

# Diversity and Dynamics of Microbial Ecosystem on Berry Surface During the Ripening of Ecolly Grape in Wuhai, China

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## Research Article

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## Abstract

The structural and functional diversity of the microbial ecosystem on the grape surface affect the health of berries and the flavor of wines, which are also changed by many factors such as climate, weather conditions, agronomic practices, and physiological development. To understand and explore the natural characteristics of grape surface microbial ecosystem during ripening, the species composition and dynamics of fungi and bacteria communities on the skin of Ecolly grape were determined by Illumina Novaseq platform sequencing. The results showed that 2146 fungal OTUs and 4175 bacterial OTUs were obtained, belonging to 4 fungal phyla and 20 bacterial phyla, and Shannon index indicated that the fungus community had the highest species diversity at the véraison stage and the bacteria community at the harvest stage. The four dominant fungal genera during grape ripening included *Alternaria*, *Naganishia*, *Filobasidium*, and *Aureobasidium*, which accounted for 82.8% of the total fungal community, and the dominant bacterial genera included *Sphingomonas*, *Brevundimonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and *Massilia*, which accounted for 77.9% of the total bacterial community. The species richness and diversity in the grape microbial ecosystem are constantly changing during the maturation stages, and there are complex interactions and correlations between related core microbial genera, which may have an important impact on the function and ecological role of the community. This study provides a basis for understanding the natural characteristics of the microbial ecosystem on the grape surface during the grape ripening, and the sustainable production concept of the microecology driving the viticulture management system.

## Introduction

Under natural conditions, there is a complex and dynamic microbial ecosystem on the surface of grape berries, including filamentous fungi, yeasts, and bacteria, which play an important role in grape quality and winemaking (Laforgue et al. 2009; Gao et al. 2019). The phenological period of grapes, the health of berries, and the transformation of grapes into wines are intricate biochemical processes involving functional metabolism and ecological interactions among various microbes (Kačániová et al. 2020). Several studies have shown that the microbial ecosystems on the grape surfaces are affected by temperature and humidity changes, ultraviolet radiation, nutrient use, and agrochemical treatment during ripening stages (Sabate et al. 2002; Renouf et al. 2005; Kántor et al. 2017). It is worth mentioning that the heavy use of chemicals (fungicides, pesticides, etc.) and fermentation auxiliaries (commercial pectinase, SO<sub>2</sub> additive, etc.) in viticulture and vinification, in pursuit of high-yield and controlled fermentation processes, has led to a decline in species diversity in grape micro-ecosystems during grape maturation, negatively affecting the balance of plant-pathogen/plant protectant microbial communities (Carmichael et al. 2017; Wu et al. 2021), and at the same time leading to the homogenization of wine styles, obscuring the regional terroir of wines.

At present, a related trend in grape ecosystems is to minimize the use of chemical additives in vineyards practices and winemaking processes, to use natural epiphytic microbes to characterize the microbial terroir of wine regions, to explore the succession of microbial communities from grape surface to wine fermentation, and to identify the characteristic microbiota of grapes and wines. Current studies suggest that microorganisms on the berries may be considered as beneficial, neutral, or harmful to the yield and quality of the grape, affecting the ripeness of the berries and the fermentation stability of wines (Belda et al. 2017). Grapes are susceptible to infection by a variety of pathogens at the maturation stages, causing grape rot and severe yield loss, such as *Botrytis cinerea* (gray rot), *Plasmopara viticola* (downy mildew), and *Erysiphe necator* (powdery mildew) (Martins et al. 2014; Oliveira et al. 2017). Some molds can produce toxic secondary metabolites such as mycotoxins, and the common *Aspergillus spp.* and *Penicillium spp.* secrete Ochratoxin A, which can lower wine quality and affect human health (Lasram et al. 2007). Grape surface bacteria are native to the surrounding environment, and most of the environmental bacteria do not survive under fermentation conditions, but some studies suggest that the metabolic activity of the bacterial community can have a lasting effect on grape quality and yield (Portillo et al. 2016; Zhang et al. 2018). In recent years, a lot of studies have been carried out on the yeast population on the grape surface. It was found that the percolation of berry juice can increase the colonization of yeast cells, and the number of indigenous yeasts on the healthy grape surface is about 10<sup>4</sup>-10<sup>6</sup>CFU/g, mainly *non-Saccharomyces*, while the population of *Saccharomyces cerevisiae* was less on the surface. The study of Barata et al. (2012) believes that the availability of nutrients increased with the berry ripening, and the number of oxidative or weakly fermentative ascomycetes tended to be dominant approaching harvest time, while the rupture of the grape skin can increase the population of ascomycetes with higher fermentative activity and wine spoilage yeast. Most *non-Saccharomyces* not only produce a variety of volatile secondary compounds that help to improve the aroma and complexity of wines (Comitini et al. 2017) but also act as biocontrol agents for some spoilage species (such as *Lactobacillus* and *Brettanomyces*). However, in some cases, these yeast groups can also cause the production of microbial film and odors, and even cause alcohol fermentation stuck or sluggish (Malfeito-Ferreira 2011; Garofalo et al. 2015, 2016), and the natural yeast microbiome on the grape surface have a strong influence on the quality and style of wines.

Studies on the mechanism of microbial interactions and metabolic functions can help us to understand the ecological role and substance transformation of microorganisms during grape ripening and fermentation, and to monitor microbial dynamics for grape disease protection and wine flavor prediction. It has been found that microbial communities can activate plant defense pathways, induce the accumulation of pathogenesis-related proteins, and protect grapes from fungal pathogens or other biological stresses (Pinto et al. 2014). *Aureobasidium pullulans* have also been found to have antagonistic effects on other microorganisms and have even begun to be used to control harmful species (Martins et al. 2012). The succession of the natural microbial community is critical to the balance of grape ecosystems, and unique

microbial diversity is associated with specific regions, climates, and varieties (Bokulich et al. 2016; Liu et al. 2021). Therefore, we must conduct a complete survey of grape microbial ecosystems under natural conditions to understand the composition and behavior of microbial communities at the ripening stages, and to broaden understanding of the role of microbiota, thereby improving agricultural practice and control (Andreote et al. 2014), promoting the natural and healthy growth of grapes and endowing wines with natural terroir characteristics.

As an important white grape variety in China, Ecolly has the characteristics of cold resistance and *Plasmopara viticola* resistance (Wang et al. 2017; Nan et al. 2018), so far there are few studies on the fungal and bacterial communities on the surface of this variety. High-throughput sequencing technique in our experiment was used to sequence rDNAs on the surface of grape berries to understand the species composition, dynamics, and functional distribution of fungi and bacterial communities at ripening stages, to provide a basis for the study of grape microbial ecosystem and make use of beneficial microbial species to promote the sustainable and high-quality development of grape and wine industry.

## Materials And Methods

### Vineyard sites

The experimental site is located in the vineyard of the Wuhai wine region (39°38'N, 106°76'E), which is a temperate continental climate, and the average annual accumulated temperature is about 3666 °C. The grape variety sampled is Ecolly, planted in 2016, spaced 0.6\*4 meters apart, crawled cordon training. The practice of viticulture management is not to apply chemical fertilizer or pesticides in the process of planting and growing.

### Sampling of Grapes

Samples on the berry surface were collected at four stages in the grape ripening in 2020, which are Beginning of Berry Ripening (BRB), Berry Veraison (BV), Berries not quite Ripe (BQR), and Harvest-ripe (HR) (corresponding to Stages 34, 35, 37 and 38 of the improved E-L system, respectively). The microbiota was sampled when 80% of the berries in the clusters reached each ripening stage. Five samples were collected at each stage to form a composite sample, which was immediately stored in a sterile bag, then the samples were frozen in carbon ice and transported to the laboratory. The samples were stored at -80 °C before treatment.

### DNA extraction and PCR amplification

Microbial DNA was extracted using the HiPure Soil DNA Kits (Magen, Guangzhou, China) according to the manufacturer's protocols. The purity of DNA was determined by NanoDrop microspectrophotometer (Nano Drop 2000, Somerset Technologies, USA), the integrity of DNA was detected by agarose gel electrophoresis (DYY-6C, Beijing Liuyi Instrument Factory, Beijing), and then DNA samples were preserved at -80 °C.

The V3-V4 region of 16S rDNA and the ITS2 region of ITS rDNA were amplified by PCR (94°C for 2 min, followed by 30 cycles at 98°C for 10 s, 62–66°C for 30 s, and 68°C for 30 s and a final extension at 68°C for 5 min) using primer pairs 341F (5'-CCTACGGGNGCWGAG-3') and 806R (5'-GGACTACHVGTGGATCTAAT-3'); ITS3\_KYO2 (5'- GATGAGAGYACAGYRAA-3') and ITS4 (TCCTGCTTATATGATATGC). PCR reactions were performed in triplicate 50 µL mixture containing 5 µL of 10 × KOD Buffer, 5 µL of 2 mM dNTPs, 3 µL of 25 mM MgSO<sub>4</sub>, 1.5 µL of each primer (10 µM), 1 µL of KOD Polymerase, and 100 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AMPure XP Beads (Beckman Agencourt, USA) according to the manufacturer's instructions and quantified using ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (PE250) on an Illumina Novaseq 6000 platform according to the standard protocols.

### Sequence Quality Control and Clustering

The raw reads were processed using FASTP (version 0.18.0), filtering out sequences containing more than 10% unknown bases and of low quality (Q < 20) from the 5' end of the sequence, and Paired-end clean reads were merged as raw tags using FLSAH (version 1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 20%. Noisy sequences of raw tags were filtered and clean tags were clustered into operational taxonomic units (OTUs) of ≥ 97 % similarity using the UPARSE (version 9.2.64) pipeline. All chimeric tags were removed using the UCHIME algorithm and finally obtained effective tags for further analysis. The tag sequence with the highest abundance was selected as a representative sequence within each cluster.

### Data analysis

The representative OTU sequences were classified into organisms by a naive Bayesian model using RDP classifier (version 2.2) based on SILVA database (version 132) and UNITE database (version 8.0), with the confidence threshold value of 0.8. The distribution of OTUs during grape ripening is illustrated by the Venn Diagram package in R (version 1.6.16). The stacked bar plot of the community composition was visualized in the R project ggplot2 package (version 2.2.1). The microbial α-diversity index was calculated through QIIME (version 1.9.1). The beta diversity of different microbial communities was calculated by principal coordinate analysis (PCoA) using the Unweighted Unifrac distance index, and the

statistically significant differences were determined by Anosim tests using the R project Vegan package (version 2.5.3). Using Simca13.0 and Origin2021 software to characterize the interrelated information of core microbial genera, and functional information of fungi and bacterial OTUs are predicted and analyzed through the FUNGuild (version 1.0) database and the PICRUSt 2 (version 2.1.4) database.

## Results

### Sequencing data and microbial diversity

A total number of 1,555,170 fungal high-quality reads and 1,527,292 bacterial high-quality reads were generated by Illumina Novaseq 6000 sequencing, with an average of 129,598 fungal reads and 127,274 bacterial reads per sample (**Table S1**). After further quality control of the raw reads and removal of chimeras, the clean tags were clustered to obtain 2146 fungal OTUs and 4175 bacterial OTUs under a similarity threshold of 97% (**Table S2**). According to results from the experiment, the number of fungal OTUs was the highest at the BRB stage (34.4%), followed by the HR stage (23.5%) and BV stage (22.9%), while the number of bacterial OTUs was the highest at BV Stage (28.2%), followed by HR stage (25.7%) and BQR stage (23.3%). As can be seen from **Fig. 1**, there are 28 shared OTUs in the fungal communities and 118 shared OTUs in the bacterial communities during grape ripening. It shows that there are not only shared OTUs on the surface of the grape during the ripening process, but also specific OTUs at each ripening stage. At the same time, we found that the total amount of OTUs in the bacterial community was higher than that in the fungal community. Except that the number of fungal OTUs at the BRB Stage was higher than that of bacteria, the number of bacterial OTUs in other stages was higher than that of fungi, indicating that the species richness and diversity of the bacterial community were higher than that of the fungal community.

The Shannon and Simpson indices of the alpha-diversity index were used to reflect species diversity in fungal and bacterial communities, and the Chao1 and ACE indices were used to measure species richness (**Table 1**). Sequencing data showed that the coverage rate of fungi and bacteria community reached more than 99.8%, which makes clear that the quantity of the data was sufficient and could reflect the microbial community diversity of each mature stage. The Shannon diversity index showed that the fungal community had the highest diversity at the BV stage and the lowest at the HR stage, while the bacterial community had the highest diversity at the HR stage and the lowest at the BQR stage. The species richness of bacterial communities is highest at the BV and lowest at the HR stage. The diversity of the fungal community increased from the BRB stage to BV stage, and then decreased gradually, while the diversity of bacterial community decreased gradually from the BRB stage, but increased greatly approaching harvest ripening, there was no significant difference in the diversity of fungal and bacterial communities during maturation stages.

**Table 1** Evaluation of species diversity and richness of fungal and bacterial communities at different stages of grape ripening

### Microbial community structure

The microbial ecosystem on the surface of grape during ripening is evolving dynamically, and community substitution can have an important effect on microbial community habitat and grape quality. Principal Coordinate Analysis (PCoA) based on Unweighted Unifrac distance index was used to study sample relationships and microbial community structure differences during the mature stage of berries (**Fig. 2**). The results displayed that the contribution rates of PCo1 and PCo2 in fungal communities were 30.59% and 22.16%, respectively, with a total interpretation of 52.75%, and the contribution rate of PCo1 and PCo2 in bacterial communities was 20.03% and 17.31% with a total interpretation of 37.34%, respectively.

The fungal community samples in each mature stage were separated, and there were significant differences in the structure of the fungal community (Anism,  $R = 0.898$ ,  $P = 0.001$ ). The distribution of fungal samples at the BRB stage has a certain distance compared with other stages, and the community structure is quite different from other stages. On the contrary, the fungal samples from the HR and BV stages are relatively close and have similarities in the community structure. In addition, some fungal sample points indicate that the community structure in the BV, BQR, and HR stages are similar, reflecting the succession of the fungal community structure.

The results also showed that the differences in bacterial community structure during the maturation stages are smaller than those of fungi, but the bacterial communities in each stage also reflect moderate differences (Anism,  $R = 0.676$ ,  $P = 0.001$ ). It should be noted, however, that the bacterial community structure evolved during the pre-harvest maturation stage in the direction of PCo1, but the samples from the HR stage were relatively close together with that of BRB, and the community structure may have changed significantly during the late maturation.

### Microbial community composition during maturation stages

#### Fungal community composition

Except for unclassified groups, all fungi samples belonged to 4 phyla, 132 families, and 210 genera. At the phyla level, *Ascomycota*,

Ecolly Grape		Fungi					Bacteria				
Maturation Stage	Sample ID	Shannon	Simpson	Chao1	ACE	coverage %	Shannon	Simpson	Chao1	ACE	coverage %
BRB	WEG1-1	3.3440	0.8138	266.05	271.64	99.96%	4.5316	0.9150	422.44	406.96	99.92%
	WEG1-2	3.3756	0.8156	287.64	301.56	99.94%	4.3629	0.9030	436.97	432.81	99.92%
	WEG1-3	3.5844	0.8308	496.76	490.95	99.90%	4.3801	0.9104	607.32	561.37	99.88%
BV	WEG2-1	4.5576	0.9406	200.05	204.94	99.94%	4.1172	0.8455	722.81	799.47	99.82%
	WEG2-2	3.2503	0.7733	177.81	170.14	99.94%	4.0150	0.8370	614.74	626.51	99.86%
	WEG2-3	3.4598	0.8496	228.00	228.83	99.93%	4.0896	0.8408	782.85	698.39	99.85%
BQR	WEG3-1	4.1221	0.9233	192.75	177.23	99.95%	2.6844	0.5929	650.76	694.73	99.85%
	WEG3-2	2.9405	0.7771	167.67	139.57	99.95%	5.8616	0.9684	586.07	503.30	99.91%
	WEG3-3	3.9211	0.9227	169.50	167.90	99.95%	2.7506	0.5981	520.40	529.55	99.87%
HR	WEG4-1	3.0063	0.8153	230.68	245.81	99.94%	4.6748	0.9313	456.10	447.17	99.90%
	WEG4-2	3.7589	0.8668	178.27	176.25	99.96%	4.6615	0.9294	501.02	505.80	99.90%
	WEG4-3	3.0912	0.8317	205.25	215.01	99.92%	4.7162	0.9310	488.00	494.83	99.91%

*Basidiomycota*, *Mucoromycota*, and *Mortierellomycota* were found, and *Ascomycota* and *Basidiomycota* were the main fungal phyla in the maturation stages (Fig. 3a). The relative abundance of *Ascomycota* was dominant in the pre-harvest ripening stages but decreased from 99.8% at the BRB stage to 70.5% at the BQR stage and 46.7% at the HR stage. Inversely, the relative abundance of *Basidiomycetes* showed a gradually increasing trend during the maturation process. Although the abundance was less than 1% during the BRB stage, it continued to increase thereafter, and the relative abundance reached 52.1% during the HR stage, becoming the dominant fungal genus.

At the fungal genera level, the top 8 genera in relative abundance include *Alternaria*, *Naganishia*, *Filobasidium*, *Aureobasidium*, *Fusarium*, *Acremonium*, *Aspergillus*, and *Styachybotrys* (Fig. 3c). It can be seen that *Alternaria* is the dominant fungal genus on the grape surface before harvest ripening, but its relative abundance continues to decrease throughout the maturation, from 36.8% at the BRB stage to 15.4% at the HR stage. We also observed that the relative abundance of *Naganishia*, *Filobasidium*, and *Aureobasidium* increased gradually during maturation, reaching 26.3%, 24.8%, and 7.1% respectively at the HR, which distinctly became the dominant fungal microbiota. In addition, *Aspergillus* mainly existed at the BV stage with a relative abundance of 5.93%, while a small amount of *Fusarium* was detected at the BQR stage with a relative abundance of 8.9%.

### Bacterial community composition

All bacterial samples belong to 20 phyla, 210 families, and 404 genera except for unclassified taxa. The bacterial phyla with the highest relative abundance rankings were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* (Fig. 3b). *Proteobacteria* was the dominant bacteria in each stage, the relative abundance reached 65.9%-93.8%, and the number of *Firmicutes* was relatively high during the BQR stage, and the relative abundance reached 23.8%. *Bacteroides* and *Actinomycetes* existed in a small amount before grape ripening, but *Bacteroidetes* disappeared at the HR stage.

At the bacterial genus level, the top 8 genera in relative abundance are *Sphingomonas*, *Brevundimonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Massilia*, *Roseomonas*, *Methylobacterium*, *Caulobacter*, and *Luteimonas* (Fig. 3d). The distribution of the bacterial community on the grape surface during the BRB stage was relatively uniform, and *Brevundimonas* (22.2%), *Sphingomonas* (13.5%), *Roseomonas* (10.8%), and *Luteimonas* (8.9%) were detected. However, the relative abundance of *Sphingomonas* during the BV and BQR periods reached 46.3% and 42.8%, which had a clear advantage before harvest. In addition, there were also some *Methylobacteria* (6.4%) and *Massilia*

(6.7%) in these two stages. At harvest ripening, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, which had previously been less abundant, suddenly became the dominant genus, with a relative abundance of 26%, and some bacterial genera such as *Brevundimonas* (14.3%), *Sphingomonas* (10.4%), and *Caulobacter* (8.7%) were still existing.

### Correlation analysis of core fungal and bacterial genera

The shared genera in the microbial community that rank the top 10 in relative abundance are defined as the core microbiota on the berry surface, with 21 shared genera of fungi and 63 shared genera of bacteria present during ripening stages were found (Fig. 4a, b) (Table S3). The relative abundance of related core genera shows significant differences, and *Alternaria* and *Sphingomonas* are the most important core fungi and bacteria during mature stages and they are not only ubiquitous, but also abundant (Fig. 4c, d). Other core fungal genera include *Naganishia*, *Filobasidium*, *Aureobasidium*, *Fusarium*, *Aspergillus*, *Comoclathris*, *Candida*, *Chaetomium*, and *Penicillium*, while other core bacterial genera include *Brevundimonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Massilia*, *Roseomonas*, *Methylobacterium*, *Caulobacter*, *Ralstonia*, *Chryseobacterium*, and *Acinetobacter*.

The association between core fungi and bacteria was analyzed using an O2PLS model to investigate the interactions between core microbiota that play a major role in grape maturation (Fig. 5). Biplot is a centralized display of the score scatter plot and loading scatter plot, which can be used to judge whether the combination of the two sets of data can reflect the characteristics of samples and to judge the relevance and influence degree of different core genera. Biplot combined with the correlation matrix (Table S4) shows that the distribution of fungal genera is scattered and mainly negatively correlated, but the negative correlation is weak, whereas the three fungal genera, *Filobasidium*, *Naganishia*, and *Aureobasidium*, all clustered together, have a stronger positive correlation, while the negative correlation between *Aureobasidium* and *Comoclathris* was the strongest. For Bacteria genera, the distance between *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Caulobacter* is the closest, and the positive correlation between them is more than 0.99. *Ralstonia* and *Acinetobacter* had a positive correlation of 0.98, and the same was true for *Roseomonas* and *Chryseobacterium*. However, the two bacteria with the highest relative abundance, *Sphingomonas*, and *Brevundimonas*, showed the strongest negative correlation relationship in bacterial community, but the degree of negative correlation was only 0.63.

Figure 5 also reflects the correlation between the fungal genera and the bacterial genera. We can observe that *Fusarium* and *Massilia* are relatively close in position, and the positive correlation between them reaches 0.86. Moreover, the correlation between the fungal genus *Filobasidium* and two bacterial genera including *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Caubacloter* reached 0.9, showing a high positive correlation. However, the negative correlation between *Filobasidium* and *Methylobacterium* is greater than 0.6, which may show a certain antagonistic effect. On the whole, the positive correlation between the microbial community is stronger than the negative correlation, and the synergy among some genera may be more significant.

### Functional prediction of microbial communities

The fungal and bacterial OTUs were compared to the FUNGuild and PICRUSt2 databases to evaluate the potential function information of fungal and bacterial communities at different maturity stages. The results indicated that FUNGuild provides 7 trophic and 51 guild analyses of the fungal community at different stages, and Animal pathogens-endophytes-phytopathogens-wood saprophytic fungi are common fungal community functions during the maturation stage, but their relative abundance decreases gradually (Fig. 6). Conversely, the functional abundance of Undefined Saprotroph and Animal Pathogen-Endophyte-Epiphyte-Plant Pathogen-Undefined Saprotroph showed an increasing trend, and the functional abundance was higher at the HR stage. At the same time, it is found that the functional abundance of Animal Pathogen-Endophyte-Fungal Parasite-Plant Pathogen-Wood Saprotroph and Wood Saprotroph was higher at the BRB stage, and Animal Pathogen was the main functional type of the fungal community during the BV stage. However, Undefined Saprotroph is the main functional one of the fungal communities in the HR stage, and the changes of the fungal community function in different maturation stages affect grape berries quality and health.

Based on 16S rRNA, the PICRUSt2 database was used to predict the function of bacterial communities, and 32 functional types of Level 2 in bacterial communities were found at different ripening stages (Fig. 7). The relative abundance of the main bacterial functional types showed a trend of first decreasing and then increasing as grape mature. Metabolism is the main functional type of bacterial communities, mainly including amino acid metabolism, carbohydrate metabolism, and metabolism of cofactors and vitamins, and there is also the metabolism of terpenoids and polyketides, lipid metabolism and energy metabolism, etc. In addition, some bacterial functions are important at different times, such as Replication and Repair, Cell Motility, and Signal Transduction are more abundant at the HR stage, while the relative abundance of Transport and catabolism is lowest at HR and highest at the BRB stage. At the same time, it was found that Environmental adaptation had the lowest functional abundance during the BV stage and the highest at the HR stage.

## Discussion

High-throughput sequencing was used to reveal the diversity and dynamics of microbes on the surface of the Ecolly grape during ripening, which is of great significance to understand the relationship between grape micro-ecosystem and berry quality and the balance between plant pathogens and beneficial microbiota. The dynamic information of the epidermal microbiome of this hybrid grape variety widely planted in China is seldom studied before. Understanding the characteristics of grape microbial ecosystems in their natural state can guide viticulture practices, manage the epiphytic microflora of berries, promote grape berry health and wine production, and thus highlight the natural terroir of grapes and wines (Gilbert et al. 2014; Bokulich et al. 2014).

The Shannon index showed that the diversity of the fungal community is highest at the BV stage and lowest at HR stage, while the bacterial community diversity is highest at the HR stage and lowest at the BQR stage, but there was no significant difference between fungal and bacterial community diversity, which consistent with Kecskeméti's research on the microbial community of Riesling grapes during grape ripening (Kecskeméti et al. 2016). However, in our study, the community structure of fungi and bacteria are separated at each ripening stage and have significant differences. We suggest that the fungal and bacterial communities may respond differently to physiological development during the maturation process, and the microbial community is susceptible to the influence of the external environment. Véraison is indeed a critical time point for grape metabolism and growth, when anthocyanin production and accumulation, pectin and cellulose degradation, acidity reduction and sugar accumulation, and berry softening, all of these factors create a more favorable environment for grape epidermal microbial colonization (Liu et al. 2020). Furthermore, heavy rainfall during the late-ripening may have led to a decrease in fungal and bacterial abundance on the grape surface and a significant recovery in bacterial abundance at the HR stage, but this weather change may have had a lasting impact on the fungal community. Many studies have shown that the climate, soil, farming practices, and grape physiology of vineyards in specific regions may affect the microbial composition and quantity on the grape surface. However, few studies have been conducted on the microbial ecosystems of grape vineyards in China (Kioroglou et al. 2019; Mezzasalma et al. 2018). As we all know, grapes are susceptible to filamentous fungi when they are immature, but the growing season in the Wuhai region is drier, reducing the colonization of common pathogenic fungi such as *Plasmoviticola* (downy mildew), *Erysinecph* (powdery mildew), and *Botrytis cinerea* (grey rot) on the surface. The absence of *Saccharomyces cerevisiae* on the surface of the berries during grape ripening suggests that the number of this species on the intact and healthy berries is indeed small (Kántor et al. 2016).

The sequencing results showed that *Alternaria* is ubiquitous during ripening and is the most abundant core fungus, but its number is decreasing, which is consistent with the Bau et al. (2005) study of ochratoxin production during grape growth in Spain. The spores of *Alternaria* are more likely to survive in arid climates, so the higher abundance of *Alternaria* in Wuhai is a reasonable phenomenon. Studies have shown that *Alternaria sp.* can secrete mycotoxins such as alternariol monomethyl ether (AME) and tenuazonic acid (TA) (Prendes et al. 2021), which can cause grape black spot disease-causing decay and damage to grape berries, but *Alternaria* is not the dominant fungus at HR stage in this experiment, and grape berries have no obvious disease. *Naganishia* and *Filobasidium* are both oxidized *Basomidiycota* yeasts that are common on the berry surface and are neutral and harmless to the grape, and they have extracellular enzyme-producing activity. For example, *Filobasidium capsuligenum* produces pectinase and remains active in the wine environment (Merín et al. 2014), both of which tend to increase in number during grape ripening and become dominant during the HR stage. *Aspergillus* and *Penicillium*, which are abundant in humid climates, were able to metabolize the toxic secondary metabolite ochratoxin A, a toxin that affects the succession of fermented species and can persist in wines to harm consumers (Cordero-Bueso et al. 2017), but their numbers were small in this study. The relative abundance of *Aureobasidium* increases during grape ripening, and *Aureobasidium pullulans* is the dominant species in the genus and a beneficial fungus of grapes. *Aureobasidium pullulans* not only can be used as an effective biological control agent against post-harvest pathogens (such as *Botrytis cinerea*), but also can produce pectinase,  $\beta$ -glucosidase, and tannase in the fermentation process, significantly affecting the color, aroma characteristics, and clarification efficiency of wine (Onetto et al. 2020).

*Sphingomonas* is the core dominant bacterial genus in grape ripening, which has higher abundance at the BV and BQR stage and lower abundance at the HR stage. Bokulich et al. (2016) found that *Sphingomonas* and *Methylobacterium* can survive in wine fermentation environments and can predict with high accuracy the presence of C6 acid, ester, and lactone in Chardonnay wines, but the effect of bacteria on fruit and wine fermentation remains unclear, except acetic bacteria and *Oenococcus oeni*. *Brevundimonas* are abundant during the BRB and HR stage, and some species of *Brevundimonas* can secrete extracellular protease (Chaia et al. 2000). *Allorhizobium-neorhizobium-pararhizobium-rhizobium*, which belongs to *Rhizobiaceae*, mainly exists in the soil of vineyard and has the effects of nitrogen fixation and antibiotic production (Zarraonaindia et al. 2015). This bacterial genus is the dominant genus at the HR stage, which may be due to the soil splashing on the berry surface caused by the pre-harvest rainfall, leading to changes in the microbial habitat of grape skin, resulting in changes in the composition of the bacterial community. This also indicates that the soil of the vineyard may be the main source of grape-related bacteria, and the microbial ecosystem is affected by the vineyard environment (Ma et al. 2018; Ramírez et al. 2019).

The interaction between microbes affects the survival and balance of grape probiotics and pathogenic microorganisms, and the cooperation and antagonism of different species and their metabolites regulate the function and phenotypic characteristics of the grape ecosystem. As the grapes mature, the increase in the surface area of the berry and the penetration of effective nutrients increase the competition between the fungi and bacterial communities that colonize the surface of the grape, which makes the abundance of some microbiota dominant. The core

microbial association analysis of this study shows that the negative correlation between fungi and bacterial communities is weak, while the positive correlation between some genera is strong, and there may be a synergistic effect between these genera. Biocontrol agents are part of the natural microbial community present in the grape, understanding the structure and functional diversity of microbial communities on fruit surface is the basis for promoting the application of local microbial antagonists in viticulture practices or management systems (Kecskeméti et al. 2016). The dynamic changes of fungi and bacteria on the surface of the fruit are affected by external biotic and abiotic factors, which also affect the quality of the grape health and the wine fermentation process (Mezzasalma et al. 2017). In this study, the diversity and dynamic change of grape epidermal microbial ecosystem in the Wuhai region is a preliminary study at the mature stage, but the dynamic characteristics of natural microbial needs to be analyzed in combination with the climatic conditions of the region to fully understand the terroir characteristics of grapes and wines. Therefore, we will further study the relationship between climate and soil factors and grape microbial communities, explore the metabolic mechanism of core microorganisms and the interaction between berries and epiphytic microorganisms to manage the planting system and practice of vineyards, promote the production of good metabolites by beneficial microorganisms and achieve sustainable and high-quality development of the grape industry.

## Declarations

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### Conflicts of interest/Competing interests:

The authors declare that they have no conflict of interest.

### Availability of data and material:

All data generated or analysed during this study are included in this published article [and its supplementary information files].

**Code availability:** Not applicable

### Authors' contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Yinting Ding], [Ruteng Wei], [Lin Wang] and [Chenlu Yang]. The first draft of the manuscript was written by [Yinting Ding] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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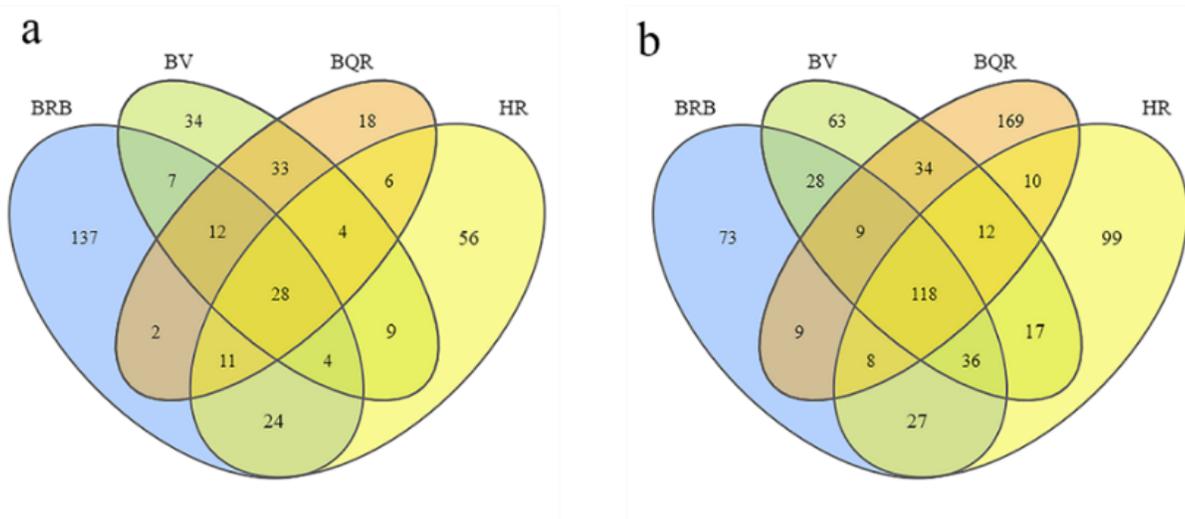
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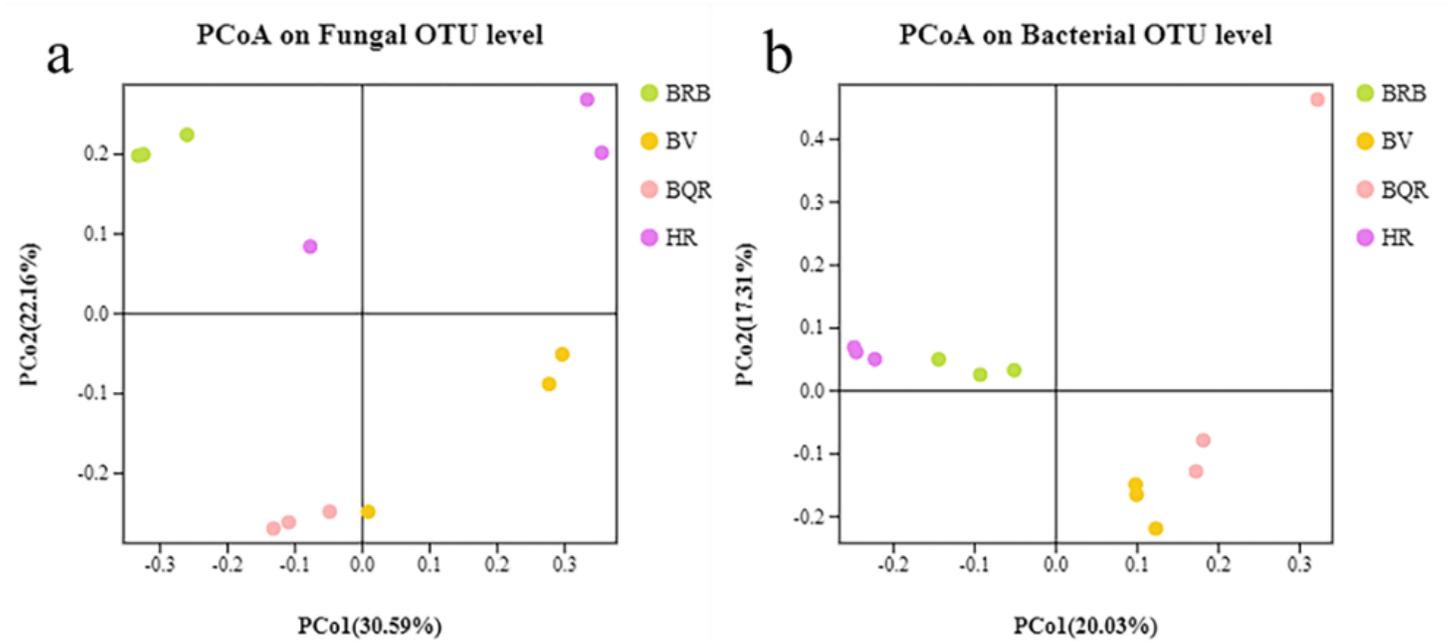
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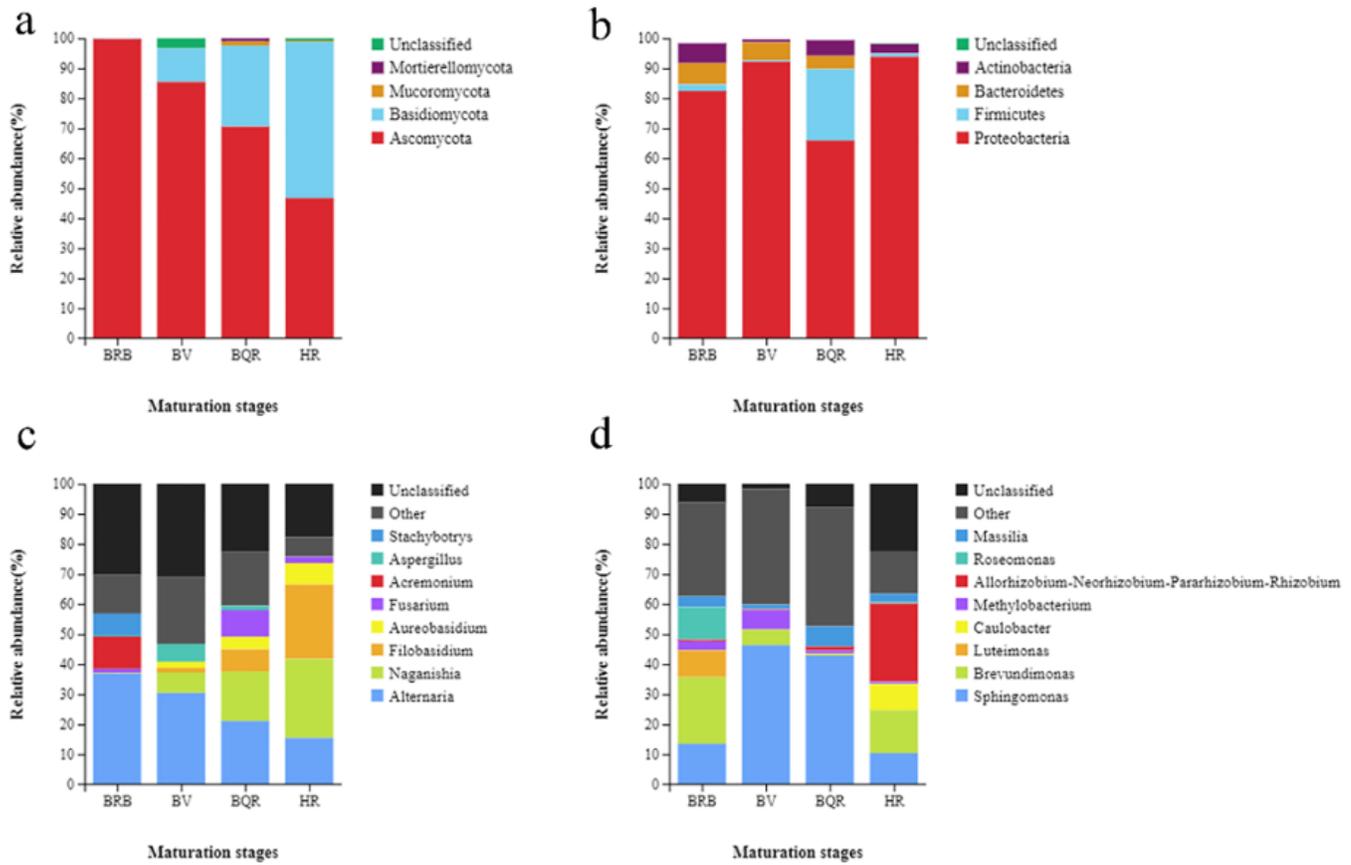
## Figures



**Figure 1**  
 Venn diagram of the number and distribution relationship of OTUs on the surface of Ecolly grape at different maturation stages. a: Fungi; b: Bacteria

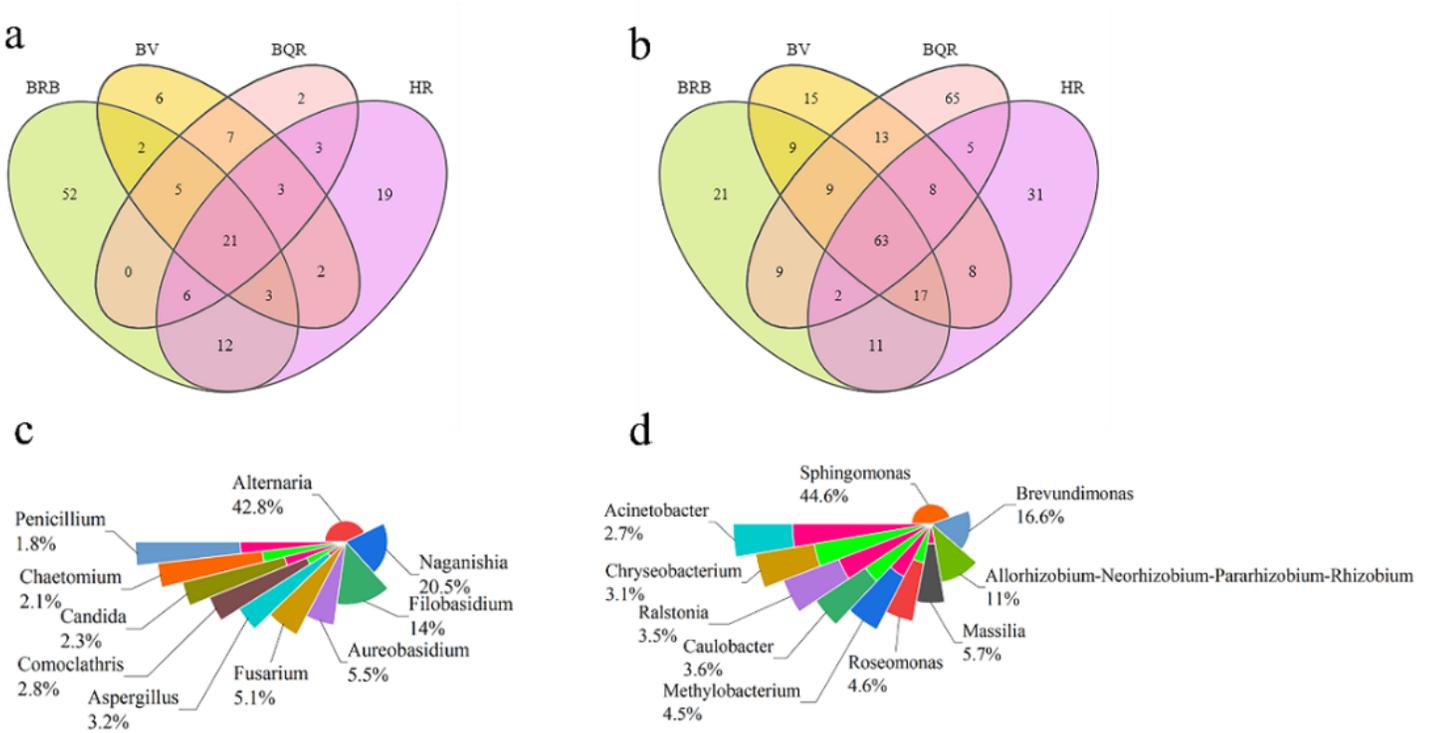


**Figure 2**  
 Principal coordinate analysis (PCoA) based on unweighted unifrac index of the microbial community structure on the surface of Ecolly grape at different stages of maturity. a: Fungi; b: Bacteria



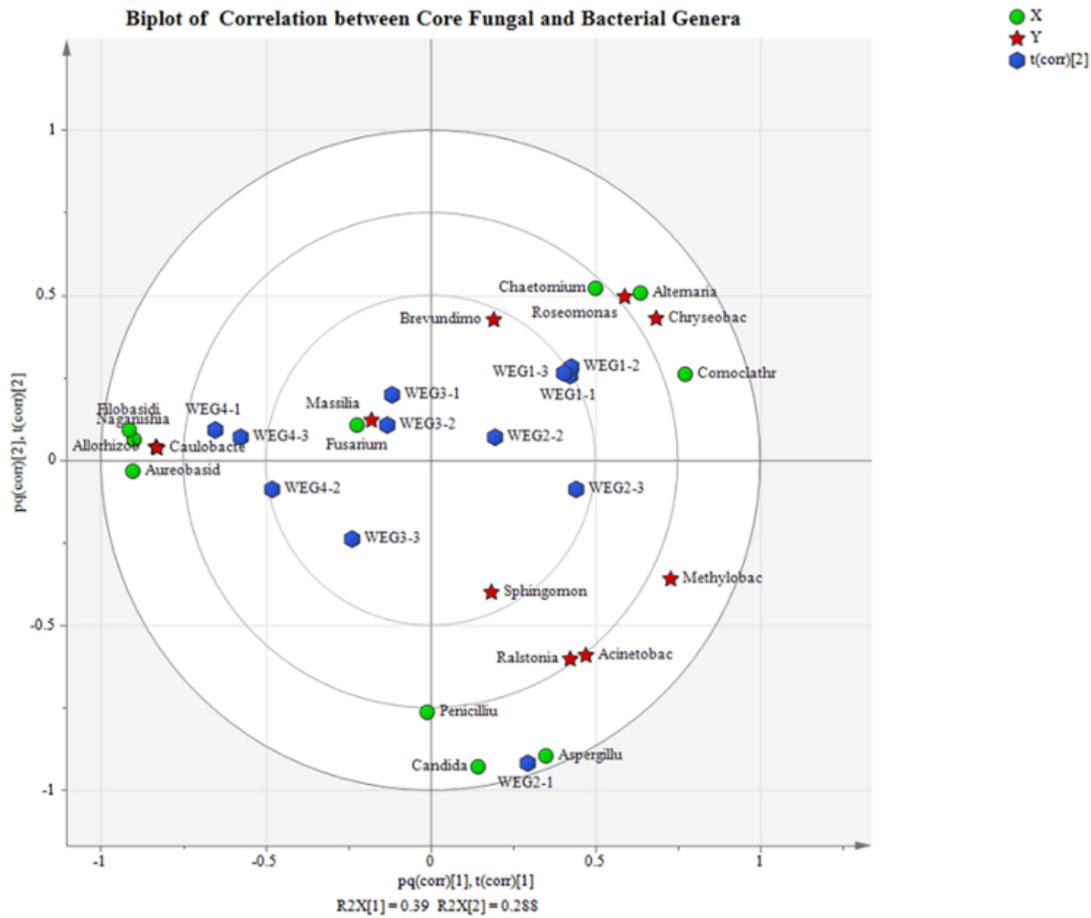
**Figure 3**

The relative abundance of microbes on the surface of Ecolly grape from different maturation stages at the Phyla (a/b) and genus (c/d) level. (a/c: fungi; b/d: bacteria)



**Figure 4**

Venn diagrams of epiphytic fungi and bacterial genera of Ecolly grape berries at different stages of maturity and the corresponding core microbial community composition. (a) Venn diagram of fungal genus at the mature stages; (b) Venn diagram of bacterial genus at mature stages; (c) Rose diagram of the composition of core fungal community during maturity; and (d) Rose diagram of the composition of the core bacterial community during maturity



**Figure 5**

Biplot diagram of the correlation based on O2PLS between core fungi and bacterial genera during maturity. WEG: Ecolly grape in Wuhai; WEG 1: Beginning of Berry Ripening; WEG 2: Berry Veraison; WEG 3: Berries not quite Ripe; WEG 4: Harvest-ripe. - 1, - 2 and - 3, replicates, 1, 2 and 3. The blue hexagon represents the samples of the microbial community, the green cycle represents the core fungus genus, and the red 5-point star represents the core bacterial genus

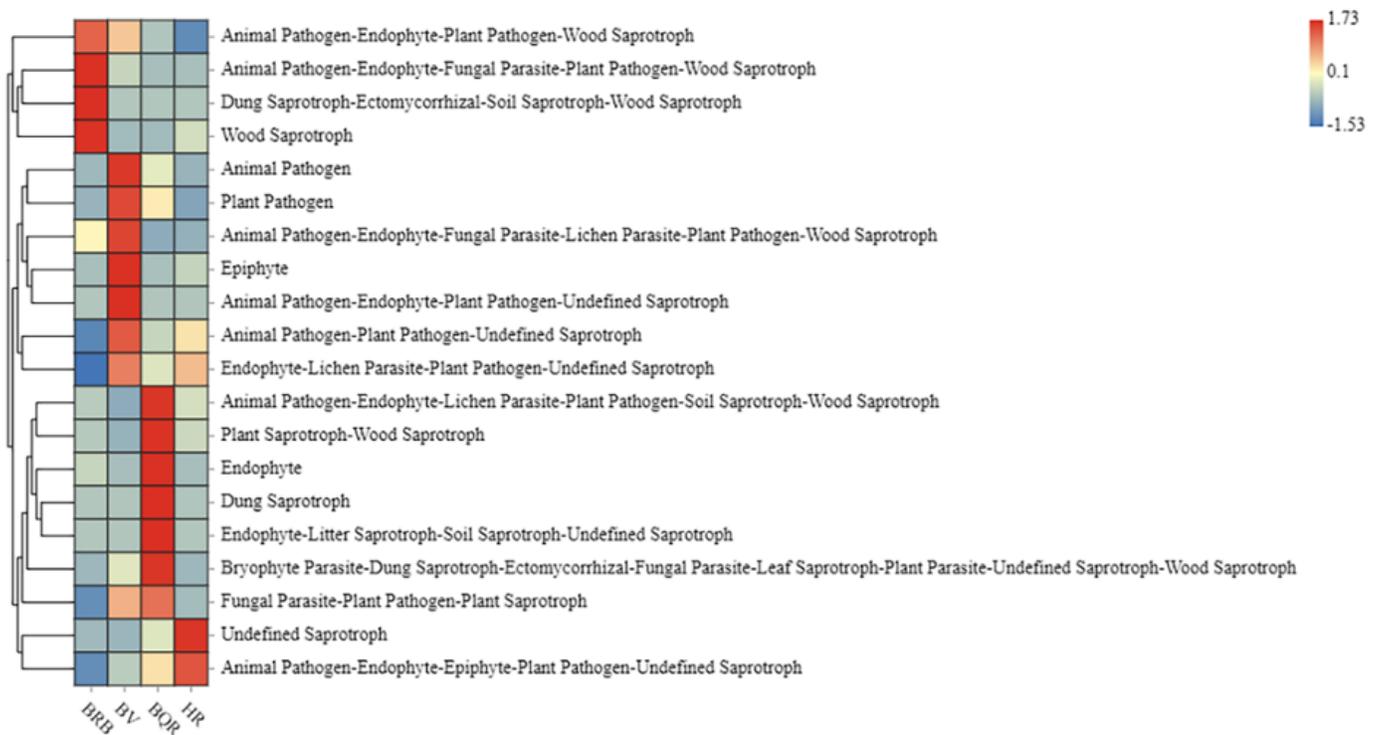


Figure 6

Heat map of representing functional abundance distribution of fungal communities at different stages of maturity

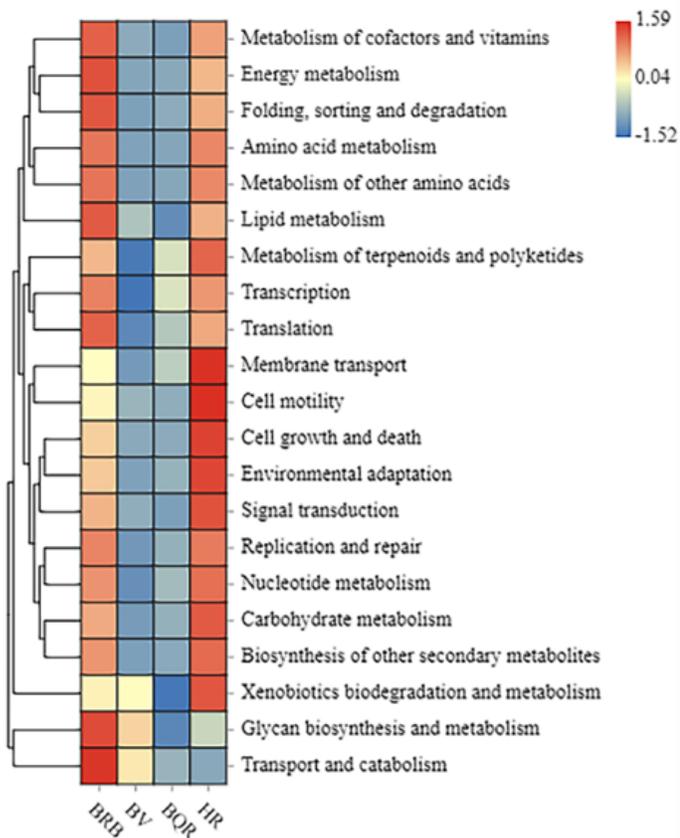


Figure 7

Heat map of representing functional abundance distribution of bacterial communities at different stages of maturity

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