible sensory and chemical variations in the properties of the resulting wine product.

Overall, wines produced from both environments were similar, but not identical, and some trends were uncovered. Due to the similarities of the yeast and fungi populations present in both wines, particularly with *Saccharomyces* accounting for over 44% in both (Supplementary Table 7), this must have had an equal impact on the sensory profiles of each of the wines. This is in line with already known facts that the majority of changes from grape must to wine are accomplished by the wine yeast, *Saccharomyces* (Swiegers et al., 2005). Our GC-MS data supports this in that 3-Methylbutan-1-ol displayed the largest peak in both wines (Supplementary Table 10). 3-Methylbutan-1-ol also known as isoamyl alcohol (Scutarasu et al., 2022) are synthesized through the Ehrlich pathway in yeast cells (Swiegers et al., 2005). Additionally, the spontaneous fermentation style employed here may also be an added factor. The native fungal populations present on the grapes often impact winemaking and specifically, wine quality (Jolly et al., 2014; Wang et al., 2021). The concentrations of fusel alcohols, isoacids and their ethyl esters, fusel alcohol acetates, all of which are linked to yeast amino acid metabolism, are related to the varietal origin of the must (Ferreira, 2010). With both fermentations having started out with the same grapes, the starting yeast community would be consistent across the two environments.

Benzeneethanol, the second most abundant volatile compound, had a larger peak in the outdoor-fermented wine when compared with the indoor-fermented wine (Supplementary Tables 9 and 10). This compound is known to contribute to the rosy and floral aroma notes of wines, and is mainly produced by wine yeast during fermentation (Ou et al., 2010). With *Saccharomyces* having a slightly higher relative abundance in the outdoor (vineyard) wine (Supplementary Fig. 3A), this perhaps accounts for its larger Benzeneethanol peak area.

The non-Saccharomyces yeast, *Hanseniaspora* was also significantly abundant in the indoor (winery) wine as compared to the outdoor (vineyard) wine (Supplementary Fig. 3E). This is particularly noteworthy since some species have been linked with the fruity and honey aroma of wines due to an increase in the production of acetate esters (Carpena et al., 2020; Zhang et al., 2022). Some volatile compounds were uniquely associated with one wine while others were found in both wines but were more abundant in one than the other. Isobutyl acetate for example was found only in the indoor wine (Supplementary Table 10). This may be related to the presence of *T. delbrueckii*, which we detected only in the indoor wine (Supplementary Table 7). Our result is supported by Renault et al. (2015) who reported increased concentrations of Isobutyl acetate due to the positive interactions between *T. delbrueckii* and *S.cerevisiae.*

Likewise, 4-Ethoxy-4-oxobutanoic acid was 17 times much higher in the indoor (winery) wine as compared to the outdoor (vineyard) wine (Supplementary Table 9). Vigentini et al. (2016) has previously reported high concentrations of 4-ethoxy-4-oxobutanoic acid and ethyl lactate in wine samples produced by inoculating *T. delbrueckii* and *K. marxianus.* This might suggest that the presence of *T. delbrueckii* in the indoor (winery) wine could have possibly enhanced some volatiles. Not surprisingly, its positive contributions to the aromatic properties of wines

have been reported (Belda, Ruiz, Beisert, et al., 2017; Benito, 2018).

In terms of attribute correlations (Fig. 1A and B; Supplementary Table 4), the Oak characters of aroma correlated significantly with other attributes in one wine as compared to the other. For instance, the positive correlation between aroma – oak characters and aroma – overall intensity. The same was seen with aroma – oak characters and aroma – spice, as seen in the indoor-fermented (winery) wine. For the outdoor fermented (vineyard) wine, no such observation was made (Supplementary Table 4). This suggests that the indoor wine fermentation, being in a surrounding filled with oak barrels might have had an impact and possibly enhanced these sensory attributes to an extent. The above may also be related to Ethyl 3-methylbutylsuccinate. Supplementary Table 9 shows the peak area of this volatile compound to be twice as abundant in the indoor (winery) wine than it was in the outdoor (vineyard) wine. This compound have previously been associated with commercial Pinot Noir studies involving barrel maturation (Schueuermann et al., 2016). More studies will be required to confirm this in the future.

A volatile compound, 2-Hexen-1-ol, (Z)-, was found only in the outdoor wine (Supplementary Table 10). This compound have been associated with the herbaceous aromatic series as they mainly contribute to the herbaceous odour of fruits (Yu et al., 2019). Indeed, Fig. 3 corroborates this in that the Flavour (Herbaceous) – Fla-H and Aroma (Herbaceous) – Aro-H attributes also correlated significantly with the outdoor (vineyard) wine. Other volatiles were present in both wines though with slight fluctuations in abundance. Such little variations in volatile aroma compounds could be the difference between an excellent wine and an average wine (Swiegers et al., 2005). In relation to the microbiome, this possibly indicates that some of these bacteria and fungi, which were significantly different in abundance, might have direct roles in the synthesis of these volatiles that were uniquely identified in each of the wines here.

From the analysis of the output of DESeq2 (Supplementary Table 5), the genus *Tatumella*, *Gluconobacter, Leuconostoc* and some species of *Metschnikowia* might have influenced the attributes of the outdoor (vineyard) wine as these were amongst the ASVs that were differentially expressed. The significant presence of *Gluconobacter* in the outdoor wine likely contributed to the mouth feel (astringency/tannin) and taste (bitterness) attributes observed in this wine. These attributes correlated significantly with the outdoor (vineyard) wine and *Gluconobacter* is well established as an acetic acid producing bacteria and can give rise to a vinegar like and bitter aroma (Campaniello & Sinigaglia, 2017; Swiegers et al., 2005). For the indoor (winery) wine, the genera *Fructobacillus, Lactococcus* and *T.delbrueckii* were differentially expressed (Supplementary Table 5). The microbial community found here might have contributed to the sensory attributes that characterized the indoor (winery) wine (Supplementary file 3).

We do acknowledge that fermentations, flavour and aroma characteristics of wines involve complex interactions that could enhance or suppress one another. In fact, the most influence on wine aroma might be due to the combined effect of all compounds present (Ferreira, 2010). This might possibly explain why the sensory panel did not report any statistically significant difference despite the variations in volatile compounds we detected via GC-MS. Furthermore, a good number of these compounds might be below the threshold value and may not necessarily be important in the sensory attributes of wines (Ferreira, 2010). Nevertheless, while our study was limited (due to production conditions and commercial limitations as described earlier), our results indicate that the sensory and volatile components of wines can be impacted by fermentation styles and environmental conditions. Our results contribute to a deeper understanding of how the microbiome links with the sensory and chemical attributes of the final wine product.

5. Conclusion

Using grapes harvested from an organic vineyard and fermented spontaneously in two different environments, we related the microbial differences observed to the sensory and volatile compounds of the resulting wines. Here we show that both wines were comparable in many sensory attributes, but they were not the same. These small but important differences may be explained by the variability in the bacterial community. We suggest that the genus *Gluconobacter* might influence the mouthfeel (astringency/tannin) and taste (bitterness) attributes of wine.

We also report the presence of different volatile profiles found in the two wines studied here. This might mean that the fungi and bacteria, which were differentially abundant, could have direct roles in their synthesis. Admittedly, the GC-MS used here was carried out using a qualitative approach (full scan mode) to explore all compounds detected. In the future, we aim to utilize a quantitative approach (using the SIM mode) to quantify the variation in wine chemistry.

Our results also suggest the concept of "*terroir*" being extended to the fermentation stage. Despite being derived from the same batch of grapes, musts fermented indoors or outdoors differed with respect to their individual microbiomes. We determined variations in the volatile components of the respective wines, and some variation in sensory profiles, with some correlation to key members of the microbial community. In an era where commercial success in the wine industry may depend on the ability to produce diverse products for different markets (Menghini, 2015), our observations could pave the way for a novel approach based on a sound understanding of the wine microbiome; and how to manipulate it.

CRediT authorship contribution statement

Aghogho Ohwofasa: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis. **Bin Tian:** Writing – review & editing, Supervision, Resources, Investigation. **Damir Torrico:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Manpreet Dhami:** Writing – review & editing, Supervision, Methodology, Investigation. **Christopher Winefield:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Stephen L.W. On:** Writing – review & editing, Supervision, Resources, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

All supplementary figures and analysis codes used can be assessed on Github via: https://github.com/Ohwofasa1/Wine_Sensory_analysis.git

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.104562.

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