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ORIGINAL RESEARCH ARTICLE

Grapevine treatment with bagasse vermicompost changes the microbiome of Albariño must and wine and improves wine quality

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ABSTRACT

Winemaking is a well-known process comprising several steps to produce must and wine. Grape marc is a byproduct of wine production that can be vermicomposted and used as organic fertiliser. Grape marc vermicompost has a richer and more stable microbiome than grape marc alone and when added to the soil of vineyards it can improve grape production and wine quality. We compared Albariño must and wine microbiotas from grapevines treated with vermicompost derived from Albariño grape marc and controls (standard fertilisation). We hypothesised that observed microbial changes are connected to improved organoleptic properties observed in fertilised must and wine. Treated Albariño vines showed increased grape production and the final wine showed improved organoleptic properties. Metataxonomic analyses of the 16S rRNA and ITS gene regions showed that the Albariño must and wine microbiome varied in their taxonomic composition. Must bacteriotas showed no significant ($p < 0.05$) variation in alpha or beta-diversity, while wine bacteriotas and must and wine mycobiotas showed significant differences in richness and evenness, as well as in microbial structure (beta-diversity) between treated and control grapevines. Must and wine bacteriotas also showed significant ($p < 0.05$) changes in their predicted metabolic pathways. Our study suggests that changes in the abundance of specific bacterial and fungal taxa and the metabolic processes they carry out during Albariño winemaking can improve the productivity of the grapevine and the organoleptic properties of the wine.

KEYWORDS: Albariño, Microbiome, ITS, 16S rRNA, earthworms, wine

ABBREVIATIONS:
[a]Bios: Biosynthesis;
DUA: Degradation/Utilization/Assimilation;
GPME: Generation of Precursor Metabolites and Energy.

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INTRODUCTION

Winemaking is a thousand-year-old well-developed industry based on the growth of grapevines with different traits. Those traits can vary depending on the type of grape, soil composition and climate (Gilbert *et al.*, 2014b). Winemaking involves both the management of the vineyard and the elaboration of the wine in the winery. The vinification process starts with the pruning, harvesting, crushing and de-stemming of the grapes to produce the must, followed by the fermentation process, sediment decanting, maturation and stabilisation to produce the finished wine. During wine production, grape marc (a byproduct that contains the skin, stalks and seeds that remain after pressing) is generated, representing up to 25 % of the processed grape's weight (Beres *et al.*, 2017). Despite being hazardous to the environment due to the high content of the organic matter that contributes to environmental chemical and biological imbalance, the great majority of grape marc is wasted (Bordiga, 2016). Vermicomposting of grape marc has proven to be a very useful procedure that yields an organic fertiliser and grape seeds as source of bioactive compounds (Domínguez *et al.*, 2014; Domínguez *et al.*, 2017; Gómez-Brandón *et al.*, 2020). Additionally, vermicomposting of grape marc can result in soil additives that comprise richer and more stable microbial communities that significantly aid the functioning of the fertilised plants (Gómez-Brandón *et al.*, 2020; Kolbe *et al.*, 2019).

The microbial terroir of a given vineyard contributes significantly to the availability of organic matter and essential nutrients in the soil, as well as to stress mitigation and pathogen inhibition in the plant (Belda *et al.*, 2017). The microbial terroir is also unique, with different bacterial and fungal microbiomes associated with specific biogeographic regions (Bokulich *et al.*, 2014). Additionally, the microbial terroir influences the diversity of microorganisms, which, in turn, affect the fermentation processes and wine maturation (Barata *et al.*, 2012). In this regard, it serves as a reservoir of the grapevine microbiome, which exhibits complex interactions and is thought to have key influence on grape traits and wine quality (Liu *et al.*, 2017); for example, it has been suggested that the microbial communities of a vine can influence traits such as productivity and grape flavour, impacting the organoleptic characteristics of the wine (Gilbert *et al.*, 2014b).

The bioclimatic region of Galicia, located in the northwest part of the Iberian Peninsula, is characterised by mild temperatures and regular rainfall throughout the year (Lorenzo *et al.*, 2013). Albariño is the most important white grape variety of *Vitis vinifera* grown in the southwest of Galicia, the Rías Baixas region. This region currently has more than 200 wineries, yielding 34.2 million kg of grapes per year, resulting in 23.5 million liters of wine (Rías Baixas Albariño) in 2020 (Xunta de Galicia, 2021).

Given the influence of the microbial terroir on the organoleptic properties of the wine and the impact of vermicomposting on the soil and plant microbiotas, we explored a novel approach to vine fertilisation. We collected and vermicomposted the grape marc of Albariño vines and used it to supplement the soil of the same grapevines the following year. Fertilization with vermicompost improved Albariño grape production by 14 and 14.5 % in 2018 and 2019 respectively, as well as wine quality (unpublished data). Wine blind tastings carried out by the wineries also showed noticeable differences in organoleptic properties (e.g., overall increased complexity, expression, freshness and balance with better visual intensity and taste persistency), resulting in the treated wine receiving better ratings and reviews (unpublished data).

Here we used a metataxonomic approach to characterising the bacteriota and mycobiota of the must and finished wine from Albariño grapevines treated with vermicompost derived from grape marc and with controls (standard fertilisation) in two consecutive years. We tested for significant differences in the composition, structure and predicted metabolic functions of those microbiotas. We hypothesised that the microbial communities change between treatments and that these changes can improve the organoleptic properties of the fertilised must and wine. These new insights could improve our understanding of microbe-grapevine interactions and the beneficial role of vermicompost supplementation in winemaking.

MATERIALS AND METHODS

1. Experimental design, sampling and processing

The vineyard experiment was conducted in the commercial Albariño vineyard Terras Gauda located in O Rosal, Pontevedra, Spain (Latitude 41.93783, Longitude: -8.79173) in 2018 and 2019. The vines, *V. vinifera* cultivar Albariño, were 25 years old, with 1.5 m × 2.75 m intra- and inter-row spacing and trained on a trellised system. Albariño grape marc was vermicomposted and used as soil supplement for the same vines in four consecutive years (2016 to 2019). We used a complete block design, including 72 blocks of 5 plants in the same parcels. Thus, a total of 360 grapevines were divided into two groups: a) a control group, subject to the standard management practices of the commercial winery (n = 36 blocks × 5 samples), and b) a treatment group, the same as the controls but with the addition of vermicompost derived from grape marc to the surrounding soil of the vines (n = 36 blocks × 5 samples).

Terras Gauda made i) experimental Albariño must and wine (1,000 L) from the grapes of the vines fertilised with Albariño vermicompost derived from grape marc, and ii) control must and wine (1,000 L) from the grapes of vines in the same experimental plot, but without vermicompost. Both wines were made following the same standard procedures of the winery.

Three replicates of each treatment (control and treated grapevines) were taken in two consecutive harvests in 2018 and 2019, resulting in twelve samples (50 mL) of must and twelve samples of wine. Thus, a total of 24 samples was processed using the VINEO™ Extract DNA Kit

(Bio-Rad Laboratories, California, USA), following manufacturer instructions. Each sample was amplified and sequenced for the V4 region of the 16S rRNA gene (~250 bp) to characterise the bacteriota, and for the Internal Transcribed Spacer (hereon ITS) rRNA gene (250 bp) to characterise the mycobiota. We followed the Earth Microbiome Project protocols for amplification and amplicon sequencing (https://www.protocols.io/workspaces/earth-microbiome-project; Gilbert *et al.*, 2014a). Amplicon libraries were created using primers for the V4 hypervariable region of the 16S rRNA gene (forward GTGYCAGCMGCCGCGGTAA and reverse GGACTACNVGGGTWTCTAAT) and a fragment of the ITS rRNA gene (ITS1f forward primer CTTGGTCATTTAGAGGAAGTAA and ITS2 reverse primer GCTGCGTTCTTCATCGATGC). All amplicon libraries were pooled and sequenced in a single run of the Illumina MiSeq sequencing platform at the Argonne National Laboratory, Illinois. The raw sequences are available at NCBI Sequence Read Archive (SRA) database within the BioProject ID PRJNA819376.

2. Data processing and statistical analysis

DADA2 (version 1.16) was used to estimate the amplicon sequence variants (ASVs) present in each sample (Callahan *et al.*, 2016). Bioinformatics processing followed the DADA2 pipeline tutorial (https://benjjneb.github.io/dada2/tutorial.html). Briefly, we inspected the read quality profiles, and filtered, trimmed and dereplicated the reads. 16S rRNA gene forward and reverse read pairs were truncated at 145 nt, no ambiguous bases allowed, and each read was required to have less than two expected errors based on their quality scores. A midpoint rooted tree of ASVs was estimated in FastTree2 (Price *et al.*, 2010). ASVs were independently inferred from the forward and reverse of each sample using the run-specific error rates, and then the read pairs were merged. Chimeras were identified in each sample, following the DADA2 pipeline tutorial; they were removed when they were identified within a sufficient fraction of the samples in which they were present. We processed ITS reads in a similar way, but we did not trim them. Taxonomic assignment was performed against the Silva v138 and UNITE v8.2 databases for 16S rRNA gene and ITS, respectively, using the available training fastas of the databases and implementation of the RDP naive Bayesian classifier available in the DADA2 R package (Quast *et al.*, 2013; Wang *et al.*, 2007). We normalised ASV abundances using the negative binomial distribution (McMurdie and Holmes, 2014), which accounts for library size differences and biological variability. Microbial taxa showing a mean relative proportion ≥ 2 % were considered as part of the most abundant taxa in the bacteriota and mycobiota of each group. Differential abundances of ASVs between selected sample pairs were estimated using the Wald test with Cook's distance correction for outliers (DESeq2 package), rare ASVs ($N \leq 10$) removed and p-values adjusted by a false discovery rate of < 0.01.

Microbial alpha-diversity was calculated at the ASV level using Shannon, Faith's phylogenetic (PD) and Chao1 diversity indices as applied in the R phyloseq package v 1.32.0 (McMurdie and Holmes, 2013). Microbiome structure (beta-diversity) was also estimated at the ASV level using phylogenetic UniFrac weighted and Bray–Curtis distances as applied in the R phyloseq package v 1.32.0 (McMurdie and Holmes, 2013). Variation in microbial alpha-diversity between groups was assessed using linear models (lm) as applied in the R stats package (R Studio Team, 2012). Our goal was to assess whether microbial diversity varied between fertilisation methods (predictor) in must and wine across harvest years (random factor). However, since there were only two harvest years (2018 and 2019), microbial diversity was fitted as a fixed effect to control for its effect on fertilisation; hence the final lm formula was expressed as: microbial diversity ~ fertilization + harvest years. Variation in microbial beta-diversity was assessed using permutational multivariate analysis of variance (PERMANOVA) through the adonis function of the R vegan package v 2.5.7 (Oksanen *et al.*, 2008). As before, we used both cultivation and harvest year as fixed factors and ran 1,000 permutations. Dissimilarity between samples was explored through principal coordinates analysis (PCoA). Venn diagrams were done using the function venn.diagram in R software. All analyses were performed in R-studio v4.0.2 (R Studio Team, 2012) with R v 4.0.2.

Bacterial metabolic functions were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States software (hereon PICRUSt2) v 2.5.0 (Douglas *et al.*, 2019) and applying a weighted nearest sequenced taxon index (NSTI) cutoff of 0.03. Predicted functions were collapsed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway metadata (Kanehisa *et al.*, 2019). The software Linear Discriminant Analysis Effect Size (hereon LEfSe) was used to identify which predicted metabolic pathways were differentially abundant between fertilisation methods (treatment and control) in the bacteriota of must and wine. We used fertilisation method as a class, a P-value cut-off of 0.05 and a LDA effect size cutoff of 2 (Segata *et al.*, 2011).

RESULTS

1. Differences in composition of the bacteriota and mycobiota of vermicomposted and control Albariño must and wine

The total number of sequences retrieved for all samples was 503,942, with a minimum of 4,601 and a maximum of 38,577 per sample. There was a total of 23 bacterial phyla and 256 bacterial genera, and 2 fungal phyla and 75 fungal genera across all samples. Proteobacteria (63 ± 20 %) and Firmicutes (14 ± 19 %) were the most abundant bacterial phyla in all groups, while Cyanobacteria (22 ± 23 %) was highly abundant in all but one group (Figure 1A). Additionally, the phylum Bacteroidota was highly abundant in both control and treatment must groups in 2019 (2 %, Figure 1A). In the mycobiota, the phylum Ascomycota was highly abundant (96 ± 4 %) in all groups, while Basidiomycota dominated all must (7 ± 4 %) groups (Figure 1B).

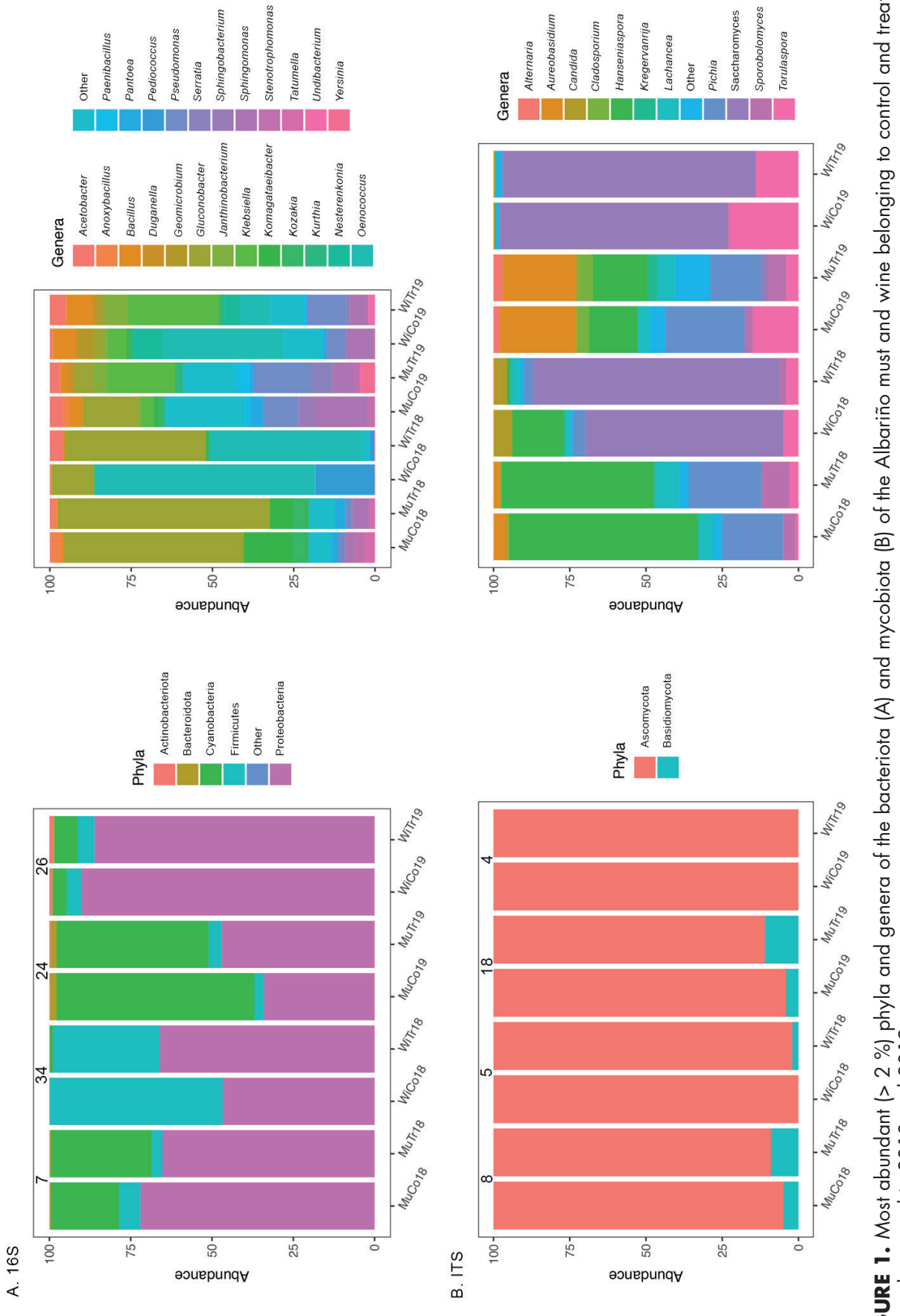

FIGURE 1. Most abundant (> 2 %) phyla and genera of the bacteriota (A) and mycobiota (B) of the Albariño must and wine belonging to control and treatment groups harvested in 2018 and 2019.

[a]Distinct bars represent the relative abundance of each taxa. Taxa that represent < 2 % of the sequences were collapsed into "Others". Numbers above bars indicate the number of shared ASVs between control and treatment groups for each grape state and harvest year, according to Wald test results. Each group of samples (n = 3) is labeled according to the state of the grape (Mu – must; Wi – wine), cultivation method (Co – control; Tr – treatment), and harvest year (18 – 2018; 19 – 2019).

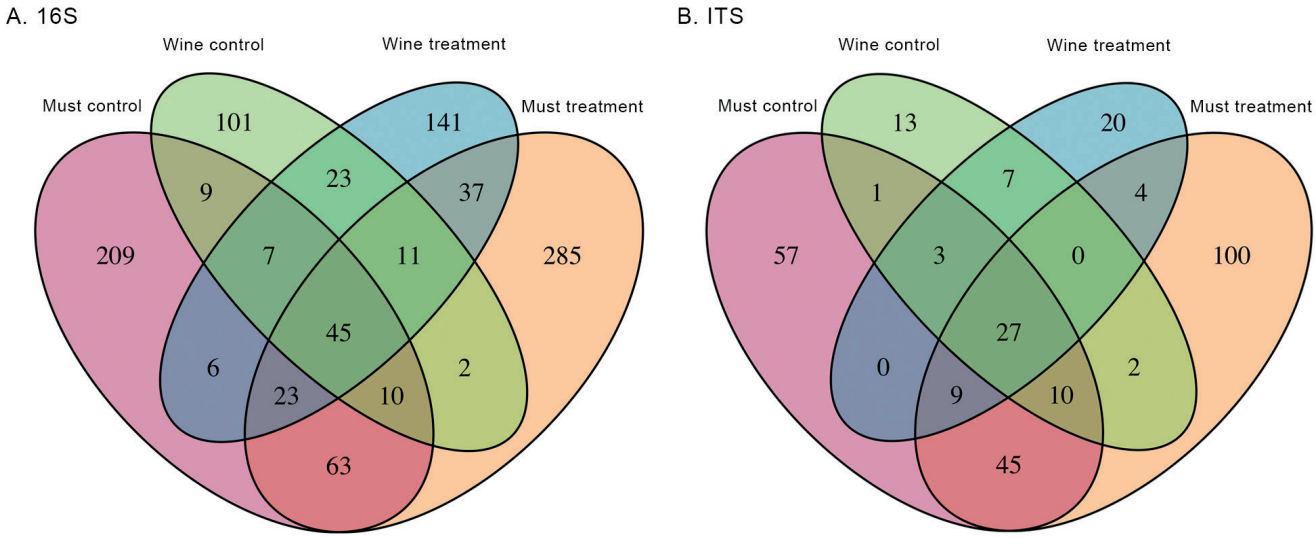

FIGURE 2. Venn diagrams showing the number of shared bacterial (A) and fungal (B) ASVs between control and treatment groups of the must and wine Albariño microbiome (n = 6).

[a]Graphs are shown for both harvest years combined.

There was high variation in genera abundances between groups in both the bacteriota and mycobiota (Figure 1). Nevertheless, it was possible to observe a distinction in bacterial genera dominance when comparing cultivation strategies (control *vs.* treatment): 4 and 10 different dominant genera in must and wine respectively (Figure 1). Different bacterial genera dominance was also observed when comparing i) grape stages (must *vs.* wine): 9 and 18 genera in 2018 and 2019 respectively (Figure 1), and ii) harvest year (2018 *vs.* 2019): 17 and 11 genera in must and wine respectively (Figure 1); see sample codes in Figure 1 legend.

Results from the Wald test revealed that between 7 and 34 bacterial ASVs and between 4 and 18 fungal ASVs varied significantly between control and treatment paired groups, depending on the grape state and harvest year ($p < 0.05$; Figure 1). When comparing fertilisation methods, the must contained 231 (62 %) and 335 (70 %) unique ASVs in the control and treatment groups respectively for the bacteriota, and 61 (40 %) and 106 (54 %) unique ASVs in the control and treatment groups respectively for the mycobiota (Figure 2). In the must bacteriota, the top unique ASVs belonged to the genera *Flavobacterium*, *Herbaspirillum*, *Novosphingobium*, *Paenibacillus*, *Pedobacter*, *Pseudomonas*, *Sphingomonas* and *Stenotrophomonas* in the treatment group, and to the genera *Acetobacter*, *Flavobacterium*, *Massilia*, *Paenibacillus*, *Pseudomonas* and *Sphingobacterium* in the control group. In the must mycobiota, the top unique ASVs belonged to genera *Zygosaccharomyces* and *Pichia* in the control and treatment groups respectively. Within the wine microbiome, there were 122 (59 %) and 207 (71 %) unique ASVs in the bacterial control and treatment groups respectively; and 26 (41 %) and 33 (47 %) unique ASVs in the mycobiota control and treatment groups respectively (Figure 2). In the wine bacteriota, the top unique ASVs belonged to the genera *Acetobacter*, *Paenibacillus*, *Pseudomonas* and *Stenotrophomonas* in the treatment group, and to the genus *Neokomagataea* in the control group. In the wine mycobiota, the top unique ASVs belonged to the genera *Saccharomyces* and *Wickerhamomyces* in the control group, and to the genera *Cladosporium*, *Filobasidium*, *Sporobolomyces* and *Wickerhamomyces* in the treatment group.

2. Differences in diversity of the bacteriota and mycobiota of control and vermicomposted Albariño must and wine

Must bacteriota and mycobiota showed higher mean values of alpha-diversity for all indices compared to the corresponding wine microbiotas (Figure 3). This was not surprising given that directed fermentation during the winemaking process is likely to reduce bacterial diversity (Bordiga, 2016). Bacterial alpha-diversity estimates did not vary significantly between the control and treatment must samples, but they varied significantly for all the indices between the control and treatment wine groups (Shannon, $F = 179$, $P = 3\text{-}7$; Faith's PD, $F = 7$, $P = 0.02$; Chao1, $F = 24$, $P = 0.001$, Figure 3A; Supplementary Table 1). Fungal alpha-diversity varied significantly for the Shannon ($F = 30$, $P = 0.0004$, Figure 3B; Supplementary Table 1) and Chao1 ($F = 7$, $P = 0.02$, Figure 3B; Supplementary Table 1) indices between control and treatment must groups, while only the Shannon diversity index showed significant differences in the wine linear model analysis ($P = 0.01$, Figure 3B; Supplementary Table 1). Accordingly, alpha-diversity mean estimates were higher in the treated groups for most of the comparisons (Figure 3B).

PCoAs showed a clear separation of the control and treatment samples along both Axis 1 and 2 for different indices (Figure 4); for example, PCoAs of wine bacteriota placed samples belonging to control and treatment groups on opposite sides of Axis 2 (Figure 4A). The same was observed for the UniFrac weighted distance of must and wine mycobiota (Figure 4B). Bacterial beta-diversity estimates did not vary significantly between control and treatment must samples, but they

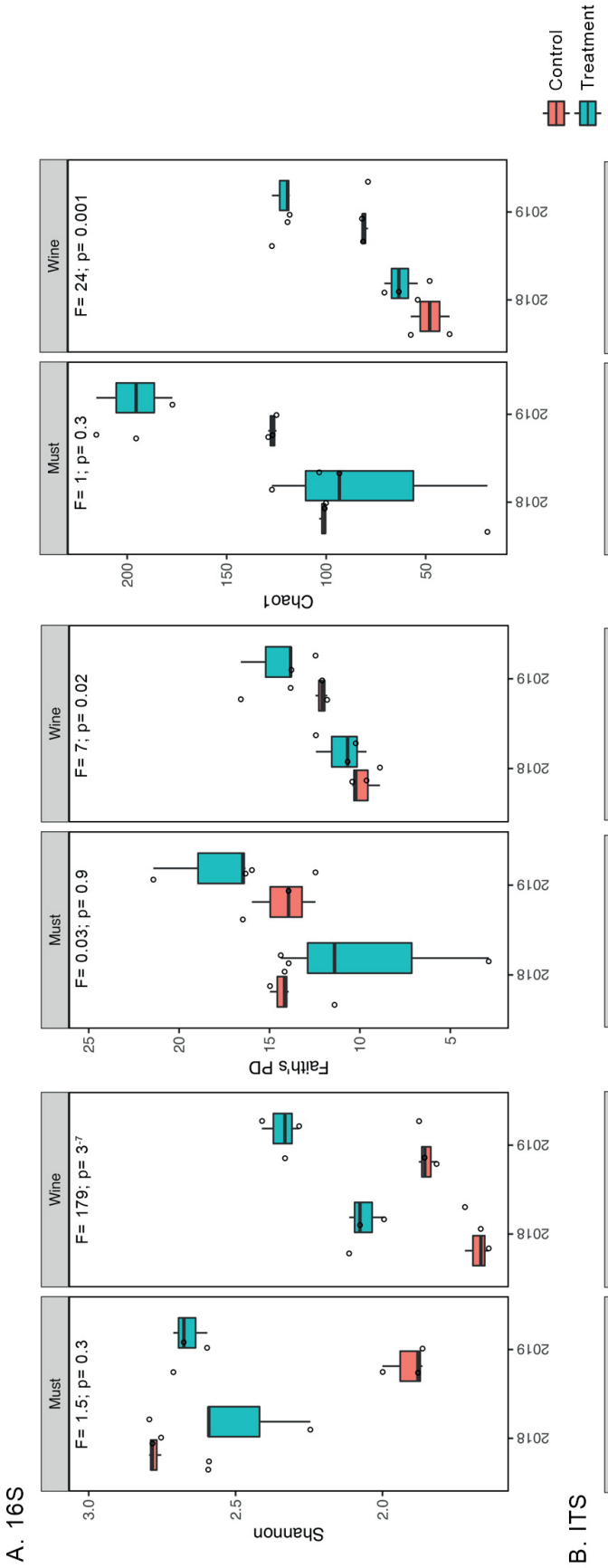

FIGURE 3. Mean values and standard deviations of Shannon, Faith's PD, and Chao1 bacterial (A) and fungal (B) alpha-diversity estimates plotted for the control and treatment groups of each grape state of Albariño in each harvest year (n = 3).

[a]Results of alpha-diversity comparisons for cultivation method are presented for each grape state, showing the F value and significance (p value).

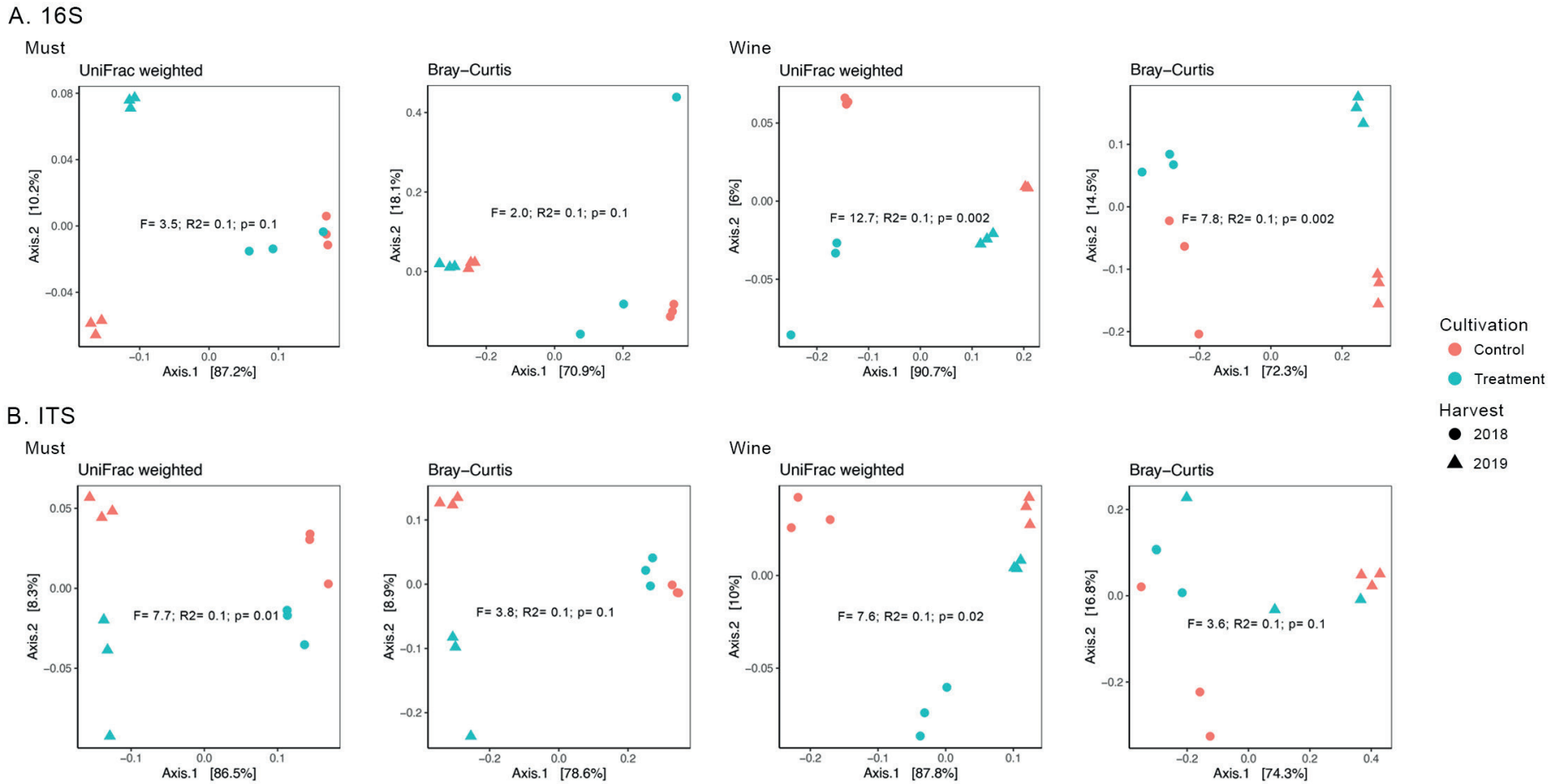

FIGURE 4. PCoA plots computed using weighted UniFrac and Bray-Curtis distances. Each dot represents a bacterial (A) and fungal (B) microbiome sample of the Albariño must and wine and is colored by cultivation method.

[a]Different shapes represent different harvest years. Results of the PERMANOVA tests for beta-diversity comparisons of the cultivation method are presented, showing the F and R2 values and significance (p value).

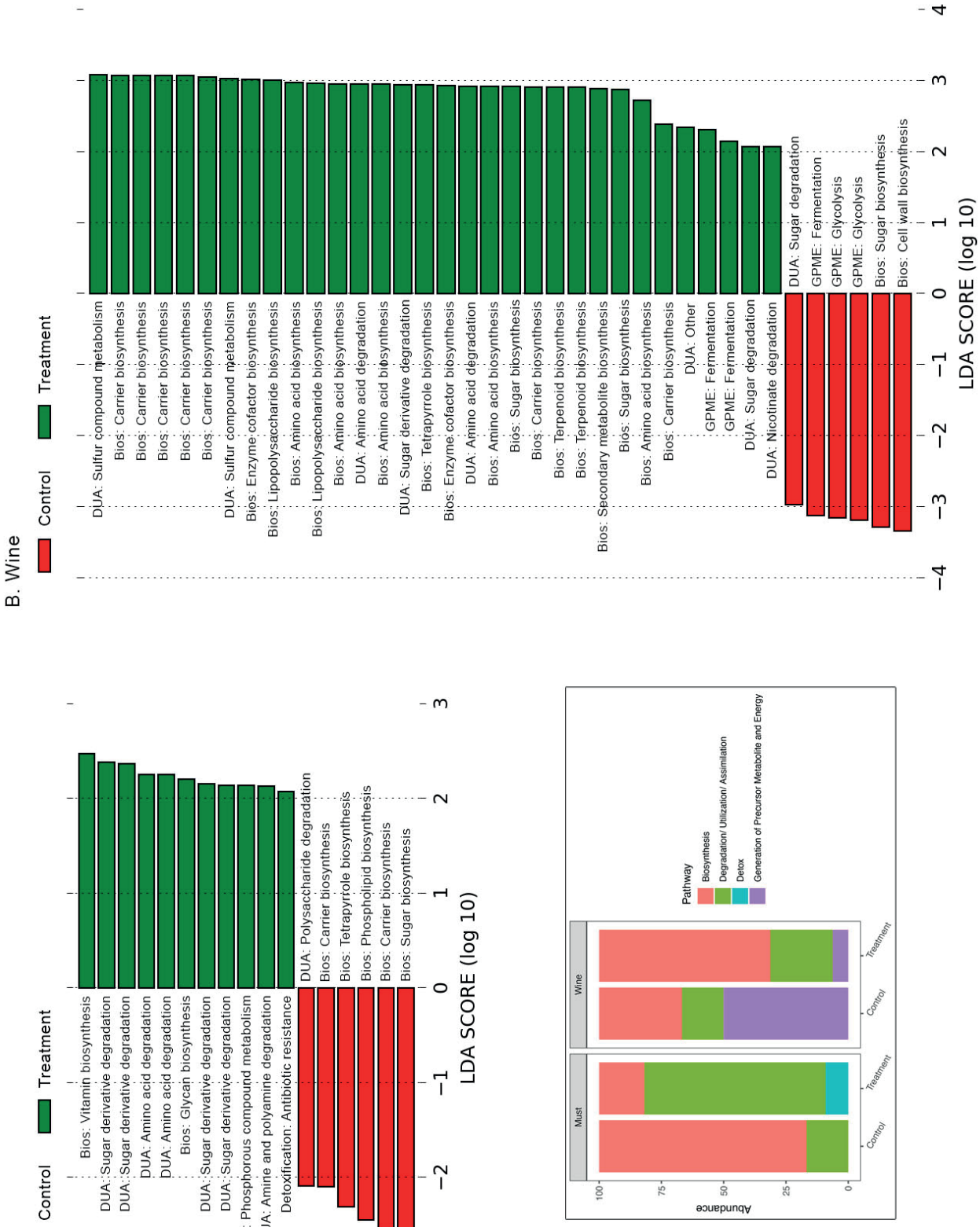

FIGURE 5. LDA score and relative frequency (inlet) of differentially abundant enriched pathways in the control and treatment groups of the Albariño must (A) and wine (B).

[a]Bios: Biosynthesis; DUA: Degradation/Utilization/Assimilation; GPME: Generation of Precursor Metabolites and Energy.

showed significant differences for both distances between the same two groups for the wine samples (PERMANOVA, $F \geq 7.8$, $P = 0.002$, Figure 4A; Supplementary Table 1). UniFrac weighted distances varied significantly between control and treatment samples in both must and wine mycobiotas (PERMANOVA, $F \geq 7.6$, $P \leq 0.02$; Supplementary Table 1), while Bray-Curtis did not (Figure 4B; Supplementary Table 1).

3. Differences in predicted bacterial functional profiles of the bacteriota of control and vermicomposted Albariño must and wine

There were 397 KEGG predicted pathways inferred in the Albariño must and wine grapes each. Linear discriminant analysis Effect Size (LEfSe) showed that 17 and 38 predicted pathways were significantly enriched in the Albariño must and wine respectively (Figure 5). Six predicted pathways were significantly enriched in the control groups of both must and wine, while 11 and 32 predicted pathways were significantly enriched in the treatment groups of must and wine, respectively (Figure 5).

Specific enriched predicted pathway categories varied between control and treatment samples in Albariño must and wine (Figure 5 inlet). Degradation/utilisation/assimilation was more abundant in the must-control, while biosynthesis was more abundant in the must-treatment. However, biosynthesis was more abundant in the wine-control, while generation of precursor metabolite and energy was more abundant in the wine-treatment. Additionally, the must-treatment group showed one enriched predicted pathway related to detoxification.

DISCUSSION

In this study, we compare taxonomic and predicted bacterial functional microbial profiles in must and wine between Albariño grapevines supplemented with vermicompost derived from grape marc and controls (standard fertilisation). In order to do this, we used 16S rRNA and ITS metataxonomics to characterise the bacteriota and mycobiota, respectively, of Albariño must and finished wine harvested in two consecutive years.

1. Vermicompost derived from grape marc changes the composition, structure and diversity of the bacterial and fungal communities in Albariño must and wine

An increasing number of studies have demonstrated the effectiveness of vermicomposting in stabilising grape marc, making it a technology readily applicable to production systems (Gómez-Brandón *et al.*, 2020). Additionally, the final vermicompost can be used as organic fertiliser, since it can promote plant growth and suppress plant diseases (Pathma & Sakthivel, 2012). In this regard, it has been shown that the microbial communities present in the vermicompost were more diverse and were associated with increases in processes related to cellulose metabolism, and synthesis of antibiotics and hormones (e.g., Domínguez *et al.*, 2019; Gómez-Brandón *et al.*, 2019; Kolbe *et al.*, 2019). The specific microbial terroir of a vineyard is thought to influence grape traits such as productivity and flavour, as well as the organoleptic characteristics of the wine, thereby improving its quality (Gilbert *et al.*, 2014b; Liu *et al.*, 2017). Thus, the microbial qualities of vermicompost used as a soil supplement in the grapevines will likely later impact the microbiome of must and finished wine during wine production. The present study has demonstrated that the bacterial and fungal communities of the Albariño wine and must were significantly altered in the grapevines treated with vermicompost derived from grape marc. Specifically, the composition, diversity and predicted function of the Albariño must and wine microbiome changed when vermicompost was used as part of the fertilisation treatment. Additionally, the majority of the ASVs retrieved from the treated Albariño must and wine microbiome were unique (61 ± 12 %), indicating that the vermicompost led to a turnover of the microbial composition during the winemaking process.

Albariño grapevines fertilised with vermicompost derived from grape marc increased their grape yield by ~14 % in 2018 and 2019 and the finished wines showed better organoleptic properties, which improved their ratings and reviews in the blind tastings carried out by the wineries (unpublished data). Interestingly, treated grapevines showed slightly different values for all the measured key chemical characteristics of the must and wine compared to standard grapevines (controls). In short, must from the treated grapevines was richer in sugars (glucose and fructose), and had lower total acidity, a slightly higher pH, lower malic acid and slightly higher readily assimilable nitrogen. Wine from the treated grapevines was slightly poorer in sugars, had slightly higher total acidity, slightly lower pH, slightly lower alcohol content, slightly lower malic acid, slightly higher volatile acidity in 2019 but slightly higher malic acid and slightly lower volatile acidity in 2018, and slightly higher both free and total sulfur dioxide. This suggests that using bagasse vermicompost as a soil additive improves wine quality without altering important chemical properties. Therefore, given that the microbial profiles of the Albariño wine and must were so strikingly different between control and treated grapevines, it seems plausible to us that changes in the number and abundance of specific microbial taxa were responsible for an increase in grape production and the subtle improvement in quality noticed in the finished wine. Microbial supplementation is a standard procedure in the winemaking industry. For example, one of the most important steps in wine production is to lower the high acidity of the must and wine (Vilela, 2017); this can be accomplished through biological deacidification by adding lactic acid bacteria or non-*Saccharomyces* yeast strains to degrade the malic acid (Van Rooyen and Tracey, 1987). Thus, adding *Schizosaccharomyces pombe* and *Lachancea thermotolerans* can help the wine to reach its potential acidity and tastiness, thus producing a final wine with more fruity notes than the control (Benito *et al.*, 2015). Our metataxonomic analysis has detected an increase in the abundance of the fungal genus *Lachancea* in the treated Albariño must, which could help to enhance the organoleptic properties of the wine.

Another study showed that application of plant growth-promoting rhizobacteria *Pseudomonas putida* to the biochar (a soil amendment) can improve the yield and quality of the *Vitis vinifera* grape (Wei *et al.*, 2020). We also observed an increase in *Pseudomonas* in the Albariño must and wine treated with vermicompost, which again underlies a positive association between certain bacterial species and grape production and wine quality. Similarly, the bacterium *Paenibacillus alvei* has been effectively used as a biocontrol agent against the tracheomycotic fungus *Phaeomoniella chlamydospora* associated with grapevine trunk disease (Gkikas *et al.*, 2021). Hence, the growth of *Paenibacillus* observed in the bacteriome of the must and wine may also represent a benefit to using grape marc vermicompost. These are just three examples of taxa that are known to positively impact winemaking; but other species of the differentially abundant genera observed here in wine and must (see Figure 1 and Results section) may also exert a positive effect on the final wine and need to be studied.

Although harvest year is not a variable of interest in this study, our analyses have also revealed its strong effect on the bacterial and fungal communities (Figures 1, 3 and 4). The climate in the Rías Baixas in 2018 and 2019 differed. Precipitation from March (sprouting) to September (harvest) in the experimental parcel was 660 L m^{-2} in 2018 and 415 L m^{-2} in 2019. Mean temperature (16.6 ºC in 2018 and 16.3 ºC in 2019) and relative humidity (78.83 % in 2018 and 76.66 % in 2019) were quite similar in both years. It is well known that microbial composition and diversity of must and wine can be also influenced by different environmental factors, such as temperature, humidity and precipitation (Gilbert *et al.*, 2014b). Hence, the observed differences in bacterial and fungal composition, structure and diversity between harvest years are likely a result of the climatic differences seen in those or previous years. Ongoing longitudinal studies by our group will help to further assess the impact of climate variation in the microbiotas and winemaking.

2. Vermicompost derived from grape marc changes the predicted metabolic function of the bacterial communities in Albariño must and wine

The changes in bacterial composition and diversity observed in the wine and must of vermicomposted grapevines were also coupled to an increase and diversification in predicted metabolic pathway diversity in both the must and wine. The differences were more remarkable in the wine, as were the taxonomic changes. Specifically, in the must, an increase in and a diversification of degradation and metabolic processes were observed in the vermicompost-treated group, suggesting a higher microbial activity during the fermentation processes in those samples. There are important compounds that are metabolised by bacteria during fermentation that produce flavour-active metabolites associated with final wine taste, such as amino acids, amines and sugars (Bartowsky and Pretorius, 2009). The observed increase in degradation of these compounds in vermicomposted must might be associated with the improved flavour of the wine described in the blind tests (unpublished data). Additionally, must with vermicompost treatment showed predicted bacterial functions related to antibiotic resistance, in line with studies showing that vermicompost has the ability to mitigate or suppress plant diseases (Atiyeh *et al.*, 2002; Lazcano and Domínguez, 2011). This benefit has been hypothesised to be provided by soil microbes through the production of antibiotic compounds (Hoitink and Boehm, 1999).

In the wine from Albariño grapevines treated with bagasse vermicompost, we observed an increase in and a diversification of predicted bacterial functions related to biosynthesis, including the biosynthesis of terpenoids and carbohydrates. Terpenoids are thought to play roles in pathogen defense and pollinator attraction in grape berries, as well as to create complex flavour and aroma in finished wines (Schwab and Wüst, 2015; Wedler *et al.*, 2015). Furthermore, carbohydrates are active substances with a general beneficial effect on live organisms due to, for example, their antibacterial and antitumor effects (e.g., Ojeda *et al.*, 2007; Rondanelli *et al.*, 2009). Thus, this evidence also suggests that an increase in predicted bacterial functional activity related to these compounds may be associated with grape marc vermicomposting during wine cultivation.

CONCLUSION

This study identified significant differences in the bacteriomes and mycobiomes of Albariño must and wine from grapevines supplemented with Albariño grape marc vermicompost compared to their control counterparts; i.e., grapevines fertilised following standard procedures only. We can conclude that the observed changes in microbial composition, diversity and predicted metabolic function in the treated Albariño grapevines contribute to increasing grape production and improving wine quality. We identified some key bacterial and fungal taxa and metabolic pathways that could act as biomarkers of wine quality or benefit winemaking if, for example, added as biological additives or via microbial selection. This has important implications for the wine industry in the shift to a circular economy and sustainable and regenerative viticulture. Thus, our results demonstrate the potential of developing an integrated cycle that allows the conversion of the bagasse produced in the wine industry into high quality vermicompost with biostimulant and vine defense properties. Sustainability is based on strategies that allow valuable products (vermicompost) to be recovered with a minimum disposal of waste streams. Application of the vermicompost to the vineyard ultimately results in the production of more "natural" wines with a distinctive character.

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